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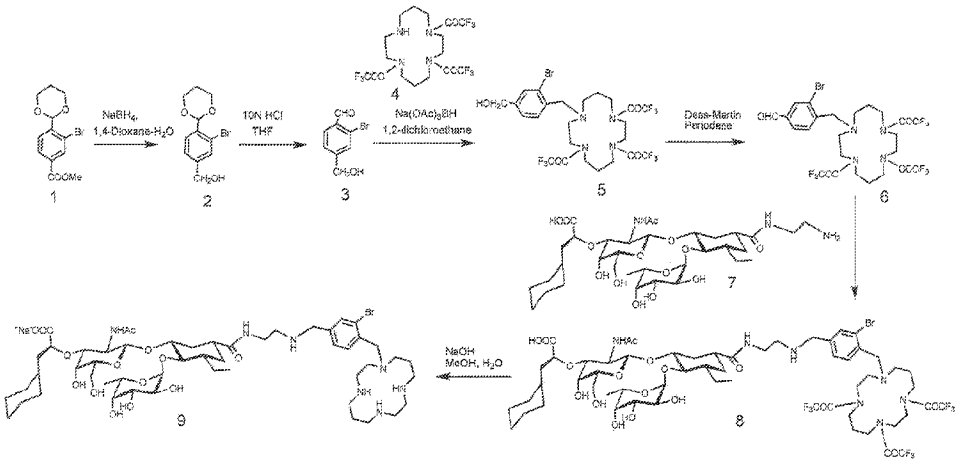
(54) Title: HETEROBIFUNCTIONAL INHIBITORS OF E-SELECTINS AND CXCR4 CHEMOKINE RECEPTORS



(57) Abrégé/Abstract:
Compounds, compositions, and methods for treatment and/or prevention of cancer and inflammatory diseases, and for releasing cells such as stem cells (e.g., bone marrow progenitor cells) into circulating blood and enhancing retention of the cells in the blood are disclosed. For example, heterobifunctional compounds that inhibit both E-selectins and CXCR4 chemokine receptors are described and pharmaceutical compositions comprising at least one of the same.

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(54) Title: HETEROBIFUNCTIONAL INHIBITORS OF E-SELECTINS AND CXCR4 CHEMOKINE RECEPTORS

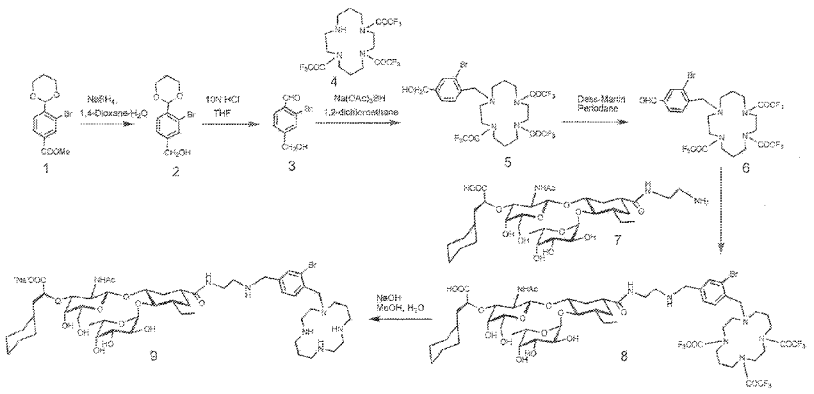

FIG. 1A

(57) Abstract: Compounds, compositions, and methods for treatment and/or prevention of cancer and inflammatory diseases, and for releasing cells such as stem cells (e.g., bone marrow progenitor cells) into circulating blood and enhancing retention of the cells in the blood are disclosed. For example, heterobifunctional compounds that inhibit both E-selectins and CXCR4 chemokine receptors are described and pharmaceutical compositions comprising at least one of the same.

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HETEROBIFUNCTIONAL INHIBITORS OF E-SELECTINS AND CXCR4 CHEMOKINE RECEPTORS

[0001]

FIELD OF INVENTION

[0002] Compounds, compositions, and methods for treating cancer and inflammatory diseases and for enhancing retention of cells after releasing into circulating blood are disclosed herein. For example, heterobifunctional compounds and compositions that inhibit E–selectins and CXCR4 chemokine receptors, and uses thereof are disclosed.

BACKGROUND OF THE INVENTION

[0003] A number of cancers are treatable before the cancer has moved beyond the primary site. However, once the cancer has spread beyond the primary site, the treatment options may be limited and the survival statistics may decline dramatically. Bones are a common location for cancer to infiltrate once leaving the primary tumor location. Breast and prostate cancer are examples of cancers that migrate to bones. Even leukemic cells that arise in the bloodstream may home to the bone marrow. Once cancer resides in bone, it may cause pain in an individual. Furthermore, once in the bone marrow, the cancer cells may also become resistant to chemotherapy. In addition, if the particular bone affected produces blood cells in the bone marrow, the individual may develop a variety of blood cell related disorders. Thus, it may be desirable to prevent cancer cells from leaving the primary site and/or to prevent extravasation of cancer cells from the bloodstream and infiltration into other tissues. Retention of cancer cells in the bloodstream makes the cells more susceptible to treatment, such as chemotherapy.

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[0004] Some cancers originate all or in part in bone. For such cancers, it may be desirable to mobilize cancer cells from bone to the bloodstream and/or to prevent those cells (as well as any cancer cells already in the bloodstream) from homing to bone or otherwise leaving the bloodstream. Retention of cancer cells in the bloodstream (or mobilization of cancer cells into the bloodstream and then retention therein) makes the cells more susceptible to treatment, such as chemotherapy.

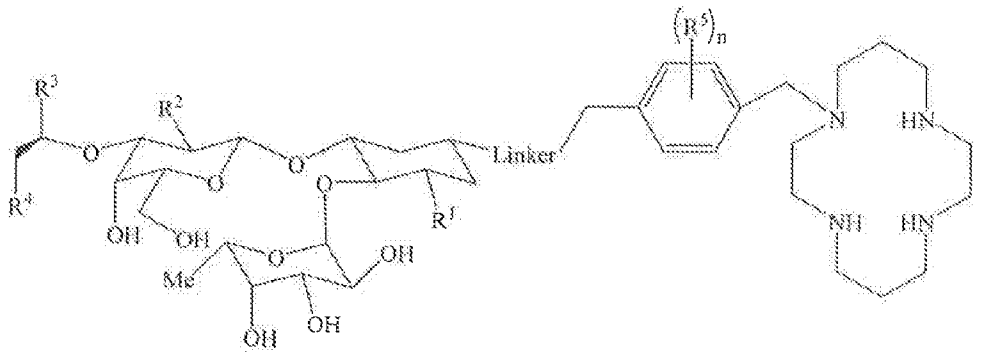
[0005] Hematopoietic stem cells (HSCs) also reside in the bone marrow and are a source of material for cellular therapy. HSCs adhere to the stroma within the bone marrow and in order to be harvested must break these adhesions and mobilize out of the bone marrow. Improved agents for increasing the number of HSCs available for harvesting may be desirable. Such HSCs may be useful for engraftment.

[0006] Accordingly, there is a need in the art for the treatment of cancers that may leave the primary site and cancers that originate all or in part in bone, and for improved methods to aid in the preparation of therapeutic–grade stem cells. The present disclosure may fulfill one or more of these needs and/or may provide other advantages.

SUMMARY OF THE INVENTION

[0007] Briefly stated, compounds, compositions, and methods for treating diseases and for improving methods in which an E–selectin and a CXCR4 chemokine receptor may play a role are disclosed. Compounds disclosed herein are heterobifunctional, wherein an E–selectin inhibitor is linked to a CXCR4 chemokine receptor inhibitor. The compounds may be used to treat cancer in which the cancer cells may leave the primary site, to treat an inflammatory disease in which the adhesion or migration of cells occurs in the disease, and/or to release cells such as stem cells (*e.g.*, bone marrow progenitor cells) into circulating blood and enhance retention of the cells in the blood (*e.g.*, to mobilize cells out of bone marrow and maintain the cells in the peripheral bloodstream).

[0008] In some embodiments, heterobifunctional inhibitors of Formula (I) are disclosed:



(I)

prodrugs of Formula (I), and pharmaceutically acceptable salts of any of the foregoing, wherein

R^1 is chosen from H, C_{1-8} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{1-8} haloalkyl, C_{2-8} haloalkenyl, and C_{2-8} haloalkynyl groups;

R^2 is chosen from –OH, $–NH_2$, $–OC(=O)Y^1$, $–NHC(=O)Y^1$, and $–NHC(=O)NHY^1$ groups, wherein Y^1 is chosen from C_{1-8} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{1-8} haloalkyl, C_{2-8} haloalkenyl, C_{2-8} haloalkynyl, C_{6-18} aryl, and C_{1-13} heteroaryl groups;

R^3 is chosen from -CN, $-CH_2CN$, and $-C(=O)Y^2$ groups, wherein Y^2 is chosen from C_{1-8} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, $-OZ^1$, -NHOH, $-NHOCH_3$, -NHCN, and $–NZ^1Z^2$ groups, wherein Z^1 and Z^2, which may be identical or different, are independently chosen from H, C_{1-8} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{1-8} haloalkyl, C_{2-8} haloalkenyl, and C_{2-8} haloalkynyl groups, wherein Z^1 and Z^2 may join together to form a ring;

R^4 is chosen from C_{3-8} cycloalkyl groups;

R^5 is independently chosen from H, halo, C_{1-8} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{1-8} haloalkyl, C_{2-8} haloalkenyl, and C_{2-8} haloalkynyl groups, with the proviso that at least one R^5 is not H;

n is chosen from integers ranging from 1 to 4; and

L is chosen from linker groups.

[0009] As used herein, 'compound of Formula (I)' includes heterobifunctional inhibitors of Formula (I), pharmaceutically acceptable salts of heterobifunctional inhibitors of Formula (I), prodrugs of heterobifunctional inhibitors of Formula (I), and pharmaceutically acceptable salts of prodrugs of heterobifunctional inhibitors of Formula (I).

[0010] In some embodiments, pharmaceutical compositions comprising at least one compound of Formula (I) and optionally at least one additional pharmaceutically acceptable ingredient are presented.

[0011] In some embodiments, a compound of Formula (I) and/or a pharmaceutical composition comprising at least one compound of Formula (I) may be used for the preparation and/or manufacture of a medicament for use in treating at least one of the diseases, disorders, and conditions described herein.

[0012] In some embodiments, a method for treatment and/or prevention of at least one cancer in which the cancer cells may leave the primary site is disclosed, the method comprising administering to a subject in need thereof an effective amount of at least one compound of Formula (I) and/or a pharmaceutical composition comprising at least one compound of Formula (I) and optionally at least one additional pharmaceutically acceptable ingredient.

[0013] In some embodiments, a method for treatment and/or prevention of at least one cancer in which it is desired to mobilize cancer cells from a site into the bloodstream and/or retain the cancer cells in the bloodstream is disclosed, the method comprising administering to a subject in need thereof an effective amount of at least one compound of Formula (I) and/or a pharmaceutical composition comprising at least one compound of Formula (I) and optionally at least one additional pharmaceutically acceptable ingredient.

[0014] In some embodiments, at least one compound of Formula (I) and/or a pharmaceutical composition comprising at least one compound of Formula (I) may be used in methods described herein for treatment and/or prevention of tumor metastasis. In some embodiments, the tumor metastasis arises from pancreatic cancer. In some emobidments, the tumor metastasis arises from prostate cancer. In some embodiments, the tumor metastasis arises from pancreatic cancer. In some embodiments, the tumor metastasis arises from breast cancer. In some embodiments, at least one additional chemotherapy agent such as gemcitabine is administered to the individual.

[0015] In some embodiments, a method for releasing cells into circulating blood and enhancing retention of the cells in the blood comprising administering to a subject in need

thereof an effective amount of at least one compound of Formula (I) and/or a pharmaceutical composition comprising at least one compound of Formula (I) and optionally at least one additional pharmaceutically acceptable ingredient is disclosed. In some embodiments, the method further includes collecting the released cells. In some embodiments, collecting the released cells utilizes apheresis. In some emobdiments, the released cells are stem cells (e.g., bone marrow progenitor cells). In some embodiments, G-CSF is administered to the individual.

[0016] In some embodiments, a method for the treatment and/or prevention of an inflammatory disease is presented in which the adhesion and/or migration of cells occurs in the diseases comprising administering to a subject in need thereof an effective amount of at least one compound of Formula (I) and/or a pharmaceutical composition comprising at least one compound of Formula (I) and optionally at least one additional pharmaceutically acceptable ingredient.

[0017] In the following description, certain specific details are set forth in order to provide a thorough understanding of various embodiments. However, one skilled in the art will understand that the disclosed embodiments may be practiced without these details. In other instances, well-known structures have not been shown or described in detail to avoid unnecessarily obscuring descriptions of the embodiments. These and other embodiments will become apparent upon reference to the following detailed description and attached drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] Figure 1 (Fig. 1A and Fig. 1B) is a diagram illustrating the synthesis of heterobifunctional Compound 9 and Compound 16.

[0019] Figure 2 shows the 400MHz ^{1}H NMR spectrum of Compound 9

[0020] Figure 3 shows the 600MHz ^{1}H NMR spectrum of Compound 16

[0021] Figure 4 depicts the results of the inhibition of SDF-1-induced chemotaxis assay by heterobifunctional Compounds 9 and 16.

[0022] Figure 5 (Fig. 5A and Fig. 5B) depicts the results of an E–selectin assay in which heterobifunctional Compounds 9 and 16 are used as the inhibitor.

[0023] Figure 6 depicts the results of a CXCR4 assay by heterobifunctional Compound 9.

[0024] Figure 7 depicts the results of a lymphatic and vacular endothelial migration toward tumor-associated fibroblasts assay by heterobifunctional Compound 9.

[0025] Figure 8 depicts the results of a PDAC cell binding to lymphatic monolayers assay by heterobifunctional Compound 9.

[0026] Figure 9 depicts the results of an intratibial tumor assay by heterobifunctional Compound 9.

DETAILED DESCRIPTION

[0027] Disclosed herein are compounds, compositions, and methods for treating diseases in which an E–selectin and a CXCR4 chemokine receptor play a role, and for enhancing retention of cells after releasing into circulating blood. The compounds have a variety of uses *in vitro* and *in vivo*.

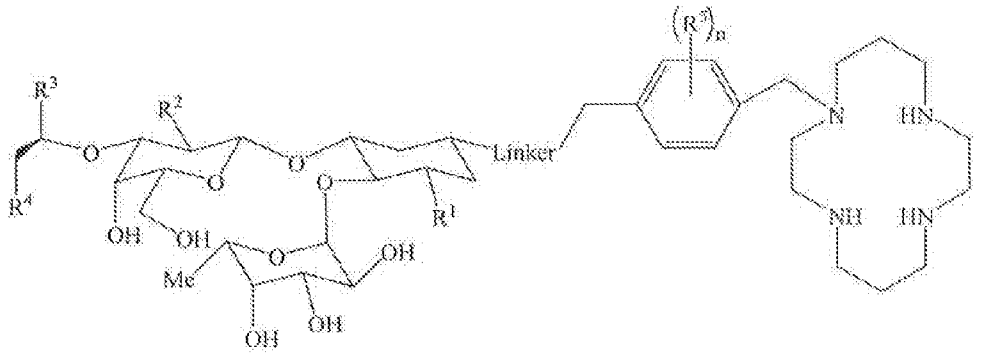
[0028] E–selectin inhibitors are known in the art. Some E–selectin inhibitors are specific for E–selectin only. Other E–selectin inhibitors have the ability to inhibit not only E–selectin but additionally P–selectin or L–selectin or both P–selectin and L–selectin. Examples of E–selectin inhibitors (specific for E–selectin or otherwise) are disclosed in U.S. Patent No. 7,060,685; U.S. Application Publication No. US–2007–0054870; U.S. Application Publication No. US–2008–0161546; and references cited in any of these patent or published application documents. Those examples are small organic molecules. Other known E–selectin inhibitors are amino acid–based, such as antibodies. For example, the humanized monoclonal antibody CDP850 is an E–selectin inhibitor.

[0029] CXCR4 chemokine receptor inhibitors are known in the art. Such inhibitors will typically prevent the binding of stromal derived factor–1 (SDF–1) to a CXCR4 receptor. Examples of CXCR4 chemokine receptor inhibitors are AMD–3100 (Hendrix et al., Antimicrob. Agents Chemother. 44:1667–1673, 2000); ALX40–4C (Doranz et al., AIDS

Research and Human Retroviruses 17:475–486, 2001); and T134 (Arakaki et al., J. Virol. 73:1719–1723, 1999). These examples include a small organic molecule and amino acid–based molecules, such as the T22 peptide. AMD–3100 is a bicyclam. Each of the two cyclam rings is attached to the same phenyl ring (each cyclam ring is para to the other) via a methylene group.

[0030] Heterobifunctional compounds for inhibition of E-selectin and the CXCR4 chemokine receptor comprising E-selectin inhibitor-Linker-CXCR4 chemokine receptor inhibitor are known in the art. Examples are disclosed, for example, in U.S. Patent No. 8,410,066.

[0031] In some embodiments, presented are heterobifunctional inhibitors of Formula (I):



(I)

prodrugs of Formula (I), and pharmaceutically acceptable salts of any of the foregoing, wherein

R^1 is chosen from H, C_{1-8} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{1-8} haloalkyl, C_{2-8} haloalkenyl, and C_{2-8} haloalkynyl groups;

R^2 is chosen from –OH, $–NH_2$, $–OC(=O)Y^1$, $–NHC(=O)Y^1$, and $–NHC(=O)NHY^1$ groups, wherein Y^1 is chosen from C_{1-8} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{1-8} haloalkyl, C_{2-8} haloalkenyl, C_{2-8} haloalkynyl, C_{6-18} aryl, and C_{1-13} heteroaryl groups;

R^3 is chosen from -CN, $-CH_2CN$, and $-C(=O)Y^2$ groups, wherein Y^2 is chosen from C_{1-8} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, $-OZ^1$, -NHOH, $-NHOCH_3$, -NHCN, and $–NZ^1Z^2$ groups, wherein Z^1 and Z^2, which may be identical or different, are independently chosen from H, C_{1-8} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{1-8} haloalkyl, C_{2-8} haloalkenyl, and C_{2-8} haloalkynyl groups, wherein Z^1 and Z^2 may join together to form a ring;

R^4 is chosen from C_{3-8} cycloalkyl groups;

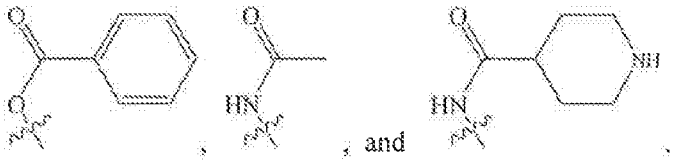
each R^5 is independently chosen from H, halo, C_{1-8} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{1-8} haloalkyl, C_{2-8} haloalkenyl, and C_{2-8} haloalkynyl groups, with the proviso that at least one R^5 is not H;

n is chosen from integers ranging from 1 to 4; and

L is chosen from linker groups.

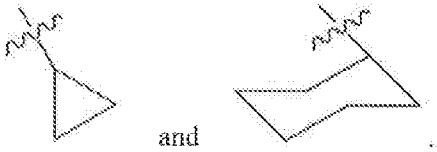
[0032] In some embodiments, R^1 is chosen from H, C_{1-4} alkyl, and C_{1-4} haloalkyl groups. In some embodiments, R^1 is chosen from H, methyl, ethyl, $-CH_2F$, $-CHF_2$, $-CF_3$, $-CH_2CH_2F$, $-CH_2CHF_2$, and $-CH_2CF_3$. In some embodiments, R^1 is H. In some embodiments, R^1 is chosen from methyl and ethyl. In some embodiments, R^1 is methyl. In some embodiments, R^1 is ethyl.

[0033] In some embodiments, R^2 is chosen from $-OC(=O)Y^1$ and $-NHC(=O)Y^1$ groups, wherein Y^1 is chosen from C_{1-8} alkyl, C_{1-8} haloalkyl, C_{6-18} aryl, and C_{1-13} heteroaryl groups. In some embodiments, R^2 is chosen from



[0034] In some embodiments, R^3 is $-C(=O)Y^2$, wherein Y^2 is chosen from $-OZ^1$ and $-NZ^1Z^2$ groups, wherein Z^1 and Z^2, which may be identical or different, are independently chosen from H, C_{1-8} alkyl, and C_{1-8} haloalkyl, wherein Z^1 and Z^2 may join together to form a ring. In some embodiments, R^3 is $-C(=O)OH$.

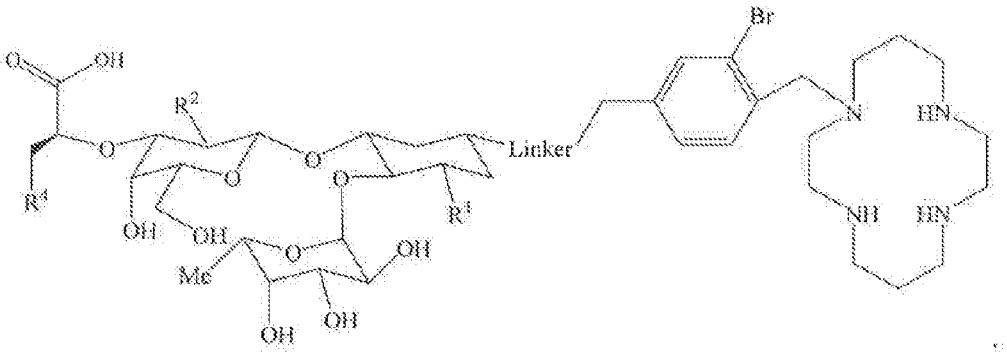
[0035] In some embodiments, R^4 is chosen from cyclopropyl and cyclohexyl groups. In some embodiments, R^4 is chosen from



[0036] In some embodiments, each R^5 is independently chosen from H, halo, C_{1-8} alkyl, and C_{1-8} haloalkyl groups, with the proviso that at least one R^5 is not H. In some embodiments, at least one R^5 is halo. In some embodiments, at least one R^5 is fluoro. In some embodiments, at least one R^5 is chloro. In some embodiments, at least one R^5 is bromo. In some embodiments, at least one R^5 is iodo.

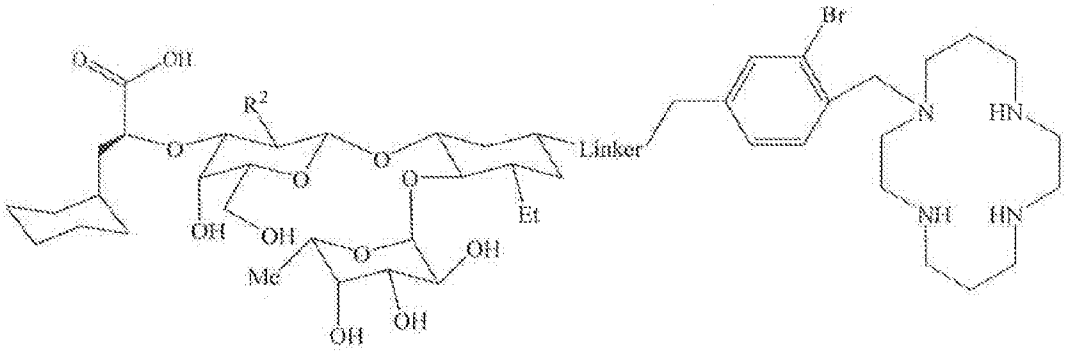
[0037] In some embodiments, n is 2. In some embodiments, n is 2 and R^5 is halo. In some embodiments, n is 2 and R^5 is bromo. In some embodiments, n is 1. In some embodiments, n is 1 and R^5 is halo. In some embodiments, n is 1 and R^5 is bromo.

