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(19) **United States**(12) **Patent Application Publication** (10) **Pub. No.: US 2020/0062771 A1**  
Movassaghi et al. (43) **Pub. Date: Feb. 27, 2020**(54) **POTENT AGELASTATIN DERIVATIVES AS MODULATORS FOR CANCER INVASION AND METASTASIS****Related U.S. Application Data**

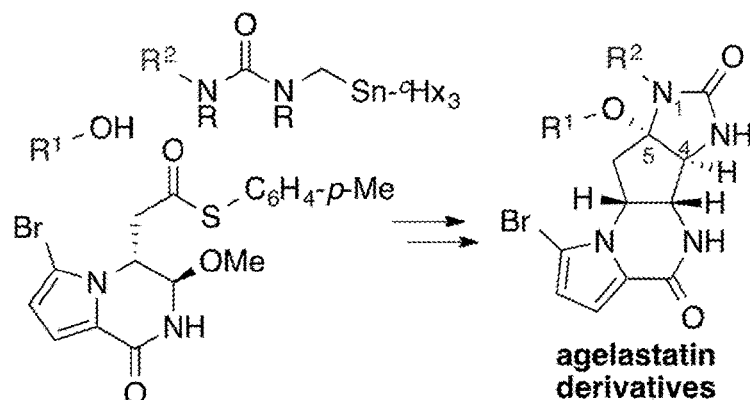
(60) Provisional application No. 62/504,877, filed on May 11, 2017.

(71) Applicants: **Massachusetts Institute of Technology**, Cambridge, MA (US); **Tufts Medical Center, Inc.**, Boston, MA (US)**Publication Classification**(72) Inventors: **Mohammad Movassaghi**, Lincoln, MA (US); **Alyssa H. Antropow**, Boston, MA (US); **Rachel J. Buchsbaum**, Winchester, MA (US); **Kun Xu Xu**, Dorchester, MA (US)(51) **Int. Cl.**  
*C07D 487/14* (2006.01)  
*C07D 498/22* (2006.01)  
*A61P 35/04* (2006.01)  
(52) **U.S. Cl.**  
CPC ..... *C07D 487/14* (2013.01); *A61P 35/04* (2018.01); *C07D 498/22* (2013.01)(73) Assignees: **Massachusetts Institute of Technology**, Cambridge, MA (US); **Tufts Medical Center, Inc.**, Boston, MA (US)(57) **ABSTRACT**(21) Appl. No.: **16/612,468**(22) PCT Filed: **May 11, 2018**(86) PCT No.: **PCT/US2018/032327**

§ 371 (c)(1),

(2) Date: **Nov. 11, 2019**

The present disclosure relates to derivatized agelastatin compounds and methods for the treatment, prevention, or delay of cancer, comprising administering a therapeutically effect amount of the derivatized agelastatin compounds, a pharmaceutically acceptable salt thereof, or a composition thereof to a subject in need thereof. Methods for making the derivatized agelastatin compounds are also provided.

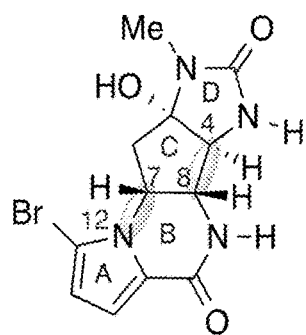
**Specification includes a Sequence Listing.****Strategic Control of Structural Motifs**

- Efficient N1-substitution
- Final-stage C5-derivatization

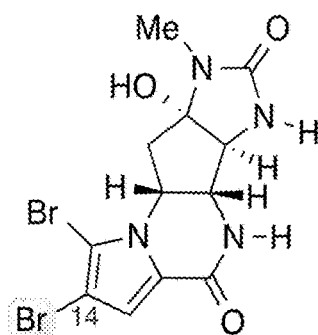
**Unique Biological Assays**

- 3D assay mimicking breast cancer microenvironment
- Potent modulators for cancer invasion and metastasis

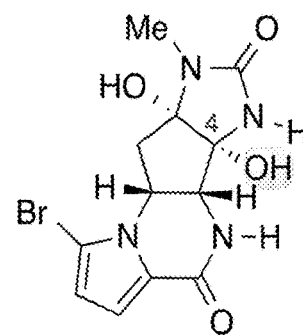
FIG. 1



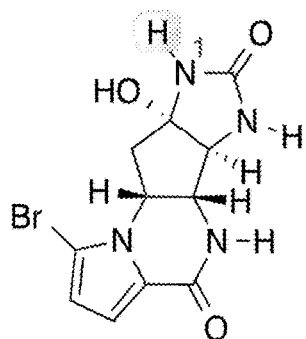
(-)-agelastatin A (1)



