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Anti-c-Met antibody drug conjugates

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ABSTRACT

The present disclosure provides c-Met antibody drug conjugates (ADCs), including compositions and methods of using such ADCs.

ANTI-C-MET ANTIBODY DRUG CONJUGATES

1. CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application is a divisional application of Australian Patent Application No. 2022267394, which is the national phase of International Patent Application No. PCT/US2022/072008, filed on 29 April 2022, which in turn claims priority from U.S. Provisional Application Ser. No. 63/181,963, filed 29 April 2021. The contents of each of the aforementioned applications are incorporated by cross reference in their entireties herein.

2. SEQUENCE LISTING

[0002] Preceding applications contained a Sequence Listing which was originally submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on April 28, 2022, is named 632WO_SL_ST25.txt and is 22,918 bytes in size. The present application contains a sequence listing which has been submitted electronically as an XML document in the ST.26 format and is hereby incorporated by reference in its entirety. Said XML copy, created on March 16, 2026, is named "P0066205AU – Sequence Listing.xml" and is 36,597 bytes in size.

3. TECHNICAL FIELD

[0003] The present application pertains to, among other things, novel topoisomerase inhibitor drugs, drug linkers, anti-c-Met antibody drug conjugates (ADCs), and methods of making the same.

4. BACKGROUND

[0004] c-Met is a signaling tyrosine kinase receptor expressed on the surface of epithelial and endothelial cells. Activation of c-Met by hepatocyte growth factor (HGF), its only known ligand, has been shown to control cell proliferation, angiogenesis, survival, and cellular motility.

[0005] Non-small cell lung cancer (NSCLC) represents 85% of all lung cancers and is the leading cause of cancer-related death worldwide. Aberrant c-Met signaling is common in NSCLC and is believed to occur via multiple mechanisms. Deregulated c-Met signaling has been associated with poor prognosis, tumorigenesis, resistance to chemotherapy/radiotherapy, and acquired resistance to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKI).

[0006] Antibody drug conjugates (ADCs) represent a relatively new class of therapeutics comprising an antibody conjugated to a cytotoxic drug via a chemical linker. The therapeutic concept of ADCs is to combine binding capabilities of an antibody with a drug, where the antibody is used to deliver the drug to a tumor cell by means of binding to a target surface antigen, including target surface antigens that are overexpressed or amplified in the tumor cells.

[0007] However, no antibody drug conjugates have been approved for the treatment of non-small cell lung cancer. There remains a need in the art for antibody drug conjugates that can be used for therapeutic purposes, such as in the treatment of non-small cell lung cancer.

5. SUMMARY

[0008] The present disclosure provides antibody drug conjugates that specifically bind to human c-Met. The amino acid sequences of exemplary CDRs, as well as the amino acid sequence of the V_H and V_L regions of the heavy and light chains of the antibody of exemplary anti-c-Met ADCs are provided in the Detailed Description below.

[0009] In another aspect, the present disclosure provides compositions including the anti-c-Met ADCs described herein. The compositions generally comprise one or more anti-c-Met ADC as described herein, and one or more excipients, carriers, or diluents.

[0010] The present disclosure provides methods of treating subjects, such as human subjects, having NSCLC comprising administering an effective amount of an anti-c-Met ADCs disclosed herein. An anti-c-Met ADC is typically administered as an intravenous infusion and/or injection.

6. BRIEF DESCRIPTION OF THE DRAWINGS

[0011] FIG. 1 shows a synthetic pathway for the topoisomerase I (TOP1) inhibitor of Formula I.

[0012] FIGS. 2A-2B show cell proliferation following administration of TOP1 inhibitor compounds for Calu-6 (lung adenocarcinoma; FIG. 2A) and A375 (malignant melanoma; FIG 2B).

[0013] FIGS. 3A-3J show in vitro activity of an ADC of the present disclosure (ADC-1) on cell lines, including (A) SNU-5 (human gastric carcinoma), (B) Hs 746T (gastric adenocarcinoma), (C) EBC1 (human lung squamous cell carcinoma), (D) NCI-H441 (human lung adenocarcinoma), (E) NCI-H1573 (lung adenocarcinoma), (F) HCC827 (lung adenocarcinoma), (G) NCI-H820 (lung papillary adenocarcinoma), (H) Calu-3 (lung adenocarcinoma), (I) MIA PaCa-2 (pancreatic ductal adenocarcinoma), and (J) HEK-293.

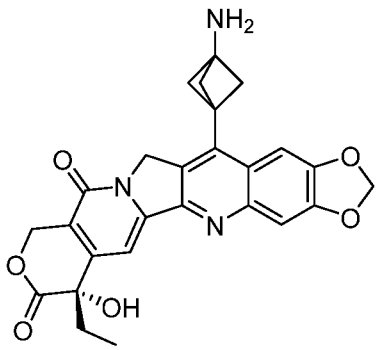
[0014] FIGS. 4A-4B show in vivo activity of ADC-1 in the NCI-H441 (human adenocarcinoma, FIG. 4A), and Hs 746T (gastric adenocarcinoma, FIG. 4B) xenograft tumor models.

7. DETAILED DESCRIPTION

[0015] Various aspects of the invention relate to topoisomerase I (TOP1) inhibitors and anti-c-Met antibody drug conjugates comprising such TOP1 inhibitors. In certain embodiments, the invention provides anti-c-Met ADCs, including anti-c-Met ADCs comprising TOP1 inhibitors, synthons useful for synthesizing the ADCs, methods of making the ADCs, and various methods of using the ADCs.

7.1. Topoisomerase 1 Inhibitors (TOP1i)

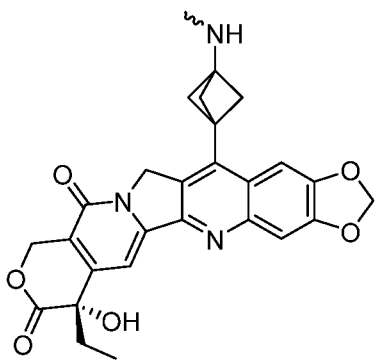
[0016] Topoisomerase 1 (TOP1) removes supercoils formed during DNA replication. TOP1 inhibitors (TOP1i) can bind and stabilize TOP1-DNA complexes, inducing DNA strand breakage and apoptosis. Presented herein is a topoisomerase I inhibitor drug ("TOP1i drug") according to structural formula (I), which may be purposed for targeted delivery to cells by conjugation to an antibody.



(I)

[0017] In embodiments, a TOP1i drug is a compound according to formula (I). In embodiments, the TOP1i drug is (7*S*)-14-(3-aminobicyclo[1.1.1]pentan-1-yl)-7-ethyl-7-hydroxy-2*H*,10*H*-[1,3]dioxolo[4,5-*g*]pyrano[3',4':6,7]indolizino[1,2-*b*]quinoline-8,11(7*H*,13*H*)-dione, or a structurally equivalent form thereof.

[0018] TOP1i drugs as contemplated herein may be conjugated to an antibody in an ADC as shown in structural formula (II):



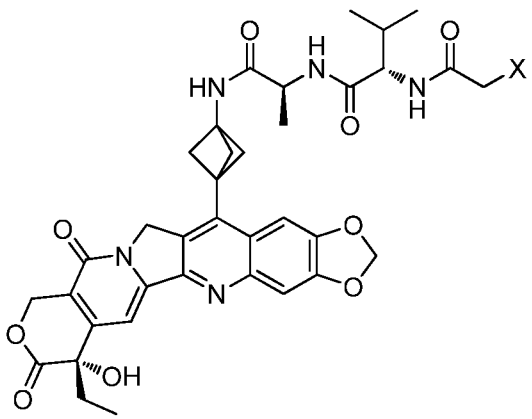
(II)

wherein  represents the point of attachment of a linker to the TOP1i drug.

7.2. Anti-c-Met ADCs

[0019] Topoisomerase inhibitors as described herein may be conjugated to an anti-c-Met antibody to form an anti-c-Met TOP1i antibody drug conjugate (ADC). Antibody-drug conjugates may increase the therapeutic efficacy of antibodies in treating disease due to the ability of the ADC to selectively deliver one or more drug moiety(s) to target tissues, such as a tumor-associated antigen, e.g., c-Met expressing tumors. Thus, in embodiments, the present disclosure provides anti-c-Met TOP1i ADCs for therapeutic use, e.g., in the treatment of non-small cell lung cancer.

[0020] In certain embodiments, a TOP1i drug is conjugated to an antibody by way of linker according to structural formula (III):



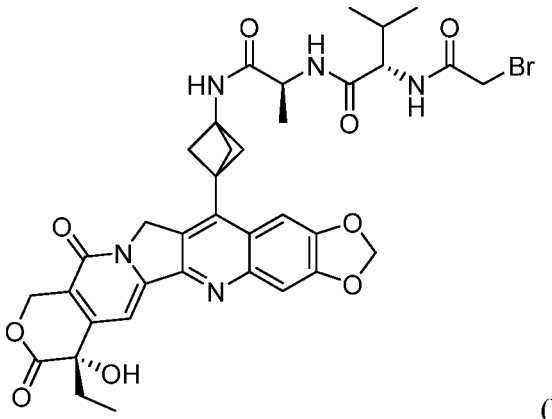
(III)

wherein X represents bromo or an N-linked maleimide.

[0021] In the anti-c-Met ADCs described herein, TOP1i drugs are conjugated to the anti-c-Met antibody by way of a linker moiety. As will be appreciated by skilled artisans, the linkers connect the TOP1i drug to the anti-c-Met antibody by forming a covalent linkage to the TOP1i drug at one location and a covalent linkage to the antibody at another. The covalent linkages are formed by reaction between functional groups on the linker and functional groups on the TOP1i drug and the anti-c-Met antibody.

[0022] Synthetic intermediate compounds that may be used to form ADCs may include:

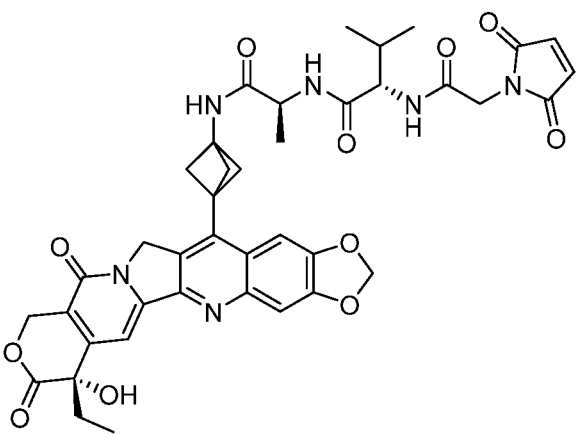
7.2.1. Linker Drug LD1 (Structural Formula (V))



(V)

(2*S*)-2-(2-bromoacetamido)-*N*-[(2*S*)-1-({3-[(7*S*)-7-ethyl-7-hydroxy-8,11-dioxo-7,8,11,13-tetrahydro-2*H*,10*H*-[1,3]dioxolo[4,5-*g*]pyrano[3',4':6,7]indolizino[1,2-*b*]quinolin-14-yl]bicyclo[1.1.1]pentan-1-yl}amino)-1-oxopropan-2-yl]-3-methylbutanamide

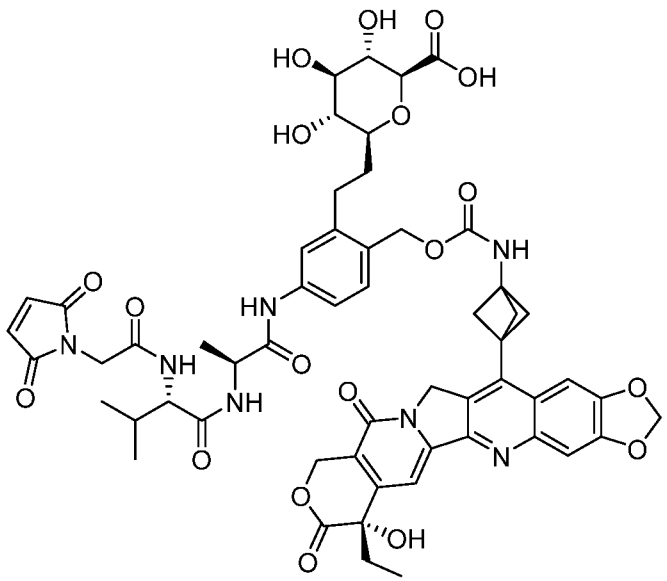
7.2.2. Linker Drug LD2 (Structural Formula (VI))



(VI)

(2*S*)-2-[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)acetamido]-*N*-[(2*S*)-1-({3-[(7*S*)-7-ethyl-7-hydroxy-8,11-dioxo-7,8,11,13-tetrahydro-2*H*,10*H*-[1,3]dioxolo[4,5-*g*]pyrano[3',4':6,7]indolizino[1,2-*b*]quinolin-14-yl]bicyclo[1.1.1]pentan-1-yl}amino)-1-oxopropan-2-yl]-3-methylbutanamide

7.2.3. Linker Drug LD3 (Structural Formula (VII))



(VII)

(2*S*,3*S*,4*R*,5*R*,6*S*)-6-[2-(5-{[(2*S*)-2-({(2*S*)-2-[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)acetamido]-3-methylbutanoyl}amino)propanoyl]amino}-2-{[({3-[(7*S*)-7-ethyl-7-hydroxy-8,11-dioxo-7,8,11,13-tetrahydro-2*H*,10*H*-[1,3]dioxolo[4,5-*g*]pyrano[3',4':6,7]indolizino[1,2-*b*]quinolin-14-yl]bicyclo[1.1.1]pentan-1-yl}carbamoyl)oxy]methyl}phenyl)ethyl]-3,4,5-trihydroxyoxane-2-carboxylic acid

7.2.4. Number of Linked Drugs

[0023] The ADCs disclosed herein comprise drug molecules linked to antibody moieties in various stoichiometric molar ratios depending on the configuration of the antibody and, at least in part, the method used to effect conjugation.

[0024] The terms "drug load" or "drug loading" refer to the number of drug molecules per antibody in an individual ADC molecule. The number of TOP1i drugs linked to an anti-c-Met ADC can vary and will be limited by the number of available attachments sites on the anti-c-Met antibody. As contemplated for the anti-c-Met ADCs of the invention, the linker will link a single TOP1i drug to the antibody an anti-c-Met ADC. As long as the anti-c-Met ADC does not exhibit unacceptable levels of aggregation under the conditions of use and/or storage, anti-c-Met ADCs (i.e., structural formula (III)) having an *n* of up to 10 are contemplated. In some embodiments, the anti-c-Met ADCs have an *n* of in the range of from 1-10. In some embodiments, the anti-c-Met ADCs have an *n* selected from

1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In embodiments, *n* is 2, 4, 6, 8, or 10. In embodiments, n is 6. In embodiments, the drug loading may comprise 1 drug molecule, 2 drug molecules, 3 drug molecules, 4 drug molecules, 5 drug molecules, 6 drug molecules, 7 drug molecules, 8 drug molecules, 9 drug molecules, or 10 drug molecules.

7.2.5. Antibody AbA

[0025] AbA is a humanized version of mouse monoclonal antibody 224G11, which was first disclosed and embodied in U.S. Patent No. 8,329,173. The murine antibody m224G11 has a variable heavy domain set forth as SEQ ID NO: 13 and a variable light domain set forth as SEQ ID NO: 14:

m224G11 Heavy Chain Variable Domain (CDRs underlined, and set forth as SEQ ID NOs: 15, 16, and 17)

EVQLQQSGPELVKPGASVKISCKTSGYIFTAYTMHWVRQSLGESLDWIGGIKPNNGLAN YNQKFKGKATLTVDKSSSTAYMDLRSLTSEDSAVYYCARSEITTEFDYWGQGTALTVSS (SEQ ID NO: 13)

m224G11 Light Chain Variable Domain (CDRs underlined, and set forth as SEQ ID NOs: 18, 19, and 20):

DIVLTQSPASLAVSLGQRATISCRASESVDSYANSFMHWYQQKPGQPPKLLIYRASNLES GIPARFSGSGSRTDFTLTINPVEADDVATYYCQQSKEDPLTFGSGTKLEMK (SEQ ID NO: 14)

[0026] AbA is a humanized recombinant IgG1κ (disclosed as 224G11 [TH7 Hz3] in U.S. Patent No. 8,741,290) that targets a unique epitope of c-Met located within the immunoglobulin-plexin-transcription factor homology (IPT) domain 1, resulting in blockade of both HGF-dependent and HGF-independent c-Met signaling.

[0027] As defined under the IMGT nomenclature, the CDR sequences of AbA comprise the following sequences:

CDR-H1: GYIFTAYT (SEQ ID NO: 1)
CDR-H2: IKPNNGLA (SEQ ID NO: 2)
CDR-H3: ARSEITTEFDY (SEQ ID NO: 3)
CDR-L1: ESVDSYANSF (SEQ ID NO: 4)
CDR-L2: RAS (SEQ ID NO: 5)
CDR-L3: QQSKEDPLT (SEQ ID NO: 6)

[0028] In some embodiments, the anti-c-Met antibodies composing an ADC of this disclosure comprise a CDR-H1 having the amino acid sequence shown as SEQ ID NO: 1, a CDR-H2 having the amino acid sequence shown as SEQ ID NO: 2; a CDR-H3 having the amino acid sequence shown as SEQ ID NO: 3, a CDR-L1 having the amino acid sequence shown as SEQ ID NO: 4, a CDR-L2 having the amino acid sequence shown as SEQ ID NO: 5; and a CDR-L3 having the amino acid sequence shown as SEQ ID NO: 6.

[0029] In some embodiments, the anti-c-Met antibodies composing an ADC of this disclosure comprise a heavy chain variable region comprising the amino acid sequence shown as SEQ ID NO: 7:

QVQLVQSGAEVKKPGASVKVSCKASGYIFTAYTMHWVRQAPGQGLEWMGWIKPNNGLAN
YAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARSEITTEFDYWGQGTLVTVSS
(SEQ ID NO: 7);

and a light chain variable region comprising the amino acid sequence shown as SEQ ID NO: 8:
DIVMTQSPDSLAVSLGERATINCKSSESVDSYANSFLHWYQQKPGQPPKLLIYRASTRESGVP
DRFSGSGSGTDFTLTISSLQAEDVAVYYCQQSKEDPLTFGGGTKVEIK (SEQ ID NO: 8).