[0038] In some embodiments, the compound is chosen from compounds of Formula (Ia):



(Ia)

[0039] In some embodiments, the compound is chosen from compounds of the following Formulae:



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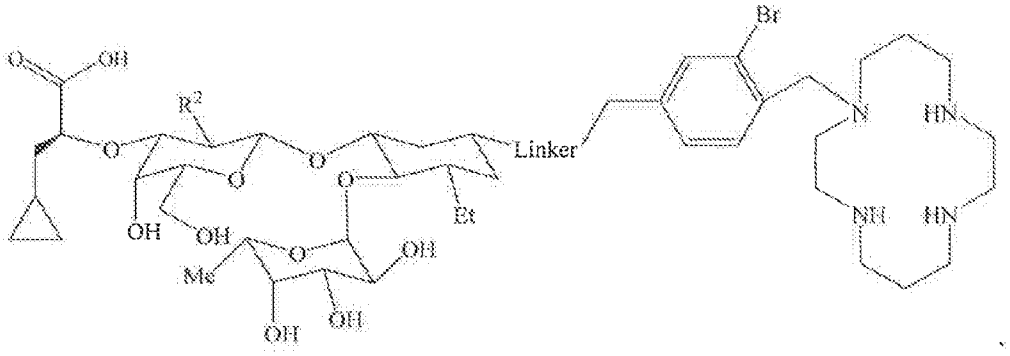
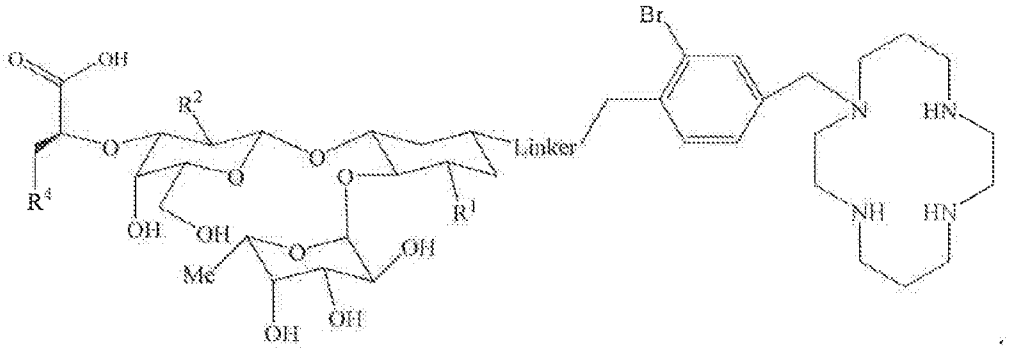
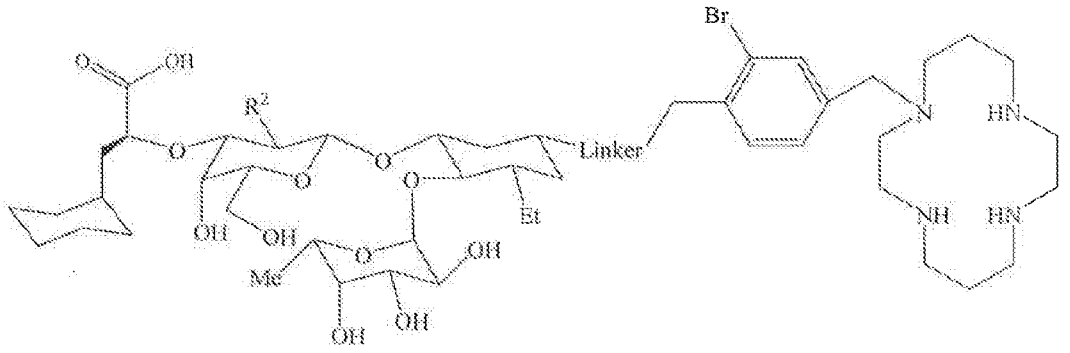


[0040] In some embodiments, the compound is chosen from compounds of Formula (Ib):



(Ib)

[0041] In some embodiments, the compound is chosen from compounds of the following Formulae:



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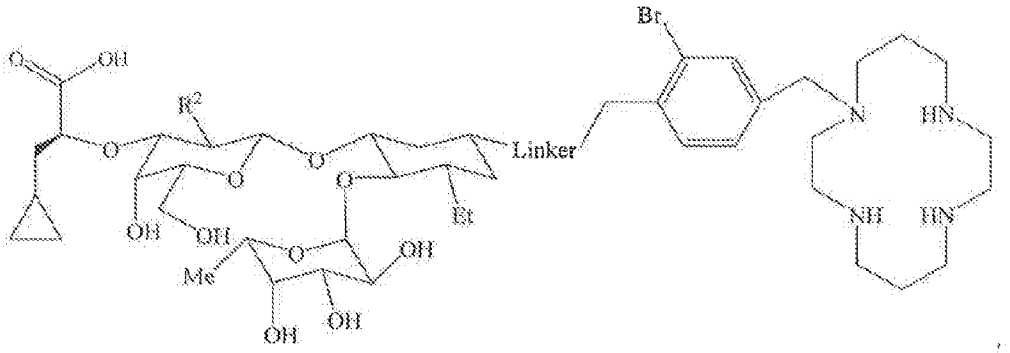
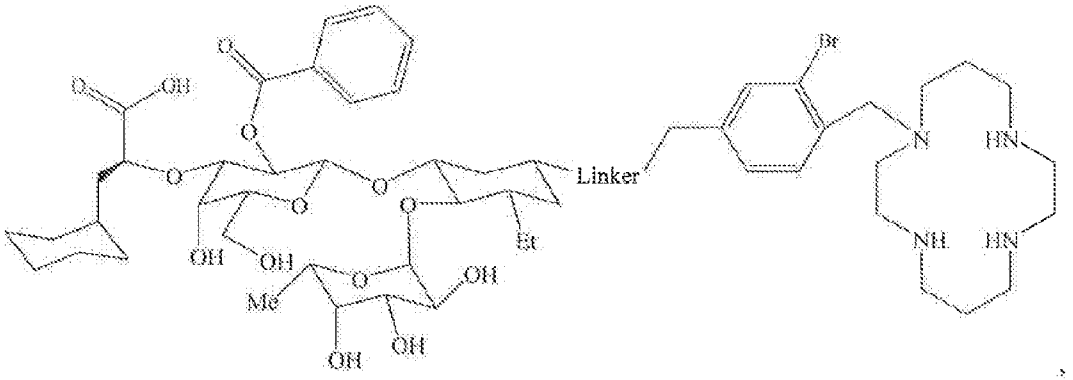
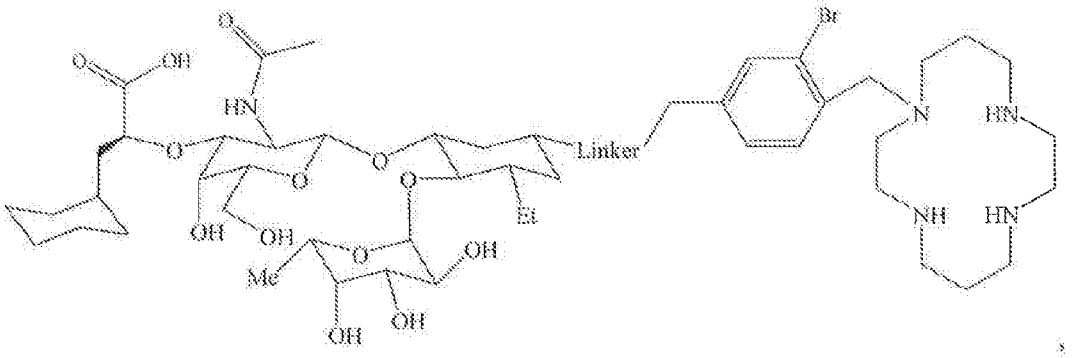
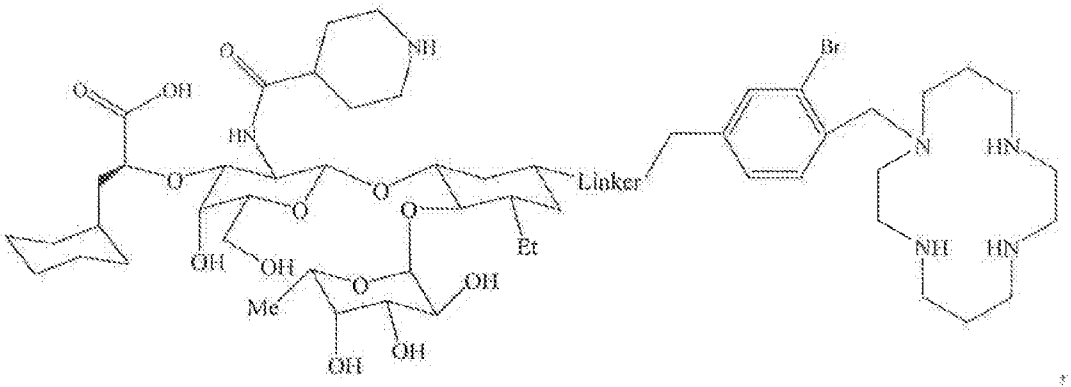


[0042] In some embodiments, the compound is chosen from compounds of the following Formulae:



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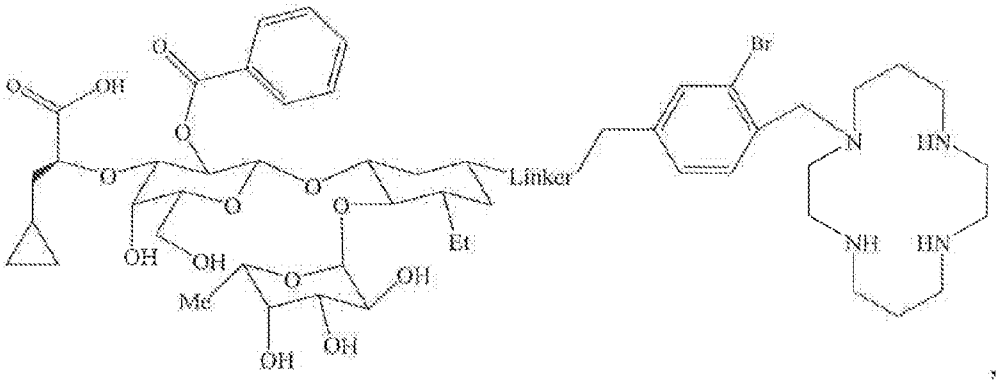
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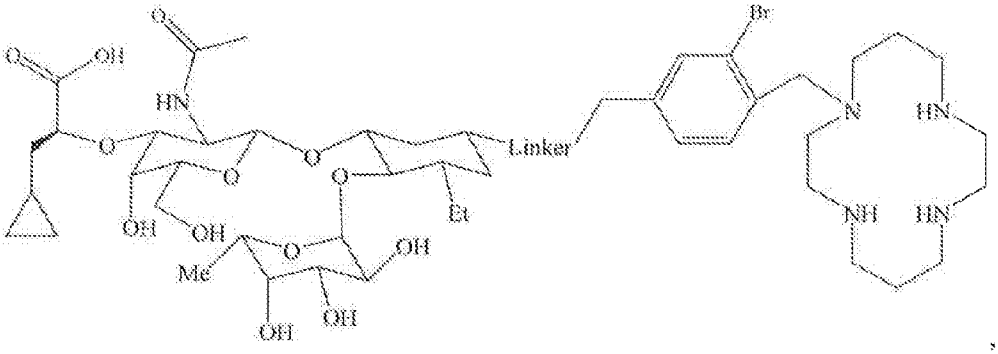
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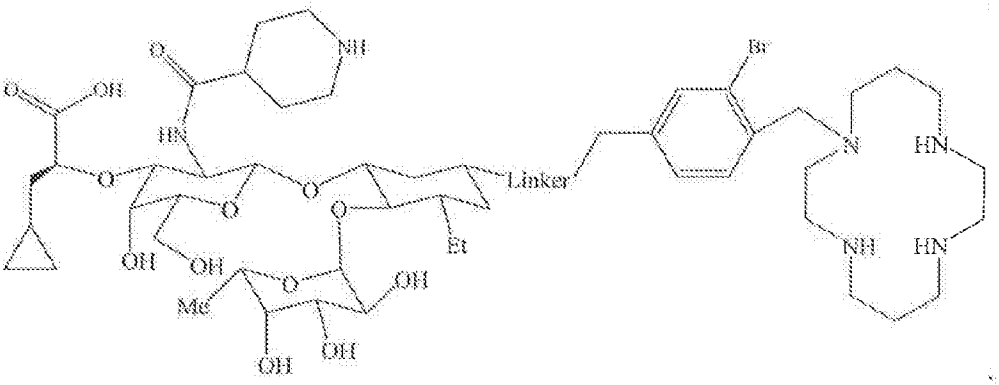
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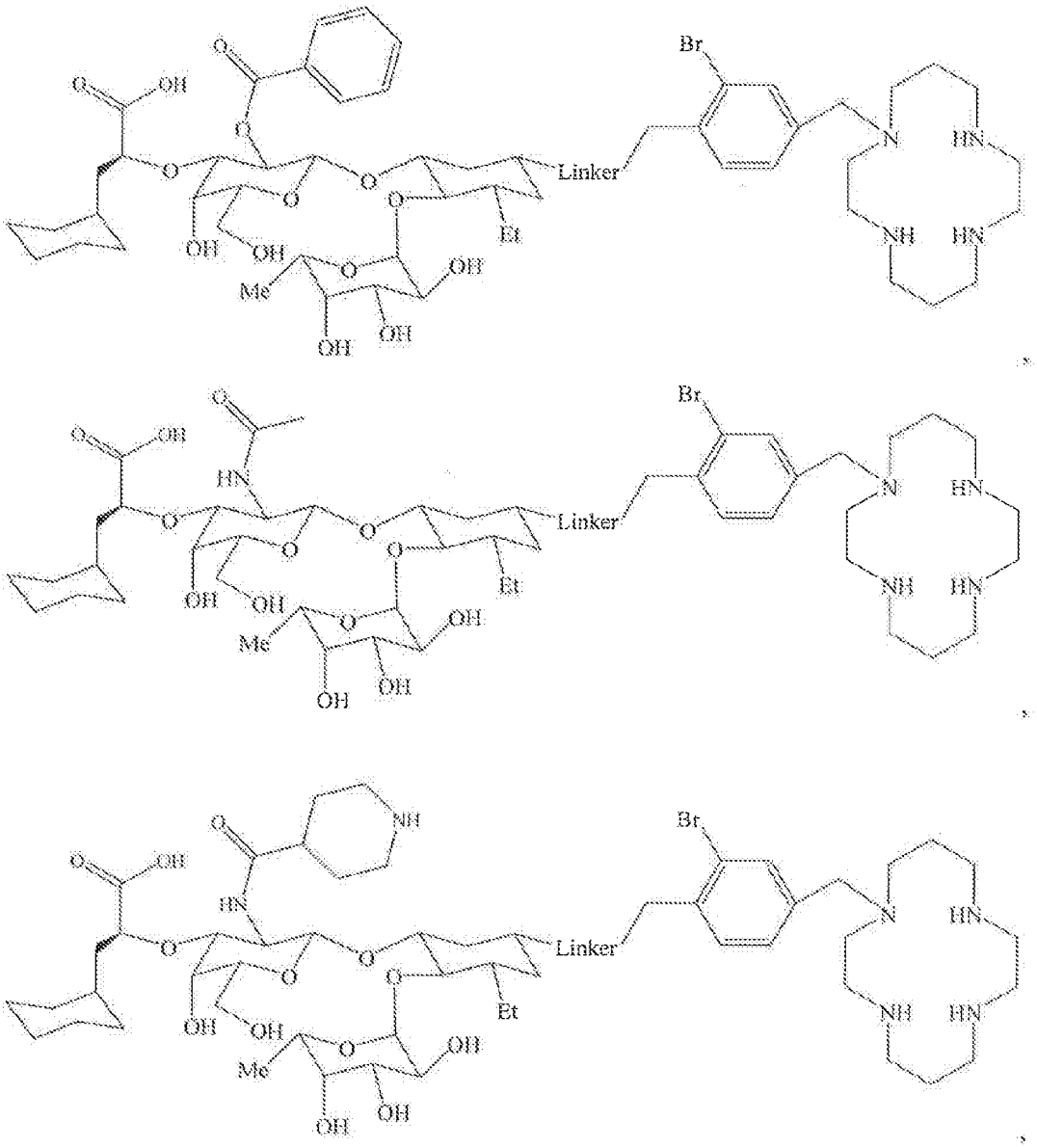
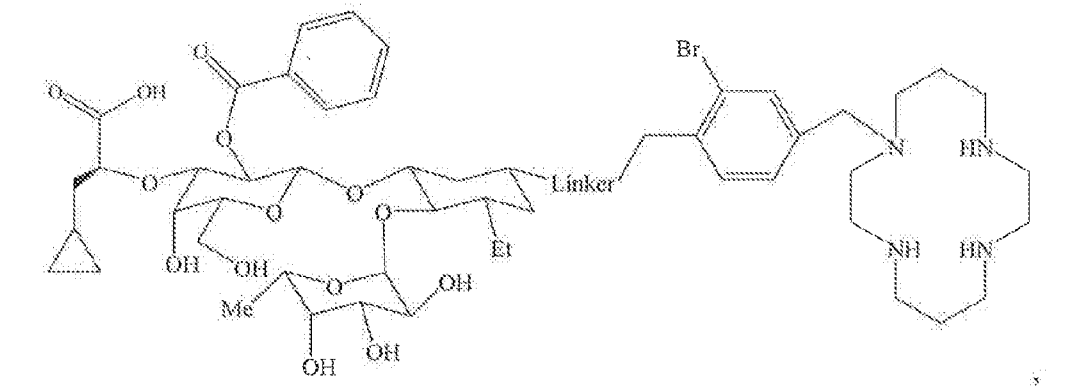
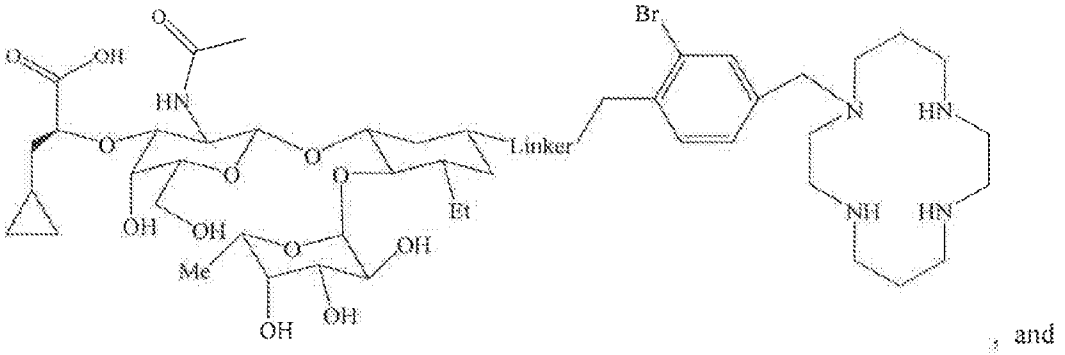
[0043] In some embodiments, the compound is chosen from compounds of the following Formulae:







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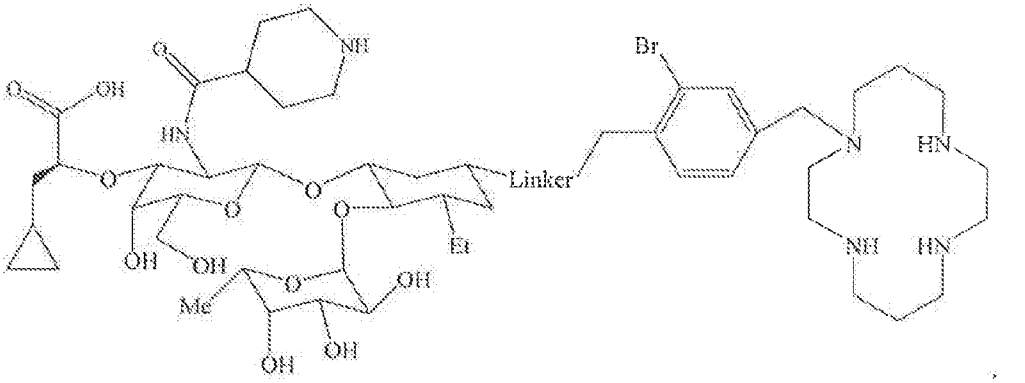
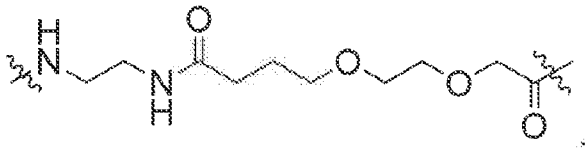
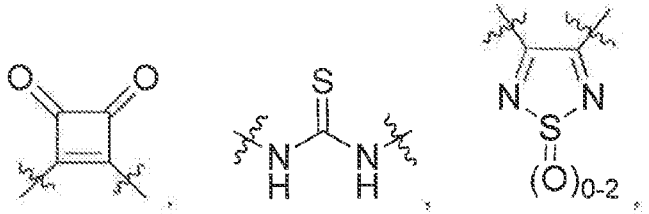
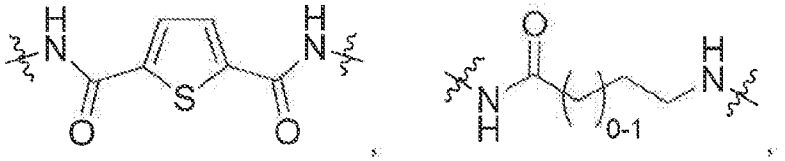


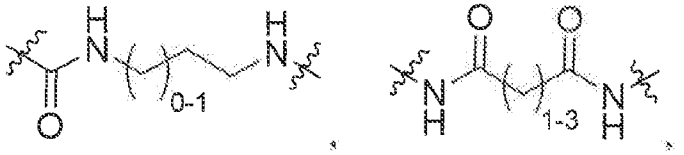
[0044] In some embodiments, linker groups may be chosen from groups comprising spacer groups, such spacer groups as, for example, -$(CH_2)_p$- and -$O(CH_2)_p$-, wherein p is chosen from integers ranging from 1 to 20. Other non-limiting examples of spacer groups include carbonyl groups and carbonyl-containing groups such as, for example, amide groups. A non-limiting example of a spacer group is

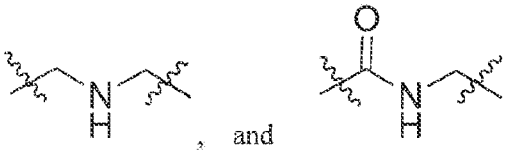


[0045] In some embodiments, the linker group is chosen from





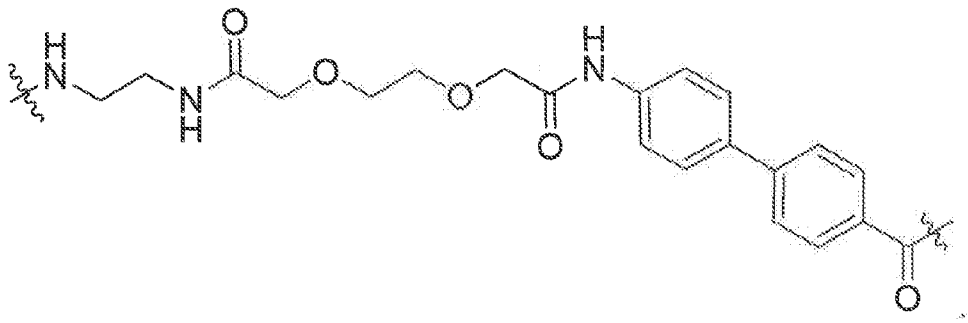




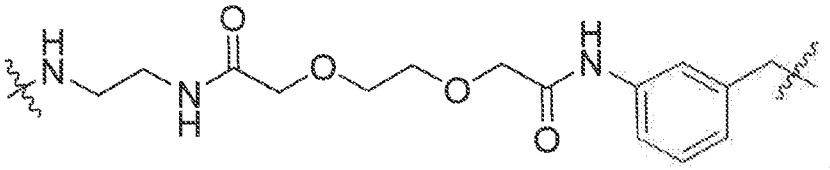
, and

[0046] Other linker groups, such as, for example, polyethylene glycols (PEGs) and -C(=O)-NH-$(CH_2)_p$-C(=O)-NH-, wherein p is chosen from integers ranging from 1 to 20, will be familiar to those of ordinary skill in the art and/or those in possession of the present disclosure.

[0047] In some embodiments, the linker group is

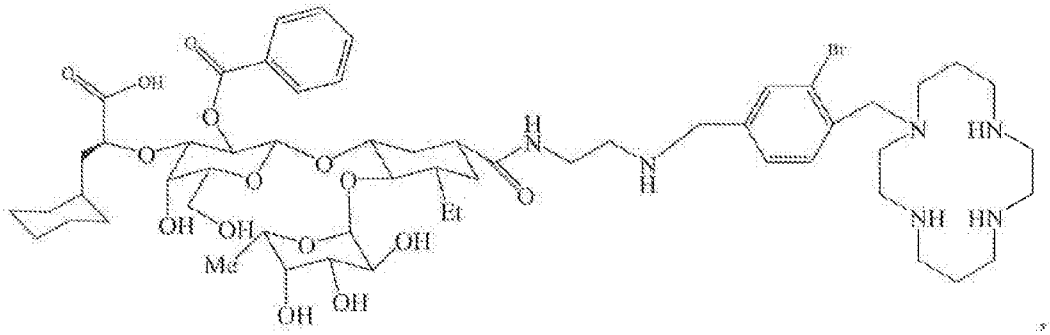


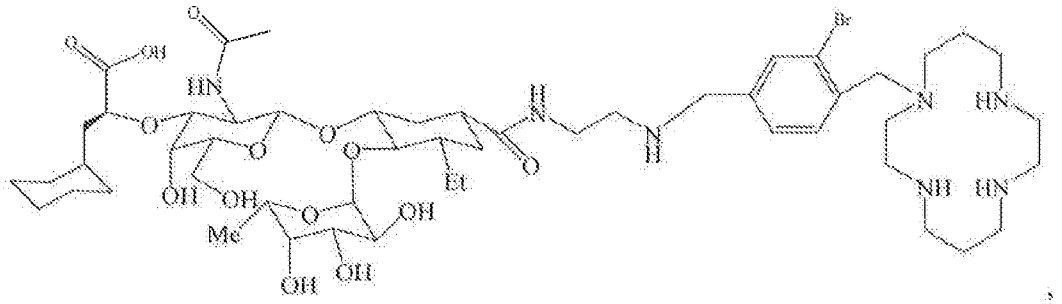
[0048] In some embodiments, the linker group is



[0049] In some embodiments, the linker group is chosen from –C(=O)NH(CH$_2$)$_2$NH-, -CH$_2$NHCH$_2$-, and –C(=O)NHCH$_2$-. In some embodiments, the linker group is -C(=O)NH(CH$_2$)$_2$NH-.

[0050] In some embodiments, the compound is chosen from compounds of the following Formulas:





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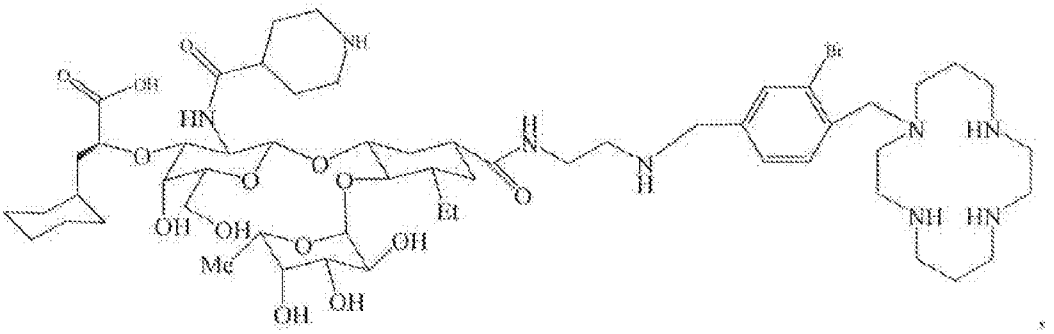

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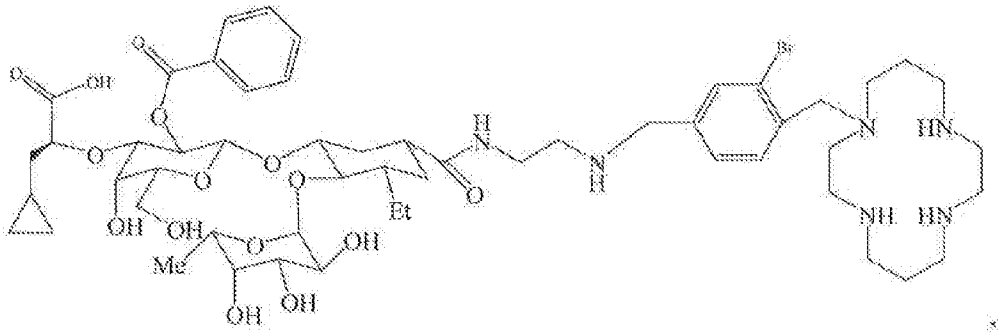

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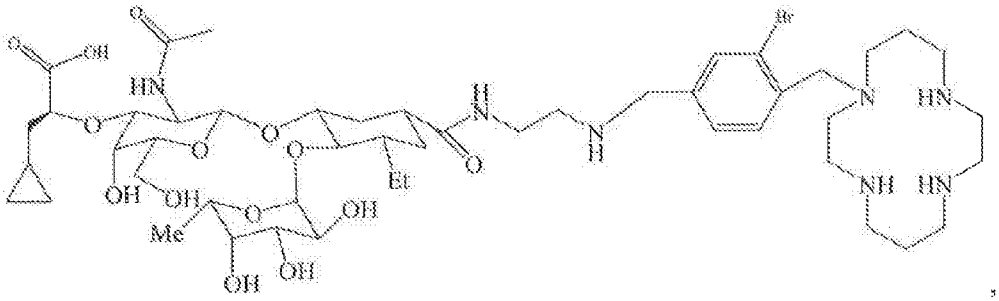

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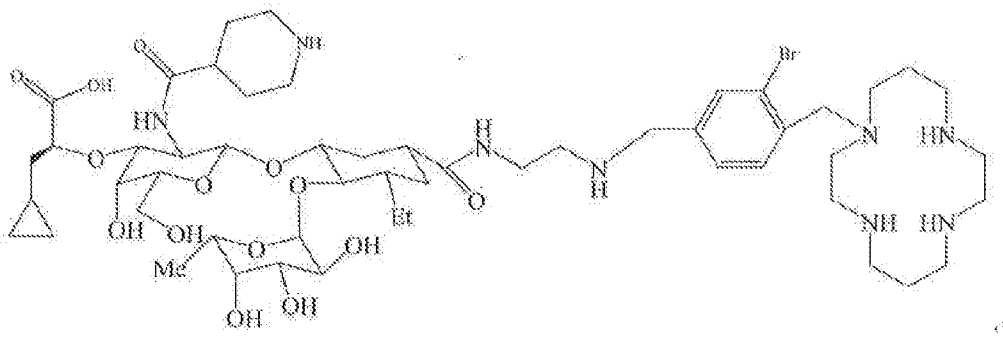
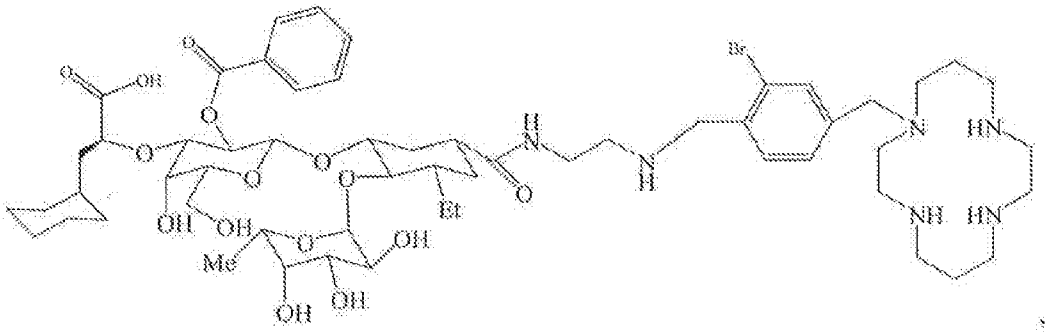
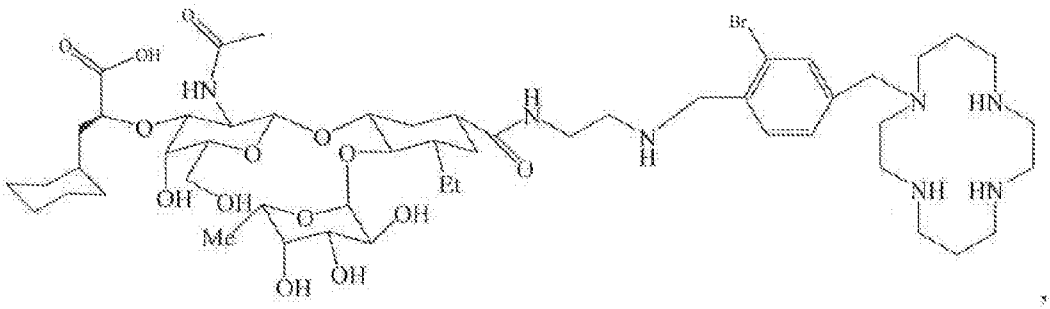
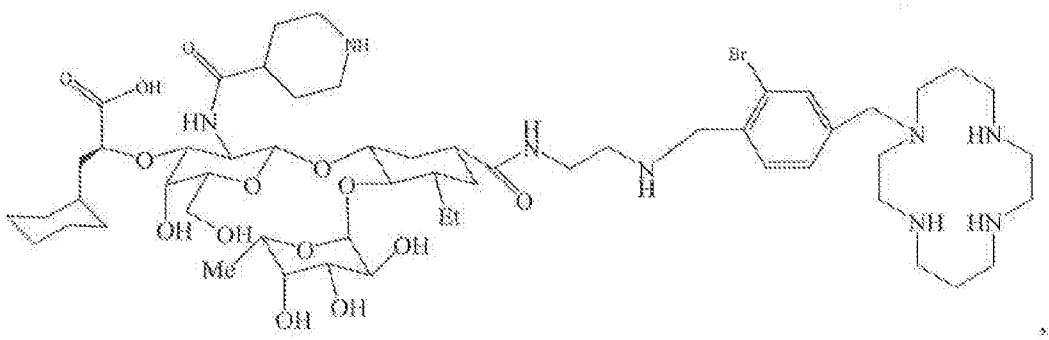
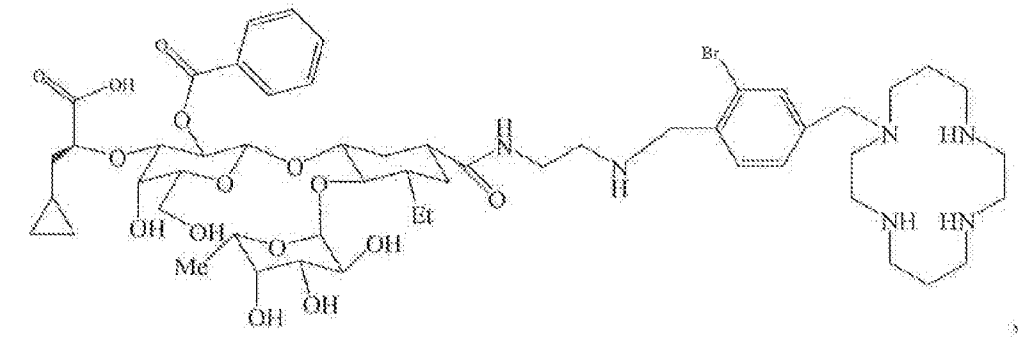


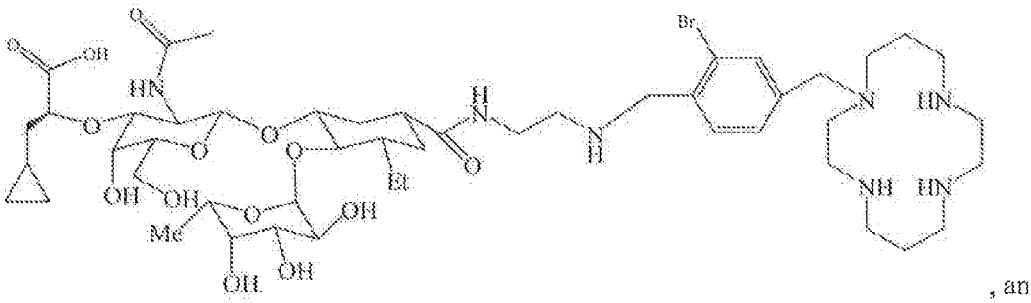
[0051] In some embodiments, the compound is chosen from compounds of the following Formulae:











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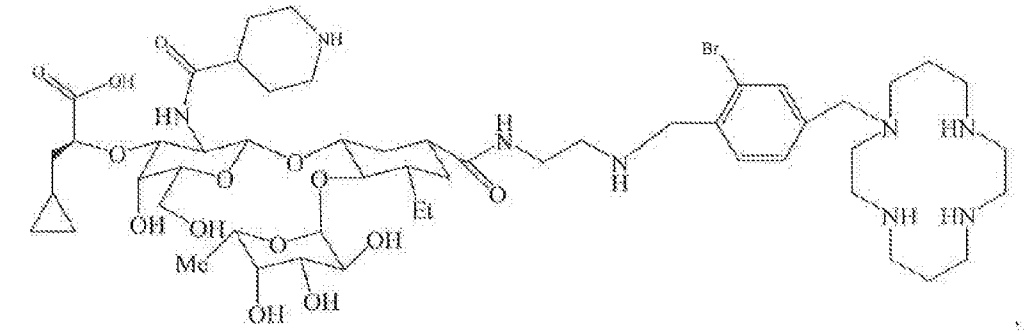


[0052] Also provided are pharmaceutical compositions comprising at least one compound of Formula (I). Such pharmaceutical compositions are described in greater detail herein. These compounds and compositions may be used in the methods described herein.

[0053] In some embodiments, at least one compound of Formula (I) and/or a pharmaceutical composition comprising at least one compound of Formula (I) may be used in methods described herein for treatment and/or prevention of a cancer in which the cancer cells may leave the primary site. A primary site may be, for example, solid tissue (e.g., breast, prostate, or pancreatic) or the bloodstream.

[0054] In addition to breast cancer, prostate cancer, and pancreatic cancer, other examples of infiltrating diseases include lung cancer and melanoma, as well as the

hematological malignancies (*e.g.*, leukemias and myelomas). As used herein, the term "treatment" (including variations such as "treating") includes for the disease or a complication associated with the disease. For example, a complication associated with the cancer may not have presented itself in an individual with the disease, and a compound may be administered to prevent presentation of the complication in the individual. Complications associated with a cancer in which the cancer cells may leave the primary site include, for example, metastasis and infiltration of cancer cells to other tissues. For example, acute myelogenous leukemia (AML) and multiple myeloma (MM) cells migrate to the endosteal region of the bone marrow where the cells become quiescent and are protected from chemotherapy–induced apoptosis. Administration of a compound described herein may prevent adhesion or migration of cancer cells. Such prevention can result in making the cancer cells more susceptible to treatment with chemotherapy. Administration of a compound described herein in the context of prevention may be to an individual who is at risk of occurrence of a cancer for the first time, or for recurrence of a cancer. For example, while a brain cancer such as glioblastoma multiforme is typically treated with another type of therapy (such as radiation or chemotherapy) for the first occurrence, such therapy is usually not effective to prevent recurrence.

[0055] In some embodiments, at least one compound of Formula (I) and/or a pharmaceutical composition comprising at least one compound of Formula (I) may be used in methods described herein for treatment and/or prevention of a cancer in which it is desired to mobilize cancer cells from a site into the bloodstream and retain the cancer cells in the bloodstream.

[0056] Examples of cancers for such treatment include leukemias and myelomas (*e.g.*, AML and MM). Mobilizing cancer cells into the bloodstream from a site and retaining the cells therein can result in making the cancer cells more susceptible to treatment with chemotherapy. An example of a site from which to mobilize cancer cells is bone. Cancer cells may, for example, be in circulation and then home to bone. Once in bone, the cancer cells are protected from chemotherapy. A compound described herein may be used, for example, to mobilize cancer cells from bone into the bloodstream and prevent cancer cells from homing to bone, thereby retaining the cancer cells in the bloodstream. Administration of a compound described herein in the context of prevention may be to an individual who is

at risk of occurrence of a cancer for the first time, or for recurrence of a cancer. For example, while a brain cancer such as glioblastoma multiforme is typically treated with another type of therapy (such as radiation or chemotherapy) for the first occurrence, such therapy is usually not effective to prevent recurrence.

[0057] In some embodiments, at least one compound of Formula (I) and/or a pharmaceutical composition comprising at least one compound of Formula (I) may be used in methods for relasing cells (cush as hematopoietic stem cells) into circulating blood and enhancing retention of the cells in the blood.

[0058] One use of the method is, for example, for stem cell harvesting. Stem cells may be needed, for example, after high–dose chemotherapy treatment. Many chemotherapies suppress bone marrow which disrupts the production of certain components of blood in an individual. As a result, the individual may develop a variety of blood cell related disorders and continuation of chemotherapy may be compromised. A compound described herein may be used, for example, to release stem cells into circulating blood and enhance retention of the stem cells in the blood. The method may include a further step of collecting cells that are released. For example, released stem cells may be collected. A variety of techniques are known in the art for collecting cells. For example, apheresis may be utilized. An example of a stem cells is a bone marrow progenitor cell. The release of such cells from bone marrow into circulating blood and retention therein has a variety of uses. For example, the mobilized bone marrow progenitor cells may be collected from the blood. A use of such collected cells is to obtain healthy bone marrow progenitor cells from an individual prior to treatment of the individual in a manner such that bone marrow is suppressed. Following treatment, the individual can receive a bone marrow transplantation utilizing the bone marrow progenitor cells collected prior to treatment. This is useful, for example, where an individual needs to be subjected to a chemotherapy protocol that will suppress bone marrow.

[0059] It can be desirable to additionally treat an individual with at least one (*i.e.*, one or more) colony stimulating factor. Such a factor may be administered, for example, before or simultaneous with administration of at least one of the above–described compounds. Where administration is simultaneous, the combination may be administered from a single container or two (or more) separate containers. An example of a suitable colony stimulating factor is

granulocyte–colony stimulating factor (G–CSF). G–CSF induces the bone marrow to grow and produce more stem cells. A compound described herein aids in releasing stem cells into circulating blood. Stem cells produced in bone marrow and released into circulating blood, as a result of the combination of the administration (separately or together) of a compound described herein and G–CSF, may be collected as described above. Such collected stem cells may be, for example, administered to the individual after chemotherapy. The stem cells return to the bone marrow and produce blood cells. Application of a compound described herein to mobilization and harvesting of healthy bone marrow progenitor cells from bone marrow treated with G–CSF provides cells useful, for example, for bone marrow transplantation.

[0060] In some embodiments, at least one compound of Formula (I) and/or a pharmaceutical composition comprising at least one compound of Formula (I) may be used in methods described herein for treatment and/or prevention of tumor metastasis. In some embodiments, the tumor metastasis arises from pancreatic cancer. In some emobidments, the tumor metastasis arises from prostate cancer. In some embodiments, the tumor metastasis arises from pancreatic cancer. In some embodiments, the tumor metastasis arises from breast cancer. In some embodiments, at least one additional chemotherapy agent such as gemcitabine is administered to the individual.

[0061] In some embodiments, at least one compound of Formula (I) and/or a pharmaceutical composition comprising at least one compound of Formula (I) may be used in methods for treatment and/or prevention of an inflammatory disease in which the adhesion or migration of cells occurs in the disease.

[0062] Examples of inflammatory diseases include inflammatory skin disorders such as atopic dermatitis and psoriasis. The treatment may reduce (partially or totally) the disease or a complication associated therewith, such as pain. The treatment may be used in conjunction with one or more other therapies for such an inflammatory disease or a complication associated therewith.

[0063] In some embodiments, a compound of Formula (I) and/or a pharmaceutical composition comprising at least one compound of Formula (I) may be used for treating at least one of the diseases, disorders, and conditions described herein or for the preparation or

manufacture of a medicament for use in treating at least one of the diseases, disorders, and/or conditions described herein. Each of these methods and uses is described in greater detail.

Definitions

[0064] Whenever a term in the specification is identified as a range (*e.g.*, C_{1-4} alkyl), the range independently discloses and includes each element of the range. As a non-limiting example, C_{1-4} alkyls includes, independently, C_1 alkyls, C_2 alkyls, C_3 alkyls, and C_4 alkyls.

[0065] The term "at least one" refers to one or more, such as one, two, etc. For example, the term "at least one C_{1-4} alkyl" refers to one or more C_{1-4} alkyl groups, such as one C_{1-4} alkyl group, two C_{1-4} alkyl groups, etc.

[0066] The term "alkyl" includes saturated straight, branched, and cyclic (also identified as cycloalkyl), primary, secondary, and tertiary hydrocarbon groups. Non-limiting examples of alkyl groups include methyl, ethyl, propyl, *iso*propyl, cyclopropyl, butyl, secbutyl, isobutyl, *tert*butyl, cyclobutyl, 1-methylbutyl, 1,1-dimethylpropyl, pentyl, cyclopentyl, isopentyl, neopentyl, cyclopentyl, hexyl, isohexyl, and cyclohexyl. Unless stated otherwise specifically in the specification, an alkyl group may be optionally substituted.

[0067] The term "alkenyl" includes straight, branched, and cyclic hydrocarbon groups comprising at least one double bond. The double bond of an alkenyl group can be unconjugated or conjugated with another unsaturated group. Non-limiting examples of alkenyl groups include vinyl, allyl, butenyl, pentenyl, hexenyl, butadienyl, pentadienyl, hexadienyl, 2-ethylhexenyl, and cyclopent-1-en-1-yl. Unless stated otherwise specifically in the specification, an alkenyl group may be optionally substituted.

[0068] The term "alkynyl" includes straight and branched hydrocarbon groups comprising at least one triple bonds. The triple bond of an alkynyl group can be unconjugated or conjugated with another unsaturated group. Non-limiting examples of alkynyl groups include ethynyl, propynyl, butynyl, pentynyl, and hexynyl. Unless stated otherwise specifically in the specification, an alkynyl group may be optionally substituted.

[0069] The term "aryl" includes hydrocarbon ring system group comprising 6 to 18 carbon ring atoms and at least one aromatic ring. The aryl group may be a monocyclic,

bicyclic, tricyclic or tetracyclic ring system, which may include fused or bridged ring systems. Non-limiting examples of aryl groups include aryl groups derived from aceanthrylene, acenaphthylene, acephenanthrylene, anthracene, azulene, benzene, chrysene, fluoranthene, fluorene, *as*-indacene, *s*-indacene, indane, indene, naphthalene, phenalene, phenanthrene, pleiadene, pyrene, and triphenylene. Unless stated otherwise specifically in the specification, an aryl group may be optionally substituted.

[0070] The term "arylalkyl" or "aralkyl" includes aryl groups, as described herein, appended to the parent molecular moiety through an alkyl group, as defined herein. Non-limiting examples of an arylalkyl or aralkyl group include benzyl, phenethyl, and diphenylmethyl. Unless stated otherwise specifically in the specification, an arylalkyl or aralkyl group may be optionally substituted.

[0071] The term "cycloalkyl" or "carbocyclic ring" includes saturated monocyclic or polycyclic hydrocarbon group, which may include fused or bridged ring systems. Non-limiting examples of a cycloalkyl group include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, adamantyl, and norbornyl. Unless otherwise stated specifically in the specification, a cycloalkyl group may be optionally substituted.

[0072] The term "E-selectin antagonist" includes inhibitors of E-selectin only, as well as inhibitors of E-selectin and either P-selectin or L-selectin, and inhibitors of E-selectin, P-selectin, and L-selectin.

[0073] The term "fused" includes any ring structure described herein which is fused to an existing ring structure. When the fused ring is a heterocyclyl ring or a heteroaryl ring, any carbon atom on the existing ring structure which becomes part of the fused heterocyclyl ring or the fused heteroaryl ring may be replaced with a nitrogen atom.

[0074] The term "halo" or "halogen" includes fluoro, chloro, bromo, and iodo.

[0075] The term "haloalkyl" includes alkyl groups, as defined herein, substituted by at least one halogen, as defined herein. Non-limiting examples include trifluoromethyl, difluoromethyl, trichloromethyl, 2,2,2-trifluoroethyl, 1,2-difluoroethyl, 3-bromo-2-fluoropropyl, and 1,2-dibromoethyl. A "fluoroalkyl" is a haloalkyl that is

substituted with at least one fluoro group. Unless stated otherwise specifically in the specification, a haloalkyl group may be optionally substituted.

[0076] The term "haloalkenyl" includes alkenyl groups, as defined herein, substituted by at least one halogen, as defined herein. Non-limiting examples include fluoroethenyl, 1,2-difluoroethenyl, 3-bromo-2-fluoropropenyl, and 1,2-dibromoethenyl. A "fluoroalkenyl" is a haloalkenyl substituted with at least one fluoro group. Unless stated otherwise specifically in the specification, a haloalkenyl group may be optionally substituted.

[0077] The term "haloalkynyl" includes alkynyl groups, as defined herein, substituted by at least one halogen, as defined herein. Non-limiting examples include fluoroethynyl, 1,2-difluoroethynyl, 3-bromo-2-fluoropropynyl, and 1,2-dibromoethynyl. A "fluoroalkynyl" is a haloalkynyl substituted with at least one fluoro group. Unless stated otherwise specifically in the specification, a haloalkynyl group may be optionally substituted.

[0078] The term "heterocyclyl" or "heterocyclic ring" includes 3- to 18-membered saturated or partially unsaturated non-aromatic ring groups comprising 2 to 12 ring carbon atoms and 1 to 6 ring heteroatom(s) each independently chosen from N, O, and S. Unless stated otherwise specifically in the specification, the heterocyclyl groups may be a monocyclic, bicyclic, tricyclic or tetracyclic ring system, which may include fused or bridged ring systems; and the nitrogen, carbon or sulfur atoms in the heterocyclyl group may be optionally oxidized; the nitrogen atom may be optionally quaternized; and the heterocyclyl group may be partially or fully saturated. Non-limiting examples include dioxolanyl, thienyl[1,3]dithianyl, decahydroisoquinolyl, imidazolinyl, imidazolidinyl, isothiazolidinyl, isoxazolidinyl, morpholinyl, octahydroindolyl, octahydroisoindolyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolidinyl, oxazolidinyl, piperidinyl, piperazinyl, 4-piperidonyl, pyrrolidinyl, pyrazolidinyl, quinuclidinyl, thiazolidinyl, tetrahydrofuryl, trithianyl, tetrahydropyranyl, thiomorpholinyl, thiamorpholinyl, 1-oxo-thiomorpholinyl, and 1,1-dioxo-thiomorpholinyl. Unless stated otherwise specifically in the specification, a heterocyclyl group may be optionally substituted.

[0079] The term "heteroaryl" includes 5- to 14-membered ring groups comprising 1 to 13 ring carbon atoms and 1 to 6 ring heteroatom(s) each independently chosen from N, O, and S, and at least one aromatic ring. Unless stated otherwise specifically in the specification, the

heteroaryl group may be a monocyclic, bicyclic, tricyclic or tetracyclic ring system, which may include fused or bridged ring systems; and the nitrogen, carbon or sulfur atoms in the heteroaryl radical may be optionally oxidized; the nitrogen atom may be optionally quaternized. Non-limiting examples include azepinyl, acridinyl, benzimidazolyl, benzothiazolyl, benzindolyl, benzodioxolyl, benzofuranyl, benzooxazolyl, benzothiazolyl, benzothiadiazolyl, benzo[*b*][1,4]dioxepinyl, 1,4-benzodioxanyl, benzonaphthofuranyl, benzoxazolyl, benzodioxolyl, benzodioxinyl, benzopyranyl, benzopyranonyl, benzofuranyl, benzofuranonyl, benzothienyl (benzothiophenyl), benzotriazolyl, benzo[4,6]imidazo[1,2-a]pyridinyl, carbazolyl, cinnolinyl, dibenzofuranyl, dibenzothiophenyl, furanyl, furanonyl, isothiazolyl, imidazolyl, indazolyl, indolyl, indazolyl, isoindolyl, indolinyl, isoindolinyl, isoquinolyl, indolizinyl, isoxazolyl, naphthyridinyl, oxadiazolyl, 2-oxoazepinyl, oxazolyl, oxiranyl, 1-oxidopyridinyl, 1-oxidopyrimidinyl, 1-oxidopyrazinyl, 1-oxidopyridazinyl, 1-phenyl-1*H*-pyrrolyl, phenazinyl, phenothiazinyl, phenoxazinyl, phthalazinyl, pteridinyl, purinyl, pyrrolyl, pyrazolyl, pyridinyl, pyrazinyl, pyrimidinyl, pyridazinyl, quinazolinyl, quinoxalinyl, quinolinyl, quinuclidinyl, isoquinolinyl, tetrahydroquinolinyl, thiazolyl, thiadiazolyl, triazolyl, tetrazolyl, triazinyl, and thiophenyl (i.e. thienyl). Unless stated otherwise specifically in the specification, a heteroaryl group may be optionally substituted.

[0080] The term "pharmaceutically acceptable salts" includes both acid and base addition salts. Non-limiting examples of pharmaceutically acceptable acid addition salts include chlorides, bromides, sulfates, nitrates, phosphates, sulfonates, methane sulfonates, formates, tartrates, maleates, citrates, benzoates, salicylates, and ascorbates. Non-limiting examples of pharmaceutically acceptable base addition salts include sodium, potassium, lithium, ammonium (substituted and unsubstituted), calcium, magnesium, iron, zinc, copper, manganese, and aluminum salts. Pharmaceutically acceptable salts may, for example, be obtained using standard procedures well known in the field of pharmaceuticals.

[0081] The term "prodrug" includes compounds that may be converted, for example, under physiological conditions or by solvolysis, to a biologically active compound described herein. Thus, the term "prodrug" includes metabolic precursors of compounds described herein that are pharmaceutically acceptable. A discussion of prodrugs can be found, for example, in Higuchi, T., et al., "Pro-drugs as Novel Delivery Systems," A.C.S. Symposium

Series, Vol. 14, and in Bioreversible Carriers in Drug Design, ed. Edward B. Roche, American Pharmaceutical Association and Pergamon Press, 1987. The term "prodrug" also includes covalently bonded carriers that release the active compound(s) as described herein *in vivo* when such prodrug is administered to a subject. Non-limiting examples of prodrugs include ester and amide derivatives of hydroxy, carboxy, mercapto and amino functional groups in the compounds described herein.

[0082] The term "substituted" includes the situation where, in any of the above groups, at least one hydrogen atom is replaced by a non-hydrogen atom such as, for example, a halogen atom such as F, Cl, Br, and I; an oxygen atom in groups such as hydroxyl groups, alkoxy groups, and ester groups; a sulfur atom in groups such as thiol groups, thioalkyl groups, sulfone groups, sulfonyl groups, and sulfoxide groups; a nitrogen atom in groups such as amines, amides, alkylamines, dialkylamines, arylamines, alkylarylamines, diarylamines, N-oxides, imides, and enamines; a silicon atom in groups such as trialkylsilyl groups, dialkylarylsilyl groups, alkyldiarylsilyl groups, and triarylsilyl groups; and other heteroatoms in various other groups. "Substituted" also includes the situation where, in any of the above groups, at least one hydrogen atom is replaced by a higher-order bond (e.g., a double- or triple-bond) to a heteroatom such as oxygen in oxo, carbonyl, carboxyl, and ester groups; and nitrogen in groups such as imines, oximes, hydrazones, and nitriles.

[0083] The term "thioalkyl" includes $-SR_a$ groups wherein R_a is chosen from alkyl, alkenyl, and alkynyl groups, as defined herein. Unless stated otherwise specifically in the specification, a thioalkyl group may be optionally substituted.