[0030] In some embodiments, the anti-c-Met antibodies composing an ADC of this disclosure comprise a heavy chain comprising the amino acid sequence shown as SEQ ID NO: 9 (constant regions are **bold**; CDRs are underlined (disclosed as SEQ ID NOS: 1-3, respectively, in order of appearance)):

QVQLVQSGAE VKKPGASVKV SCKASGYIFT AYTMHWVRQA PGQGLEWMGW 050
IKPNNGLANY AQKFQGRVTM TRDTSISTAY MELSRLRSDD TAVYYCARSE 100
ITTEFDYWGQ GTLVTVSS**AS TKGPSVFPLA PSSKSTSGGT AALGCLVKDY 150**
FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTQTYI 200
CNVNHKPSNT KVDKRVEPKS CDCHCPPCPA PELLGGPSVF LFPPKPKDTL 250
MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR 300
VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG QPREPQVYTL 350
PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDSD 400
GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPG 445
(full-length sequence disclosed as SEQ ID NO: 9)

and a light chain comprising the amino acid sequence shown as SEQ ID NO: 10 (CDR sequences disclosed as SEQ ID NOS: 4-6, respectively, in order of appearance):

DIVMTQSPDS LAVSLGERAT INCKSSESVD SYANSFLHWY QQKPGQPPKL 050
LIYRASTRES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQSKEDPL 100

IFGGGTKVEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV 150
QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV 200
THQGLSSPVT KSFNRGEC 218

(full-length sequence disclosed as SEQ ID NO: 10).

[0031] In embodiments, an antibody of the present disclosure comprises a heavy chain of SEQ ID NO: 9 and a light chain of SEQ ID NO: 10.

[0032] In one embodiment, the heavy chain of an anti-c-Met antibody composing an ADC of this disclosure is encoded by the following nucleotide sequence (full-length sequence disclosed as SEQ ID NO: 21):

ATGGGATGGTCTTGGATCTTTCTGCTGTTTCTGTCTGGTACTGCTGGTGTGCTGAG
Ccaggtccagctggtgcaatccggcgcagaggtgaagaagccaggcgcttccgtgaaggtgagctgtaaggcctctggctacatcttcacagca
tacaccatgcactgggtgaggcaagctcctgggcagggactggagtggatgggatggattaaacccaacaatgggctggccaactacgcccag
aaattccagggtagggtcactatgacaagggataccagcatcagcaccgcatatatggagctgagcaggctgaggtctgacgacactgctgtctat
tattgcgccaggagcgaaattacaacagaattcgattactgggggcagggcaccctggtgaccgtgtcctct**gccagcaccaagggcccaagc**
gtgttccccctggcccccagcagcaagagcaccagcggcggcacagccgccctgggctgcctggtgaaggactacttccccgagcccgt
gaccgtgtcctggaacagcggagccctcacttctggagttcataccttcccagcagtattgcagagcagtggcctgtattcactgtcttccgt
cgtaacagttccatcctccagcctcgggacacagacttacatttgtaacgtgaatcacaagcctagcaacaccaaggtcgacaagagagt
tgaaccaaagagttgtgattgccactgtcctccctgcccagctcctgagctgcttggcggtcccagtgtcttcttgtttccccctaaacccaaa
gacaccctgatgatctcaaggactcccgaggtgacatgcgtggtggtggatgtgtctcatgaggacccagaggtgaagttcaactggtac
gtggacggcgtggaggtgcacaacgccaagaccaagcccagagaggagcagtacaacagcacctacagggtggtgtccgtgctgacc
gtgctgcaccaggactggctgaacggcaaggagtacaagtgtaaggtgtccaacaaggccctgccagccccaatcgaaaagaccatca
gcaaggccaagggccagccaagagagccccaggtgtacaccctgccacccagcagggaggagatgaccaagaaccaggtgtccctga
cctgtctggtgaagggcttctacccaagcgacatcgccgtggagtgggagagcaacggccagcccgagaacaactacaagaccacccc
cccagtgctggacagcgacggcagcttcttcctgtacagcaagctgaccgtggacaagagcagatggcagcagggcaacgtgttcagct
gctccgtgatgcacgaggccctgcacaaccactacacccagaagagcctgagcctgtccccaggctga (SEQ ID NO: 21)

Secretion signal peptide in **bold CAPITAL letters**; includes final stop codon (TGA); constant region is **bold;** CDRs are underlined (CDR sequences disclosed as SEQ ID NOS: 22-24, respectively, in order of appearance)

[0033] In one embodiment, the light chain of an anti-c-Met antibody composing an ADC of this disclosure is encoded by the following nucleotide sequence (full-length sequence disclosed as SEQ ID NO: 25):

ATGGAAACTGATACACTGCTGCTGTGGGTCCTGCTGCTGTGGGTCCCTGGAAGCACAGGGgacattgtgatgac
ccagtctcccgatagcctggccgtgtccctgggcgagagggctaccatcaactgtaaaagctccgaatctgtgg
actcttacgcaaacagctttctgcactggtatcagcaaaagccaggccaacctccaaagctgctgatttacagg
gcttctaccagggagagcggcgtgcccgataggttcagcggatctggcagcggcaccgactttacactgaccat

ctccagcctgcaggccgaagatgtggcagtctattactgc<u>cagcagtccaaggaggacccctgact</u>ttcgggg
gtggtactaaagtggagatcaag**cgtacggtggccgctcccagcgtgttcatcttcccccaagcgacgagcag**
ctgaagagcggcaccgccagcgtggtgtgtctgctgaacaacttctaccccagggaggccaaggtgcagtggaa
ggtggacaacgccctgcagagcggcaacagccaggagagcgtcaccgagcaggacagcaaggactccacctaca
gcctgagcagcaccctgaccctgagcaaggccgactacgagaagcacaaggtgtacgcctgtgaggtgacccac
cagggcctgtccagccccgtgaccaagagcttcaacaggggcgagtgctga (SEQ ID NO: 25)

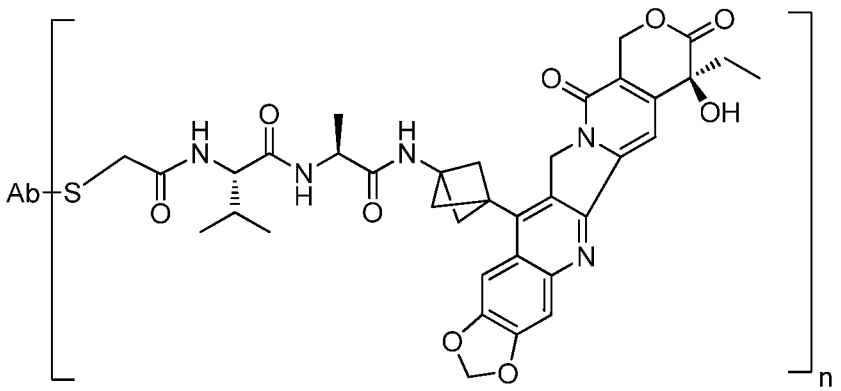
Secretion signal peptide in **bold CAPITAL** letters; includes final stop codon (TGA); constant region is **bold**; CDRs are underlined (CDR sequences disclosed as SEQ ID NOS: 26-28, respectively, in order of appearance).

7.2.6. Exemplary Anti-c-Met ADCs

[0034] In particular embodiments, anti-c-Met ADCs of the invention comprise an anti-c-Met antibody comprising six complementarity determining regions (CDRs) corresponding to the CDRs of antibody AbA, which is conjugated to a TOP1i drug through a cleavable valine alanine (va) linker. In certain embodiments, the linker comprises a bromoacetamide functional group for conjugation to a sulfhydryl of a reduced cysteine from the anti-c-Met antibody of the ADC. In other embodiments, the linker comprises a maleimide functional group for conjugation to a sulfhydryl of a reduced cysteine from the anti-c-Met antibody of the ADC. In some embodiments, the linker further comprises a self-immolating spacer, preferably p-aminobenzylcarbonyl (PABC) or an analogue thereof.

[0035] In embodiments, an anti-c-Met ADC comprises an anti-c-Met antibody comprising six complementarity determining regions (CDRs) corresponding to the CDRs of antibody AbA, which is conjugated to linker drug LD1 (structural formula (V)) via a linkage formed with a sulfhydryl group of a cysteine residue of the anti-c-Met antibody. In some embodiments, the anti-c-Met antibody comprises the variable heavy (VH) chain and variable light (VL) chain region sequences of antibody AbA. In some embodiments, the anti-c-Met antibody comprises the heavy chain (HC) and light chain (LC) sequences of antibody AbA.

[0036] In embodiments, an anti-c-Met ADC has the following structural formula (VIII):



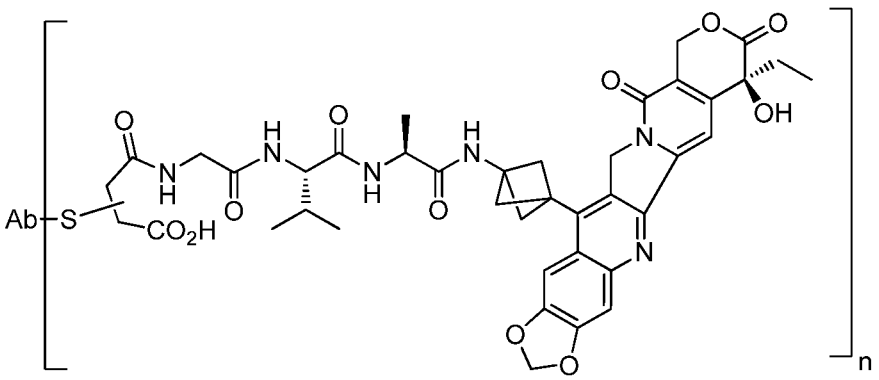
(VIII)

wherein n is an integer from 1-10, and wherein Ab is an IgG_1 anti-c-Met antibody comprising a heavy chain CDR1 shown as SEQ ID NO: 1, a heavy chain CDR2 shown as SEQ ID NO: 2, a heavy chain CDR3 shown as SEQ ID NO: 3, a light chain CDR1 shown as SEQ ID NO: 4, a light chain CDR2 shown as SEQ ID NO: 5, and a light chain CDR3 shown as SEQ ID NO: 6. In embodiments, the antibody Ab is an IgG_1 anti-c-Met antibody comprising a heavy chain variable region comprising the amino acid sequence shown as SEQ ID NO: 7 and a light chain variable region comprising the amino acid sequence shown as SEQ ID NO: 8. In embodiments, the antibody Ab is an anti-c-Met antibody comprising a heavy chain comprising the amino acid sequence shown as SEQ ID NO: 9 and a light chain comprising the amino acid sequence shown as SEQ ID NO: 10. In embodiments, conjugation of the linker-drug to the antibody is via a linkage formed with a sulfhydryl group of a cysteine residue of the antibody. In embodiments, n has a value of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In embodiments, n has a value of 2, 4, 6, 8, or 10. In embodiments, n is 6. In an embodiment, anti-c-Met ADC of structural formula (VIII) comprises an antibody Ab having a heavy chain comprising the amino acid sequence shown as SEQ ID NO: 9 and a light chain comprising the amino acid sequence shown as SEQ ID NO: 10 and n has a value of 2, 4, 6, 8, or 10. In an embodiment, anti-c-Met ADC of structural formula (VIII) comprises an antibody Ab having a heavy chain comprising the amino acid sequence shown as SEQ ID NO: 9 and a light chain comprising the amino acid sequence shown as SEQ ID NO: 10 and n has a value of 6.

[0037] In certain embodiments, an anti-c-Met ADC comprises an anti-c-Met antibody comprising six complementarity determining regions (CDRs) corresponding to the CDRs of antibody AbA, which is conjugated to linker drug LD2 (structural formula (VI)) via a linkage formed with a sulfhydryl group of a cysteine residue of the anti-c-Met antibody. In some embodiments, the anti-c-Met antibody

comprises the variable heavy (VH) chain and variable light (VL) chain region sequences of antibody AbA. In some embodiments, the anti-c-Met antibody comprises the heavy chain (HC) and light chain (LC) sequences of antibody AbA.

[0038] In some embodiments, an anti-c-Met ADC has the following structural formula (IX):



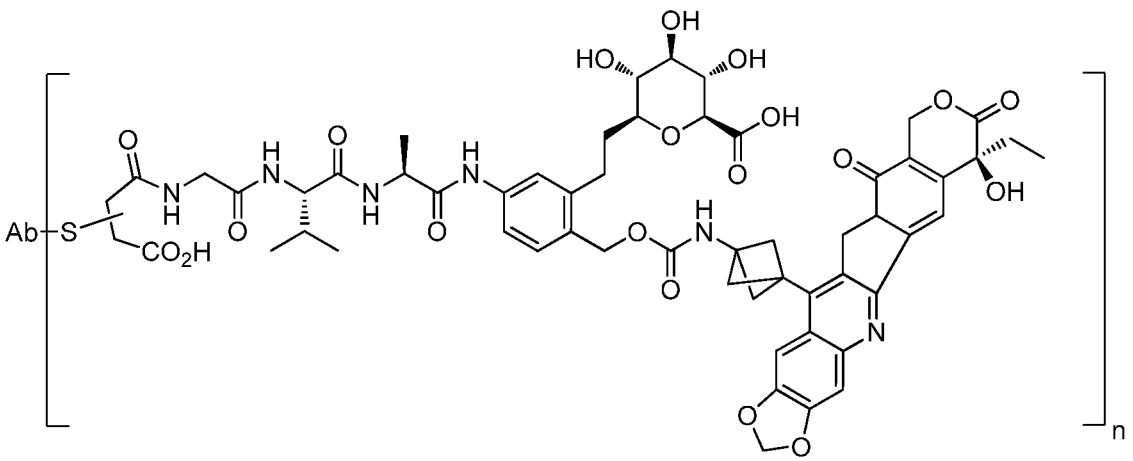
(IX)

wherein n is an integer from 1-10, and wherein Ab is an IgG_1 anti-c-Met antibody comprising a heavy chain CDR1 shown as SEQ ID NO: 1, a heavy chain CDR2 shown as SEQ ID NO: 2, a heavy chain CDR3 shown as SEQ ID NO: 3, a light chain CDR1 shown as SEQ ID NO: 4, a light chain CDR2 shown as SEQ ID NO: 5, and a light chain CDR3 shown as SEQ ID NO: 6. In embodiments, the antibody Ab is an IgG_1 anti-c-Met antibody comprising a heavy chain variable region comprising the amino acid sequence shown as SEQ ID NO: 7 and a light chain variable region comprising the amino acid sequence shown as SEQ ID NO: 8. In embodiments, the antibody Ab is an anti-c-Met antibody comprising a heavy chain comprising the amino acid sequence shown as SEQ ID NO: 9 and a light chain comprising the amino acid sequence shown as SEQ ID NO: 10. In embodiments, conjugation of the linker-drug to the antibody is via a linkage formed with a sulfhydryl group of a cysteine residue of the antibody. In embodiments, n has a value of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In embodiments, n has a value of 2, 4, 6, 8, or 10. In embodiments, n is 6. In an embodiment, anti-c-Met ADC of structural formula (IX) comprises an antibody Ab having a heavy chain comprising the amino acid sequence shown as SEQ ID NO: 9 and a light chain comprising the amino acid sequence shown as SEQ ID NO: 10, and n has a value of 2, 4, 6, 8, or 10. In an embodiment, anti-c-Met ADC of structural formula (IX) comprises an antibody Ab having a heavy chain comprising the amino acid sequence

shown as SEQ ID NO: 9 and a light chain comprising the amino acid sequence shown as SEQ ID NO: 10 and n has a value of 6.

[0039] In certain embodiments, an anti-c-Met ADC comprises an anti-c-Met antibody comprising six complementarity determining regions (CDRs) corresponding to the CDRs of antibody AbA, which is conjugated to linker drug LD3 (structural formula (VII)) via a linkage formed with a sulfhydryl group of a cysteine residue of the anti-c-Met antibody. In some embodiments, the anti-c-Met antibody comprises the variable heavy (VH) chain and variable light (VL) chain region sequences of antibody AbA. In some embodiments, the anti-c-Met antibody comprises the heavy chain (HC) and light chain (LC) sequences of antibody AbA.

[0040] In some embodiments, an anti-c-Met ADC has the following structural formula (X):



(X)

[0041] wherein n is an integer from 1-10, and wherein Ab is an IgG_1 anti-c-Met antibody comprising a heavy chain CDR1 shown as SEQ ID NO: 1, a heavy chain CDR2 shown as SEQ ID NO: 2, a heavy chain CDR3 shown as SEQ ID NO: 3, a light chain CDR1 shown as SEQ ID NO: 4, a light chain CDR2 shown as SEQ ID NO: 5, and a light chain CDR3 shown as SEQ ID NO: 6. In embodiments, the antibody Ab is an IgG_1 anti-c-Met antibody comprising a heavy chain variable region comprising the amino acid sequence shown as SEQ ID NO: 7 and a light chain variable region comprising the amino acid sequence shown as SEQ ID NO: 8. In embodiments, the antibody Ab is an anti-c-Met antibody comprising a heavy chain comprising the amino acid sequence shown as SEQ ID NO: 9 and a light chain comprising the amino acid sequence shown as SEQ ID NO: 10. In

embodiments, conjugation of the linker-drug to the antibody is via a linkage formed with a sulfhydryl group of a cystine residue of the antibody. In embodiments, n has a value of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In embodiments, n has a value of 2, 4, 6, 8, or 10. In embodiments, n is 6. In an embodiment, anti-c-Met ADC of structural formula (X) comprises an antibody Ab having a heavy chain comprising the amino acid sequence shown as SEQ ID NO: 9 and a light chain comprising the amino acid sequence shown as SEQ ID NO: 10 and n has a value of 2, 4, 6, 8, or 10. In an embodiment, anti-c-Met ADC of structural formula (X) comprises an antibody Ab having a heavy chain comprising the amino acid sequence shown as SEQ ID NO: 9 and a light chain comprising the amino acid sequence shown as SEQ ID NO: 10 and n has a value of 6.

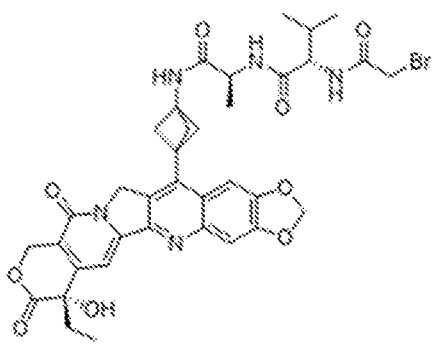
[0042] The ADCs of this disclosure may be provided as a composition suitable for administration to a subject. In some embodiments, the ADC composition is a pharmaceutical composition, comprising an ADC of this disclosure and a pharmaceutically acceptable carrier. A given formulation of the ADCs disclosed herein may comprise a distribution of antibodies having differing drug loading, i.e., differing values of *n*.

7.3. Methods of Use

[0043] In embodiments, the methods described herein involve treating patients who have non-squamous NSCLC with the anti-c-Met ADCs of the invention.