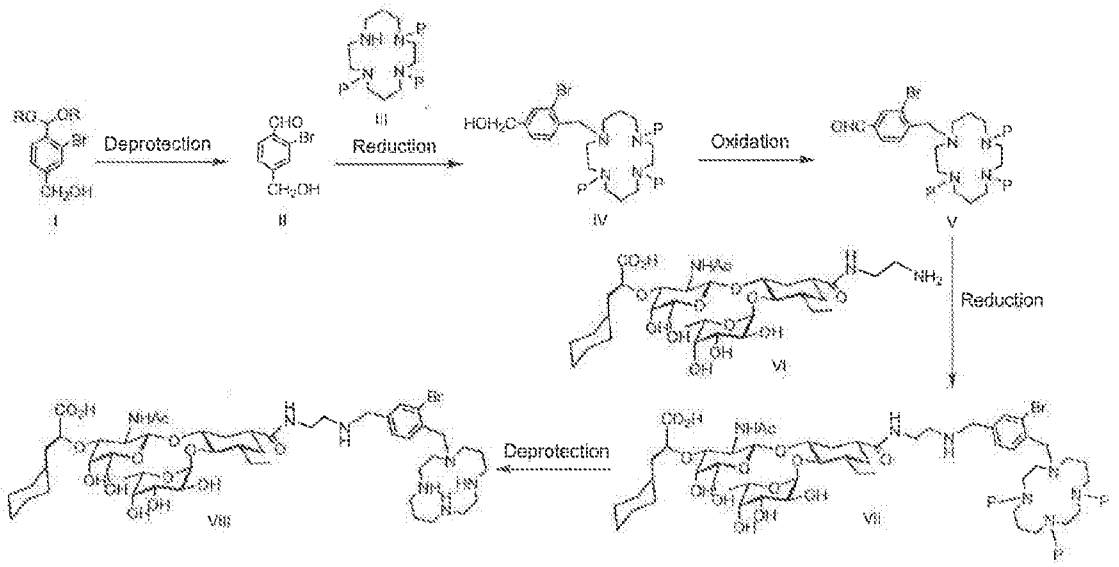
[0084] The present disclosure includes within its scope all the possible geometric isomers, *e.g.*, Z and E isomers (*cis* and *trans* isomers), of the compounds as well as all the possible optical isomers, e.g. diastereomers and enantiomers, of the compounds. Furthermore, the present disclosure includes in its scope both the individual isomers and any mixtures thereof, e.g. racemic mixtures. The individual isomers may be obtained using the corresponding isomeric forms of the starting material or they may be separated after the preparation of the end compound according to conventional separation methods. For the separation of optical isomers, *e.g.*, enantiomers, from the mixture thereof conventional resolution methods, e.g. fractional crystallization, may be used.

[0085] The present disclosure includes within its scope all possible tautomers. Furthermore, the present disclosure includes in its scope both the individual tautomers and any mixtures thereof.

Compound Synthesis Procedures

[0086] Compounds of Formula (I) may be prepared according to General Reaction Schemes I and II below. It is understood that one of ordinary skill in the art may be able to make these compounds by similar methods or by combining other methods known to one of ordinary skill in the art. It is also understood that one of ordinary skill in the art would be able to make, in a similar manner as described below, other compounds of Formula (I) not specifically illustrated herein by using appropriate starting components and modifying the parameters of the synthesis as needed. In general, starting components may be obtained from sources such as Sigma Aldrich, Lancaster Synthesis, Inc., Maybridge, Matrix Scientific, TCI, and Fluorochem USA, etc. and/or synthesized according to sources known to those of ordinary skill in the art (see, for example, Advanced Organic Chemistry: Reactions, Mechanisms, and Structure, 5th edition (Wiley, December 2000)) and/or prepared as described herein.

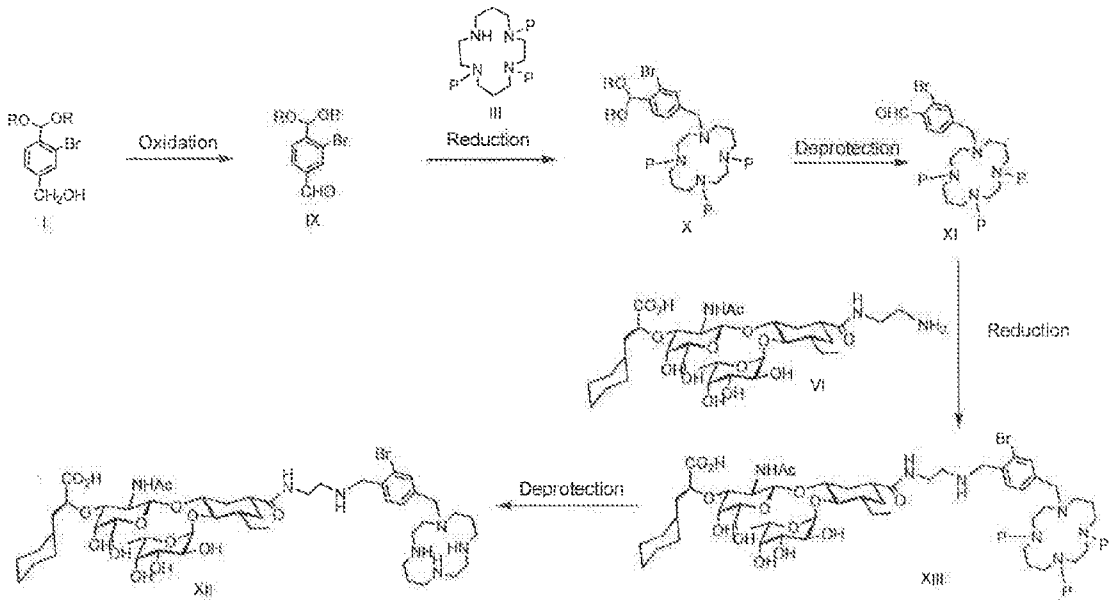
General Reaction Scheme I



[0087] Deprotection of compound I gives brominated hydroxymethyl aldehyde II. Reductive amination with a suitably tri-protected cyclam generates compound IV. Oxidation gives aldehyde V which can be coupled to compound VI (WO 2013/096926) via reductive amination. Deprotection then gives a compound of the invention.

[0088] Alternatively, the regioisomeric bromide can be prepared according to Scheme II. Oxidation of compound I gives the aldehyde IX. Reductive amination with a suitably tri-protected cyclam gives intermediate X. Deprotection provides XI which can be coupled with compound VI via reductive amination to provide XIII. Deprotection then gives a compound of the invention.

General Reaction Scheme II



[0089] Those of ordinary skill in the art will understand that, in processes described herein, the functional groups of intermediate compounds may need to be protected by at least one suitable protecting group. Non-limiting examples of such functional groups include, hydroxyl groups, aldehyde groups, amino groups, mercapto groups, and carboxylic acid groups. Non-limiting examples of suitable protecting groups for hydroxy groups include trialkylsilyl and diarylalkylsilyl groups (for example, t-butyldimethylsilyl, t-

butyldiphenylsilyl or trimethylsilyl), tetrahydropyranyl, and benzyl. Non-limiting examples of suitable protecting groups for aldehyde groups include 1,3-dioxanes and 1,3-dioxolanes. Non-limiting examples of suitable protecting groups for amino, amidino and guanidino include t-butoxycarbonyl, benzyloxycarbonyl, allyloxycarbonyl, and trifluoracetyl groups. Non-limiting examples of suitable protecting groups for mercapto include -C(O)-R" (where R" is alkyl, aryl or arylalkyl), p-methoxybenzyl, and trityl groups. Non-limiting examples of suitable protecting groups for carboxylic acid include alkyl, aryl and arylalkyl esters. Protecting groups may be added or removed in accordance with standard techniques, which are known to one of ordinary skill in the art and as described herein. The use of protecting groups is, for example, described in detail in Green, T.W. and P.G.M. Wutz, Protective Groups in Organic Synthesis (1999), 3rd Ed., Wiley. As one of ordinary skill in the art would appreciate, the protecting group may also be a polymer resin such as a Wang resin, Rink resin or a 2-chlorotrityl-chloride resin.

Methods for Characterizing Heterobifuctional Compounds

[0090] Biological activity of a heterobifuctional compound described herein may be determined, for example, by performing at least one *in vitro* and/or *in vivo* study routinely practiced in the art and described herein or in the art. *In vitro* assays include without limitation binding assays, immunoassays, competitive binding assays and cell based activity assays.

[0091] An inhibition assay may be used to screen for antagonists of E-selectin. For example, an assay may be performed to characterize the capability of a compound described herein to inhibit (*i.e.*, reduce, block, decrease, or prevent in a statistically or biologically significant manner) interaction of E-selectin with sLe^a or sLe^x. The inhibition assay may be a competitive binding assay, which allows the determination of IC_{50} values. By way of example, E-selectin/Ig chimera may be immobilized onto a matrix (*e.g.*, a multi-well plate, which may be made from a polymer, such as polystyrene; a test tube, and the like); a composition may be added to reduce nonspecific binding (*e.g.*, a composition comprising non-fat dried milk or bovine serum albumin or other blocking buffer routinely used by a person skilled in the art); the immobilized E-selectin may be contacted with the candidate compound in the presence of sLe^a comprising a reporter group under conditions and for a

time sufficient to permit sLe^a to bind to the immobilized E-selectin; the immobilized E-selectin may be washed; and the amount of sLe^a bound to immobilized E-selectin may be detected. Variations of such steps can be readily and routinely accomplished by a person of ordinary skill in the art.

[0092] An inhibition assay may be used to screen for antagonism of CXCR4 mediated chemotaxis. For example, an assay may be performed to measure the ability of a glycomimetic CXCR4 antagonist to inhibit migration of CCRF-CEM cells, which express CXCR4 on their cell surfaces, across a membrane toward the CXCR4 ligand CXCL12 (SDF-1α). By way of example, CCRF-CEM cells are human T lymphoblasts that express CXCR4 on the cell surface. The cells may be labeled with 3 uM Calcein AM to enable detection by fluorescence. The cells may be treated with a CXCR4 antagonist and placed into the upper chamber of a transwell insert. The transwells may be placed into the wells of a 24-well plate with each well containing 600 ul of RPMI 1640 plus 2% FBS and 50 ng/mL CXCL12 (SDF1α). The cells may be allowed to migrate across the membrane from the upper chamber into the lower chamber for 3 hours at 37°C in 5% CO2. The transwell inserts may be removed from the 24-well plate and the fluorescence in the lower chambers measured using a Molecular Devices FlexStation 3 with an excitation wavelength of 485 nm and an emission wavelength of 538 nm.

[0093] Alternatively, an assay may be used to measure the ability of a glycomimetic CXCR4 antagonist to inhibit the binding of CXCL12 (SDF-1α) to CHO cells that have been genetically engineered to express CXCR4 on the cell surface. One skilled in the art may activate CXCR4 by ligand binding (CXCL12), causing Gi to dissociate from the CXCR4 complex. The activated CXCR4 may bind to adenylyl cyclase, thus inactivating it, resulting in decreased levels of intracellular cAMP. Intracellular cAMP is usually low, so the decrease of the low level of cAMP by a Gi-coupled receptor will be hard to detect. Forskolin is added to the CHO cells to directly activate adenylyl cyclase (bypassing all GPCRs), thus raising the level of cAMP in the cell, so that a Gi response can be easily observed. CXCL12 interaction with CXCR4 decreases the intracellular level of cAMP and inhibition of CXCL12 interaction with CXCR4 by a CXCR4 antagonist increases the intracellular cAMP level, which is measured by luminescence.

[0094] Alternatively, one skilled in the art may use an assay to measure the ability of a glycomimetic CXCR4 antagonist to block the binding of an anti-CXCR4 antibody to Jurkat cells, which express CXCR4 on the cell surface. Jurkat cells may be treated with a CXCR4 antagonist followed by a phycoerythrin-conjugated anti-CXCR4 antibody. The antibody may be allowed to bind to the cells for 1 hour at 4°C. The cells may be washed and the binding of the anti-CXCR4-PE antibody to the cells may be assessed by flow cytometry.

[0095] Conditions for a particular assay include temperature, buffers (including salts, cations, media), and other components that maintain the integrity of any cell used in the assay and the compound, which a person of ordinary skill in the art will be familiar and/or which can be readily determined. A person of ordinary skill in the art also readily appreciates that appropriate controls can be designed and included when performing the *in vitro* methods and *in vivo* methods described herein.

[0096] The source of a compound that is characterized by at least one assay and techniques described herein and in the art may be a biological sample that is obtained from a subject who has been treated with the compound. The cells that may be used in the assay may also be provided in a biological sample. A "biological sample" may include a sample from a subject, and may be a blood sample (from which serum or plasma may be prepared), a biopsy specimen, one or more body fluids (*e.g.*, lung lavage, ascites, mucosal washings, synovial fluid, urine), bone marrow, lymph nodes, tissue explant, organ culture, or any other tissue or cell preparation from the subject or a biological source. A biological sample may further include a tissue or cell preparation in which the morphological integrity or physical state has been disrupted, for example, by dissection, dissociation, solubilization, fractionation, homogenization, biochemical or chemical extraction, pulverization, lyophilization, sonication, or any other means for processing a sample derived from a subject or biological source. In some embodiments, the subject or biological source may be a human or non-human animal, a primary cell culture (*e.g.*, immune cells), or culture adapted cell line, including but not limited to, genetically engineered cell lines that may contain chromosomally integrated or episomal recombinant nucleic acid sequences, immortalized or immortalizable cell lines, somatic cell hybrid cell lines, differentiated or differentiatable cell lines, transformed cell lines, and the like.

[0097] As described herein, methods for characterizing heterobifunctional inhibitors include animal model studies. Non-limiting examples of animal models for liquid cancers used in the art include multiple myeloma (*see, e.g.,* DeWeerdt, Nature 480:S38–S39 (15 December 2011) doi:10.1038/480S38a; Published online 14 December 2011; Mitsiades et al., *Clin. Cancer Res.* 2009 15:1210021 (2009)); acute myeloid leukemia (AML) (Zuber et al., *Genes Dev.* 2009 April 1; 23(7): 877–889). Animal models for acute lymphoblastic leukemia (ALL) have been used by persons of ordinary skill in the art for more than two decades. Numerous exemplary animal models for solid tumor cancers are routinely used and are well known to persons of ordinary skill in the art.

[0098] As understood by a person of ordinary skill in the medical art, the terms, "treat" and "treatment," include medical management of a disease, disorder, or condition of a subject (*i.e.,* patient, individual) (*see, e.g.,* Stedman's Medical Dictionary). In general, an appropriate dose and treatment regimen provide at least one of the compounds of the present disclosure in an amount sufficient to provide therapeutic and/or prophylactic benefit. For both therapeutic treatment and prophylactic or preventative measures, therapeutic and/or prophylactic benefit includes, for example, an improved clinical outcome, wherein the object is to prevent or slow or retard (lessen) an undesired physiological change or disorder, or to prevent or slow or retard (lessen) the expansion or severity of such disorder. As discussed herein, beneficial or desired clinical results from treating a subject include, but are not limited to, abatement, lessening, or alleviation of symptoms that result from or are associated with the disease, condition, or disorder to be treated; decreased occurrence of symptoms; improved quality of life; longer disease-free status (*i.e.,* decreasing the likelihood or the propensity that a subject will present symptoms on the basis of which a diagnosis of a disease is made); diminishment of extent of disease; stabilized (*i.e.,* not worsening) state of disease; delay or slowing of disease progression; amelioration or palliation of the disease state; and remission (whether partial or total), whether detectable or undetectable; and/or overall survival. "Treatment" can include prolonging survival when compared to expected survival if a subject were not receiving treatment. Subjects in need of treatment include those who already have the disease, condition, or disorder as well as subjects prone to have or at risk of developing the disease, condition, or disorder, and those in which the disease, condition, or disorder is to be prevented (*i.e.,* decreasing the likelihood of occurrence of the disease, disorder, or condition).

[0099] In some embodiments of the methods described herein, the subject is a human. In some embodiments of the methods described herein, the subject is a non-human animal. A subject in need of treatment as described herein may exhibit at least one symptom or sequelae of the disease, disorder, or condition described herein or may be at risk of developing the disease, disorder, or condition. Non-human animals that may be treated include mammals, for example, non-human primates (*e.g.*, monkey, chimpanzee, gorilla, and the like), rodents (*e.g.*, rats, mice, gerbils, hamsters, ferrets, rabbits), lagomorphs, swine (*e.g.*, pig, miniature pig), equine, canine, feline, bovine, and other domestic, farm, and zoo animals.

[00100] The effectiveness of the compounds of the present disclosure in treating and/or preventing a disease, disorder, or condition described herein can readily be determined by a person of ordinary skill in the medical and clinical arts. Determining and adjusting an appropriate dosing regimen (*e.g.*, adjusting the amount of compound per dose and/or number of doses and frequency of dosing) can also readily be performed by a person of ordinary skill in the medical and clinical arts. One or any combination of diagnostic methods, including physical examination, assessment and monitoring of clinical symptoms, and performance of analytical tests and methods described herein, may be used for monitoring the health status of the subject.

Pharmaceutical Compositions and Methods of Using Pharmaceutical Compositions

[00101] Also provided herein are pharmaceutical compositions comprising at least one compound of Formula (I). In some embodiments, the pharmaceutical composition further comprises at least one additional pharmaceutically acceptable ingredient.

[00102] In pharmaceutical dosage forms, any one or more of the compounds of the present disclosure may be administered in the form of a pharmaceutically acceptable derivative, such as a salt, and/or it/they may also be used alone and/or in appropriate association, as well as in combination, with other pharmaceutically active compounds.

[00103] An effective amount or therapeutically effective amount refers to an amount of a compound of the present disclosure or a composition comprising at least one such compound that, when administered to a subject, either as a single dose or as part of a series of doses, is effective to produce at least one therapeutic effect. Optimal doses may generally be

determined using experimental models and/or clinical trials. Design and execution of pre-clinical and clinical studies for each of the therapeutics (including when administered for prophylactic benefit) described herein are well within the skill of a person of ordinary skill in the relevant art. The optimal dose of a therapeutic may depend upon the body mass, weight, and/or blood volume of the subject. In general, the amount of at least one compound of Formula (I) as described herein, that is present in a dose, may range from about 0.01 μg to about 1000 μg per kg weight of the subject. The minimum dose that is sufficient to provide effective therapy may be used in some embodiments. Subjects may generally be monitored for therapeutic effectiveness using assays suitable for the disease or condition being treated or prevented, which assays will be familiar to those having ordinary skill in the art and are described herein. The level of a compound that is administered to a subject may be monitored by determining the level of the compound (or a metabolite of the compound) in a biological fluid, for example, in the blood, blood fraction (*e.g.*, serum), and/or in the urine, and/or other biological sample from the subject. Any method practiced in the art to detect the compound, or metabolite thereof, may be used to measure the level of the compound during the course of a therapeutic regimen.

[00104] The dose of a compound described herein may depend upon the subject's condition, that is, stage of the disease, severity of symptoms caused by the disease, general health status, as well as age, gender, and weight, and other factors apparent to a person of ordinary skill in the medical art. Similarly, the dose of the therapeutic for treating a disease or disorder may be determined according to parameters understood by a person of ordinary skill in the medical art.

[00105] Pharmaceutical compositions may be administered in any manner appropriate to the disease or disorder to be treated as determined by persons of ordinary skill in the medical arts. An appropriate dose and a suitable duration and frequency of administration will be determined by such factors as discussed herein, including the condition of the patient, the type and severity of the patient's disease, the particular form of the active ingredient, and the method of administration. In general, an appropriate dose (or effective dose) and treatment regimen provides the pharmaceutical composition(s) as described herein in an amount sufficient to provide therapeutic and/or prophylactic benefit (for example, an improved clinical outcome, such as more frequent complete or partial remissions, or longer disease-free

and/or overall survival, or a lessening of symptom severity or other benefit as described in detail above).

[00106] The pharmaceutical compositions described herein may be administered to a subject in need thereof by any one of several routes that effectively delivers an effective amount of the compound. Non-limiting suitable administrative routes include topical, oral, nasal, intrathecal, enteral, buccal, sublingual, transdermal, rectal, vaginal, intraocular, subconjunctival, sublingual, and parenteral administration, including subcutaneous, intravenous, intramuscular, intrasternal, intracavernous, intrameatal, and intraurethral injection and/or infusion.

[00107] The pharmaceutical composition described herein may be sterile aqueous or sterile non-aqueous solutions, suspensions or emulsions, and may additionally comprise at least one pharmaceutically acceptable excipient (*i.e.*, a non-toxic material that does not interfere with the activity of the active ingredient). Such compositions may be in the form of a solid, liquid, or gas (aerosol). Alternatively, the compositions described herein may be formulated as a lyophilizate, or compounds described herein may be encapsulated within liposomes using technology known in the art. The pharmaceutical compositions may further comprise at least one additional pharmaceutical acceptable ingredient, which may be biologically active or inactive. Non-limiting examples of such ingredients include buffers (*e.g.*, neutral buffered saline or phosphate buffered saline), carbohydrates (*e.g.*, glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides, amino acids (*e.g.*, glycine), antioxidants, chelating agents (*e.g.*, EDTA and glutathione), stabilizers, dyes, flavoring agents, suspending agents, and preservatives.

[00108] Any suitable excipient or carrier known to those of ordinary skill in the art for use in pharmaceutical compositions may be employed in the compositions described herein. Excipients for therapeutic use are well known, and are described, for example, in *Remington: The Science and Practice of Pharmacy* (Gennaro, 21st Ed. Mack Pub. Co., Easton, PA (2005)). In general, the type of excipient is selected based on the mode of administration, as well as the chemical composition of the active ingredient(s). Pharmaceutical compositions may be formulated for the particular mode of administration. For parenteral administration, pharmaceutical compositions may further comprise water, saline, alcohols, fats, waxes, and

buffers. For oral administration, pharmaceutical compositions may further comprise at least one ingredient chosen, for example, from any of the aforementioned excipients, solid excipients and carriers, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, kaolin, glycerin, starch dextrins, sodium alginate, carboxymethylcellulose, ethyl cellulose, glucose, sucrose, and magnesium carbonate.

[00109] The pharmaceutical compositions (*e.g.,* for oral administration or delivery by injection) may be in the form of a liquid. A liquid pharmaceutical composition may include, for example, at least one the following: a sterile diluent such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils that may serve as the solvent or suspending medium, polyethylene glycols, glycerin, propylene glycol or other solvents; antibacterial agents; antioxidants; chelating agents; buffers and agents for the adjustment of tonicity such as sodium chloride or dextrose. A parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. In some embodiments, the pharmaceutical composition comprises physiological saline. In some embodiments, the pharmaceutical composition an injectable pharmaceutical composition, and in some embodiments, the injectable pharmaceutical composition is sterile.

[00110] For oral formulations, at least one of the compounds of the present disclosure can be used alone or in combination with at least one additive appropriate to make tablets, powders, granules and/or capsules, for example, those chosen from conventional additives, disintegrators, lubricants, diluents, buffering agents, moistening agents, preservatives, coloring agents, and flavoring agents. The pharmaceutical compositions may be formulated to include at least one buffering agent, which may provide for protection of the active ingredient from low pH of the gastric environment and/or an enteric coating. A pharmaceutical composition may be formulated for oral delivery with at least one flavoring agent, *e.g.*, in a liquid, solid or semi-solid formulation and/or with an enteric coating.

[00111] Oral formulations may be provided as gelatin capsules, which may contain the active compound or biological along with powdered carriers. Similar carriers and diluents may be used to make compressed tablets. Tablets and capsules can be manufactured as sustained release products to provide for continuous release of active ingredients over a

period of time. Compressed tablets can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric coated for selective disintegration in the gastrointestinal tract.

[00112] A pharmaceutical composition may be formulated for sustained or slow release. Such compositions may generally be prepared using well known technology and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain the active therapeutic dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane. Excipients for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of active component release. The amount of active therapeutic contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release, and the nature of the condition to be treated or prevented.

[00113] The pharmaceutical compositions described herein can be formulated as suppositories by mixing with a variety of bases such as emulsifying bases or water-soluble bases. The pharmaceutical compositions may be prepared as aerosol formulations to be administered via inhalation. The compositions may be formulated into pressurized acceptable propellants such as dichlorodifluoromethane, propane, nitrogen and the like.

[00114] The compounds of the present disclosure and pharmaceutical compositions comprising these compounds may be administered topically (*e.g.*, by transdermal administration). Topical formulations may be in the form of a transdermal patch, ointment, paste, lotion, cream, gel, and the like. Topical formulations may include one or more of a penetrating agent or enhancer (also call permeation enhancer), thickener, diluent, emulsifier, dispersing aid, or binder. Physical penetration enhancers include, for example, electrophoretic techniques such as iontophoresis, use of ultrasound (or "phonophoresis"), and the like. Chemical penetration enhancers are agents administered either prior to, with, or immediately following administration of the therapeutic, which increase the permeability of the skin, particularly the stratum corneum, to provide for enhanced penetration of the drug through the skin. Additional chemical and physical penetration enhancers are described in, for example, Transdermal Delivery of Drugs, A. F. Kydonieus (ED) 1987 CRL Press;

Percutaneous Penetration Enhancers, eds. Smith et al. (CRC Press, 1995); Lenneräs et al., *J. Pharm. Pharmacol.* 54:499-508 (2002); Karande et al., *Pharm. Res.* 19:655-60 (2002); Vaddi et al., *Int. J. Pharm.* 91:1639-51 (2002); Ventura et al., *J. Drug Target* 9:379-93 (2001); Shokri et al., *Int. J. Pharm.* 228(1-2):99-107 (2001); Suzuki et al., *Biol. Pharm. Bull.* 24:698-700 (2001); Alberti et al., *J. Control Release* 71:319-27 (2001); Goldstein et al., *Urology* 57:301-5 (2001); Kiijavainen et al., *Eur. J. Pharm. Sci.* 10:97-102 (2000); and Tenjarla et al., *Int. J. Pharm.* 192:147-58 (1999).

[00115] Kits comprising unit doses of at least one compound of the present disclosure, for example in oral or injectable doses, are provided. Such kits may include a container comprising the unit dose, an informational package insert describing the use and attendant benefits of the therapeutic in treating the pathological condition of interest, and/or optionally an appliance or device for delivery of the at least one compound or composition comprising the same.

EXAMPLES

EXAMPLE 1

HETEROBIFUNCTIONAL INHIBITOR OF E-SELECTIN AND CXCR4 CHEMOKINE RECEPTOR (COMPOUNDS 9 AND 16)

[00116] Exemplary heterobifunctional compounds of Formula (I) were synthesized as described in Examples 1-2 and as shown in the exemplary synthesis schemes set forth in Figure 1.

[00117] Synthesis of compound 2: Compound 1 (2.5g, 8.3mmol, Qian et al, Nature Communications, 2, 2011, 495) was dissolved in dioxane (30ml) and H_2O (20ml) was added slowly with stirring at room temperature. The solution was cooled to 0 °C (ice bath) and $NaBH_4$ (3g, 79.3mmol) was added slowly with stirring. The reaction mixture was stirred at 64 °C for 16h. The reaction mixture was cooled to 0 °C and quenched with 5N HCl. A solid mass precipitated out of solution which was removed by filtration. The filtrate was diluted with EtOAc (125ml) and transferred to a separatory funnel. The phases were separated. The

organic phase was washed with saline (100ml), dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography using hexanes-EtOAc as mobile phase to give compound 2 (1.8g, 6.6mmol, 79.3%).

[00118] Synthesis of compound 3: Compound **2** (1.7g, 6.2mmol) was dissolved in THF (32ml) and 10N HCl (30ml) was added with stirring at room temperature. The reaction mixture was stirred at room temperature 4.5h. The reaction mixture was diluted with H_2O (150ml) and extracted with EtOAc (3x125ml). Combined organic phases were with washed saturated solution of $NaHCO_3$ (1x125ml) and brine (1x125ml), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by column chromatography using hexanes and EtOAc as mobile phase to give compound **3** (1.22g, 5.7mmol, 91.7%).

[00119] Synthesis of compound 5: A mixture of compound **4** (3.7g, 7.58mmol, Tetrahedron Letters, 2003, **44**, 2481-2483) and compound 3 (2.05g, 9.53mmol) was co-evaporated with toluene (2 x 40 ml) and kept under vacuum for 30 min. The mixture was dissolved in 1, 2-dichloroethane, 40ml) and stirred at room temperature for 30 min under argon, $Na(OAc)_3BH$ (3.2g, 15 mmol) was added and the reaction mixture stirred overnight at room temperature under argon. Water (60ml) was added followed by CH_2Cl_2 (80ml). The reaction mixture was transferred to a seperatory funnel and organic phase was collected. Aqueous phase was washed with CH_2Cl_2 (2x60ml). Combined organic phases were washed successively with cold saturated solution of $NaHCO_3$ (80ml) and brine (80ml), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by column chromatography using Hexanes and EtOAc as mobile phase to give compound **5** (4.5g, 6.54mmol, 86.3%).

[00120] Synthesis of compound 6: Compound **5** (4.5g, 6.54mmol) was dissolved in CH_2Cl_2 (50ml) under argon and cooled on an ice-bath. Dess-Martin reagent (3.6g, 8.49mmol) was added and the reaction mixture was stirred for 3h under argon during which time the reaction mixture attained the room temperature slowly. The reaction mixture was diluted with CH_2Cl_2 (40ml) and washed with cold saturated solution of $NaHCO_3$ and cold brine. Organic phase was dried (Na_2SO_4), filtered, and concentrated. The residue was purified by column chromatography using Hexanes-EtOAc as mobile phase to give compound **5** (3.6g, 5.25mmol, 80.28%).

[00121] Synthesis of compound 8: A mixture of compound **6** (3.5g, 5.11mmol) and compound **7** (2.8g, WO2013/096926) was co-evaporated with MeOH (3x50ml) and dried under vacuum. The residue was dissolve in MeOH (50ml) and stirred under argon for 1h at room temperature. $Na(OAc)_3BH$ (3.6g, 16.99mmol) was added and the reaction mixture was stirred under argon for 17h at room temperature. The reaction mixture was concentrated. The solid residue was suspended in $CHCl_3$ (100ml), H_2O (250ml) was added with stirring. The mixture was stirred for 10 min at room temperature during which time the solid product precipitated. The solid product was collected by filtration, washed with water, and dried under vacuum to give compound **8** (4.4g, 3.14mmol, 82.2% based on compound **6**).

[00122] Synthesis of compound 9: To a solution of compound **8** (4.2g, 3mmol) in MeOH (100ml) was added an aqueous solution of 1N NaOH (50ml) with stirring at room temperature. The reaction mixture (pH 12.9) was stirred for 2h at room temperature. The pH of resulting reaction mixture was adjusted to 8.9 by adding AcOH (3ml). Solvent was evaporated off and then lyophilized. The solid mass was dissolve in H_2O (20ml) and pH of the solution was adjusted to 9.5 by adding NaOH solution. Desalting was performed by using pre-packed Sep-Pak™ C18 column (2x10g) using H_2O (150ml each column), 50% MeOH in H_2O (60ml each column), 70% MeOH in H_2O (100ml each column), and 80% MeOH in H_2O (50ml each column). Desired compound eluted in 50-80% MeOH in H_2O. They were combined and concentrated to ¼ of the total volume. The resulting solution was lyophilized to give compound **8** (2.9g, 2.6mmol, 86.7%). m/z calculated for $C_{52}H_{88}BrN_7O_{14}$ [M+H]: 1116.2; found: 1116.4.

[00123] Synthesis of compound 10: To a solution of compound **2** (0.225g, 0.73mmol) in CH_2Cl_2 was added Celite™ followed by pyridinium chlorochomate (0.28g, 1.3mmol) with stirring at room temperature. The reaction mixture was stirred at room temperature for 2h and filtered through a bed of silica and celite™. The filtrate was evaporated to dryness and purified by column chromatography to give compound **10** (0.2g).

[00124] Synthesis of compound 12: To a suspension of cyclam (5g, 25mmol,) in anhydrous CH_2Cl_2 (150ml) was added a solution of diallyldicarbonate (12ml, d 0.991g/ml, 83.7mmol) in CH_2Cl_2 (100ml) drop-wise with stirring. The reaction mixture was stirred at room temperature overnight during which the reaction turn light green and gave a clear

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solution. The solvent was removed and the residue was purified by column chromatography using CH_2Cl_2 and MeOH as mobile phase to give compound **12** (10.5g, 23.2mmol, 92.9%). TLC: CH_2Cl_2-MeOH (95:5).

[00125] Synthesis of compound 13: A solution of compound **10** (0.19g, 0.7mmol) and compound **12** (0.45g, 1mmol) in MeOH (1 ml and THF 0.5 ml) was stirred at room temperature for 30min. To this solution was added $Na(OAc)_3BH$ (0.36g, 1.6mmol) and the reaction mixture was stirred room temperature for overnight. The solution was diluted with EtOAc and washed with H_2O. Organic layer was dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was purified by column chromatography to give compound **13** (0.2g).

[00126] Synthesis of compound 14: To a solution of compound **13** (0.24g, 0.34mmol) in THF (7 ml) was added concentrated HCl (5 ml) and the reaction mixture was stirred at room temperature for 10h. The reaction mixture was diluted with H_2O (20ml) and extracted with EtOAc (3x16ml). The combined organic phases were dried (Na_2SO_4), filtered, and concentrated. The residue was purified by column chromatography to give compound **14** (0.15g).

[00127] Synthesis of compound 15: A mixture of compound **7** (0.1g, 0.14mmol, WO2013/096926) and compound **14** (0.15g, 0.23mmol) in MeOH (1.5ml) was stirred at room temperature for 30min. followed by the addition of $Na(OAc)_3BH$ (0.096g, 0.45mmol). The reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated and the residue was suspended in MeOH. The resulting solid was removed by filtration and the filtrate was concentrated. The residue was purified by column chromatography to give compound **15** (35mg).

[00128] Synthesis of compound 16: To a solution of compound **15** (0.028g, 0.02mmol) in CH_2Cl_2 (2ml) was added AcOH (0.005ml, 0.09mmol) followed by $Pd(PPh_3)_4$ (0.003g, 0.003mmol) and Bu_3SnH (0.017ml, 0.06mmol) and the reaction mixture was stirred at room temperature for 3h. The reaction mixture was diluted with CH_2Cl_2 (10ml) and extracted with H_2O (8ml). The aqueous layer was lyophilized, dissolved in H_2O and purified by Sep-Pak C-18 Column. Fraction corresponding to the product was concentrated and dissolved in H_2O. The pH of the solution was adjusted 9.5 by a solution of NaOH and lyophilized to give

compound 16 (6.5mg) as Na-Salt. m/z calculated for $C_{52}H_{88}BrN_7O_{14}$ [M+H]: 1116.2; found: 1116.6

EXAMPLE 2

CXCR4 Assay to Assess Inhibition of SDF-1 Induced Chemotaxis

[00129] A chemotaxis assay was used to measure the ability of a glycomimetic CXCR4 antagonist to inhibit migration of CCRF-CEM cells, which express CXCR4 on their cell surfaces, across a membrane toward the CXCR4 ligand CXCL12 (SDF-1α). CCRF-CEM cells are human T lymphoblasts that express CXCR4 on the cell surface. The cells were labeled with 3 uM Calcein AM for 15 minutes at 37°C to enable detection by fluorescence. Subsequently, the cells were pelleted at 250 x g for 10 minutes and resuspended to a final concentration of about 5 x 10^5 cells per mL in RPMI 1640 medium supplemented with 2% FBS. Typically, 200 ul of cells were mixed with 22 ul of a 10x concentration of the compound to be tested and placed at room temperature for 10 minutes. The treated cells were evaluated in duplicate, so 100 ul of the cells were placed into the upper chamber of each of two transwell inserts (Costar number 3421; 5.0 um pores; 6.5 mm diameter inserts). The transwells were place into the wells of a 24-well plate with each well containing 600 ul of RPMI 1640 plus 2% FBS and 50 ng/mL CXCL12. Negative control wells contained no CXCL12 in the lower chamber. The cells were allowed to migrate across the membrane from the upper chamber into the lower chamber for 3 hours at 37°C in 5% CO_2. The transwell inserts were removed from the 24-well plate and the fluorescence in the lower chambers was measured using a Molecular Devices FlexStation 3 with an excitation wavelength of 485 nm and an emission wavelength of 538 nm. See Figure 4.

EXAMPLE 3

E-Selectin Activity – Binding Assay

[00130] The inhibition assay to screen and characterize antagonists of E-selectin is a competitive binding assay, from which IC_{50} values may be determined. E-selectin/Ig chimera was immobilized in 96 well microtiter plates by incubation at 37 °C for 2 hours. To reduce nonspecific binding, bovine serum albumin was added to each well and incubated at room temperature for 2 hours. The plate was washed and serial dilutions of the test compounds

were added to the wells in the presence of conjugates of biotinylated, sLe^a polyacrylamide with streptavidin/horseradish peroxidase and incubated for 2 hours at room temperature.

[00131] To determine the amount of sLe^a bound to immobilized E-selectin after washing, the peroxidase substrate, 3,3',5,5' tetramethylbenzidine (TMB) was added. After 3 minutes, the enzyme reaction was stopped by the addition of H_3PO_4, and the absorbance of light at a wavelength of 450 nm was determined. The concentration of test compound required to inhibit binding by 50% was determined and reported as the IC_{50} value for each E-selectin antagonist as shown in the table below. IC_{50} values for exemplary compounds disclosed herein are provided in the following table. See Figure 5.

E-Selectin Antagonist Activity of Heterobifunctional Compounds

Compound	IC50 (μM)	rIC50
Compound 9	1.49	0.60
Compound 16	1.08	0.23

EXAMPLE 4

CXCR4 ASSAY - INHIBITION OF CYCLIC AMP

[00132] The CXCR4-cAMP assay measures the ability of a glycomimetic CXCR4 antagonist to inhibit the binding of CXCL12 (SDF-1α) to CHO cells that have been genetically engineered to express CXCR4 on the cell surface. Assay kits may be purchased from DiscoveRx (95-0081E2CP2M; cAMP Hunter eXpress CXCR4 CHO-K1). The G_i-coupled receptor antagonist response protocol described in the kit instruction manual was followed. GPCRs, such as CXCR4, are typically coupled to one of the 3 G-proteins: Gs, Gi or Gq. In the CHO cells supplied with the kit, CXCR4 is coupled to Gi. After activation of CXCR4 by ligand binding (CXCL12), Gi dissociates from the CXCR4 complex, becomes activated, and binds to adenylyl cyclase, thus inactivating it, resulting in decreased levels of intracellular cAMP. Intracellular cAMP is usually low, so the decrease of the low level of cAMP by a Gi-coupled receptor will be hard to detect. Forskolin is added to the CHO cells to directly activate adenylyl cyclase (bypassing all GPCRs), thus raising the level of cAMP in the cell, so that a Gi response can be easily observed. CXCL12 interaction with CXCR4

decreases the intracellular level of cAMP and inhibition of CXCL12 interaction with CXCR4 by a CXCR4 antagonist increases the intracellular cAMP level, which is measured by luminescence. See Figure 6.

EXAMPLE 5

INHIBITION OF LYMPHATIC AND VASCULAR ENDOTHELIAL MIGRATION TOWARD TUMOR-ASSOCIATED FIBROBLASTS

[00133] Plated 8.0 x 10^5 13.34 fibroblasts, S2.013 tumor cells, and Colo357 tumor cells in a T-25. Incubated overnight. Changed media to serum-free EBM-2 and allowed cells to condition media for 24 hours. Collected media and filtered to remove debris. Added 750 ul conditioned media to lower wells of a Boyden chamber migration plate (3 replicate/cell type/treatment). Plated specifications: 24 well; 8.0 um pores. Added 3.0 x 10^4 hLECs or HUVECs to the upper wells of the Boyden chamber diluted in serum-free EBM-2 (500 ul/insert). Added 100 ug/ml compound 9 to upper wells. Allowed hLECs or HUVECs to migrate overnight. After migration, washed inserts and removed non-migrated cells on the upper side of the membrane with a Q-tip. Fixed and stained migrated cells with Diff-Quik Kit. Removed membranes from the inserts and mounted on a slide. Drew quadrants over the membranes and imaged each quadrant. Quantified the number of migratory endothelial cells. See Figure 7.

EXAMPLE 6

INHIBITION OF PDAC CELL BINDING TO LYMPHATIC MONOLAYERS

[00134] Plated 4.5 x 10^4 hLECs into the wells of 8-well chamber slides. Incubated cells until a confluent monolayer of endothelial cells is achieved. Pretreated the endothelial cells for 2 hours with designated treatments: control media, 100 ug/ml an E-selectin specific antagonist, 10 ug/ml compound 9, or 100 ug/ml compound 9. Dyed S2.013 or Colo357 with CFDA-SE Cell Tracker Dye. Following endothelial cell pretreatment, added 3.0 x 10^4 S2.013 or Colo357 cells diluted in serum-free EBM-2 to the wells along with designated treatments (400 ul/well; 3 replicate wells/treatment). Incubated the tumor cells on the endothelial monolayer for 1 hour. Following binding incubation, washed each well 3X with PBS+0.5% FBS to remove non-adherent cells. Fixed with 4% PFA and coverslip slides.

Imaged 5 locations/well at 10X magnification. Quantified the number of adherent cells in each image. See Figure 8.

EXAMPLE 7

PROSTATE CANCER MODEL

[00135] Luciferase transfected PC3Luc cells were injected at 2 × 105 cells/10 µl of serum-free medium into the proximal tibiae of 4-week old male CD1 nu/nu mice. The development of metastases was monitored by using a Faxitron cabinet x-ray system and tumor burden evaluated by bioluminescence analyses (see below). The development of metastases was monitored by radiography using a Faxitron cabinet x-ray system (Faxitron x-ray corp., Wheeling, IL, USA). Radiographic analyses were performed at days 28, 35, 42 and 50 after cell injection. No Faxitron analysis was performed after the 50th day since after this time the estimated risk of anesthesia-related mortality of mice was significantly increased. However, in order to determine both cumulative incidence of bone metastases and disease free survival (DSF), Xrays were also repeated at the death of each animal or in the survived animal at the end of follow-up, that we have defined to be 170 days, when animals were sacrificed. Burden of osteolytic lesions was evaluated by digital examination of radiography (ImageJ, a public domain software by Wayne Rasband, NIH, USA). Animals were sacrificed by carbon dioxide inhalation 170 days after heart injections, or earlier if there were early signs of serious distress. All animals were subjected to an accurate post mortem examination and samples of various organs were processed for routine histological analyses.

[00136] For luminescence imaging, mice received 150 mg firefly luciferase (Synchem Ug and Co.KG, Felsberg-Altenburg, Germany) per kg body weight given intraperitoneally. Following anesthesia with ketamine/xylazine mixture mice were placed into a Hamamatsu imaging station (Hamamatsu photonics, Italian distributor, Rome Italy). Bioluminescence generated by the luciferin/luciferase reaction was used for quantification using a dedicated Living Image software on a red (high intensity/cell number) to blue (low intensity/cell number) visual scale. A digital grayscale animal image was acquired followed by acquisition and overlay of a pseudo-color image representing the spatial distribution of detected photon counts emerging from active luciferase within the animal. Signal intensity was quantified as the sum of all detected photons within the region of interest during a 1-minute luminescent

integration time. Tumor incidence was scored on a dichotomous scale as being either positive or negative if animals had at least one lesion detected in either the humeri or tibia/femur region. See Figure 9.

[00137] The various embodiments described above can be combined to provide further embodiments.

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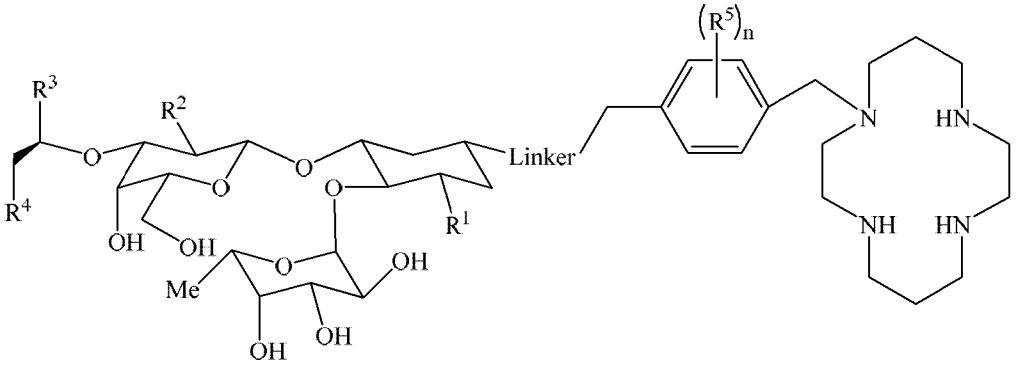
What is claimed is:

1. At least one compound selected from the group consisting of compounds of Formula (I):

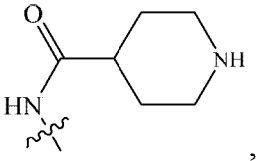


(I)

and pharmaceutically acceptable salts thereof,

wherein:

R^1 is selected from the group consisting of H, C_{1-8} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{1-8} haloalkyl, C_{2-8} haloalkenyl, and C_{2-8} haloalkynyl groups;

R^2 is selected from the group consisting of –OH, $–NH_2$, $–OC(=O)Y^1$, –NHC(=O)Y^1,  , and $–NHC(=O)NHY^1$ groups, wherein Y^1 is selected from the group consisting of C_{1-8} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{1-8} haloalkyl, C_{2-8} haloalkenyl, C_{2-8} haloalkynyl, C_{6-18} aryl, and C_{1-13} heteroaryl groups;

R^3 is selected from the group consisting of -CN, $-CH_2CN$, and $-C(=O)Y^2$ groups, wherein Y^2 is selected from the group consisting of C_{1-8} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, $-OZ^1$, -NHOH, $-NHOCH_3$, -NHCN, and $–NZ^1Z^2$ groups, wherein Z^1 and Z^2, which may be identical or different, are independently selected from the group consisting of H, C_{1-8} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{1-8} haloalkyl, C_{2-8} haloalkenyl, and C_{2-8} haloalkynyl groups, wherein Z^1 and Z^2 may join together to form a ring;

R^4 is selected from the group consisting of C_{3-8} cycloalkyl groups;

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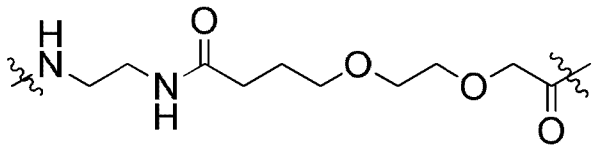
each R^5 is independently selected from the group consisting of H, halo, C_{1-8} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{1-8} haloalkyl, C_{2-8} haloalkenyl, and C_{2-8} haloalkynyl groups, with the proviso that at least one R^5 is not H;

n is selected from the group consisting of integers ranging from 1 to 4; and

Linker is selected from the group consisting of $-(CH_2)_p-$, $-O(CH_2)_p-$, $-C(=O)-NH-(CH_2)_p-C(=O)-NH-$,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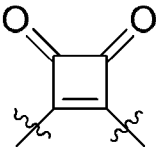
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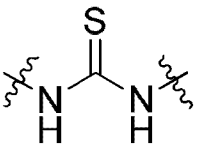
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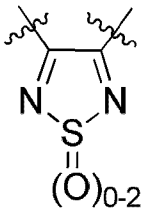
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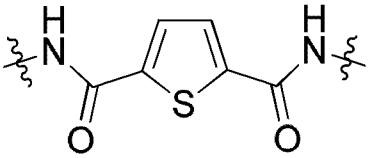
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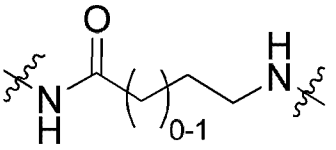
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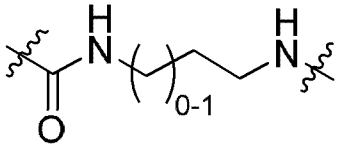
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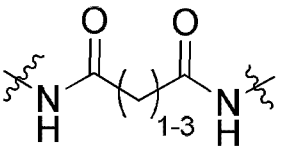
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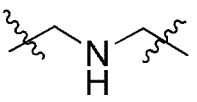
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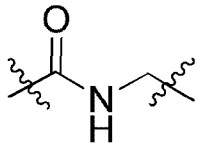
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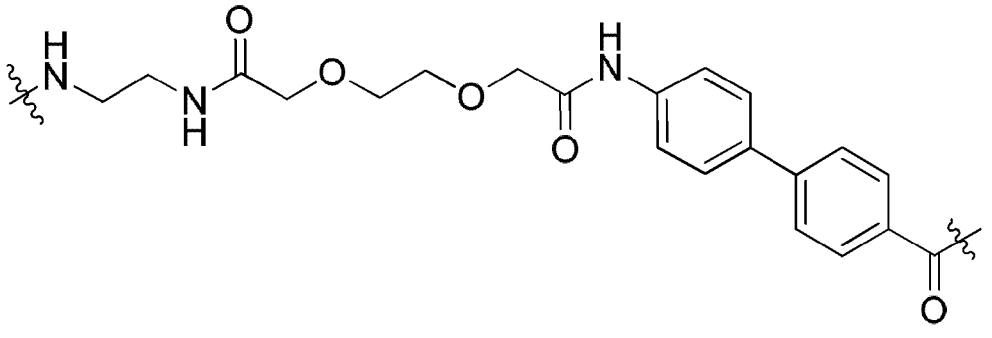
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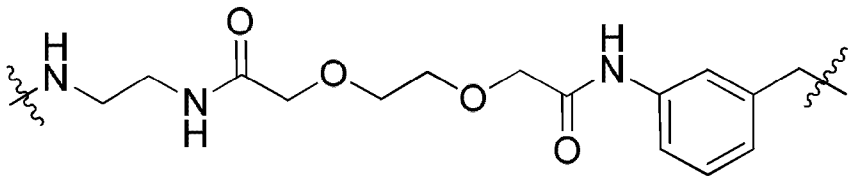

groups, wherein p is selected from the group consisting of integers ranging from 1 to 20.

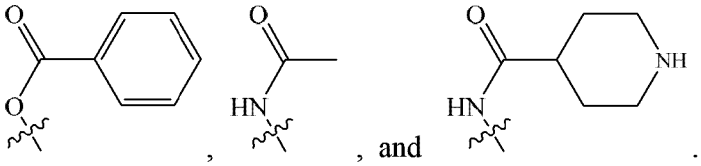
2. The at least one compound according to claim 1, wherein R^1 is selected from the group consisting of C_{1-8} alkyl groups.

3. The at least one compound according to claim 2, wherein R^1 is selected from the group consisting of ethyl and methyl.

4. The at least one compound according to claim 3, wherein R^1 is ethyl.

5. The at least one compound according to any one of claims 1-4, wherein R^2 is selected from the group consisting of –OH, $-NH_2$, $-OC(=O)Y^1$, and $–NHC(=O)Y^1$, wherein Y^1 is selected from the group consisting of C_{1-8} alkyl, C_{6-18} aryl, and C_{1-13} heteroaryl groups.

6. The at least one compound according to claim 5, wherein R^2 is selected from the group consisting of


, and .

7. The at least one compound according to any one of claims 1-6, wherein R^3 is $-C(=O)Y^2$, wherein Y^2 is selected from the group consisting of $-OZ^1$, -NHOH, $-NHOCH_3$, and $–NZ^1Z^2$ groups, wherein Z^1 and Z^2, which may be identical or different, are independently selected from the group consisting of H and C_{1-8} alkyl groups, wherein Z^1 and Z^2 may join together to form a ring.

8. The at least one compound according to claim 7, wherein R^3 is $-C(=O)OH$.

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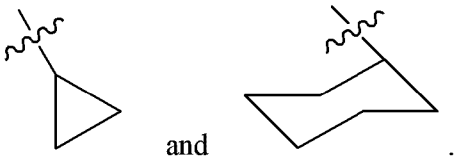
9. The at least one compound according to any one of claims 1-8, wherein R^4 is selected from the group consisting of



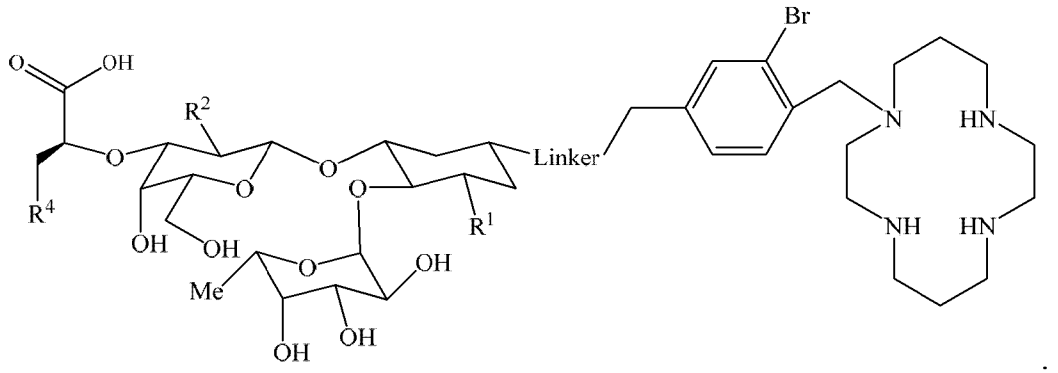
and .