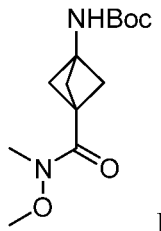
8. EXAMPLES

[0044] The following Examples, which highlight certain features and properties of the exemplary embodiments of the antibodies and binding fragments described herein are provided for purposes of illustration.



Example 1

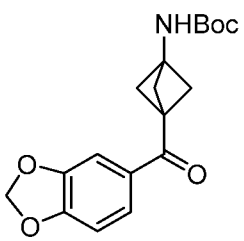
(2*S*)-2-(2-bromoacetamido)-*N*-[(2*S*)-1-({3-[(7*S*)-7-ethyl-7-hydroxy-8,11-dioxo-7,8,11,13-tetrahydro-2*H*,10*H*-[1,3]dioxolo[4,5-*g*]pyrano[3',4':6,7]indolizino[1,2-*b*]quinolin-14-yl]bicyclo[1.1.1]pentan-1-yl}amino)-1-oxopropan-2-yl]-3-methylbutanamide



Example 1A

tert-butyl (3-(methoxy(methyl)carbamoyl)bicyclo[1.1.1]pentan-1-yl)carbamate

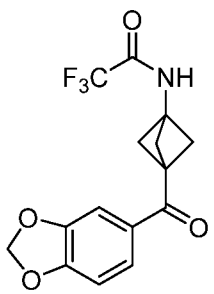
[0045] To a solution of 3-((*tert*-butoxycarbonyl)amino)bicyclo[1.1.1]pentane-1-carboxylic acid (4.9 g), *N*,*O*-dimethylhydroxylamine hydrochloride (2.2 g) and *N*,*N*-diisopropylethylamine (11.30 mL) in dichloromethane (10 mL) was added 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo [4,5-*b*]pyridinium 3-oxide hexafluorophosphate (8.61 g) in portions at 10 °C. The reaction mixture was stirred at 20 °C for 12 hours. Two additional reactions were set up and allowed to stir at 20 °C for 12 hours as described. All three reactions were combined. The reaction was diluted with dichloromethane (200 mL) and added to 1 N aqueous HCl (50 mL). The precipitate formed was filtered and the filtrate was allowed to separate. The organic layer was washed with saturated aqueous sodium bicarbonate solution (50 mL) and brine (50 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by chromatography on silica gel, eluting with 1-50% ethyl acetate in petroleum ether to give the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.97 (br s, 1H), 3.66 (s, 3H), 3.18 (s, 3H), 2.34 (s, 6H), 1.45 (s, 9H). MS (ESI+) *m/z* 271.2 $(M+H)^+$.



Example 1B

tert-butyl (3-(benzo[*d*][1,3]dioxole-5-carbonyl)bicyclo[1.1.1]pentan-1-yl)carbamate

[0046] To a solution of 5-bromobenzo[*d*][1,3]dioxole (8.83 g) in tetrahydrofuran (100 mL) was added *n*-butyllithium (17.57 mL, 2.5 M in hexane) slowly at -65 °C under nitrogen gas. The mixture was stirred at -65 °C for 30 minutes. A solution of Example 1A (4.75 g) in tetrahydrofuran (40 mL) was added slowly. The mixture was stirred at -65 °C for 3 hours. Three additional reactions were set up and allowed to stir at -65 °C for 3 hours. All four reactions were combined. The mixture was quenched with saturated aqueous ammonium chloride solution (500 mL) and extracted with ethyl acetate (3 × 500 mL). The combined organic layers were washed with brine (500 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by chromatography on silica gel, eluting with 1-50% ethyl acetate in petroleum ether to give the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.63 (dd, 1H), 7.45 (d, 1H), 6.82 (d, 1H), 6.03 (s, 2H), 5.04 (br s, 1H), 2.50 (s, 6H), 1.46 (s, 9H). MS (ESI+) *m/z* 354.2 $(M+Na)^+$.



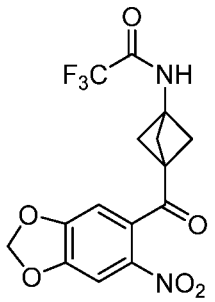
Example 1C

N-(3-(benzo[*d*][1,3]dioxole-5-carbonyl)bicyclo[1.1.1]pentan-1-yl)-2,2,2-trifluoroacetamide

[0047] Step 1: To a solution of Example 1B (6.2 g) in dichloromethane (62 mL) was added trifluoroacetic acid (62 mL) slowly at 0 °C. The reaction was stirred at 25 °C for 4 hours. Two additional reactions were set up and stirred at 25 °C for 4 hours. Each mixture was concentrated under reduced pressure. Each residue was used in the next step without further purification.

[0048] Step 2: To a solution of crude product above in dichloromethane (62 mL) was added *N*,*N*-diisopropylethylamine (16.34 mL) and trifluoroacetic anhydride (3.96 mL) dropwise at 0 °C. The mixture was stirred at 25 °C for 2 hours. Two additional reactions were set up as described and stirred at 25 °C for 2 hours. All three reactions were combined and poured into water (200 mL), extracted with dichloromethane (2 × 200 mL). The organic layer was washed with brine (200 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with 25% ethyl acetate in petroleum

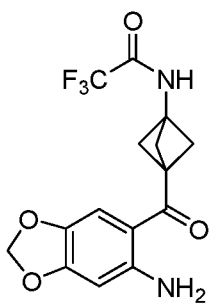
ether to give the title compound. ^{1}H NMR (501 MHz, $CDCl_3$) δ ppm 7.61 (dd, 1H), 7.43 (d, 1H), 7.00 (s, 1H), 6.85 (d, 1H), 6.05 (s, 2H), 2.62 (s, 6H). MS (ESI+) *m/z* 328.2 $(M+H)^+$.



Example 1D

2,2,2-trifluoro-*N*-(3-(6-nitrobenzo[*d*][1,3]dioxole-5-carbonyl)bicyclo[1.1.1]pentan-1-yl)acetamide

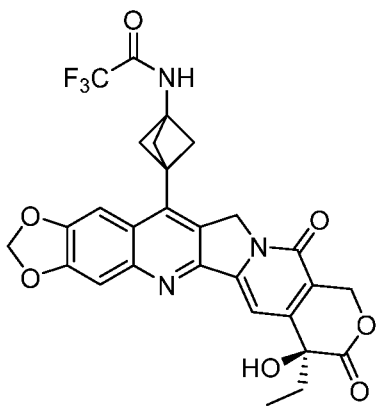
[0049] To a solution of Example 1C (4.3 g) in acetic anhydride (25 mL) was added copper(II) nitrate trihydrate (4.76 g) in portions at 0 °C. The mixture was stirred at 0 °C for 3 hours. Three additional reactions were set up and stirred at 0 °C for 3 hours as described. All four reactions were combined. The mixture was poured into water (50 mL) and extracted with ethyl acetate (5 × 100 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, concentrated under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with 75% ethyl acetate in petroleum ether to give the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.61 (s, 1H), 6.77 (br s, 1H), 6.64 (s, 1H), 6.21 (s, 2 H), 2.43 (s, 6 H). MS (APCI+) *m/z* 373.1 $(M+H)^+$.



Example 1E

N-(3-(6-aminobenzo[*d*][1,3]dioxole-5-carbonyl)bicyclo[1.1.1]pentan-1-yl)-2,2,2-trifluoroacetamide

[0050] To a solution of Example 1D (4 g) in ethanol (40 mL) and water (8 mL) was added iron (5.4 g) and ammonium chloride (5.17 g) under nitrogen. The mixture was stirred at 100 °C for 3 hours. Three additional reactions were set up and stirred at 100 °C for 3 hours as described. After cooling to ambient temperature, all four reactions were combined. The mixture was poured into water (1 L) and extracted with ethyl acetate (5 × 500 mL). The combined organic phases were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with 10-75% ethyl acetate in petroleum ether to give the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.22 (s, 1H), 6.70 (s, 1H), 6.50 (s, 2H), 6.14 (s, 1H), 5.92 (s, 2H), 2.63 (s, 6H). MS (ESI+) *m/z* 343.2 $(M+H)^+$.

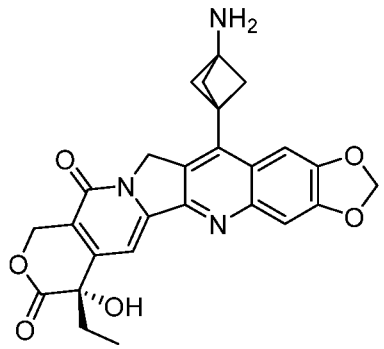


Example 1F

(*S*)-*N*-(3-(7-ethyl-7-hydroxy-8,11-dioxo-8,10,11,13-tetrahydro-7*H*-[1,3]dioxolo[4,5-*g*]pyrano[3',4':6,7]indolizino[1,2-*b*]quinolin-14-yl)bicyclo[1.1.1]pentan-1-yl)-2,2,2-trifluoroacetamide

[0051] To a suspension of Example 1E (3.5 g) and (*S*)-4-ethyl-4-hydroxy-7,8-dihydro-1*H*-pyrano[3,4-*f*]indolizine-3,6,10(4*H*)-trione (2.69 g) in toluene (140 mL) was added *para*-toluenesulfonic acid monohydrate (1.945 g). The mixture was stirred at 115 °C for 12 hours. Three additional reactions were set up and stirred at 115 °C for 12 hours as described. After cooling to ambient temperature, all four reactions were combined. The mixture was filtered and the solid collected was triturated with acetonitrile (200 mL) to give the title compound. ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 10.28 (s, 1H), 7.64 (s, 1H), 7.52 (s, 1H), 7.24 (s, 1H), 6.47 (s, 1H), 6.30

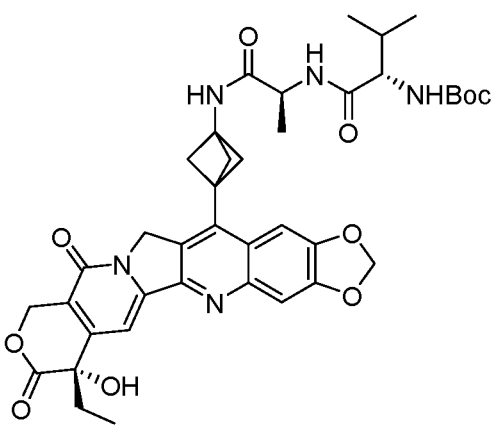
(dd, 2H), 5.42 (s, 2H), 5.36 (s, 2H), 2.87 (s, 6H), 1.94 – 1.77 (m, 2H), 0.88 (t, 3H). MS (ESI+) *m/z* 570.3 $(M+H)^+$.



(I)

Example 1G: (7*S*)-14-(3-aminobicyclo[1.1.1]pentan-1-yl)-7-ethyl-7-hydroxy-2*H*,10*H*-[1,3]dioxolo[4,5-*g*]pyrano[3',4':6,7]indolizino[1,2-*b*]quinoline-8,11(7*H*,13*H*)-dione

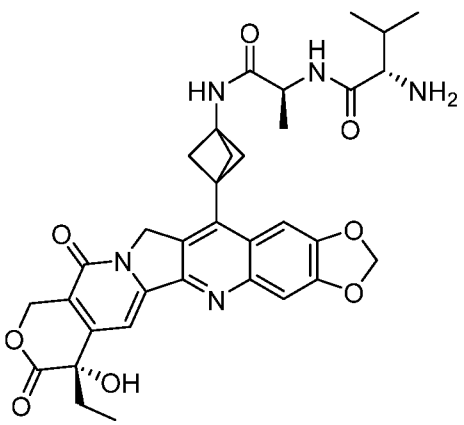
[0052] To a solution of Example 1F (3 g) in methanol (30 mL) was added HCl (60 mL, 4 M in methanol). The mixture was stirred at 65 °C for 4 hours. Four additional reactions were set up and stirred at 65 °C for 4 hours as described above. After cooling to ambient temperature, all five reactions were combined. The mixture was concentrated under reduced pressure and the residue was triturated with methanol (200 mL) to give the title compound. ^{1}H NMR (400 MHz, dimethyl sulfoxide -d_6) δ ppm 9.23 (s, 3H), 7.54 (s, 1H), 7.51 (s, 1H), 7.24 (s, 1H), 6.30 (d, 2H), 5.41 (s, 2H), 5.39 – 5.26 (m, 2H), 2.79 (s, 6H), 1.87 (hept, 2H), 0.88 (t, 3H). MS (ESI+) *m/z* 474.3 $(M+H)^+$.



Example 1H

tert-butyl ((*S*)-1-(((*S*)-1-((3-((*S*)-7-ethyl-7-hydroxy-8,11-dioxo-8,10,11,13-tetrahydro-7*H*-[1,3]dioxolo[4,5-*g*]pyrano[3',4':6,7]indolizino[1,2-*b*]quinolin-14-yl)bicyclo[1.1.1]pentan-1-yl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate

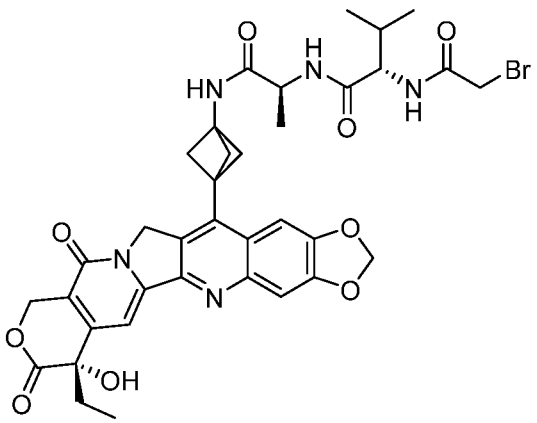
[0053] To a suspension of (*S*)-2-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methylbutanamido)propanoic acid (2.49 g), 2-hydroxypyridine 1-oxide (1.31 g), and N^1-((ethylimino)methylene)-N^3,N^3-dimethylpropane-1,3-diamine hydrochloride (2.26 g) in acetonitrile (40 mL) was added 2,6-lutidine (2.74 mL). The mixture was stirred at ambient temperature for 30 minutes. In a separate flask, Example 1G (4 g) and 2,6-lutidine (2.74 mL) were combined in *N*,*N*-dimethylformamide (40 mL) and the above solution was added. The mixture was stirred at ambient temperature overnight. The mixture was concentrated under reduced pressure and the residue was dissolved in dichloromethane (300 mL), washed with saturated aqueous ammonium chloride solution (100 mL), brine (100 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with 0-10% methanol in dichloromethane to give the title compound. ^{1}H NMR (400 MHz, dimethyl sulfoxide -d_6) δ ppm 8.65 (s, 1H), 7.89 (d, 1H), 7.61 (s, 1H), 7.49 (s, 1H), 7.22 (s, 1H), 6.77 (d, 1H), 6.46 (s, 1H), 6.29 (d, 2H), 5.41 (s, 2H), 5.32 (s, 2H), 4.29 (q, 1H), 3.87 – 3.77 (m, 1H), 2.76 (s, 6H), 1.99 (q, 1H), 1.92 – 1.81 (m, 2H), 1.41 (s, 9H), 1.25 (d, 3H), 0.92 – 0.80 (m, 9H). MS (ESI+) *m/z* 744.4 $(M+H)^+$.



Example 1I

(*S*)-2-amino-*N*-((*S*)-1-((3-((*S*)-7-ethyl-7-hydroxy-8,11-dioxo-8,10,11,13-tetrahydro-7*H*-[1,3]dioxolo[4,5-*g*]pyrano[3',4':6,7]indolizino[1,2-*b*]quinolin-14-yl)bicyclo[1.1.1]pentan-1-yl)amino)-1-oxopropan-2-yl)-3-methylbutanamide

[0054] Example 1H (5.5 g) was treated with trifluoracetic acid (30 mL) at ambient temperature for 30 minutes. The mixture was concentrated under reduced pressure and the residue was dissolved in 50% acetonitrile in water (200 mL). The solution was lyophilized to give the title compound. ^{1}H NMR (600 MHz, dimethyl sulfoxide -d_6) δ ppm 8.80 (s, 1H), 8.59 (d, 1H), 8.10 (d, 3H), 7.60 (s, 1H), 7.48 (s, 1H), 7.23 (s, 1H), 6.33 – 6.26 (m, 2H), 5.41 (d, 2H), 5.35 – 5.23 (m, 2H), 4.35 (p, 1H), 3.66 – 3.63 (m, 1H), 2.77 (s, 6H), 2.17 – 2.06 (m, 1H), 1.91 – 1.83 (m, 2H), 1.31 (d, 3H), 1.01 – 0.96 (dd, 6H), 0.89 (t, 3H). MS (ESI+) *m/z* 644.4 $(M+H)^+$.



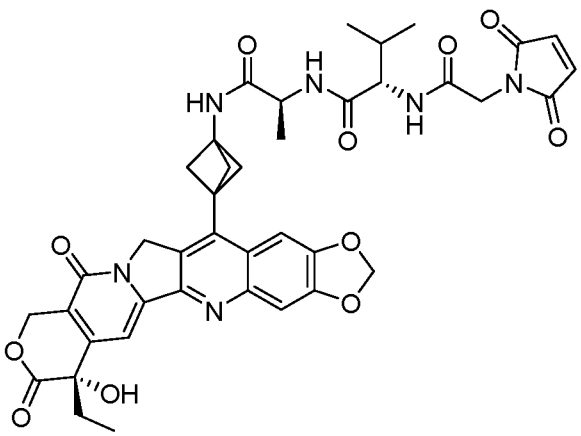
(V)

Example 1J

(2*S*)-2-(2-bromoacetamido)-*N*-[(2*S*)-1-({3-[(7*S*)-7-ethyl-7-hydroxy-8,11-dioxo-7,8,11,13-tetrahydro-2*H*,10*H*-[1,3]dioxolo[4,5-*g*]pyrano[3',4':6,7]indolizino[1,2-*b*]quinolin-14-yl]bicyclo[1.1.1]pentan-1-yl}amino)-1-oxopropan-2-yl]-3-methylbutanamide

[0055] To a solution of 2-bromoacetic acid (1.435 g) in *N*,*N*-dimethylformamide (26 mL) was added ethyl 2-ethoxyquinoline-1(2*H*)-carboxylate (2.55 g). The mixture was stirred at ambient temperature for 10 minutes. In a separate flask, Example 1I (4.5 g) and 2,6-lutidine (3.61 mL) were combined in *N*,*N*-dimethylformamide (26 mL) and the above solution was added. The mixture was stirred at ambient temperature for 30 minutes. The mixture was acidified with trifluoroacetic acid (4 mL) and purified by reversed-phase HPLC on a CombiFlash® Teledyne Isco system using a Luna column (250 × 50 mm, 10 mm), eluting with 5-75% acetonitrile in water containing 0.1% trifluoroacetic acid over 30 minutes to give the title compound after lyophilization. ^{1}H NMR (600 MHz, dimethyl sulfoxide -d_6) δ ppm 8.57 (s, 1H), 8.32 (d, 1H), 8.16 (d, 1H), 7.67 (s, 1H), 7.52 (s, 1H), 7.24 (s, 1H), 6.30 (dd,

2H), 5.42 (s, 2H), 5.38 (d, 2H), 4.28 – 4.19 (m, 2H), 4.03 – 3.91 (m, 2H), 2.76 (s, 6H), 2.02 (h, 1H), 1.86 (ddp, 2H), 1.25 (d, 3H), 0.93 – 0.84 (m, 9H). MS (ESI+) *m/z* 764.46 $(M+H)^+$.

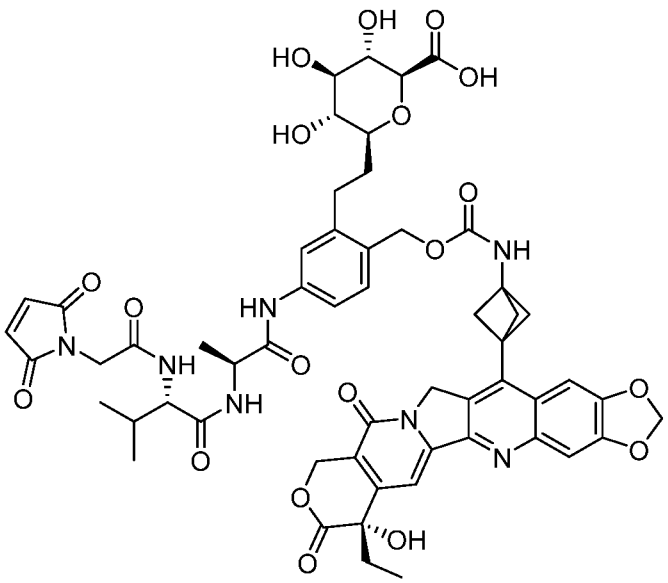


(VI)

Example 2

(2*S*)-2-[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)acetamido]-*N*-[(2*S*)-1-({3-[(7*S*)-7-ethyl-7-hydroxy-8,11-dioxo-7,8,11,13-tetrahydro-2*H*,10*H*-[1,3]dioxolo[4,5-*g*]pyrano[3',4':6,7]indolizino[1,2-*b*]quinolin-14-yl]bicyclo[1.1.1]pentan-1-yl}amino)-1-oxopropan-2-yl]-3-methylbutanamide

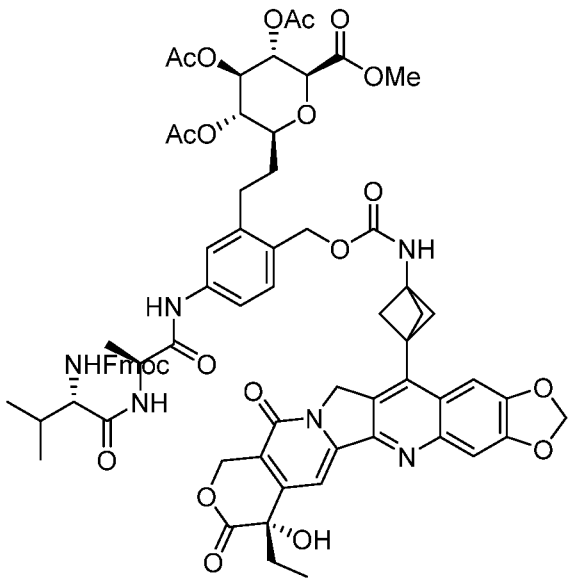
[0056] A solution of Example 1I (2 g) in *N*,*N*-dimethylformamide (54 mL) was added 2,5-dioxopyrrolidin-1-yl 2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)acetate (0.7 g) followed by *N*,*N*-diisopropylethylamine (2.3 mL). The mixture was stirred at ambient temperature for 30 minutes. The reaction was quenched with trifluoroacetic acid (2 mL) and purified by reversed-phase HPLC on a Gilson PLC 2020 system using a Luna column (250 × 50 mm, 10 mm), eluting with 5-75% acetonitrile in water containing 0.1% trifluoroacetic acid over 30 minutes to provide the title compound after lyophilization. ^{1}H NMR (500 MHz, dimethyl sulfoxide -d_6) δ ppm 8.50 (s, 1H), 8.29 (d, 1H), 8.13 (d, 1H), 7.64 (s, 1H), 7.51 (s, 1H), 7.23 (s, 1H), 7.12 (s, 2H), 6.30 (d, 2H), 5.42 (s, 2H), 5.36 (d, 2H), 4.24 (p, 1H), 4.20 – 4.14 (m, 3H), 2.75 (s, 6H), 2.01 (h, 1H), 1.86 (dp, 2H), 1.26 (d, 3H), 0.93 – 0.83 (m, 9H). MS (ESI+) *m/z* 781.14 $(M+H)^+$.



(VII)

Example 3

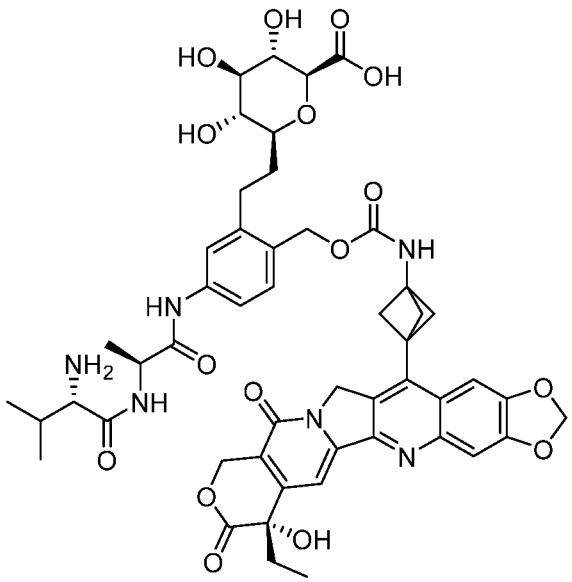
(2*S*,3*S*,4*R*,5*R*,6*S*)-6-[2-(5-{[(2*S*)-2-({(2*S*)-2-[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)acetamido]-3-methylbutanoyl}amino)propanoyl]amino}-2-{[({3-[(7*S*)-7-ethyl-7-hydroxy-8,11-dioxo-7,8,11,13-tetrahydro-2*H*,10*H*-[1,3]dioxolo[4,5-*g*]pyrano[3',4':6,7]indolizino[1,2-*b*]quinolin-14-yl]bicyclo[1.1.1]pentan-1-yl}carbamoyl)oxy]methyl}phenyl)ethyl]-3,4,5-trihydroxyoxane-2-carboxylic acid



Example 3A

(2*S*,3*S*,4*R*,5*S*,6*S*)-2-(5-((*S*)-2-((*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)-2-((((3-((*S*)-7-ethyl-7-hydroxy-8,11-dioxo-8,10,11,13-tetrahydro-7*H*-[1,3]dioxolo[4,5-*g*]pyrano[3',4':6,7]indolizino[1,2-*b*]quinolin-14-yl)bicyclo[1.1.1]pentan-1-yl)carbamoyl)oxy)methyl)phenethyl)-6-(methoxycarbonyl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate

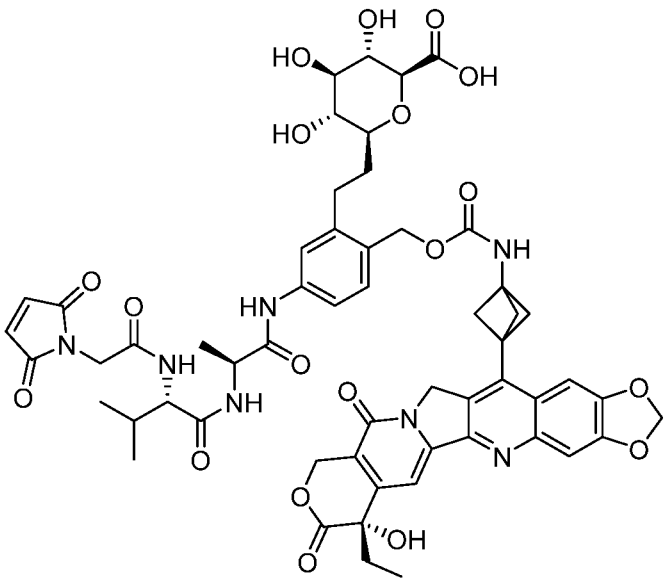
[0057] To a mixture of (2*S*,3*S*,4*R*,5*S*,6*S*)-2-(5-((*S*)-2-((*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)-2-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenethyl)-6-(methoxycarbonyl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (prepared as described in WO2016094509, 2.99 g), Example 1G (1.35 g) and 1-hydroxy-7-azabenzotriazole (0.361 g) in *N*,*N*-dimethylformamide (26.5 mL) was added *N*,*N*-diisopropylethylamine (1.39 mL). The mixture was stirred at ambient temperature for 4 hours. The mixture was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel, eluting with 0-8% methanol in dichloromethane to give the title compound. ^{1}H NMR (501 MHz, $CDCl_3$) δ ppm 8.43 (br s, 1H), 7.74 (d, 2H), 7.61 (br s, 1H), 7.57 – 7.46 (d, 4H), 7.43 (s, 1H), 7.38 (t, 3H), 7.33 – 7.27 (m, 3H), 6.43 (br s, 1H), 6.17 (s, 2H), 5.89 (br s, 1H), 5.72 (d, 1H), 5.37 – 5.02 (m, 8H), 4.94 (t, 1H), 4.67 – 4.56 (m, 1H), 4.53 – 4.44 (t, 2H), 4.19 (br s, 1H), 4.00 (d, 2H), 3.77 (s, 3H), 3.48 (dt, 1H), 2.94 (d, 1H), 2.88 – 2.66 (m, 6H), 2.16 (br s, 1H), 2.08 – 1.97 (m, 9H), 1.94 – 1.80 (ddt, 4H), 1.45 (d, 3H), 1.02 (t, 3H), 0.98 – 0.90 (m, 6H). MS (ESI+) *m/z* 1359.5 $(M+H)^+$.



Example 3B

(2*S*,3*S*,4*R*,5*R*,6*S*)-6-(5-((*S*)-2-((*S*)-2-amino-3-methylbutanamido)propanamido)-2-((((3-((*S*)-7-ethyl-7-hydroxy-8,11-dioxo-8,10,11,13-tetrahydro-7*H*-[1,3]dioxolo[4,5-*g*]pyrano[3',4':6,7]indolizino[1,2-*b*]quinolin-14-yl)bicyclo[1.1.1]pentan-1-yl)carbamoyl)oxy)methyl)phenethyl)-3,4,5-trihydroxytetrahydro-2*H*-pyran-2-carboxylic acid

[0058] To a solution of Example 3A (3.2 g) in methanol (24 mL) and tetrahydrofuran (24 mL) was added lithium hydroxide monohydrate (846 mg) in water (24 mL). The mixture was stirred at ambient temperature for 30 minutes. The reaction was quenched with trifluoracetic acid (3 mL) and extracted with heptane (5 × 30 mL). The aqueous layer was purified by reversed-phase HPLC on a CombiFlash® Teledyne Isco system using a Luna column (250 × 50 mm, 10 mm), eluting with 5-75% acetonitrile in water containing 0.1% trifluoroacetic acid over 30 minutes to provide the title compound after lyophilization. ^{1}H NMR (400 MHz, dimethyl sulfoxide -d_6) δ ppm 10.03 (s, 1H), 8.62 (d, 1H), 8.02 (br s, 4H), 7.55 (s, 1H), 7.43 (s, 1H), 7.41 (d, 2H), 7.35 (s, 1H), 7.22 (d, 1H), 7.16 (s, 1H), 6.40 (br s, 1H), 6.23 (s, 2H), 5.35 (s, 2H), 5.27 (s, 2H), 4.97 (s, 2H), 4.43 (p, 1H), 3.60 – 3.47 (m, 3H), 3.15 – 3.01 (m, 3H), 2.90 (t, 1H), 2.64 (s, 6H), 2.00 (tq, 2H), 1.79 (hept, 2H), 1.60 – 1.46 (m, 1H), 1.29 (d, 3H), 0.89 (dd, 6H), 0.80 (t, 3H). MS (ESI+) *m/z* 997.3 $(M+H)^+$.



(VII)

Example 3C

(2*S*,3*S*,4*R*,5*R*,6*S*)-6-[2-(5-{[(2*S*)-2-({(2*S*)-2-[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)acetamido]-3-methylbutanoyl}amino)propanoyl]amino}-2-{[({3-[(7*S*)-7-ethyl-7-hydroxy-8,11-dioxo-7,8,11,13-tetrahydro-2*H*,10*H*-[1,3]dioxolo[4,5-*g*]pyrano[3',4':6,7]indolizino[1,2-*b*]quinolin-14-yl]bicyclo[1.1.1]pentan-1-yl}carbamoyl)oxy]methyl}phenyl)ethyl]-3,4,5-trihydroxyoxane-2-carboxylic acid

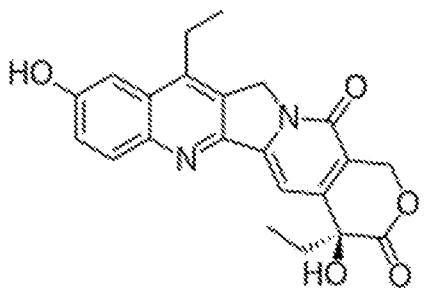
[0059] A solution of Example 3B (1.02 g) in *N*,*N*-dimethylformamide (16 mL) was added 2,5-dioxopyrrolidin-1-yl 2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)acetate (0.28 g) followed by *N*,*N*-diisopropylethylamine (0.8 mL). The mixture was stirred at ambient temperature for 30 minutes. The reaction was quenched with acetic acid (0.8 mL) and purified by reversed-phase HPLC on a Gilson PLC 2020 system using a Luna column (250 × 50 mm, 10 mm), eluting with 5-75% acetonitrile in water containing 0.1% trifluoroacetic acid over 30 minutes to provide the title compound after lyophilization. ^{1}H NMR (501 MHz, dimethyl sulfoxide -d_6) δ ppm 9.90 (s, 1H), 8.29 – 8.25 (m, 2H), 8.11 (s, 1H), 7.64 (s, 1H), 7.51 (s, 1H), 7.47 (d, 1H), 7.42 (s, 1H), 7.27 (d, 1H), 7.23 (s, 1H), 7.08 (s, 2H), 6.30 (s, 2H), 5.42 (s, 2H), 5.36 (s, 2H), 5.03 (s, 2H), 4.38 (p, 1H), 4.22 (dd, 1H), 4.13 (s, 2H), 3.58 (d, 2H), 3.15 (d, 4H), 2.97 (t, 1H), 2.71 (s, 6H), 2.10 – 1.93 (m, 2H), 1.86 (dp, 2H), 1.64 – 1.52 (m, 1H), 1.31 (d, 3H), 0.91 – 0.79 (m, 9H). MS (ESI+) *m/z* 1134.3 $(M+H)^+$.

Example 4: Cell Proliferation

[0060] The effect of TOP1i drugs on cell viability was assessed by monitoring ATP presence for metabolically active cells. Proliferation of Calu-6 cells (lung adenocarcinoma) and A375 cells (malignant melanoma) were quantified using CellTiter-Glo® luminescence. As shown in FIG. 2A and in Table 1, the compound of Formula I (Example 1G) exhibited sub-nanomolar potency for reducing viability of proliferating Calu-6 cells (0.57 nM, FIG. 2A) and A375 (0.50 nM, FIG. 2B). This sub-nanomolar potency improved over performance of the TOP1i drug SN-38 in the same assay, which demonstrated an EC_{50} of 6.58 nM (Calu-6) and 2.30 nM (A375).

Table 1: TOP1i Compound Potency

Compound	EC_{50} (nM)	
	Calu-6	A375
SN-38	6.58	2.30
Example 1G	0.57	0.50



(IV) SN-38

Example 5: Preparation and Purification of AbA

[0061] AbA was expressed in Chinese hamster ovary (CHO) cells with the heavy and light chain sequences of SEQ ID NOS: 9 and 10, respectively. AbA was isolated and purified prior to conjugation.

Example 6: Procedure for Conjugation (ADC-1)

Conjugation Step 1

[0062] Prior to reduction, a solution of anti-c-Met antibody AbA was incubated at 4°C for a minimum of 12 hours. A 0.5 M ethylenediaminetetraacetic acid disodium salt solution (EDTA, Sigma Aldrich) was then added, for a final concentration of 5 mM. Tris (2-carboxyethyl) phosphine (TCEP, 100 mM, 2.67 molar equivalents, Bond Breaker™, Thermo Scientific™) was added to the

solution (approximately 10 mg/mL in 1X DPBS (Dulbecco's phosphate-buffered saline) with gentle stir plate mixing (225 rpm) for 5 minutes. Following refrigerator incubation at 4°C for 17 hours, the solution was brought to 22°C on a stir plate mixing at 225 rpm. A 10% v/v *N,N*-dimethylacetamide (DMA, Sigma Aldrich) co-solvent was then added, followed by 20% v/v of prepared 1 M boric acid, pH 8.0 (Sigma Aldrich) and then 6 mol equivalents of 50 mM linker drug LD1 (structural formula V, Example 1J) in *N,N*-dimethylacetamide, dropwise. Mixing was discontinued after 20 minutes and the solution was incubated at 22°C for 3.3 hours. Following incubation, 2 mol equivalents of 50 mM *N*-Acetyl-L-cysteine (NAC, Sigma Aldrich) prepared in 1X DPBS, were added to the solution, and gently mixed by manually agitating the vessel. Following a 1-hour incubation at 22°C, the solution was desalted using a 691 mL G25 Fine Desalting (Cytiva™) Column, into 1X DPBS.

Conjugation Step 2

[0063] Prior to reduction, a solution of anti-c-Met antibody AbA was incubated at 4°C for a minimum of 12 hours. A 0.5 M ethylenediaminetetraacetic acid disodium salt solution (EDTA, Sigma Aldrich) was then added, for a final concentration of 5 mM. Tris (2-carboxyethyl) phosphine (TCEP, 100 mM, 1.00 mol eq, Bond Breaker™, Thermo Scientific™) was added to a solution of antibody (approximately 4 mg/mL in 1X DPBS) with gentle stir plate mixing (225 rpm) for 5 minutes. Following refrigerator incubation at 4°C for 17 hours, the solution was brought to 22°C on a stir plate mixing at 225 rpm. A 10% v/v *N,N*-dimethylacetamide (DMA, Sigma Aldrich) co-solvent was then added, followed by 20% v/v 1 M boric acid, pH 8.0 (Sigma Aldrich) and then 4 molar equivalents of 50 mM linker drug LD1 (structural formula V, Example 1J) in *N,N*-dimethylacetamide, dropwise. Mixing was discontinued after 20 minutes and the solution was incubated at 22 °C for 2.1 hours. Following incubation, 2 molar equivalents of 10 mM *N*-acetyl-L-cysteine (NAC, Sigma Aldrich), prepared in 1X DPBS, were added to the solution, and gently mixed by manually agitating the vessel. Following a 5-hour incubation at 22 °C, the solution was concentrated, and buffer exchanged via Ultrafiltration Diafiltration (UF/DF).