10. The at least one compound according to any one of claims 1-9, wherein at least one R^5 is halo.

11. The at least one compound according to claim 10, wherein at least one R^5 is Bromo.

12. The at least one compound according to claim 10 or 11, wherein n is 1.

13. The at least one compound according to any one of claims 1-6, wherein the at least one compound is selected from the group consisting of compounds of Formula (Ia):



(Ia)

14. The at least one compound according to any one of claims 1-6, wherein the at least one compound is selected from the group consisting of compounds of Formula (Ib):

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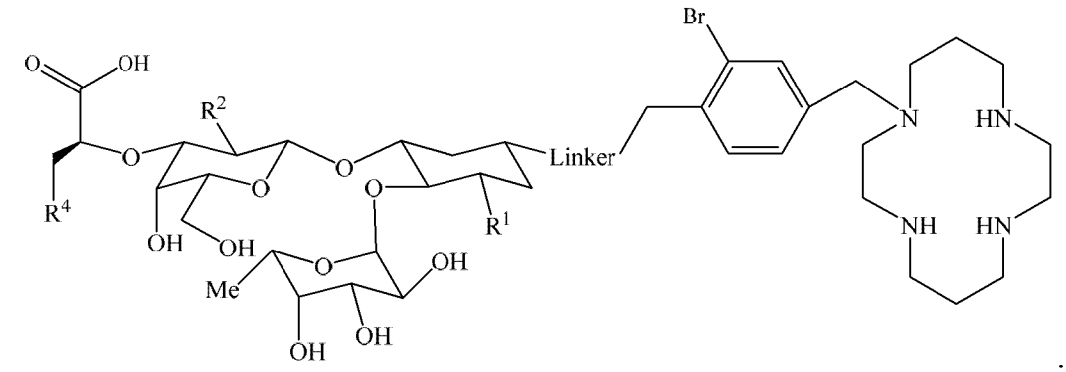
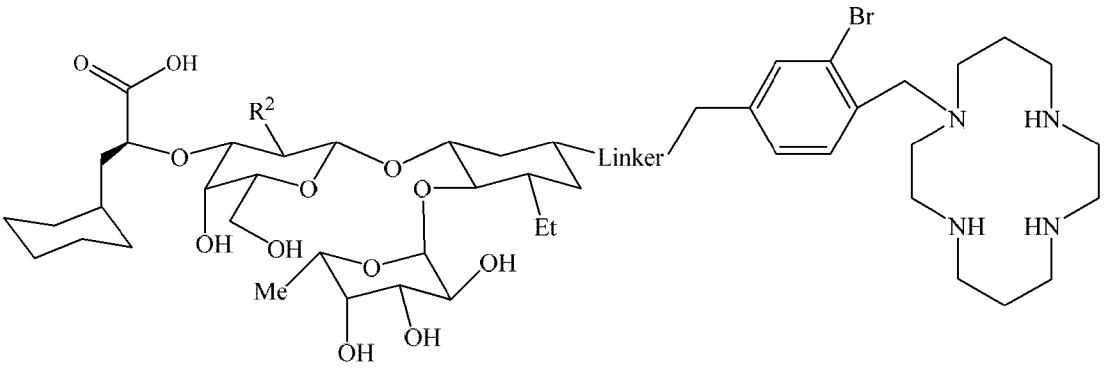


(Ib)

15. The at least one compound according to claim 13, wherein the at least one compound is selected from the group consisting of the following Formulae:



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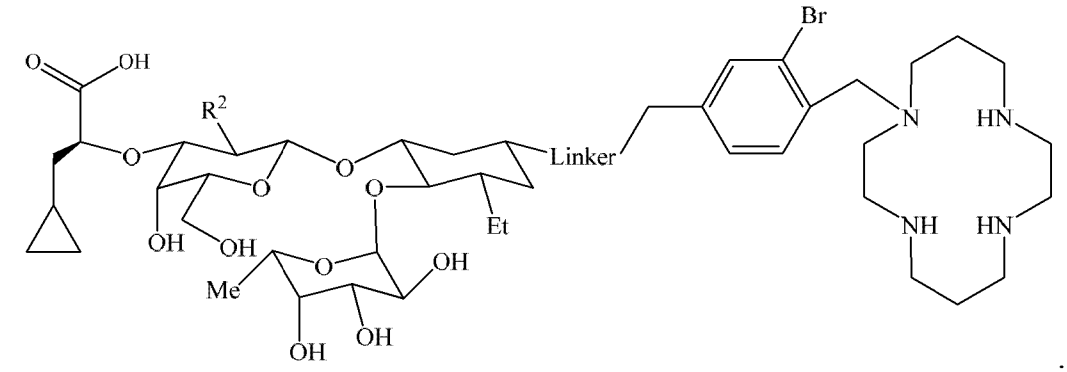

16. The at least one compound according to claim 14, wherein the at least one compound is selected from the group consisting of the following Formulae:

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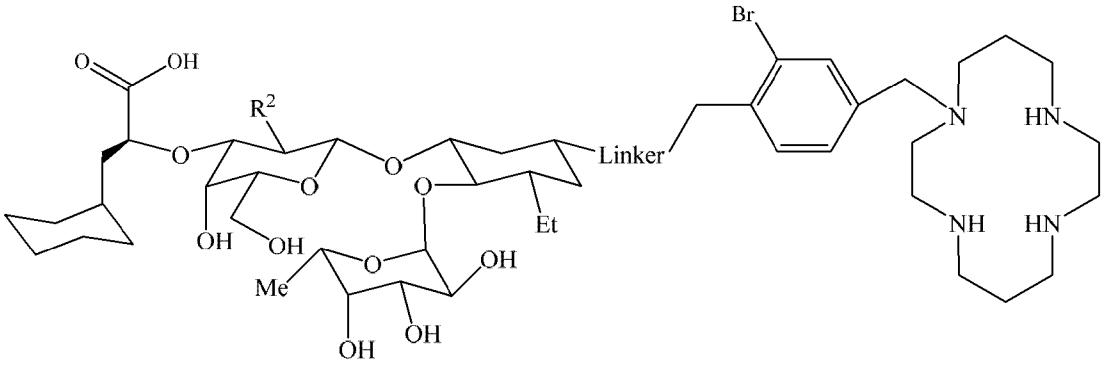
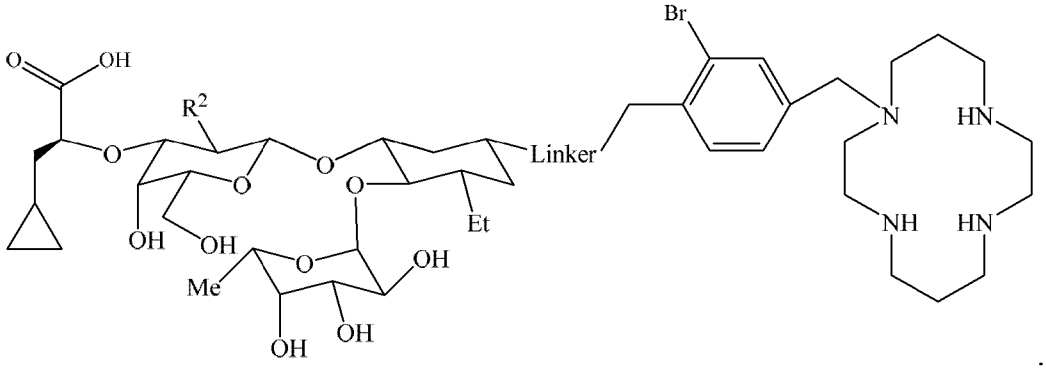
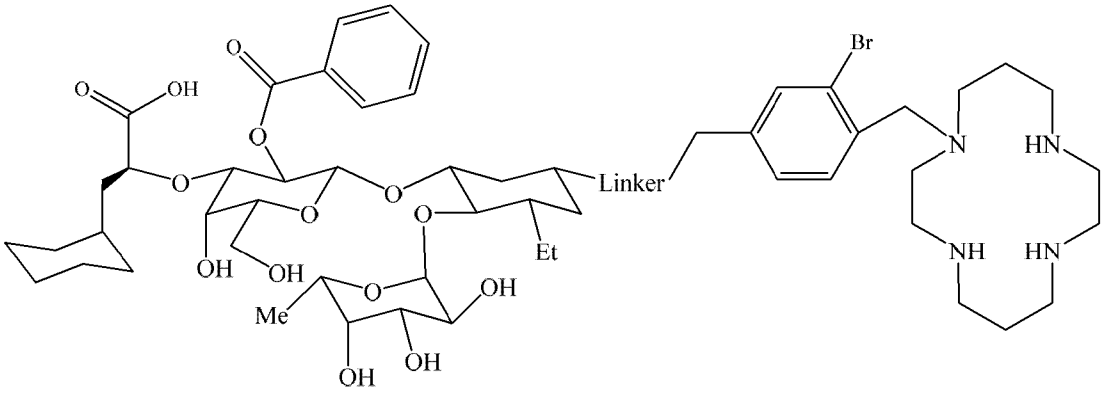


and



.

17. The at least one compound according to claim 1, wherein the at least one compound is selected from the group consisting of the following Formulae:



,

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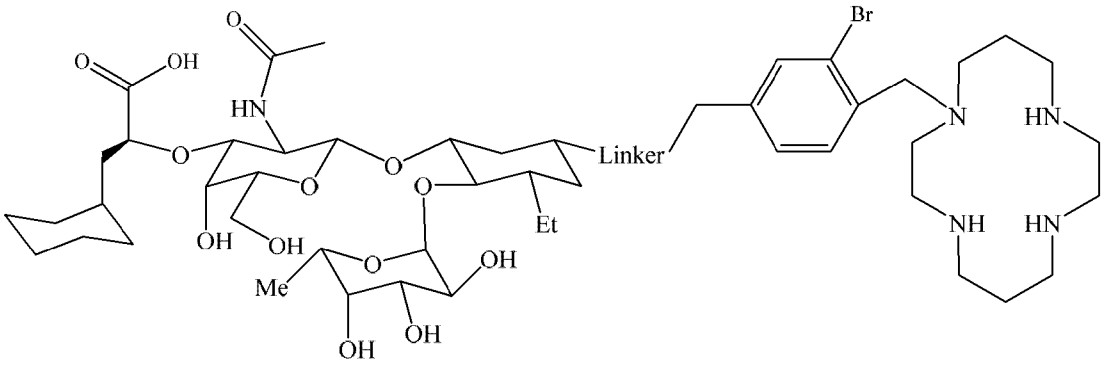

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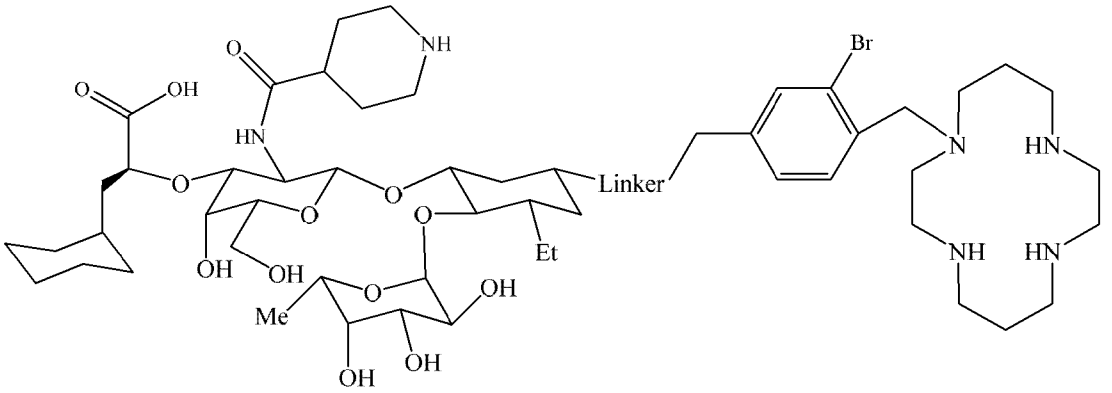

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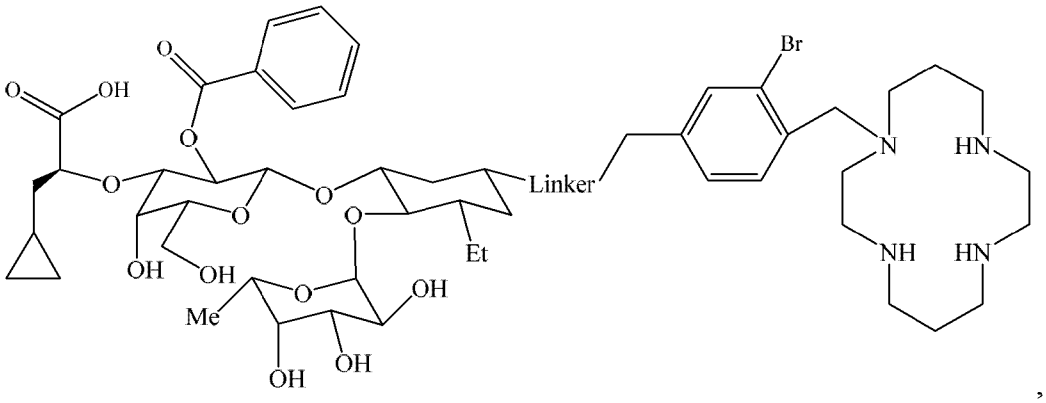

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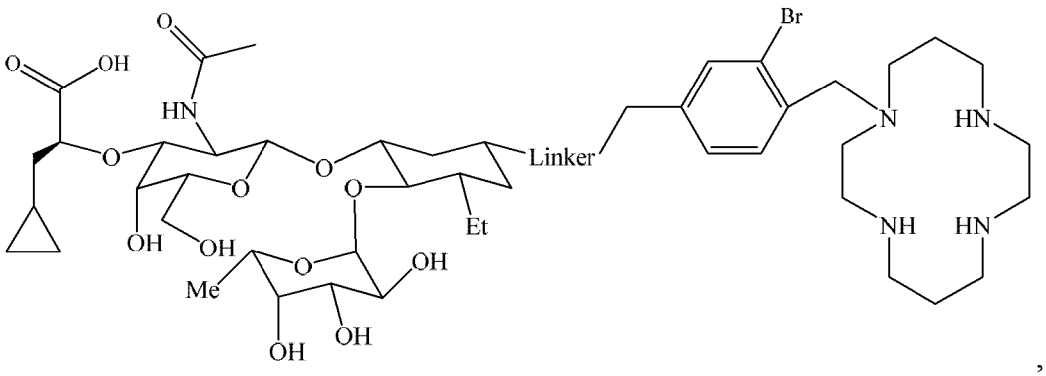
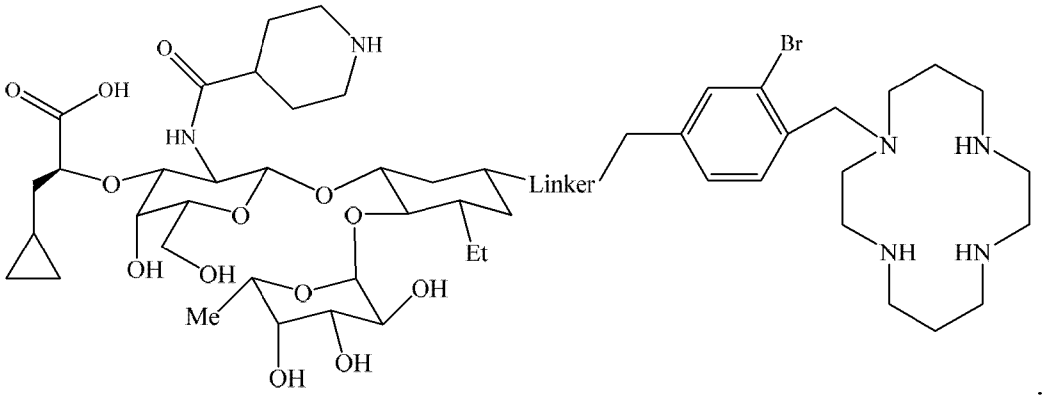
Date Reçue/Date Received 2021-07-05



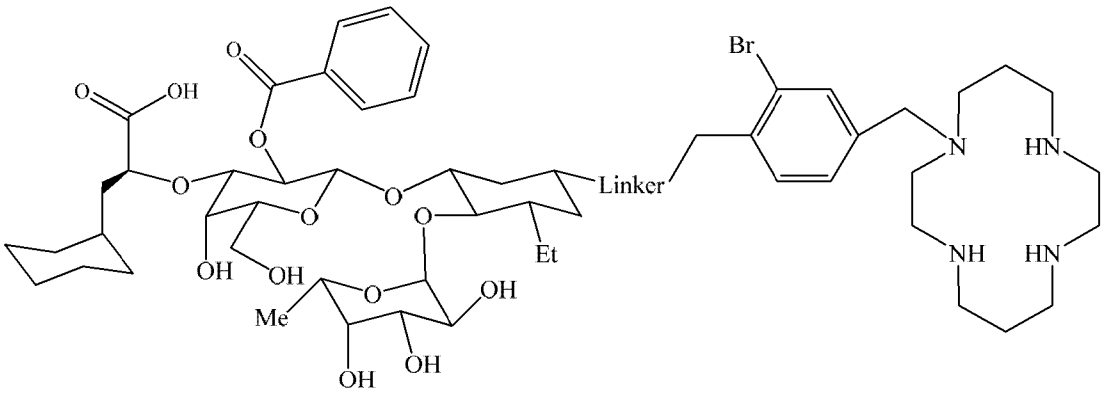
,

and



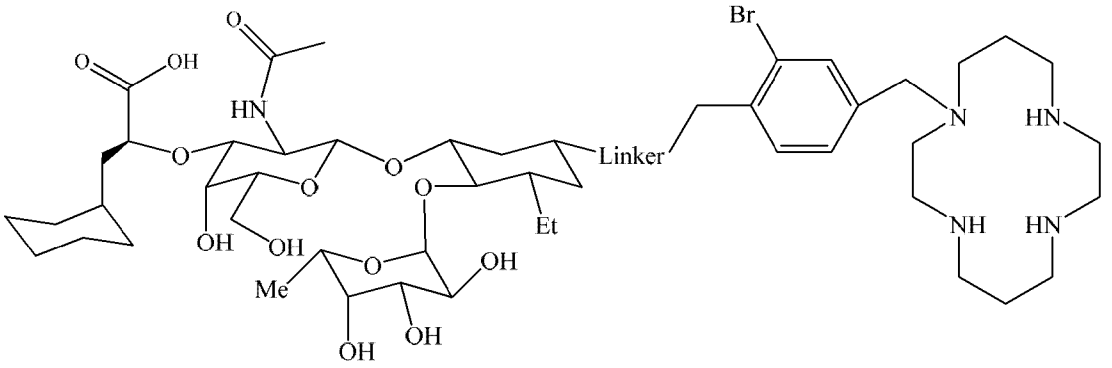
.

18. The at least one compound according to claim 1, wherein the at least one compound is selected from the group consisting of the following Formulae:



,

Date Reçue/Date Received 2021-07-05



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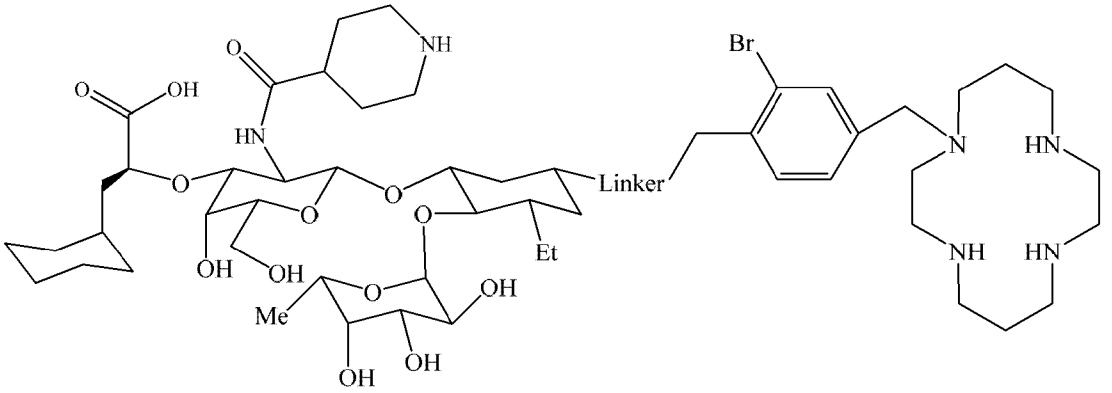

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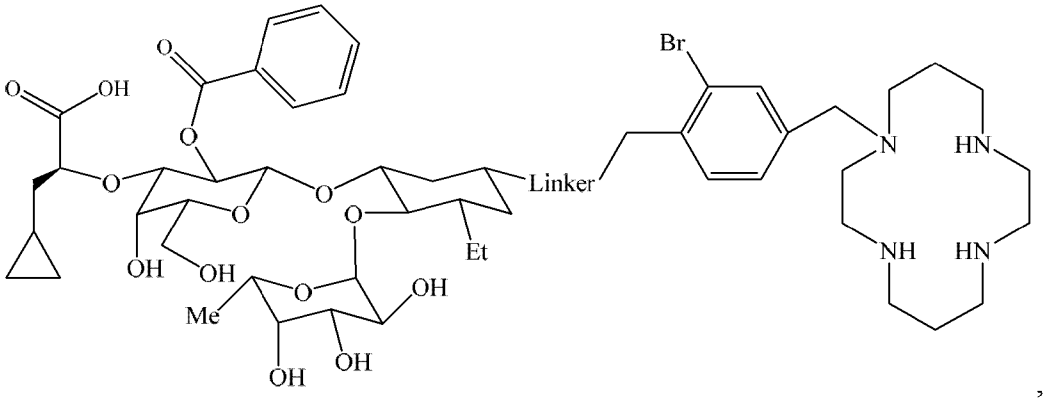

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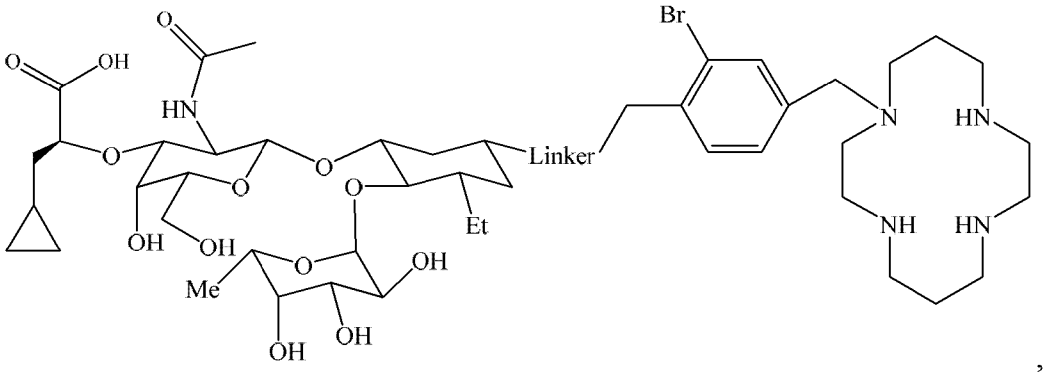
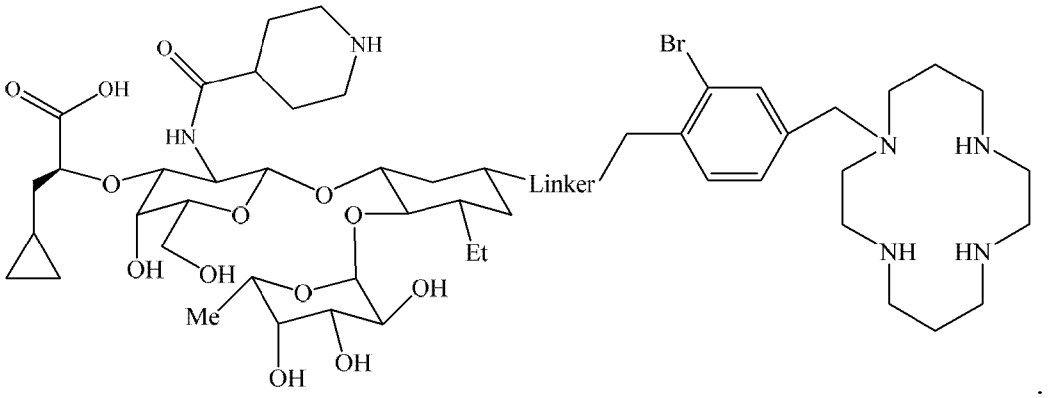
Date Reçue/Date Received 2021-07-05



,

and



.

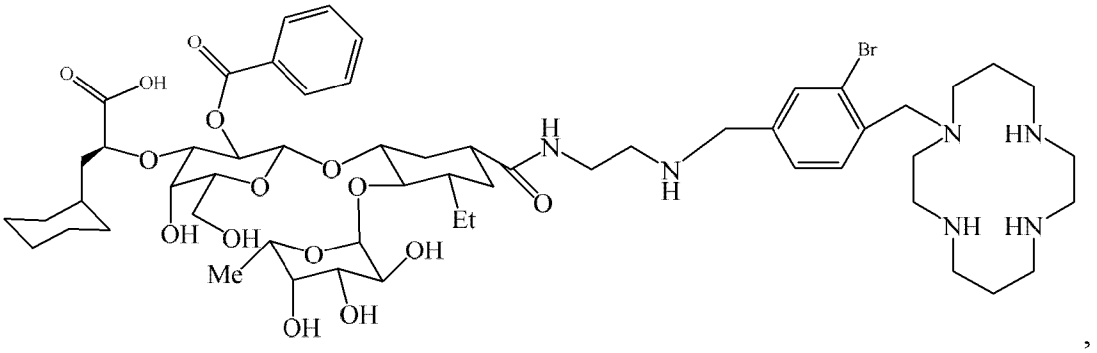
19. The at least one compound according to any one of claims 1-18, wherein the linker group is -C(=O)NH(CH_2)$_2$NH-.