UF/DF Step

[0064] A Pellicon® 3 0.22 m^2 Cassette (Millipore) was flushed with 2 L of sterile water and then 0.5 L of formulation buffer. Ultrafiltration (UF) was performed at 250 mL/minute feed flow rate, and a Transmembrane Pressure (TMP) of 12-13 psi, with a starting volume of 1.3 L. Final permeate volume was 1.1 L. Diafiltration (DF) was performed with 25 diavolumes (DV) of final formulation buffer, at the same feed flow rate and TMP as the UF step. Following DF, a second UF step was

performed to further reduce ADC solution volume to 100 mL. The ADC solution was removed, and the cassette was twice rinsed and with 125 mL of formulation buffer which was then ultrafiltered to 20 mL each. These rinses were then added to the bulk ADC solution. The final solution was sterile filtered with a 0.22 μm filter, prior to characterization.

Example 7: Procedure for Conjugation (ADC-2)

[0065] Prior to reduction, a solution of anti-c-Met antibody AbA was incubated on wet ice for 35 minutes. A 0.5 M ethylenediaminetetraacetic acid disodium salt solution (EDTA, Sigma Aldrich) was then added, for a final concentration of 5 mM. Tris (2-carboxyethyl) phosphine (TCEP, 25 mM, 3.50 molar equivalent, Bond Breaker™, Thermo Scientific™) was added to the solution (approximately 10 mg/mL in 1X DPBS) and gently mixed by slowly inverting the tube several times. The solution was then placed on wet ice and placed in the 4 °C refrigerator for 24 hours. Following incubation, 10% v/v *N,N*-dimethylacetamide co-solvent was added followed by 10 molar equivalents of 10 mM linker drug LD2 (structural formula VI, Example 2) in *N,N*-dimethylacetamide (DMA, Sigma Aldrich) solution, and gently mixed by slowly inverting the tube several times. The solution was then placed on wet ice for 30 minutes and incubated at 22 °C for 90 minutes. Following incubation, 8 molar equivalents of 10 mM *N*-acetyl-L-cysteine (NAC, Sigma Aldrich) prepared in PBS were added to the solution, and gently mixed by slowly inverting the tube several times. After a 60-minute incubation at 22 °C, the solution was desalted over a HiPrep™ 26/10 desalting column into 1X DPBS (Cytiva™). Desalted ADCs were then hydrolyzed by the addition of a prepared solution of 10% v/v 1 M boric acid, pH 8.0 (Sigma Aldrich) with a 22 °C incubation, for 96 hours. Hydrolyzed ADCs were purified through coupled affinity to desalting chromatography (2 x 1 mL HiTrap MabSelect SuRe, HiPrep™ 26/10 desalting, Cytiva™) into a final formulation of 1X DPBS. Collected fractions were pooled and sterile filtered through a 0.22 μm filter prior to characterization.

Example 8: Procedure for Conjugation (ADC-3)

[0066] Aqueous ethylenediaminetetraacetic acid disodium salt (EDTA, 0.5 M, 21.3 μL, Sigma Aldrich) was added to 5.337 mL of a solution of anti-c-Met antibody (AbA, 13.34 mg/mL in 1X DPBS, pH 7.4). Tris(2-carboxyethyl) phosphine (TCEP, 10 mM, 3.0 molar eq, 142.4 μL, Bond Breaker™, Thermo Scientific™) was added to the solution, gently stirred, and kept at 37 °C for 75 minutes. The solution was cooled to ambient temperature and linker-drug LD3 (structural formula VII, Example 3C) was added (10 eq., 527 μL of 10 mM solution of LD3 (Structural Formula VII, Example 3C in *N,N*-dimethylacetamide, 90% purity)). The conjugation was gently stirred and

allowed to stand for 1.2 hours at ambient temperature. The ADC solution was purified by desalting. After desalting, the ADC solution was filtered through 0.22 μm filter and resulting sample stored at 4 °C. The resultant ADC was hydrolyzed by adding 10% v/v 1.0 M borate buffer, pH 8.0 and incubated in a dark environment at ambient temperature for 72 hours. After hydrolysis, the resultant ADC solution was purified by adsorbing to a protein A resin column (MabSelect™ SuRe™ LX, GE Healthcare); washing with 4 column volumes of 1X DPBS, pH 7.4; eluting off resin with 5 column volumes of IgG Elution Buffer (Thermo Scientific™); and desalted into 1X DPBS, pH 7.4. After desalting, the ADC solution was filtered through a 0.22 μm filter and the resulting sample was stored at 4 °C.

Example 9: Procedure for Conjugation (ADC-4)

[0067] Aqueous ethylenediaminetetraacetic acid disodium salt (EDTA, 0.5 M, 974 μL, Sigma Aldrich) was added to 243.3 mL of a purified solution of MSL109-C6v1 antibody (12.3 mg/mL in 20 mM Tris buffer, pH 7.4). MSL109-C6v1 is a monoclonal antibody that binds to CMV glycoprotein H. MSL109-C6v1 comprises a heavy chain set forth as SEQ ID NO: 11 and a light chain set forth as SEQ ID NO: 12 and is used as a non-targeting control antibody. Tris(2-carboxyethyl) phosphine (TCEP, 500 mM, 6.0 molar eq, 242.4 μL, Bond Breaker™, Thermo Scientific™) was added to the solution of MSL109-C6v1 with 2 mM EDTA, and gently stirred and kept at 4 °C for 20 hours. Tris buffer at 4 °C (1.0 M, pH 8.0) was added to the solution (24.5 mL, 10% v/v). The linker-drug LD1 (Example 1J, (2*S*)-2-(2-bromoacetamido)-*N*-[(2*S*)-1-({3-[(7*S*)-7-ethyl-7-hydroxy-8,11-dioxo-7,8,11,13-tetrahydro-2*H*,10*H*-[1,3]dioxolo[4,5-*g*]pyrano[3',4':6,7]indolizino[1,2-*b*]quinolin-14-yl]bicyclo[1.1.1]pentan-1-yl}amino)-1-oxopropan-2-yl]-3-methylbutanamide) was added to the reduced antibody at pH 8.0 (10 equivalents, 22.5 mL of 10 mM solution of LD1, structural formula V, Example 1J, in *N*,*N*-dimethylacetamide, 90% purity). The conjugation was gently stirred and allowed to stand for 1.5 hours in an ambient temperature water bath. The conjugation was quenched with addition of 4 equivalents of an aqueous solution of *N*-acetyl-L-cysteine (NAC, Sigma Aldrich, 100 mM solution in 1X DPBS, 0.8 mL). The mixture was gently stirred and allowed to stand for 1 hour at ambient temperature. Tris buffer (15%, Sigma, 1.0 M, pH 7.4, 44 mL) was added to adjust the ADC solution to pH 7.5. The ADC solution was stored at 4 °C overnight. The ADC solution was concentrated, and buffer exchanged via Ultrafiltration Diafiltration (Pellicon® 3 0.22 m^2 Cassette (Millipore)). The final ADC solution was filtered through a 0.22 μm filter and the resulting ADC sample stored at 4 °C. Final volume of ADC was 138 mL.

Example 10: DAR (Drug Antibody Ratio) Determination

[0068] DAR was determined by LC-MS. LC-MS analysis was performed using an Agilent 1100 HPLC system interfaced to an Agilent LC/MSD TOF 6220 ESI mass spectrometer. The ADC was reduced with 5 mM (final concentration) Bond-Breaker™ TCEP solution (Thermo Scientific™, Rockford, IL), loaded onto a Protein Microtrap (Michrom Bioresources, Auburn, CA) desalting cartridge, and eluted with a gradient of 10% B to 75% B in 0.2 minutes at ambient temperature. Mobile phase A was H_2O with 0.1% formic acid (FA), mobile phase B was acetonitrile with 0.1% FA, and the flow rate was 0.2 mL/minute. Electrospray-ionization time-of-flight mass spectra of the co-eluting light and heavy chains were acquired using Agilent MassHunter™ acquisition software. The extracted intensity vs. *m/z* spectrum was deconvoluted using the Maximum Entropy feature of MassHunter™ software to determine the mass of each reduced antibody fragment. DAR was calculated from the deconvoluted spectrum by summing intensities of the naked and modified peaks for the light chain and heavy chain, normalized by multiplying intensity by the number of drugs attached. The summed, normalized intensities were divided by the sum of the intensities, and the summing results for two light chains and two heavy chains produced a final average DAR value for the full ADC. Thiosuccinimide hydrolysis of a bioconjugate can be monitored by electrospray mass spectrometry since the addition of water to the conjugate results in an increase of 18 Daltons to the observable molecular weight of the conjugate.

Example 11: Cytotoxicity Assay In Vitro

[0069] Tumor cells were plated at 2000-5000 cells/well in 180 μL growth medium containing 10% FBS (fetal bovine serum) in 96-well plates and cultured at 37 °C in a humidified incubator with 5% CO_2. After 18 to 24 hours, titrations of antibodies or ADCs in 20 μL were added and cells were incubated for 6 days except for HCC827, NCI-H820, and HEK-293 (5 days) and MIA PaCa2 (4 days). Cell viability was determined using a CellTiter-Glo® Luminescent Cell Viability Assay (Promega) according to the manufacturer's instructions. A non-binding, irrelevant negative control ADC (ADC-4, Structural Formula VIII, where Ab is MSL109-C6v1) was also included in all assays to confirm that cell killing was antigen dependent. All ADCs had an approximately equivalent average DAR.

Table 2: Antibody Drug Conjugates					
	Antibody	Antibody Heavy Chain (SEQ ID NO)	Antibody Light Chain (SEQ ID NO)	ADC Structural Formula	Conjugation Example
ADC-1	AbA	9	10	VIII	Ex. 6
ADC-2	AbA	9	10	IX	Ex. 7
ADC-3	AbA	9	10	X	Ex. 8
ADC-4	MSL109-C6v1	11	12	VIII	Ex. 9

Example 12: Determination of Receptor Density

[0070] c-Met cell surface density (antigen binding capacity per cell) was determined by indirect immunofluorescence staining of cell surface antigens on cultured cells using QIFIKIT® (Dako/ Agilent). Briefly, cells were harvested from a culture flask as described above for FACS (fluorescence activated cell sorting) analysis, added to a round bottom 96-well plate at 100 µL/well and incubated at 4 °C with 3 µg/mL c-Met antibody m224G11. Wells, treated with an irrelevant mouse monoclonal antibody of the same isotype (mIgG1) at 3 µg/mL, were included as controls. Following a one-hour incubation with primary antibody, cells were centrifuged for 3 minutes at 300 x g and washed twice with FACS buffer. For the indirect immunofluorescence staining of the QIFIKIT® beads, 100 µL of resuspended beads from Vial 1 (Set-up Beads) and Vial 2 (Calibration Beads) were added to separate wells, centrifuged for 3 minutes at 300 x g, and washed once with FACS buffer. All wells were incubated for one hour at 4 °C with 100 µL of the anti-mouse Alexa Fluor® 488 conjugated antibody (Invitrogen, cat. # A-11029) diluted 1:250 in FACS buffer. Cells were centrifuged for 3 minutes at 300 x g, washed twice with FACS buffer, and fixed with 100 µL/well of 1% formaldehyde in PBS (phosphate-buffered saline). Data were acquired on a BD™ LSRII flow cytometer and Geomean values for the 5 bead populations were recorded and used to generate a standard curve based on the lot specific antibody molecules per bead. The standard curve was used to assign ABC (Antibody Binding Capacity or number of receptors) to stained cell samples.

Results

[0071] To determine a potential correlation of c-Met expression level and sensitivity to ADC-1, a panel of 9 cell lines were tested in proliferation assays in vitro. FACS analysis demonstrated that these cell lines possess a range of c-Met expression levels as quantified via c-Met antibody binding capacity representing the number of cell surface c-Met molecules (TABLE 3). Sensitivity to ADC-1 in the cell proliferation assay was quantified as maximal killing and IC_{50} (TABLE 3). ADC-1 inhibited proliferation of cancer cells that over-express c-Met, including the *MET* amplified cell lines (TABLE 3). As a comparison, unconjugated AbA inhibited proliferation of cells with *MET* amplification i.e., Hs 746T, SNU-5, and EBC-1 (FIG. 3A-C), but not cell lines without *MET* amplification, i.e., NCI-H441, NCI-H1573, HCC827, NCI-H820, and Calu-3, and where ADC-1 was also active (FIG. 3D-H). Neither unconjugated AbA nor ADC-1 was active on low-c-Met expressing cells HEK-293 and MIA PaCa2 (TABLE 3, FIG. 3I and FIG. 3J), suggesting that there is a threshold level of c-Met expression required for significant killing by ADC-1.

TABLE 3: Cell Potency In Vitro

Tissue	c-Met receptors/ cell[a]	Cytotoxicity IC_{50} (nM)		
		ADC-3 (max[b])	ADC-2 (max)	ADC-1 (max)
NSCLC Adenocarcinoma				
NCI-H441	197,000	0.1 (60%)	0.1 (70%)	0.25 (70%)
NCI-H441	170,000	0.2 (85%)	0.25 (90%)	0.3 (90%)
NCI-H1573	116,000	1.1 (60%)	1[c] (40%)	1[c] (40%)
HCC827	94,000	0.5 (80%)	0.9 (70%)	0.85 (70%)
NCI-H820	70,000	0.06 (75%)	0.16 (75%)	0.17 (75%)
Calu-3	60,000	nd	nd	20 (30%)
Gastric				
Hs 746T	320,000	0.050 (40%)	0.1 (50%)	0.17 (50%)
SNU-5	290,000	nd[e]	nd	0.19 (90%)
Embryonic Kidney				
HEK-293	27,000	nd	nd	>60[d]
Pancreatic				
MIA PaCa-2	5000	>60[d]	>60[d]	>60[d]

[a]Approximate number of c-Met molecules on cell surface determined by FACS analysis as antibody binding capacity for m224GI I (the murine parent of AbA) binding at 0.01 mg/mL

[b]Max is maximum percent decrease relative to the untreated control in the proliferation assay.

[c]Estimated due to curve-fitting issues

[d]Not different from control

[e]nd means not determined

Example 13: c-Met-Targeting TOP1i ADC Inhibits the Growth of Cancer Xenografts In Vivo

[0072] The cell lines, Hs 746T and NCI-H441 were obtained from ATCC (American Type Culture Collection). Cells were maintained in monolayer culture for at most 3 passages according to recommendations of the supplier. A suspension of 2 x 10^6 cells in culture medium mixed with Matrigel® (1:1, volume:volume) was injected subcutaneously in the right flank of female C.B-17 SCID mice to generate xenografts from the gastric carcinoma cell line, Hs 746T. To generate xenografts from the NSCLC cell line, NCI-H441 a suspension of 5 x 10^6 cells in culture medium mixed with Matrigel (1:1, volume:volume) was injected subcutaneously in female SCID/bg mice. Treatment started when the size of the flank tumors was approximately 200 mm^3.

[0073] Vehicle (0 mg/kg) and four dose levels (Doses A–D, from lowest to highest) were administered. Each animal received a single dose.

[0074] Figure 4A and 4B show ADC-1 inhibited growth of human NSCLC grown as xenografts in immune-compromised mice. Robust growth inhibition is shown after administration of one dose of ADC-1 at Dose C and Dose D, with moderate tumor growth delay after dosing Dose B in both the Hs 746T and NCI-H441 xenografts.

Example 14: c-MET-Targeting TOP1i ADCs Inhibit the Growth of Patient-Derived Cancer Cells In Vivo

[0075] The NSCLC patient-derived xenograft (PDX) models, LU450, LU120, LU572, LU123, and LU413, were established internally by implanting patient biopsy tissue fragments subcutaneously in female immunodeficient NOD/SCID mice (Charles River Laboratories). Once tumors were established, PDX tumor cells were expanded by dissociation of tumors to cell suspensions, and 5 x 10^4 cells in culture medium were mixed with Matrigel® (1:1, volume:volume) were injected subcutaneously into the mammary fat pad region of female NOD/SCID mice. For efficacy studies, tumor bearing mice were randomized into treatment groups, with each group having equal average tumor volume (130-200 mm^3). The c-Met-ADCs were administered as a single dose at six dose levels (doses A-F, from lowest to highest) via intraperitoneal injection. c-Met-targeted efficacy was compared to a similar administration of vehicle. Tumor volumes were measured one to two times weekly, and efficacy was assessed by plotting tumor volume (mm^3) versus time to calculate the time to tumor progression (TTP). TTP, expressed in days, is the time after the single dose treatment when tumor regrow to double the size prior to treatment (at randomization). TTP for durable responses (cures) is indicated as the study length with a ">" sign in front of the number.

TABLE 4. In Vivo Activity of ADC-1 and ADC-2 in NSCLC PDX models		
PDX name	**ADC-1**[1]	**TTP (days)**
LU450	0 3 10	3 15 23
LU572	0 0.1 0.25 1 3	9 26 >78 >91 >91
LU211	0 3	4 41
PDX name	**ADC-2**	**TTP (days)**
LU450	0 5 10	4 34 45
LU123	0 5	8 > 106

TABLE 4. In Vivo Activity of ADC-1 and ADC-2 in NSCLC PDX models		
PDX name	**ADC-1**[1]	**TTP (days)**
	10	> 113
LU120	0 5 10	23 >94 >112
LU413	0 5	11 40
LU413	10	71

[1]0 = vehicle control

An ADC having a structure according to structural formula (VIII) demonstrated improved in vivo toxicity.

SEQUENCE LISTING TABLE		
SEQ ID NO:	Description	Sequence
1	AbA CDR-H1	GYIFTAYT
2	AbA CDR-H2	IKPNNGLA
3	AbA CDR-H3	ARSEITTEFDY
4	AbA CDR-L1	ESVDSYANSF
5	AbA CDR-L2	RAS
6	AbA CDR-L3	QQSKEDPLT
7	AbA Heavy Chain Variable Domain	QVQLVQSGAEVKKPGASVKVSCKASGYIFTAYTM HWVRQAPGQGLEWMGWIKPNNGLANYAQKFQG RVTMTRDTSISTAYMELSRLRSDDTAVYYCARSEI TTEFDYWGQGTLVTVSS
8	AbA Light Chain Variable Domain	DIVMTQSPDSLAVSLGERATINCKSSESVDSYANS FLHWYQQKPGQPPKLLIYRASTRESGVPDRFSGSG SGTDFTLTISSLQAEDVAVYYCQQSKEDPLTFGGG TKVEIK
9	AbA Heavy Chain	QVQLVQSGAEVKKPGASVKVSCKASGYIFTAYTM HWVRQAPGQGLEWMGWIKPNNGLANYAQKFQG RVTMTRDTSISTAYMELSRLRSDDTAVYYCARSEI TTEFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKRVEPKSCDCHCPPCPAPELLGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG
10	AbA Light Chain	DIVMTQSPDSLAVSLGERATINCKSSESVDSYANS FLHWYQQKPGQPPKLLIYRASTRESGVPDRFSGSG SGTDFTLTISSLQAEDVAVYYCQQSKEDPLTFGGG

SEQUENCE LISTING TABLE		
		TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPV TKSFNRGEC
11	MSL109-C6v1Heavy Chain	EEQVLESGGGLVKPGGSLRLSCAASGFTFSPYSVF WVRQAPGKGLEWVSSINSDSTYKYYADSVKGRFT ISRDNAENSIFLQMNSLRAEDTAVYYCARDRSYY AFSSGSLSDYYYGLDVWGQGTTVIVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK
12	MSL109-C6v1 Light Chain	DIVMTQSPLSLSVTPGEPASISCRSSQSLLHTNGYN YLDWYVQKPGQSPQLLIYLASNRASGVPDRFSGS GSGTDFTLKISRVETEDVGVYYCMQALQIPRTFGQ GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSP VTKSFNRGEA
13	m224G11 Heavy Chain Variable Domain	EVQLQQSGPELVKPGASVKISCKTSGYIFTAYTMH WVRQSLGESLDWIGGIKPNNGLANYNQKFKGKA TLTVDKSSSTAYMDLRSLTSEDSAVYYCARSEITT EFDYWGQGTALTVSS
14	m224G11-Light Chain Variable Domain	DIVLTQSPASLAVSLGQRATISCRASESVDSYANSF MHWYQQKPGQPPKLLIYRASNLESGIPARFSGSGS

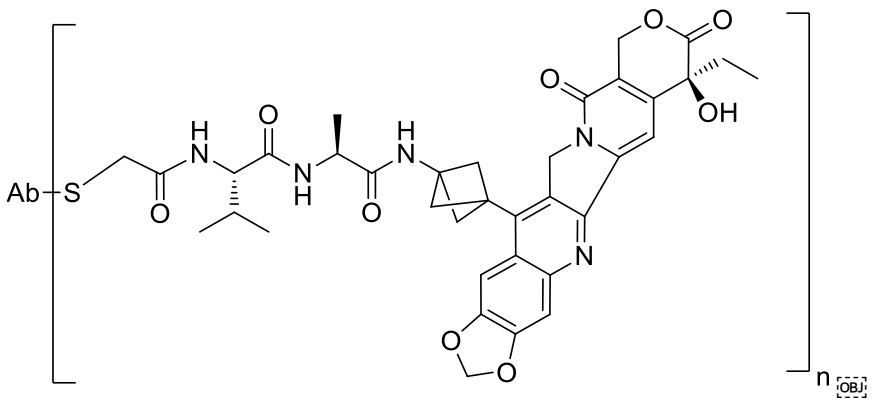
SEQUENCE LISTING TABLE		
		RTDFTLTINPVEADDVATYYCQQSKEDPLTFGSGT KLEMK
15	m224G11 HC CDR1	GYIFTAYT
16	m224G11 HC CDR2	IKPNNGLA
17	m224G11 HC CDR3	ARSEITTEFDY
18	m224G11 LC CDR1	ESVDSYANSF
19	m224G11 LC CDR2	RAS
20	m224G11 LC CDR3	QQSKEDPLT
21	DNA encoding AbA Heavy Chain	atgggatggtcttggatctttctgctgtttctgtctggtactgctggtgtgctgagc caggtccagctggtgcaatccggcgcagaggtgaagaagccaggcgcttcc gtgaaggtgagctgtaaggcctctggctacatcttcacagcatacaccatgcac tgggtgaggcaagctcctgggcagggactggagtggatgggatggattaaac ccaacaatgggctggccaactacgcccagaaattccagggtagggtcactatg acaagggataccagcatcagcaccgcatatatggagctgagcaggctgaggt ctgacgacactgctgtctattattgcgccaggagcgaaattacaacagaattcga ttactgggggcagggcaccctggtgaccgtgtcctctgccagcaccaagggc ccaagcgtgttcccccctggcccccagcagcaagagcaccagcggcggcaca gccgccctgggctgcctggtgaaggactacttccccgagcccgtgaccgtgtc ctggaacagcggagccctcacttctggagttcataccttcccagcagtattgca gagcagtggcctgtattcactgtcttccgtcgtaacagttccatcctccagcctcg ggacacagacttacatttgtaacgtgaatcacaagcctagcaacaccaaggtcg acaagagagttgaaccaaagagttgtgattgccactgtcctccctgcccagctc ctgagctgcttggcggtcccagtgtcttcttgtttccccctaaacccaaagacac cctgatgatctcaaggactcccgaggtgacatgcgtggtggtggatgtgtctca tgaggacccagaggtgaagttcaactggtacgtggacggcgtggaggtgcac aacgccaagaccaagcccagagaggagcagtacaacagcacctacagggtg gtgtccgtgctgaccgtgctgcaccaggactggctgaacggcaaggagtaca agtgtaaggtgtccaacaaggccctgccagccccaatcgaaaagaccatcag caaggccaagggccagccaagagagccccaggtgtacaccctgccacccag cagggaggagatgaccaagaaccaggtgtccctgacctgtctggtgaagggc

SEQUENCE LISTING TABLE		
		ttctacccaagcgacatcgccgtggagtgggagagcaacggccagcccgag aacaactacaagaccacccccccagtgctggacagcgacggcagcttcttcct gtacagcaagctgaccgtggacaagagcagatggcagcagggcaacgtgttc agctgctccgtgatgcacgaggccctgcacaaccactacacccagaagagcc tgagcctgtccccaggctga
22	DNA encoding AbA CDR-H1	ggctacatcttcacagcatacacc
23	DNA encoding AbA CDR-H2	attaaacccaacaatgggctggcc
24	DNA encoding AbA CDR-H3	gccaggagcgaaattacaacagaattcgattac
25	DNA encoding AbA Light Chain	atggaaactgatacactgctgctgtgggtcctgctgctgtgggtccctggaagc acaggggacattgtgatgacccagtctcccgatagcctggccgtgtccctggg cgagagggctaccatcaactgtaaaagctccgaatctgtggactcttacgcaaa cagctttctgcactggtatcagcaaaagccaggccaacctccaaagctgctgat ttacagggcttctaccagggagagcggcgtgcccgataggttcagcggatctg gcagcggcaccgactttacactgaccatctccagcctgcaggccgaagatgtg gcagtctattactgccagcagtccaaggaggacccccctgactttcgggggtggt actaaagtggagatcaagcgtacggtggccgctcccagcgtgttcatcttcccc ccaagcgacgagcagctgaagagcggcaccgccagcgtggtgtgtctgctg aacaacttctaccccagggaggccaaggtgcagtggaaggtggacaacgcc ctgcagagcggcaacagccaggagagcgtcaccgagcaggacagcaagga ctccacctacagcctgagcagcaccctgaccctgagcaaggccgactacgag aagcacaaggtgtacgcctgtgaggtgacccaccagggcctgtccagccccg tgaccaagagcttcaacaggggcgagtgctga
26	DNA encoding AbA CDR-L1	gaatctgtggactcttacgcaaacagcttt
27	DNA encoding AbA CDR-L2	agggcttct
28	DNA encoding AbA CDR-L3	cagcagtccaaggaggacccctgact

FORMS

Disclosed herein are the following forms:

1. An anti-c-Met antibody drug conjugate comprising the following structure:



wherein n is an integer from 1 to 10, and wherein Ab is an IgG_1 anti-c-Met antibody comprising

a heavy chain variable region comprising a heavy chain CDR3 domain comprising the amino acid sequence shown as SEQ ID NO: 3, a heavy chain CDR2 domain comprising the amino acid sequence shown as SEQ ID NO: 2, and a heavy chain CDR1 domain comprising the amino acid sequence shown as SEQ ID NO: 1; and

a light chain variable region comprising a light chain CDR3 domain comprising the amino acid sequence shown as SEQ ID NO: 6, a light chain CDR2 domain comprising the amino acid sequence shown as SEQ ID NO: 5, and a light chain CDR1 domain comprising the amino acid sequence shown as SEQ ID NO: 4.

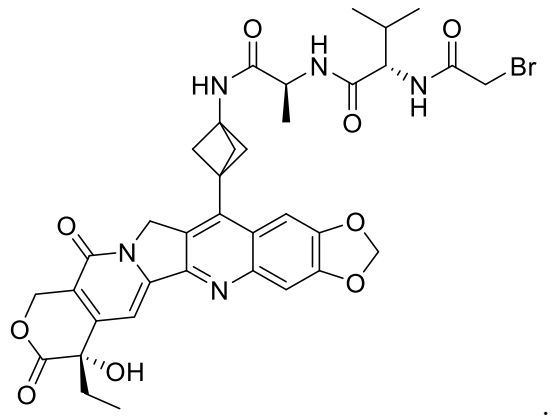
2. The anti-c-Met antibody drug conjugate according form 1, wherein the antibody Ab comprises a heavy chain variable region comprising the amino acid sequence set forth as SEQ ID NO: 7 and a light chain variable region comprising the amino acid sequence set forth as SEQ ID NO: 8.

3. The anti-c-Met antibody drug conjugate according to form 1, wherein the antibody Ab comprises a heavy chain comprising the amino acid sequence set forth as SEQ ID NO: 9 and a light chain comprising the amino acid sequence set forth as SEQ ID NO: 10.

4. A pharmaceutical composition comprising an anti-c-Met antibody drug conjugate of any of the preceding forms.

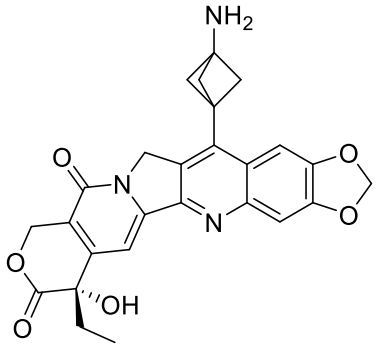
5. A method of treating non-small cell lung cancer, the method comprising administering an antibody-drug conjugate according to forms 1-3 to a patient in need thereof.

6. A compound according to the structure:



.

7. A compound according to the structure:

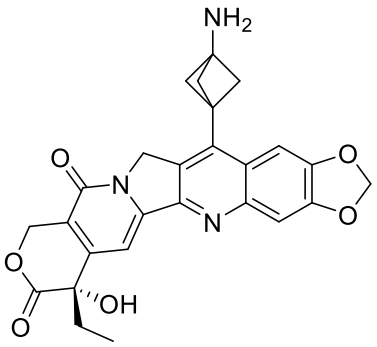


.

CLAIMS

WHAT IS CLAIMED:

1. A compound according to the structure:



.

2. A process for making a TOP1i antibody drug conjugate (ADC) comprising the steps of:

a) contacting a solution comprising an antibody with a reducing agent, and

b) conjugating the reduced antibody to the compound of claim 1 via a chemical linker;

thereby producing the ADC.

3. The process of claim 2, wherein the chemical linker is attached to the free amino group on the compound of claim 1.

4. A TOP1i antibody drug conjugate (ADC) produced by the process of claim 2.

5. A TOP1i antibody drug conjugate (ADC) produced by the process of claim 3.

6. A TOP1i antibody drug conjugate (ADC) comprising the compound of claim 1 conjugated to an antibody via a chemical linker.

7. The TOP1i antibody drug conjugate (ADC) of claim 6, wherein the chemical linker is attached to the free amino group on the compound of claim 1.

8. A pharmaceutical composition comprising the ADC of any one of claims 4 to 6 and a pharmaceutically acceptable excipient.

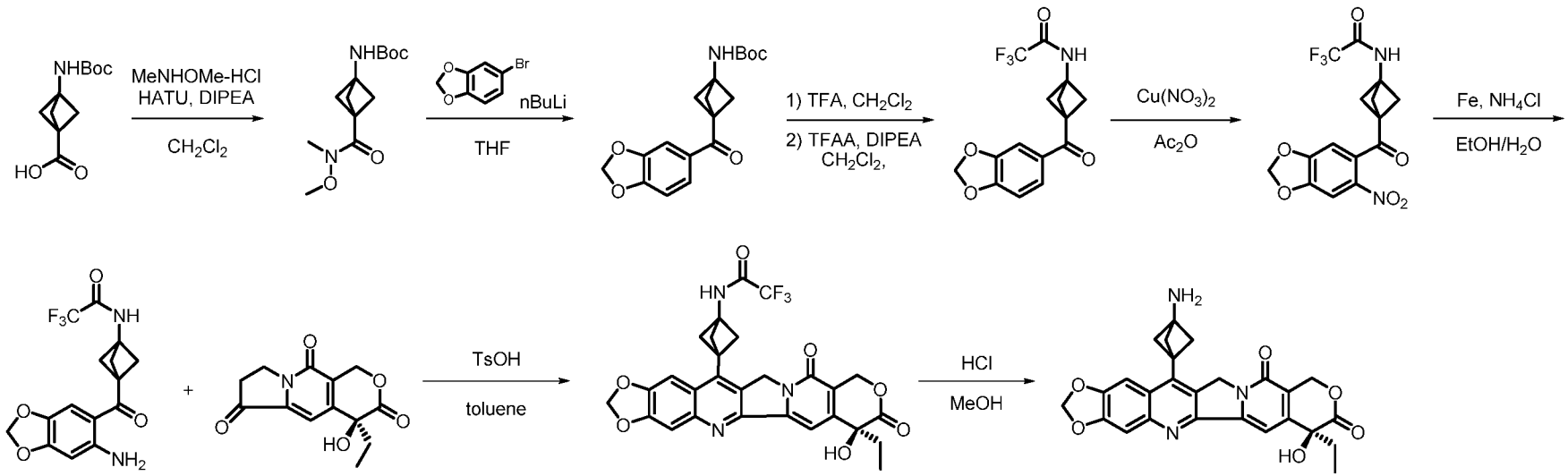

NHBoc
HO
O
MeNHOMe-HCl
HATU, DIPEA
CH2Cl2
N
O
Br
nBuLi
THF
1) TFA, CH2Cl2
2) TFAA, DIPEA
CH2Cl2,
F3C
NH
Cu(NO3)2
Ac2O
NO2
Fe, NH4Cl
EtOH/H2O
NH2
+
TsOH
toluene
HN
CF3
HO
HCl
MeOH