20. The at least one compound according to any one of claims 1-18, wherein the linker group is -CH_2NHCH_2-.

21. The at least one compound according to any one of claims 1-18, wherein the linker group is -C(=O)$NHCH_2$-.

22. The at least one compound according to claim 1, wherein the at least one compound is selected from the group consisting of the following Formulae:

Date Reçue/Date Received 2021-07-05



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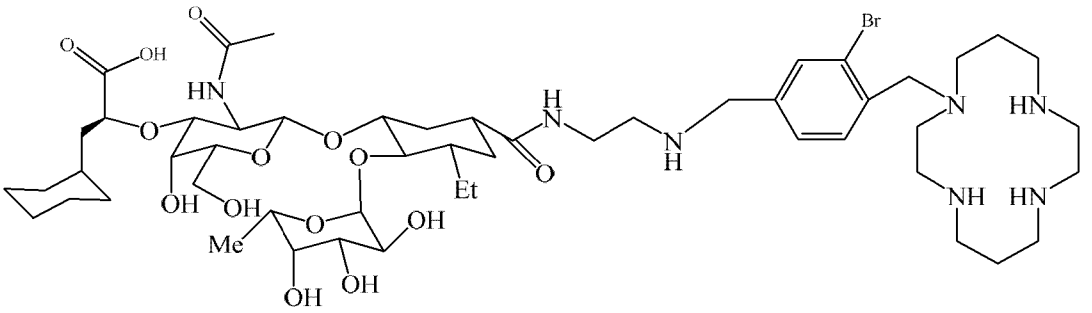

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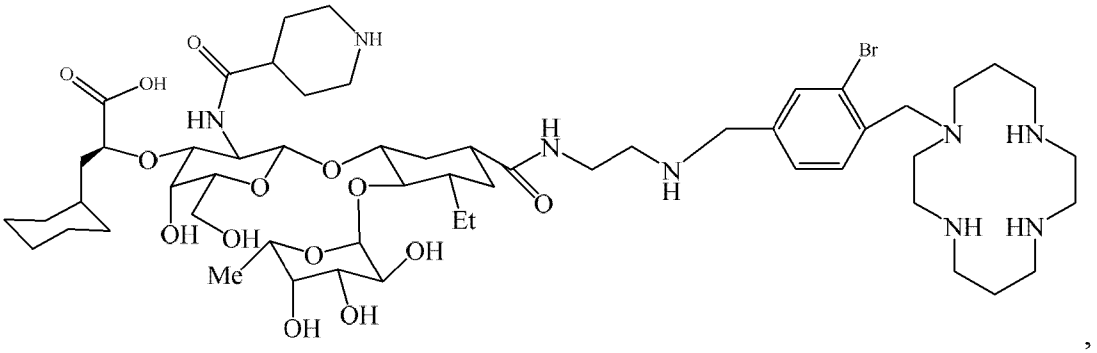

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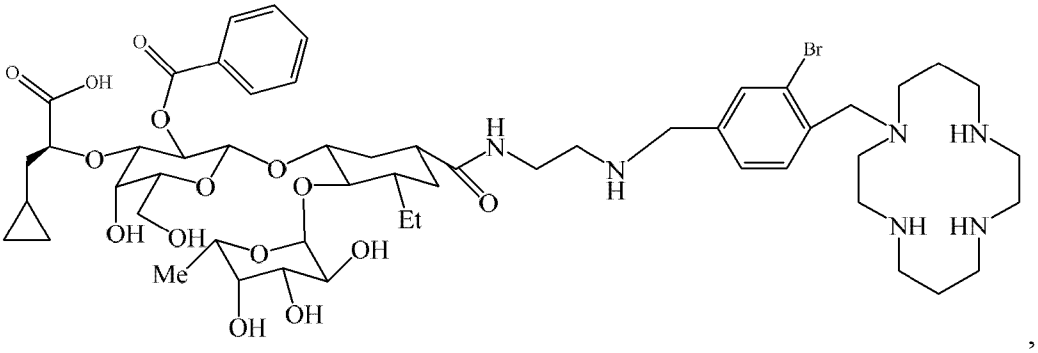

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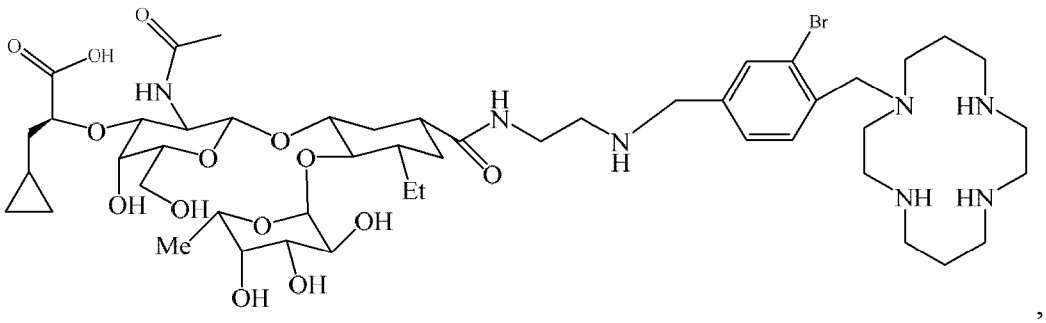
Date Reçue/Date Received 2021-07-05



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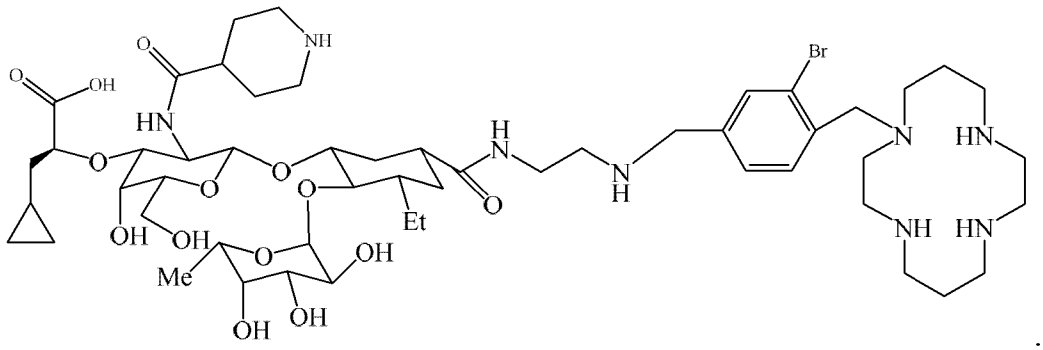
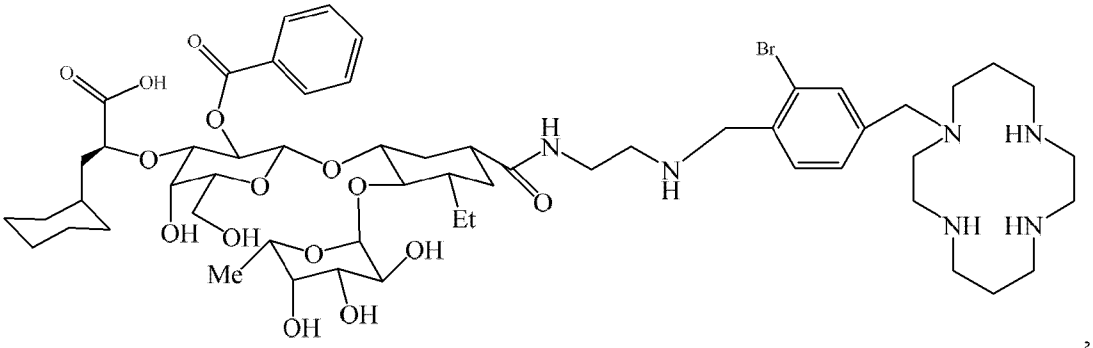
and



.

23. The at least one compound according to claim 1, wherein the at least one compound is selected from the group consisting of the following Formulae:



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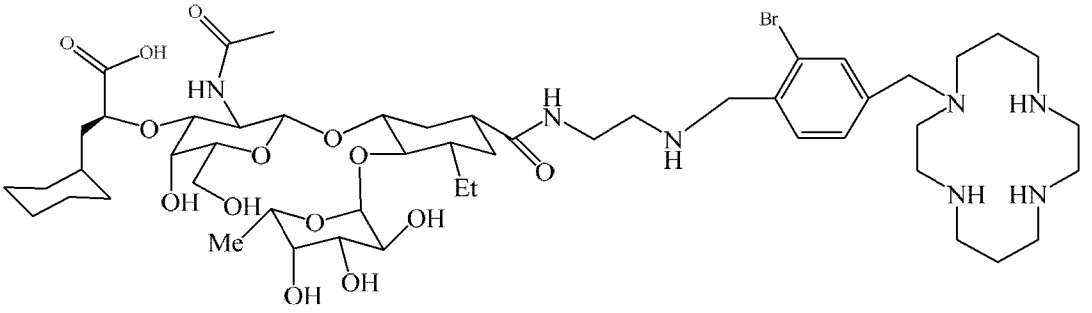

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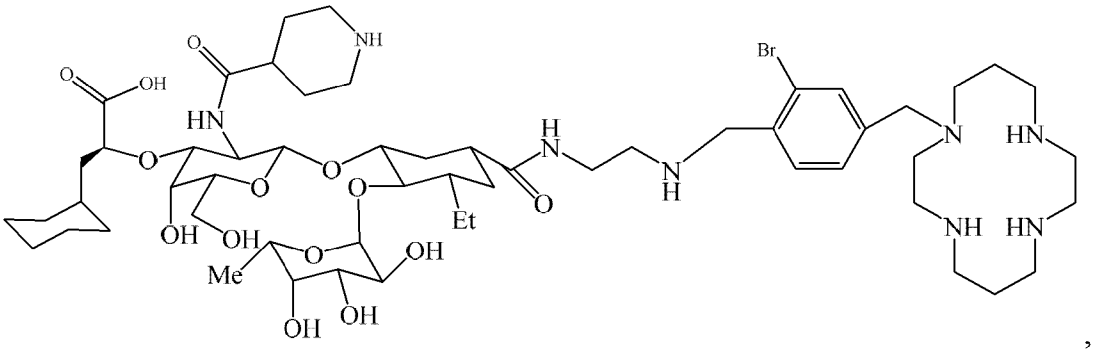
Date Reçue/Date Received 2021-07-05



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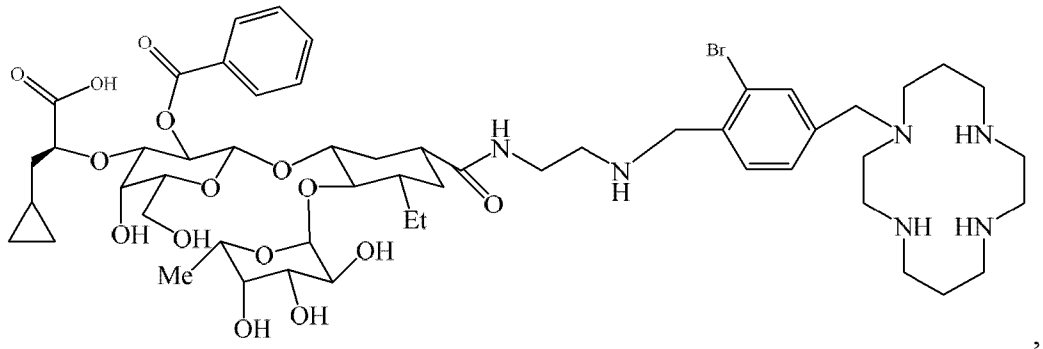

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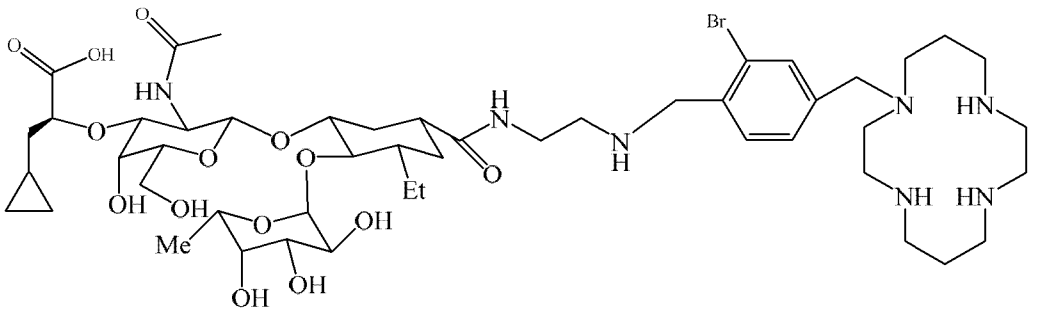

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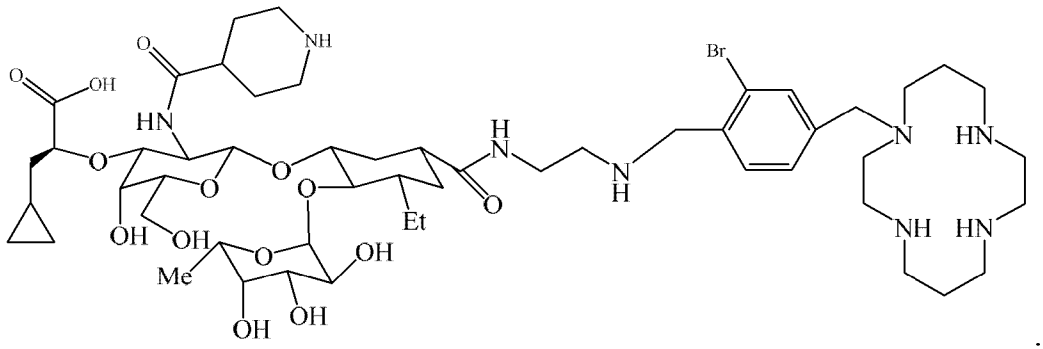
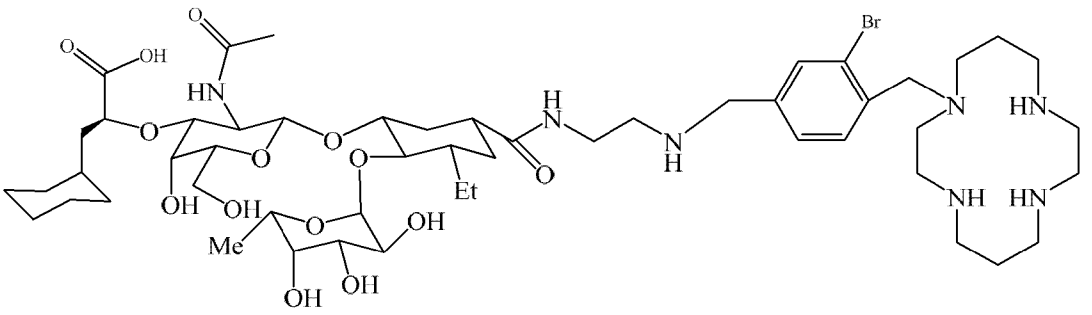
and



.

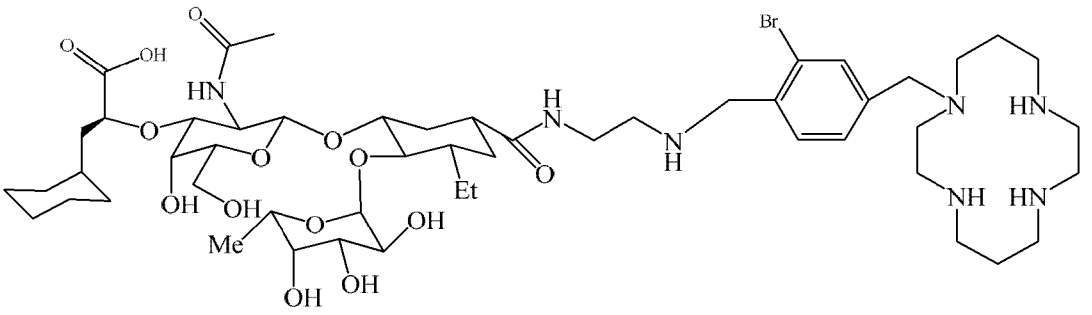
24. The at least one compound according to claim 1, wherein the at least one compound is

Date Reçue/Date Received 2021-07-05



.

25. The at least one compound according to claim 1, wherein the at least one compound is



.

26. A composition comprising at least one compound of any one of claims 1-25 and at least one additional pharmaceutically acceptable ingredient.

27. A composition for the treatment or prevention of a cancer in which the cancer cells may leave the primary site, the composition comprising at least one compound of any one of claims 1-25 and at least one additional pharmaceutically acceptable ingredient.

28. A composition for the treatment or prevention of a cancer in which it is desired to mobilize cancer cells from a site into the bloodstream and retain the cancer cells in the bloodstream, the composition comprising at least one compound of any one of claims 1-25 and at least one additional pharmaceutically acceptable ingredient.

29. A composition for releasing cells into circulating blood and enhancing retention of the cells in the blood, the composition comprising at least one compound of any one of claims 1-25 and at least one additional pharmaceutically acceptable ingredient.

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30. A composition for the treatment or prevention of tumor metastasis, the composition comprising at least one compound of any one of claims 1-25 and at least one additional pharmaceutically acceptable ingredient.

31. A composition for the treatment or prevention of an inflammatory disease in which the adhesion or migration of cells occurs in the disease, the composition comprising at least one compound of any one of claims 1-25 and at least one additional pharmaceutically acceptable ingredient.

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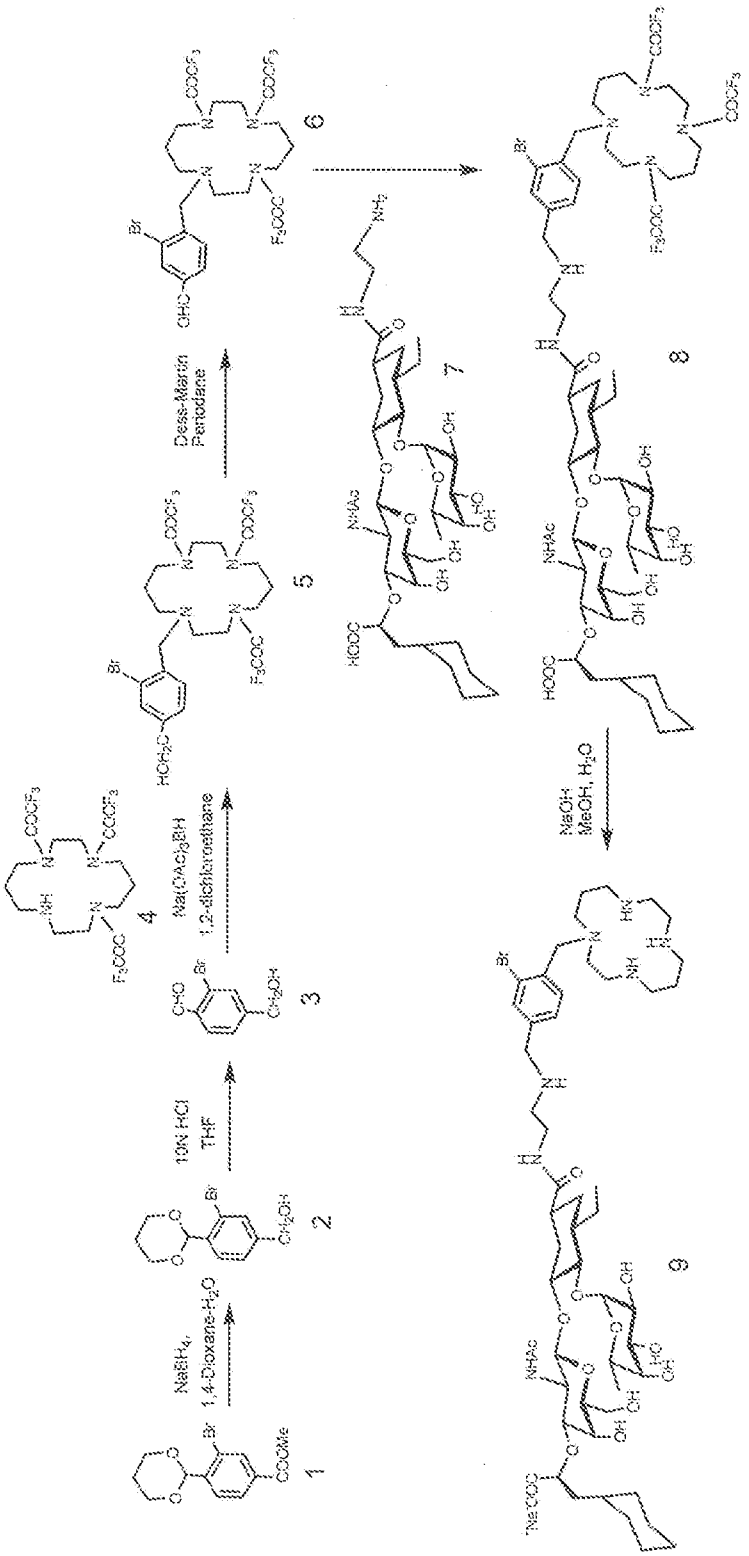


FIG. 1A

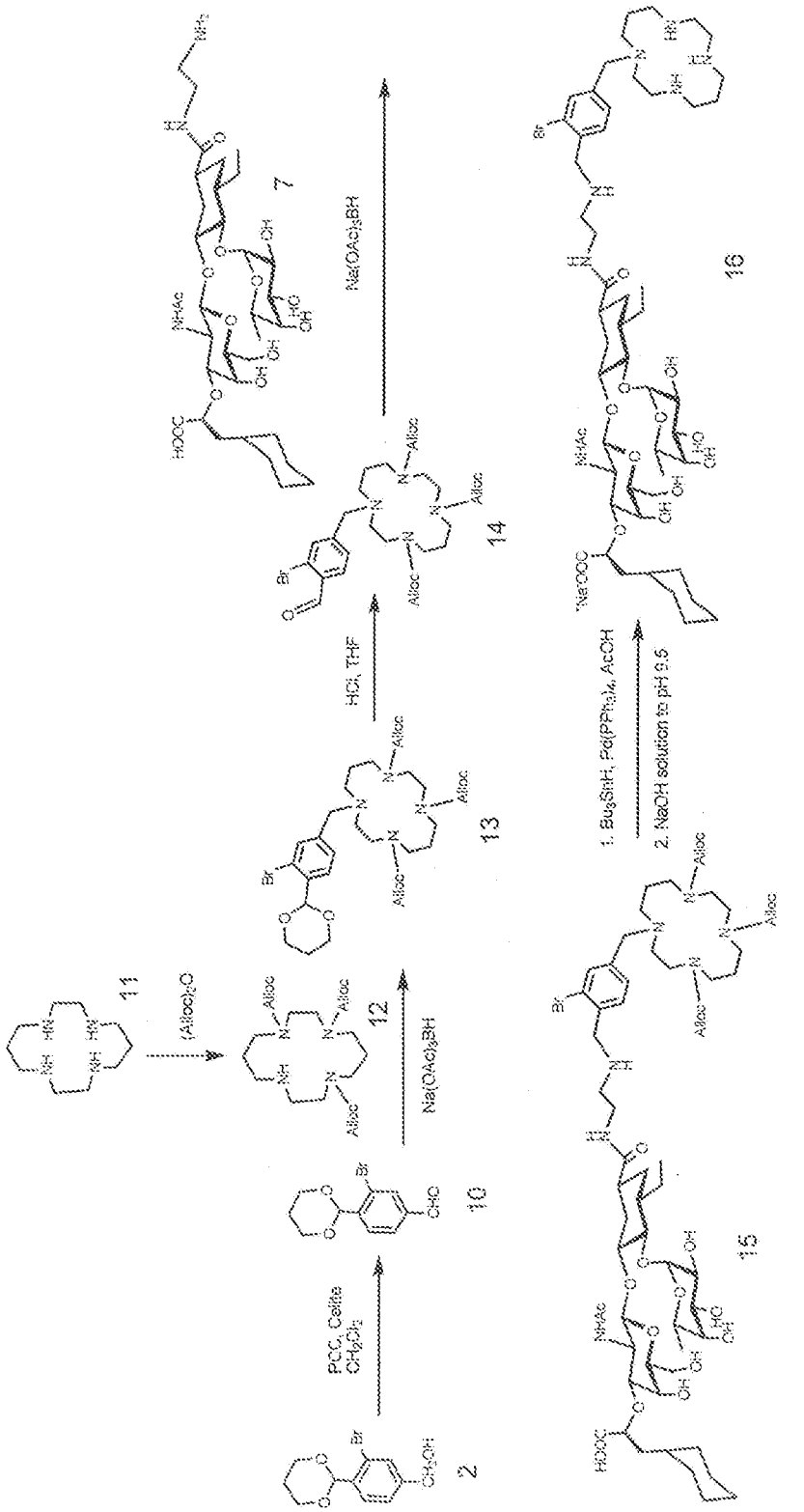

PCC, Celite
CH2Cl2
(Alloc)2O
Na(OAc)3BH
HCl, THF
Na(OAc)3BH
1. Bu3SnH, Pd(PPh3)4, AcOH
2. NaOH solution to pH 9.5
2
10
11
12
13
14
7
15
16