FIG. 1

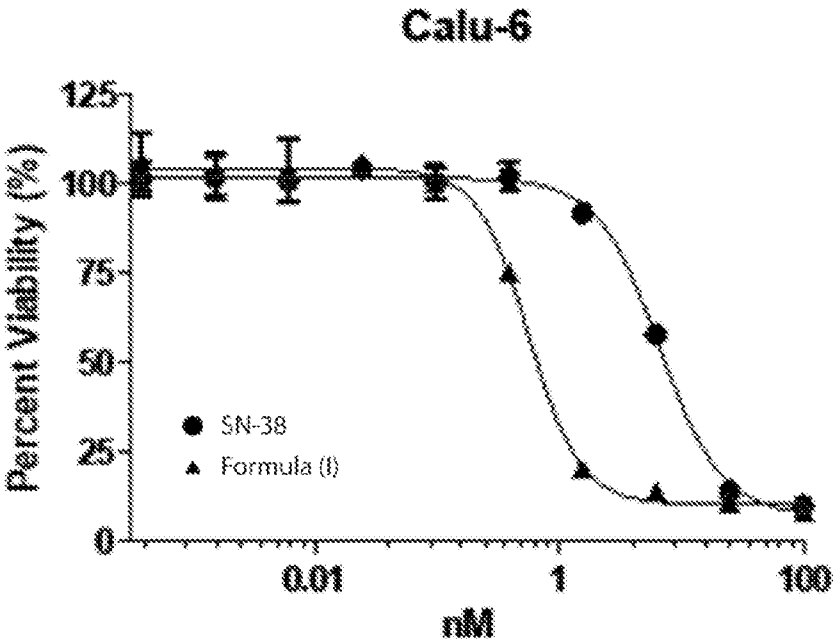

Calu-6
Percent Viability (%)
125
100
75
50
25
0
SN-38
Formula (I)
0.01
1
100
nM

FIG. 2A

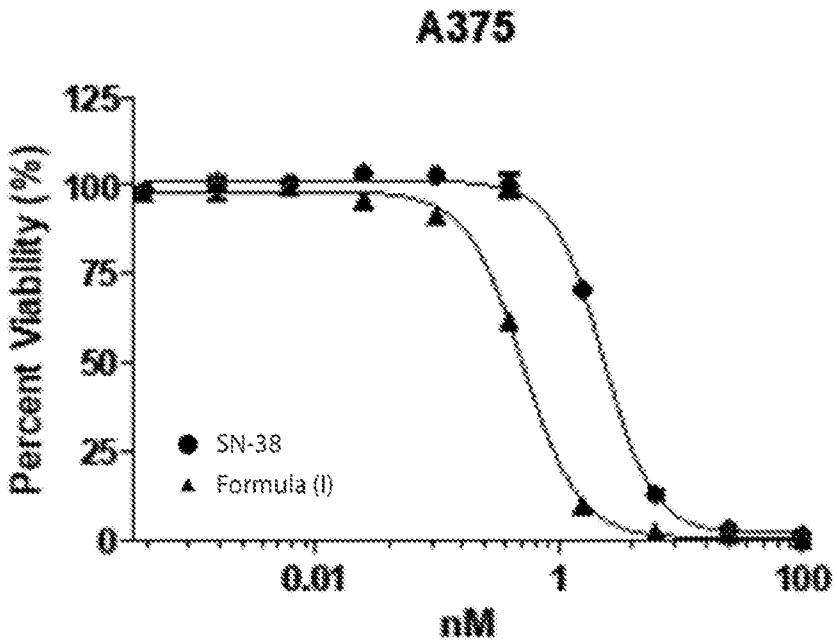

A375
Percent Viability (%)
125
100
75
50
25
0
SN-38
Formula (I)
0.01
1
100
nM

FIG. 2B

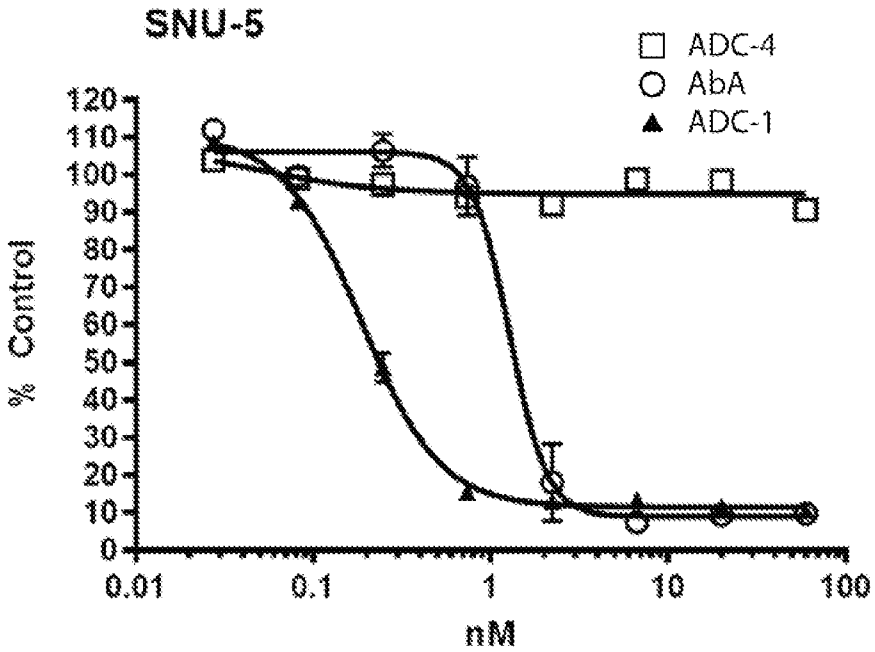


FIG. 3A

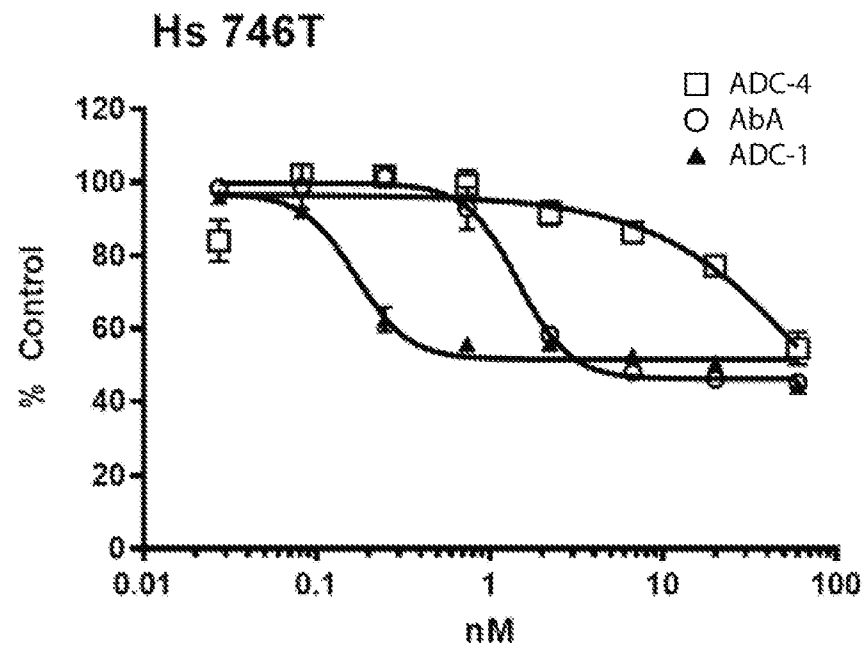


FIG. 3B

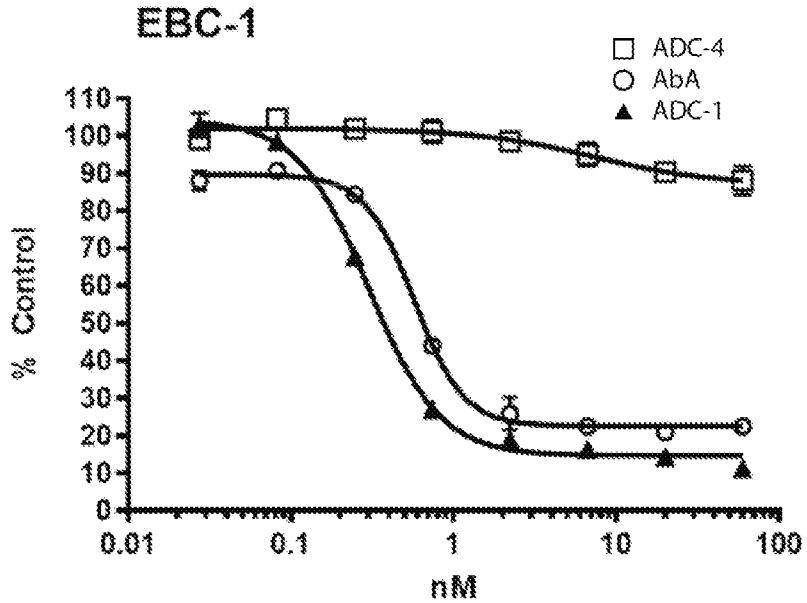


FIG 3C

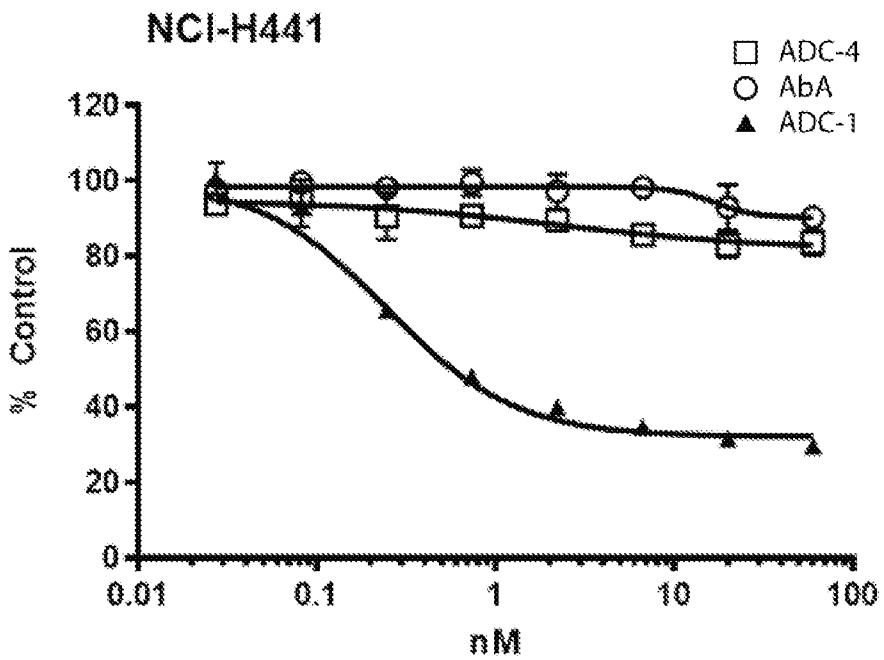


FIG. 3D

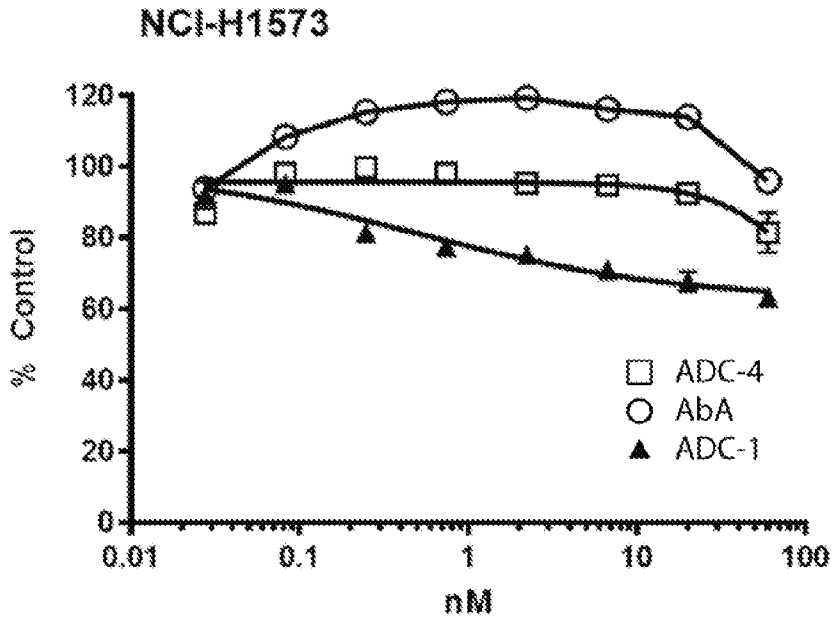


FIG. 3E

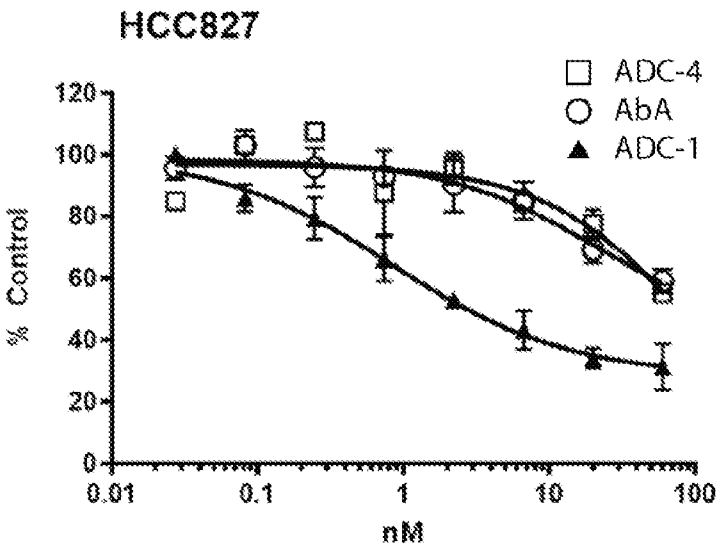


FIG. 3F

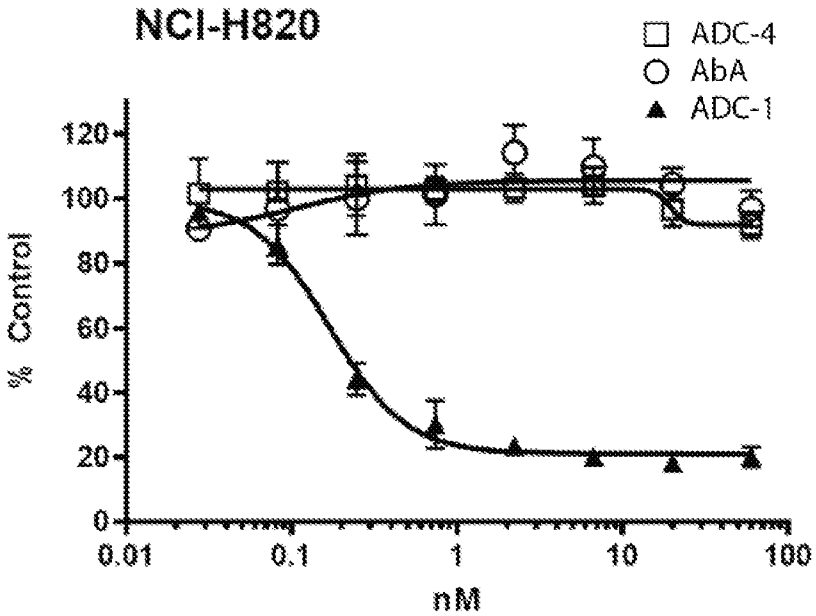


FIG. 3G

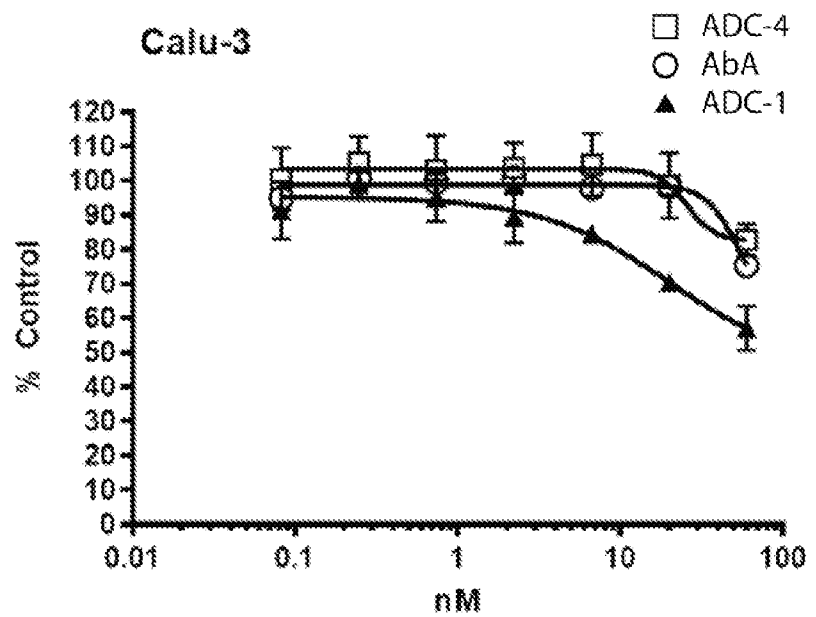


FIG. 3H

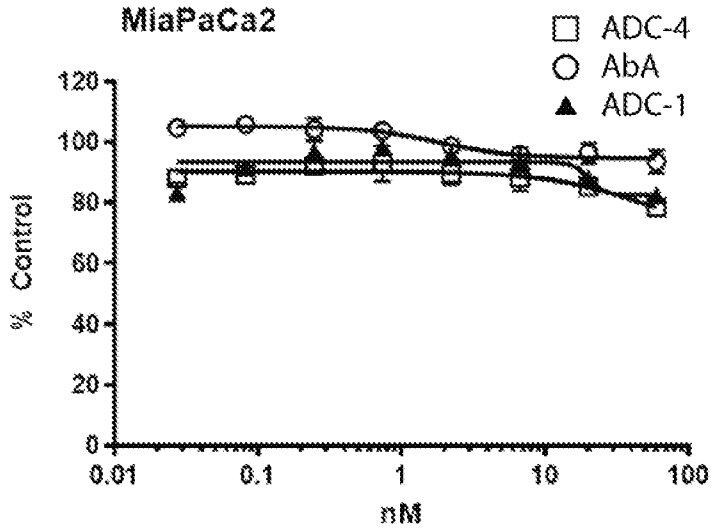


FIG. 3I

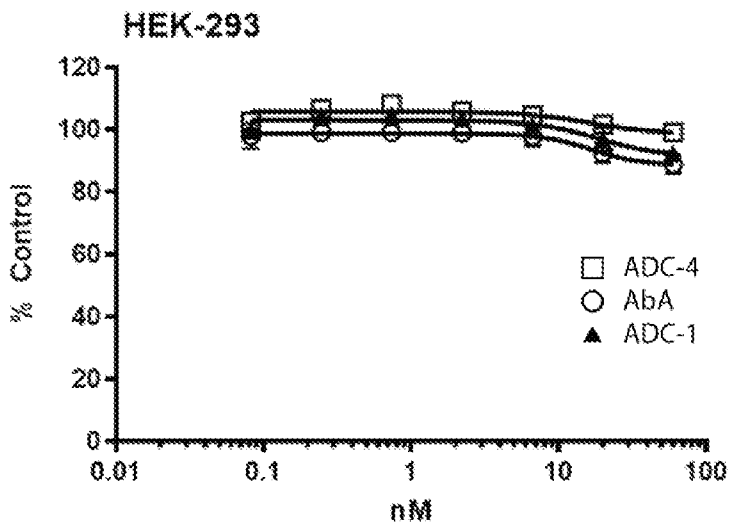


FIG. 3J

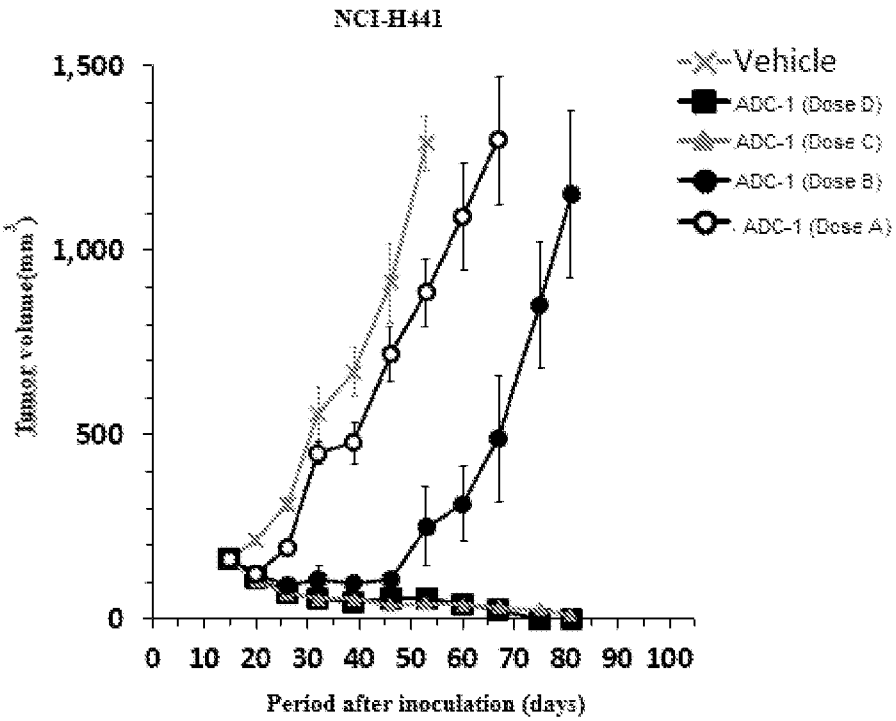


FIG. 4A

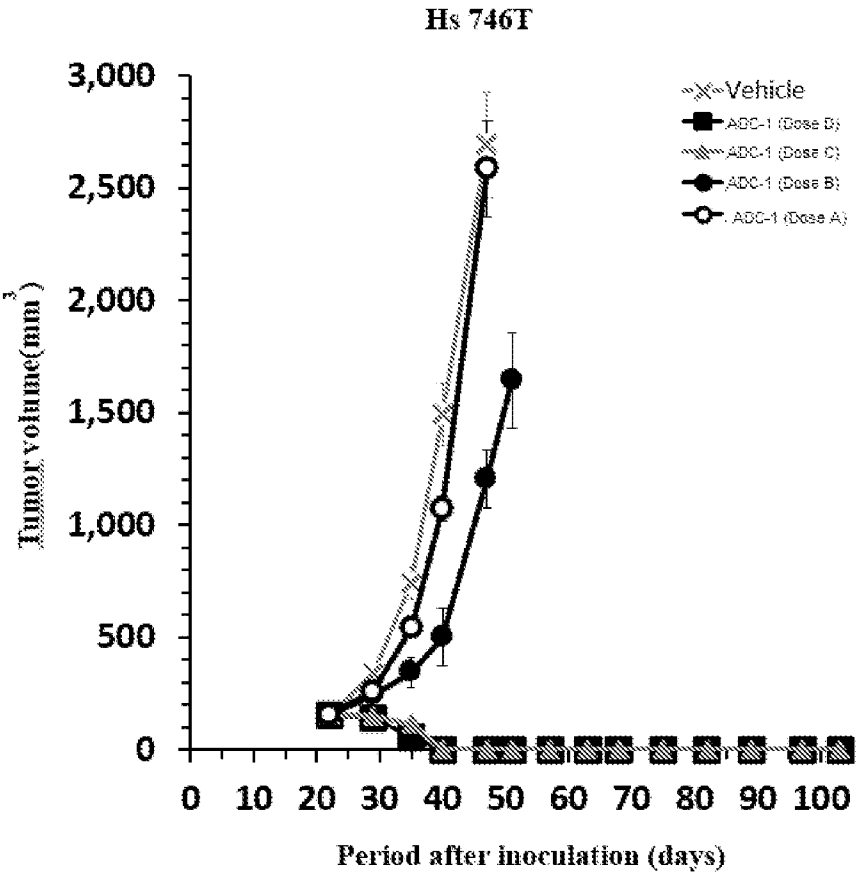

Hs 746T
Tumor volume(mm3)
3,000
2,500
2,000
1,500
1,000
500
0
0 10 20 30 40 50 60 70 80 90 100
Period after inoculation (days)
Vehicle
ADC-1 (Dose D)
ADC-1 (Dose C)
ADC-1 (Dose B)
ADC-1 (Dose A)

FIG. 4B

Sequence Listing

1	**Sequence Listing Information**	
1-1	File Name	P0066205AUD1 - Sequence Listing.xml
1-2	DTD Version	V1_3
1-3	Software Name	WIPO Sequence
1-4	Software Version	2.3.0
1-5	Production Date	2026-03-16
1-6	Original free text language code	
1-7	Non English free text language code	
2	**General Information**	
2-1	Current application: IP Office	
2-2	Current application: Application number	
2-3	Current application: Filing date	
2-4	Current application: Applicant file reference	397835-632WO
2-5	Earliest priority application: IP Office	US
2-6	Earliest priority application: Application number	63/181,963
2-7	Earliest priority application: Filing date	2021-04-29
2-8en	Applicant name	AbbVie Manufacturing Management Unlimited Company
2-8	Applicant name: Name Latin	
2-9en	Inventor name	
2-9	Inventor name: Name Latin	
2-10en	Invention title	ANTI-C-MET ANTIBODY DRUG CONJUGATES
2-11	Sequence Total Quantity	28

3-1	**Sequences**		
3-1-1	Sequence Number [ID]	1	
3-1-2	Molecule Type	AA	
3-1-3	Length	8	
3-1-4	Features	**REGION 1..8**	
	Location/Qualifiers	note=Synthetic polypeptide	
		source 1..8	
		mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-1-5	Residues	GYIFTAYT	8
3-2	**Sequences**		
3-2-1	Sequence Number [ID]	2	
3-2-2	Molecule Type	AA	
3-2-3	Length	8	
3-2-4	Features	**REGION 1..8**	
	Location/Qualifiers	note=Synthetic polypeptide	
		source 1..8	
		mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-2-5	Residues	IKPNNGLA	8
3-3	**Sequences**		
3-3-1	Sequence Number [ID]	3	
3-3-2	Molecule Type	AA	
3-3-3	Length	11	
3-3-4	Features	**REGION 1..11**	
	Location/Qualifiers	note=Synthetic polypeptide	
		source 1..11	
		mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-3-5	Residues	ARSEITTEFD Y	11
3-4	**Sequences**		
3-4-1	Sequence Number [ID]	4	
3-4-2	Molecule Type	AA	
3-4-3	Length	10	
3-4-4	Features	**REGION 1..10**	
	Location/Qualifiers	note=Synthetic polypeptide	
		source 1..10	
		mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-4-5	Residues	ESVDSYANSF	10
3-5	**Sequences**		
3-5-1	Sequence Number [ID]	5	
3-5-2	Molecule Type		
3-5-3	Length		
3-5-4	Features		
	Location/Qualifiers		
	NonEnglishQualifier Value		
3-5-5	Residues	000	3
3-6	**Sequences**		
3-6-1	Sequence Number [ID]	6	
3-6-2	Molecule Type	AA	
3-6-3	Length	9	
3-6-4	Features	**REGION 1..9**	
	Location/Qualifiers	note=Synthetic polypeptide	
		source 1..9	
		mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-6-5	Residues	QQSKEDPLT	9
3-7	**Sequences**		
3-7-1	Sequence Number [ID]	7	
3-7-2	Molecule Type	AA	
3-7-3	Length	118	
3-7-4	Features	**REGION 1..118**	

	Location/Qualifiers	note=Synthetic polypeptide
		source 1..118
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-7-5	Residues	QVQLVQSGAE VKKPGASVKV SCKASGYIFT AYTMHWVRQA PGQGLEWMGW IKPNNGLANY 60 AQKFQGRVTM TRDTSISTAY MELSRLRSDD TAVYYCARSE ITTEFDYWGQ GTLVTVSS 118
3-8	**Sequences**	
3-8-1	Sequence Number [ID]	8
3-8-2	Molecule Type	AA
3-8-3	Length	111
3-8-4	Features	**REGION 1..111**
	Location/Qualifiers	note=Synthetic polypeptide
		source 1..111
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-8-5	Residues	DIVMTQSPDS LAVSLGERAT INCKSSESVD SYANSFLHWY QQKPGQPPKL LIYRASTRES 60 GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQSKEDPL TFGGGTKVEI K 111
3-9	**Sequences**	
3-9-1	Sequence Number [ID]	9
3-9-2	Molecule Type	AA
3-9-3	Length	445
3-9-4	Features	**REGION 1..445**
	Location/Qualifiers	note=Synthetic polypeptide
		source 1..445
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-9-5	Residues	QVQLVQSGAE VKKPGASVKV SCKASGYIFT AYTMHWVRQA PGQGLEWMGW IKPNNGLANY 60 AQKFQGRVTM TRDTSISTAY MELSRLRSDD TAVYYCARSE ITTEFDYWGQ GTLVTVSSAS 120 TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL 180 YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKRVEPKS CDCHCPPCPA PELLGGPSVF 240 LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR 300 VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG QPREPQVYTL PPSREEMTKN 360 QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN 420 VFSCSVMHEA LHNHYTQKSL SLSPG 445
3-10	**Sequences**	
3-10-1	Sequence Number [ID]	10
3-10-2	Molecule Type	AA
3-10-3	Length	218
3-10-4	Features	**REGION 1..218**
	Location/Qualifiers	note=Synthetic polypeptide
		source 1..218
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-10-5	Residues	DIVMTQSPDS LAVSLGERAT INCKSSESVD SYANSFLHWY QQKPGQPPKL LIYRASTRES 60 GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQSKEDPL TFGGGTKVEI KRTVAAPSVF 120 IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS 180 STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC 218
3-11	**Sequences**	
3-11-1	Sequence Number [ID]	11
3-11-2	Molecule Type	AA
3-11-3	Length	460
3-11-4	Features	**REGION 1..460**
	Location/Qualifiers	note=Synthetic polypeptide
		source 1..460
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-11-5	Residues	EEQVLESGGG LVKPGGSLRL SCAASGFTFS PYSVFWVRQA PGKGLEWVSS INSDSTYKYY 60 ADSVKGRFTI SRDNAENSIF LQMNSLRAED TAVYYCARDR SYYAFSSGSL SDYYYGLDVW 120 GQGTTVIVSS ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV 180 HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHTCP 240 PCPAPEAAGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA 300 KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ 360 VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY 420 SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK 460
3-12	**Sequences**	

3-12-1	Sequence Number [ID]	12
3-12-2	Molecule Type	AA
3-12-3	Length	219
3-12-4	Features	**REGION 1..219**
	Location/Qualifiers	note=Synthetic polypeptide
		source 1..219
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-12-5	Residues	DIVMTQSPLS LSVTPGEPAS ISCRSSQSLL HTNGYNYLDW YVQKPGQSPQ LLIYLASNRA 60 SGVPDRFSGS GSGTDFTLKI SRVETEDVGV YYCMQALQIP RTFGQGTKVE IKRTVAAPSV 120 FIFPPSDEQL KSGTASVVCL LNNFYPREAK VQWKVDNALQ SGNSQESVTE QDSKDSTYSL 180 SSTLTLSKAD YEKHKVYACE VTHQGLSSPV TKSFNRGEA 219
3-13	**Sequences**	
3-13-1	Sequence Number [ID]	13
3-13-2	Molecule Type	AA
3-13-3	Length	118
3-13-4	Features	**REGION 1..118**
	Location/Qualifiers	note=Synthetic polypeptide
		source 1..118
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-13-5	Residues	EVQLQQSGPE LVKPGASVKI SCKTSGYIFT AYTMHWVRQS LGESLDWIGG IKPNNGLANY 60 NQKFKGKATL TVDKSSSTAY MDLRSLTSED SAVYYCARSE ITTEFDYWGQ GTALTVSS 118
3-14	**Sequences**	
3-14-1	Sequence Number [ID]	14
3-14-2	Molecule Type	AA
3-14-3	Length	111
3-14-4	Features	**REGION 1..111**
	Location/Qualifiers	note=Synthetic polypeptide
		source 1..111
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-14-5	Residues	DIVLTQSPAS LAVSLGQRAT ISCRASESVD SYANSFMHWY QQKPGQPPKL LIYRASNLES 60 GIPARFSGSG SRTDFTLTIN PVEADDVATY YCQQSKEDPL TFGSGTKLEM K 111
3-15	**Sequences**	
3-15-1	Sequence Number [ID]	15
3-15-2	Molecule Type	AA
3-15-3	Length	8
3-15-4	Features	**REGION 1..8**
	Location/Qualifiers	note=Synthetic polypeptide
		source 1..8
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-15-5	Residues	GYIFTAYT 8
3-16	**Sequences**	
3-16-1	Sequence Number [ID]	16
3-16-2	Molecule Type	AA
3-16-3	Length	8
3-16-4	Features	**REGION 1..8**
	Location/Qualifiers	note=Synthetic polypeptide
		source 1..8
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-16-5	Residues	IKPNNGLA 8
3-17	**Sequences**	
3-17-1	Sequence Number [ID]	17
3-17-2	Molecule Type	AA
3-17-3	Length	11
3-17-4	Features	**REGION 1..11**
	Location/Qualifiers	note=Synthetic polypeptide
		source 1..11
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	

3-17-5	Residues	ARSEITTEFD Y	11
3-18	**Sequences**		
3-18-1	Sequence Number [ID]	18	
3-18-2	Molecule Type	AA	
3-18-3	Length	10	
3-18-4	Features	**REGION 1..10**	
	Location/Qualifiers	note=Synthetic polypeptide	
		source 1..10	
		mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-18-5	Residues	ESVDSYANSF	10
3-19	**Sequences**		
3-19-1	Sequence Number [ID]	19	
3-19-2	Molecule Type		
3-19-3	Length		
3-19-4	Features		
	Location/Qualifiers		
	NonEnglishQualifier Value		
3-19-5	Residues	000	3
3-20	**Sequences**		
3-20-1	Sequence Number [ID]	20	
3-20-2	Molecule Type	AA	
3-20-3	Length	9	
3-20-4	Features	**REGION 1..9**	
	Location/Qualifiers	note=Synthetic polypeptide	
		source 1..9	
		mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-20-5	Residues	QQSKEDPLT	9
3-21	**Sequences**		
3-21-1	Sequence Number [ID]	21	
3-21-2	Molecule Type	DNA	
3-21-3	Length	1395	
3-21-4	Features	**misc_feature 1..1395**	
	Location/Qualifiers	note=Synthetic polynucleotide	
		source 1..1395	
		mol_type=other DNA	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-21-5	Residues	atgggatggt cttggatctt tctgctgttt ctgtctggta ctgctggtgt gctgagccag 60 gtccagctgg tgcaatccgg cgcagaggtg aagaagccag gcgcttccgt gaaggtgagc 120 tgtaaggcct ctggctacat cttcacagca tacaccatgc actgggtgag gcaagctcct 180 gggcagggac tggagtggat gggatggatt aaacccaaca atgggctggc caactacgcc 240 cagaaattcc agggtagggt cactatgaca agggatacca gcatcagcac cgcatatatg 300 gagctgagca ggctgaggtc tgacgacact gctgtctatt attgcgccag gagcgaaatt 360 acaacagaat tcgattactg ggggcagggc accctggtga ccgtgtcctc tgccagcacc 420 aagggcccaa gcgtgttccc cctggccccc agcagcaaga gcaccagcgg cggcacagcc 480 gccctgggct gcctggtgaa ggactacttc cccgagcccg tgaccgtgtc ctggaacagc 540 ggagccctca cttctggagt tcataccttc ccagcagtat tgcagagcag tggcctgtat 600 tcactgtctt ccgtcgtaac agttccatcc tccagcctcg ggacacagac ttacatttgt 660 aacgtgaatc acaagcctag caacaccaag gtcgacaaga gagttgaacc aaagagttgt 720 gattgccact gtcctccctg cccagctcct gagctgcttg gcggtcccag tgtcttcttg 780 tttcccccta aacccaaaga caccctgatg atctcaagga ctcccgaggt gacatgcgtg 840 gtggtggatg tgtctcatga ggacccagag gtgaagttca actggtacgt ggacggcgtg 900 gaggtgcaca acgccaagac caagcccaga gaggagcagt acaacagcac ctacagggtg 960 gtgtccgtgc tgaccgtgct gcaccaggac tggctgaacg gcaaggagta caagtgtaag 1020 gtgtccaaca aggccctgcc agccccaatc gaaaagacca tcagcaaggc caagggccag 1080 ccaagagagc cccaggtgta caccctgcca cccagcaggg aggagatgac caagaaccag 1140 gtgtccctga cctgtctggt gaagggcttc tacccaagcg acatcgccgt ggagtgggag 1200 agcaacggcc agcccgagaa caactacaag accacccccc cagtgctgga cagcgacggc 1260 agcttcttcc tgtacagcaa gctgaccgtg gacaagagca gatggcagca gggcaacgtg 1320 ttcagctgct ccgtgatgca cgaggccctg cacaaccact acacccagaa gagcctgagc 1380 ctgtccccag gctga 1395
3-22	**Sequences**		
3-22-1	Sequence Number [ID]	22	
3-22-2	Molecule Type	DNA	
3-22-3	Length	24	
3-22-4	Features	**misc_feature 1..24**	

	Location/Qualifiers	note=Synthetic polynucleotide	
		source 1..24	
		mol_type=other DNA	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-22-5	Residues	ggctacatct tcacagcata cacc	24
3-23	**Sequences**		
3-23-1	Sequence Number [ID]	23	
3-23-2	Molecule Type	DNA	
3-23-3	Length	24	
3-23-4	Features	**misc_feature 1..24**	
	Location/Qualifiers	note=Synthetic polynucleotide	
		source 1..24	
		mol_type=other DNA	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-23-5	Residues	attaaaccca acaatgggct ggcc	24
3-24	**Sequences**		
3-24-1	Sequence Number [ID]	24	
3-24-2	Molecule Type	DNA	
3-24-3	Length	33	
3-24-4	Features	**misc_feature 1..33**	
	Location/Qualifiers	note=Synthetic polynucleotide	
		source 1..33	
		mol_type=other DNA	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-24-5	Residues	gccaggagcg aaattacaac agaattcgat tac	33
3-25	**Sequences**		
3-25-1	Sequence Number [ID]	25	
3-25-2	Molecule Type	DNA	
3-25-3	Length	717	
3-25-4	Features	**misc_feature 1..717**	
	Location/Qualifiers	note=Synthetic polynucleotide	
		source 1..717	
		mol_type=other DNA	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-25-5	Residues	atggaaactg atacactgct gctgtgggtc ctgctgctgt gggtccctgg aagcacaggg 60 gacattgtga tgacccagtc tcccgatagc ctggccgtgt ccctgggcga gagggctacc 120 atcaactgta aaagctccga atctgtggac tcttacgcaa acagctttct gcactggtat 180 cagcaaaagc caggccaacc tccaaagctg ctgatttaca gggcttctac cagggagagc 240 ggcgtgcccg ataggttcag cggatctggc agcggcaccg actttacact gaccatctcc 300 agcctgcagg ccgaagatgt ggcagtctat tactgccagc agtccaagga ggacccctg 360 actttcgggg gtggtactaa agtggagatc aagcgtacgg tggccgctcc cagcgtgttc 420 atcttccccc caagcgacga gcagctgaag agcggcaccg ccagcgtggt gtgtctgctg 480 aacaacttct accccaggga ggccaaggtg cagtggaagg tggacaacgc cctgcagagc 540 ggcaacagcc aggagagcgt caccgagcag gacagcaagg actccaccta cagcctgagc 600 agcaccctga ccctgagcaa ggccgactac gagaagcaca aggtgtacgc ctgtgaggtg 660 acccaccagg gcctgtccag ccccgtgacc aagagcttca acaggggcga gtgctga 717
3-26	**Sequences**		
3-26-1	Sequence Number [ID]	26	
3-26-2	Molecule Type	DNA	
3-26-3	Length	30	
3-26-4	Features	**misc_feature 1..30**	
	Location/Qualifiers	note=Synthetic polynucleotide	
		source 1..30	
		mol_type=other DNA	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-26-5	Residues	gaatctgtgg actcttacgc aaacagcttt	30
3-27	**Sequences**		
3-27-1	Sequence Number [ID]	27	
3-27-2	Molecule Type		
3-27-3	Length		
3-27-4	Features		
	Location/Qualifiers		
	NonEnglishQualifier Value		
3-27-5	Residues	000	3

3-28	**Sequences**	
3-28-1	Sequence Number [ID]	28
3-28-2	Molecule Type	DNA
3-28-3	Length	27
3-28-4	Features	**misc_feature 1..27**
	Location/Qualifiers	note=Synthetic polynucleotide
		source 1..27
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-28-5	Residues	`cagcagtcca aggaggaccc cctgact` 27