FIG. 1B

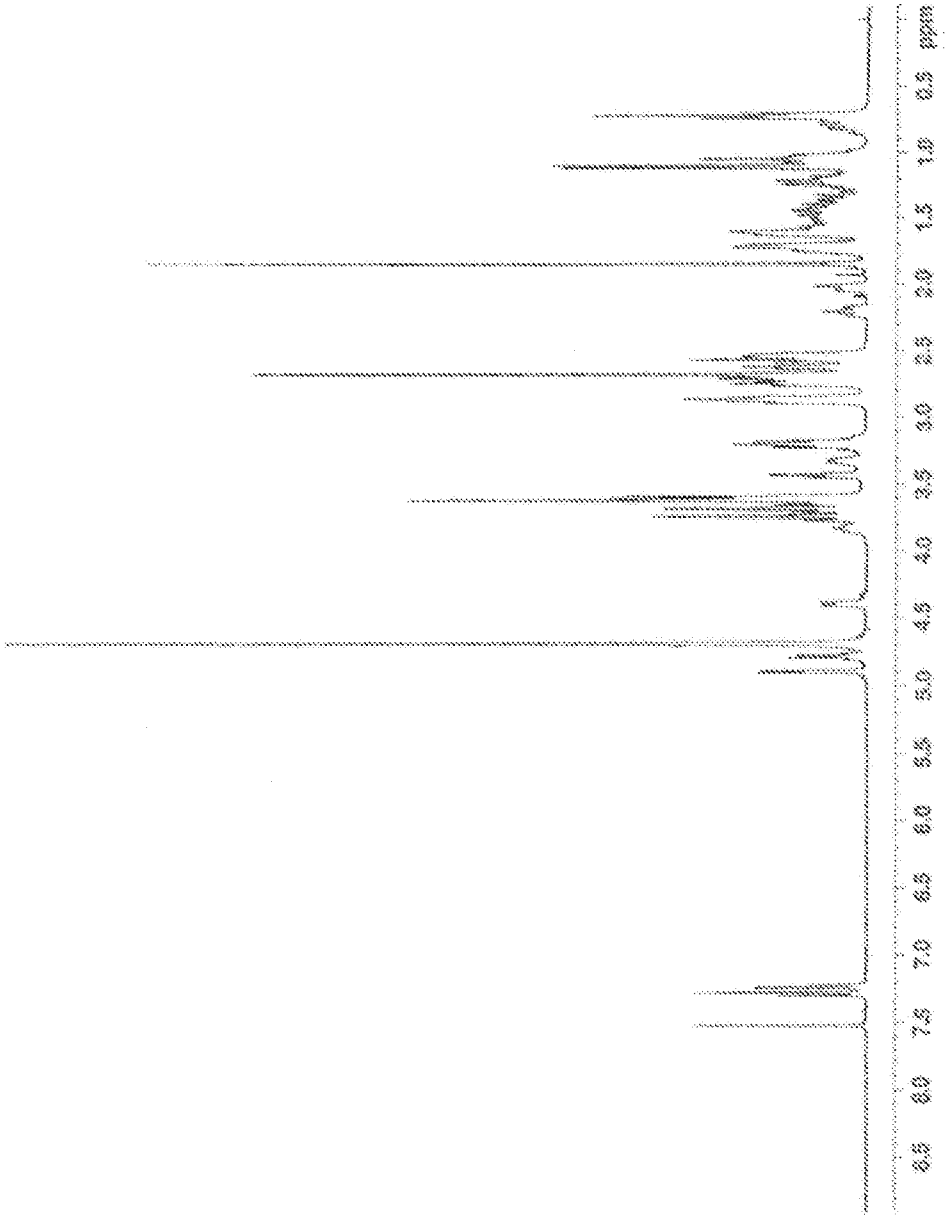


FIG. 2

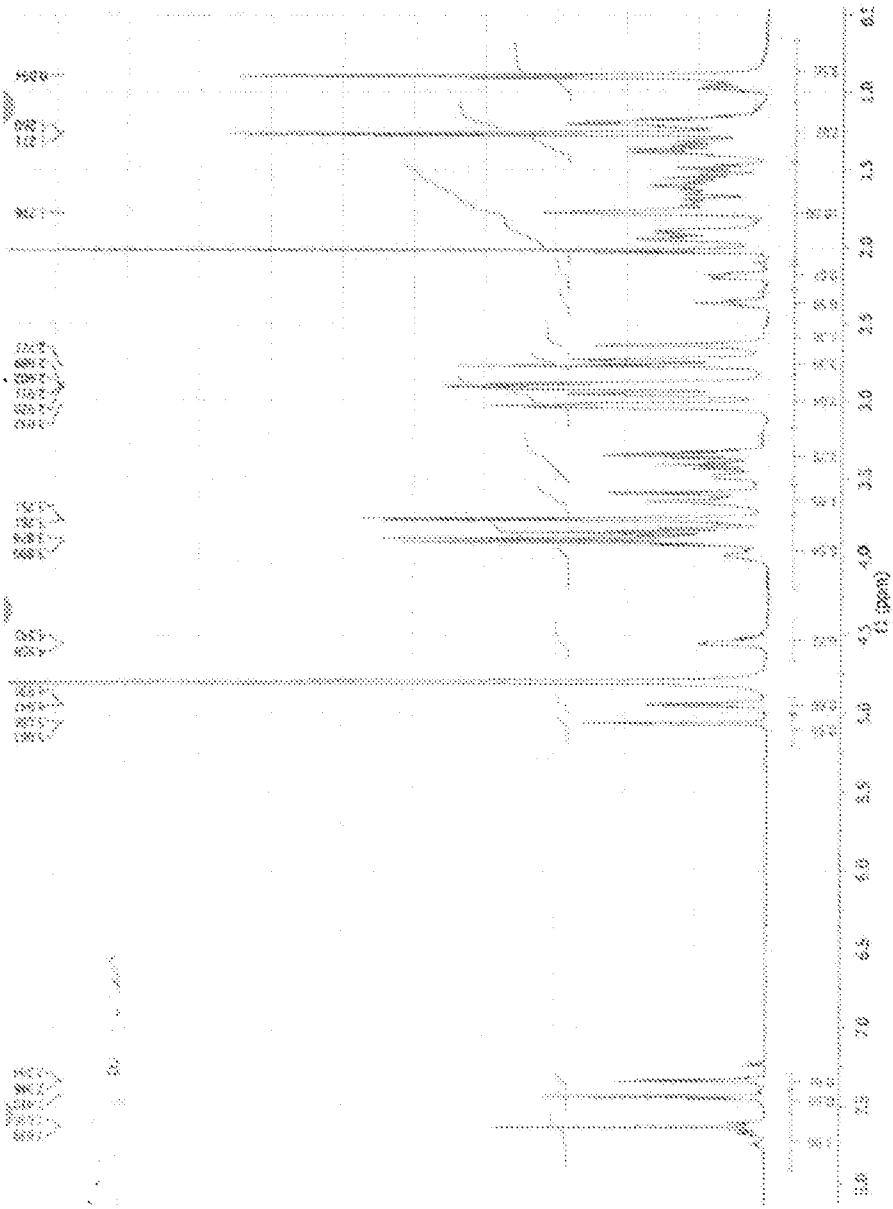


FIG. 3

Inhibition of SDF-1-Induced Chemotaxis by Compounds 9 and 16

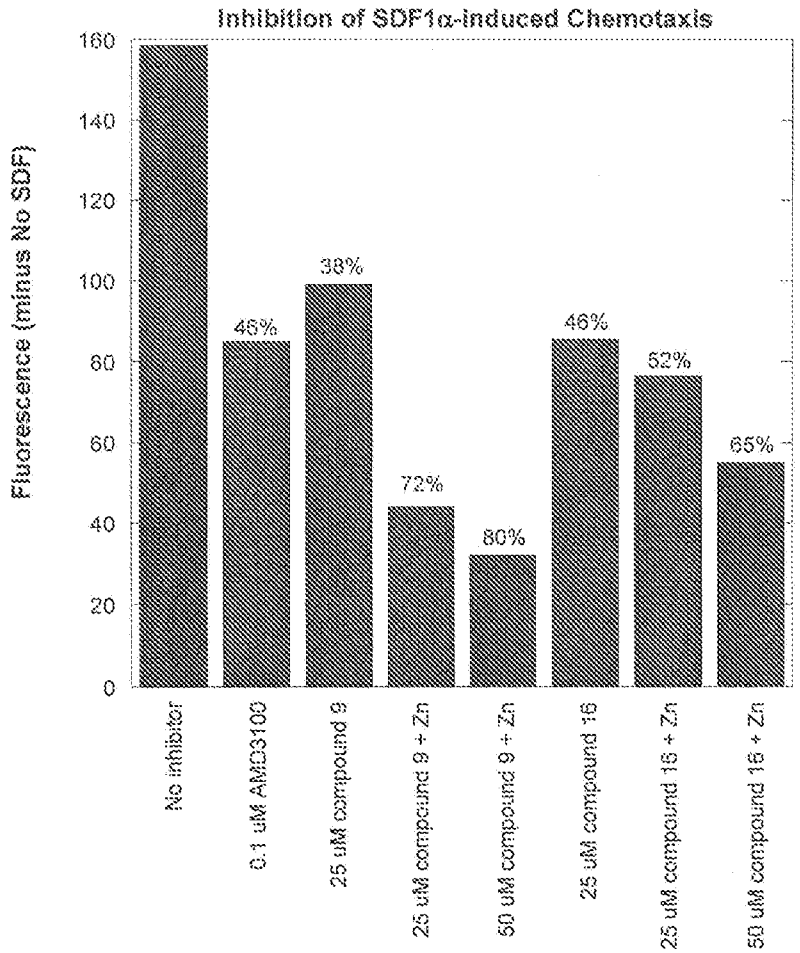


FIG. 4

Inhibition of E-Selectin by Compound 9

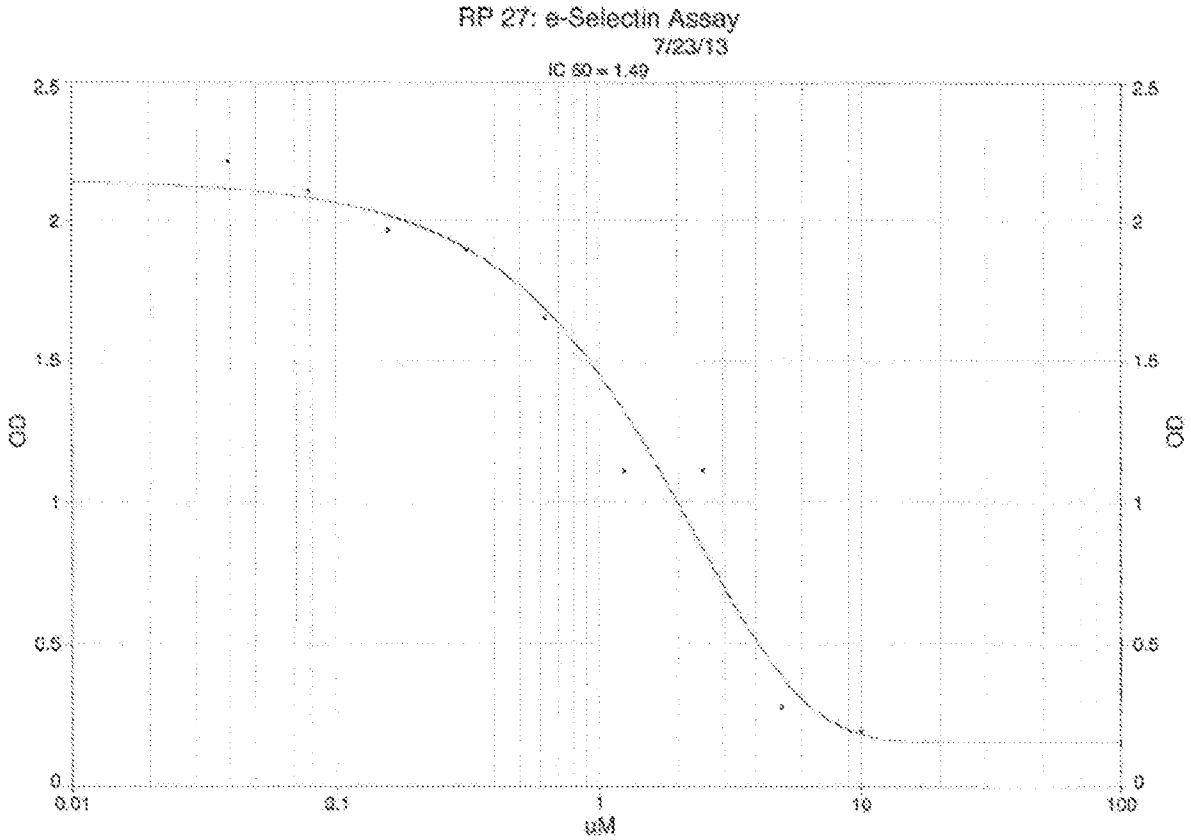


FIG. 5A

Inhibition of E-Selectin by Compound 16

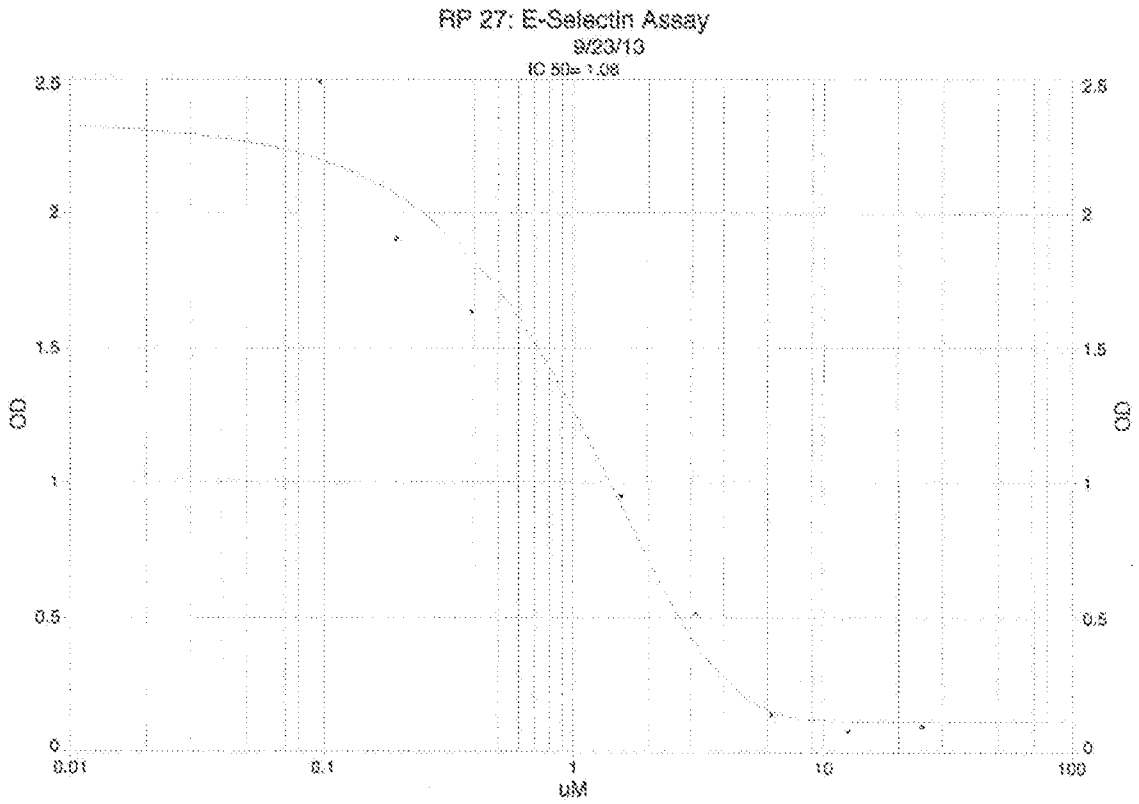


FIG. 5B

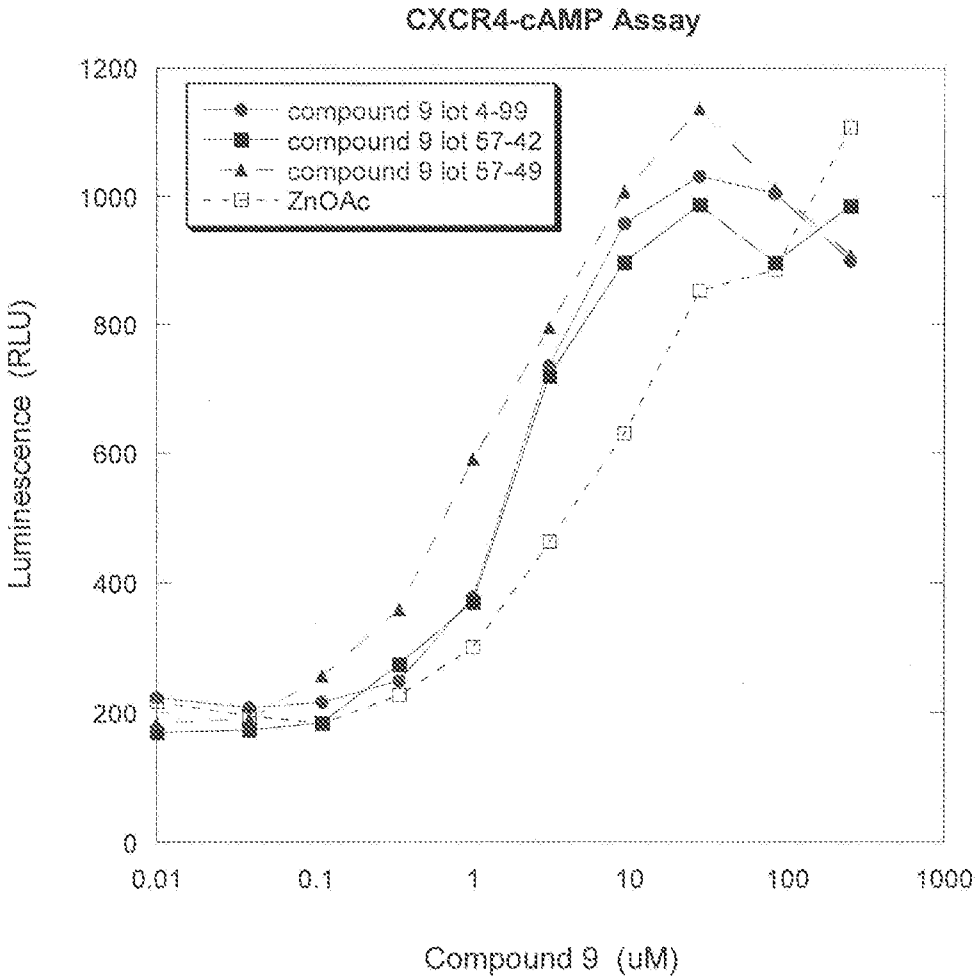

CXCR4-cAMP Assay
compound 9 lot 4-99
compound 9 lot 57-42
compound 9 lot 57-49
ZnOAc
Luminescence (RLU)
1200
1000
800
600
400
200
0
0.01
0.1
1
10
100
1000
Compound 9 (uM)

FIG. 6

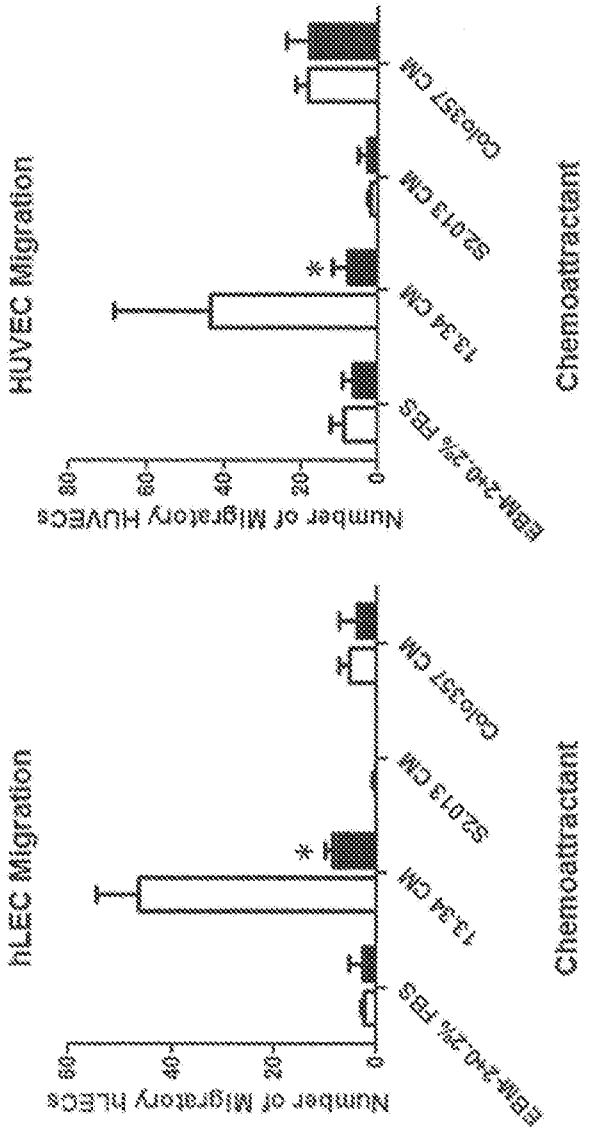
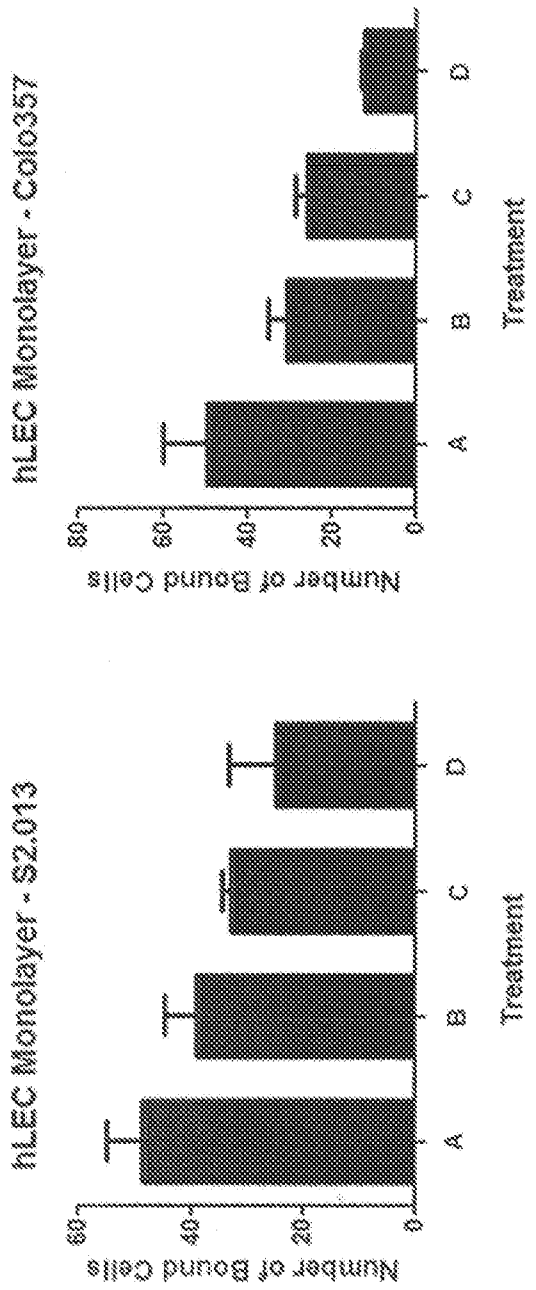

hLEC Migration
Number of Migratory hLECs
60
40
20
0
EBM-2+0.2% FBS
13.3A CM
SZ013 CM
Cob357 CM
Chemoattractant
HUVEC Migration
Number of Migratory HUVECs
80
60
40
20
0
EBM-2+0.2% FBS
13.3A CM
SZ013 CM
Cob357 CM
Chemoattractant
No Compound 9
Compound 9

FIG. 7



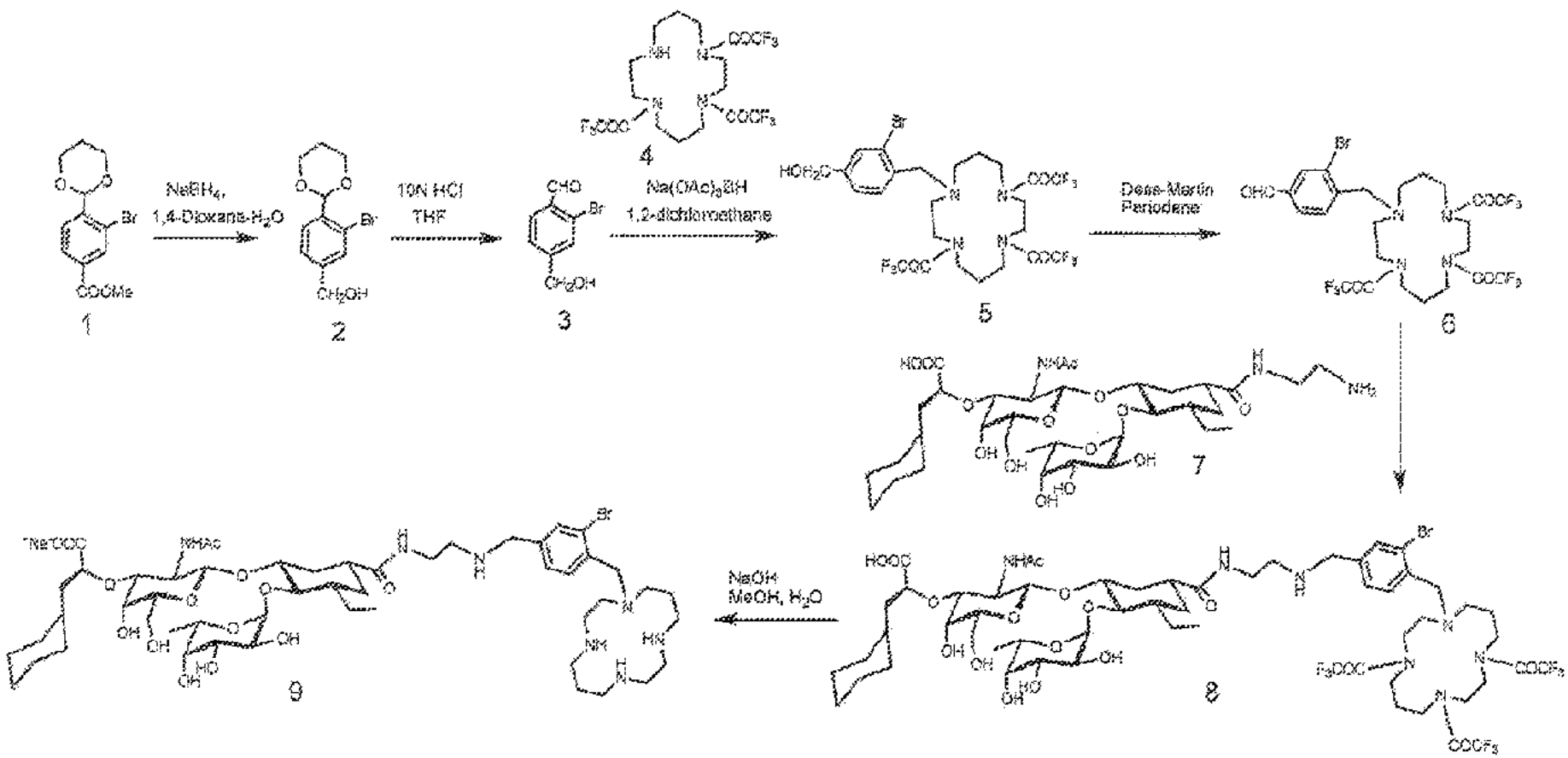
A: Control
B: E-Selectin Specific Antagonist (100 ug/ml)
C: Compound 9 (10 ug/ml)
D: Compound 9 (100 ug/ml)

FIG. 8

Data at 35 Days Post Treatment (5 days from the end of treatments)

Treatment	Animals	Tibiae	% Positive Tibiae	Lytic Area (Lytic units)
Vehicle	8	16	11/16 (69 %)	9.71 +/- 1.37
Compound 9	8	16	6/16 (37.5 %)	2.17 +/- 0.44
DTX	5	10	6/10 (60%)	6.04 +/- 0.81
Compound 9 + DTX	8	16	4/16 (25%)	1.17 +/- 0.15
E-selectin antagonist	8	16	8/16 (50%)	6.88 +/- 0.49
E-selectin antagonist + DTX	8	16	6/16 (37.5%)	4.97 +/- 1.03
AMD3100	5	10	5/10 (50%)	3.43 +/- 0.44
AMD + DTX	5	10	3/10 (30%)	1.32 +/- 0.64

FIG. 9


NaBH4,
1,4-Dioxane-H2O
10N HCl
THF
Na(OAc)3BH
1,2-dichloroethane
Dess-Martin
Periodane
NaOH
MeOH, H2O
1
2
3
4
5
6
7
8
9