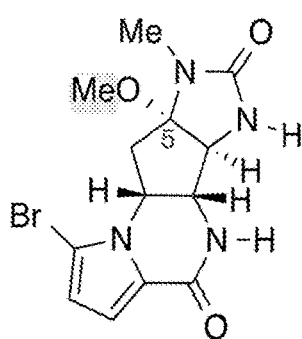
(-)-agelastatin B (2)



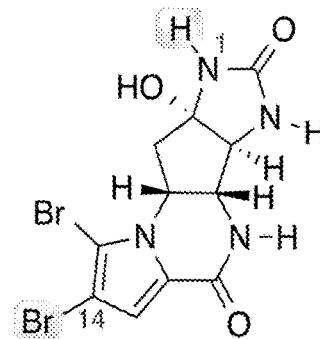
(-)-agelastatin C (3)



(-)-agelastatin D (4)



(-)-agelastatin E (5)



(-)-agelastatin F (6)

FIG. 2

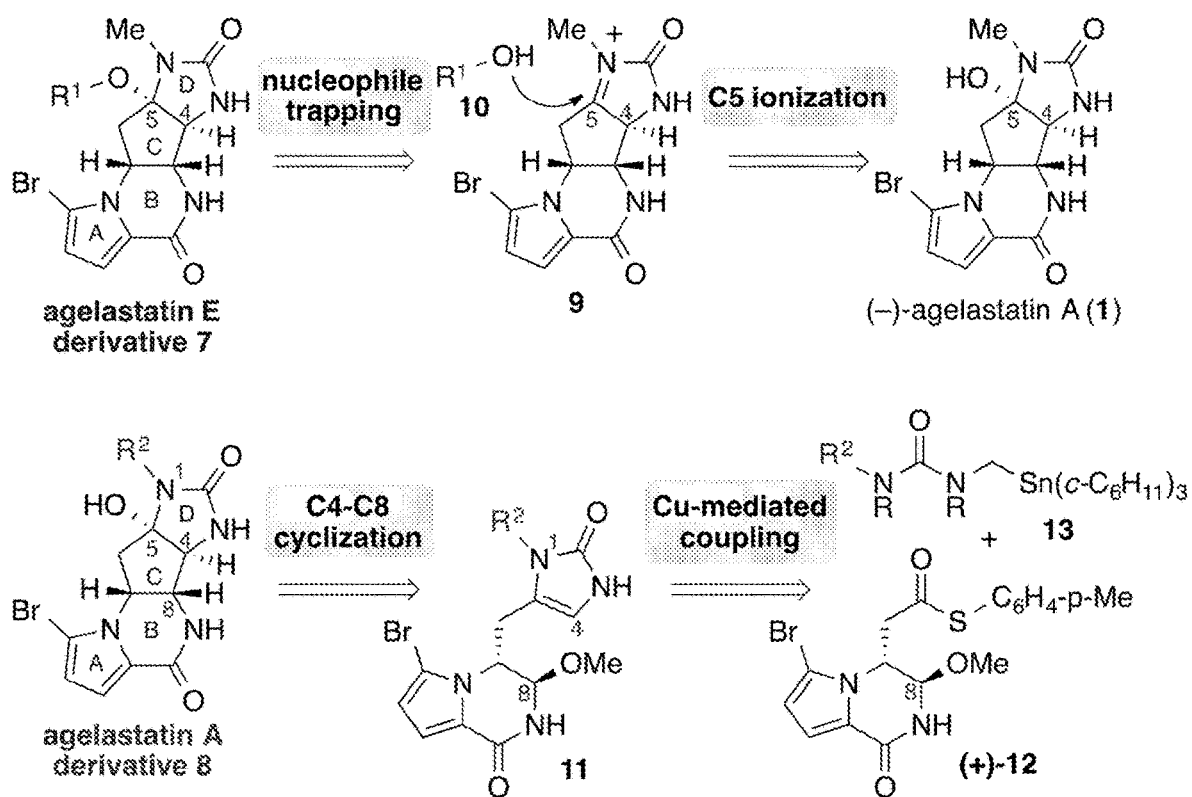


FIG. 3

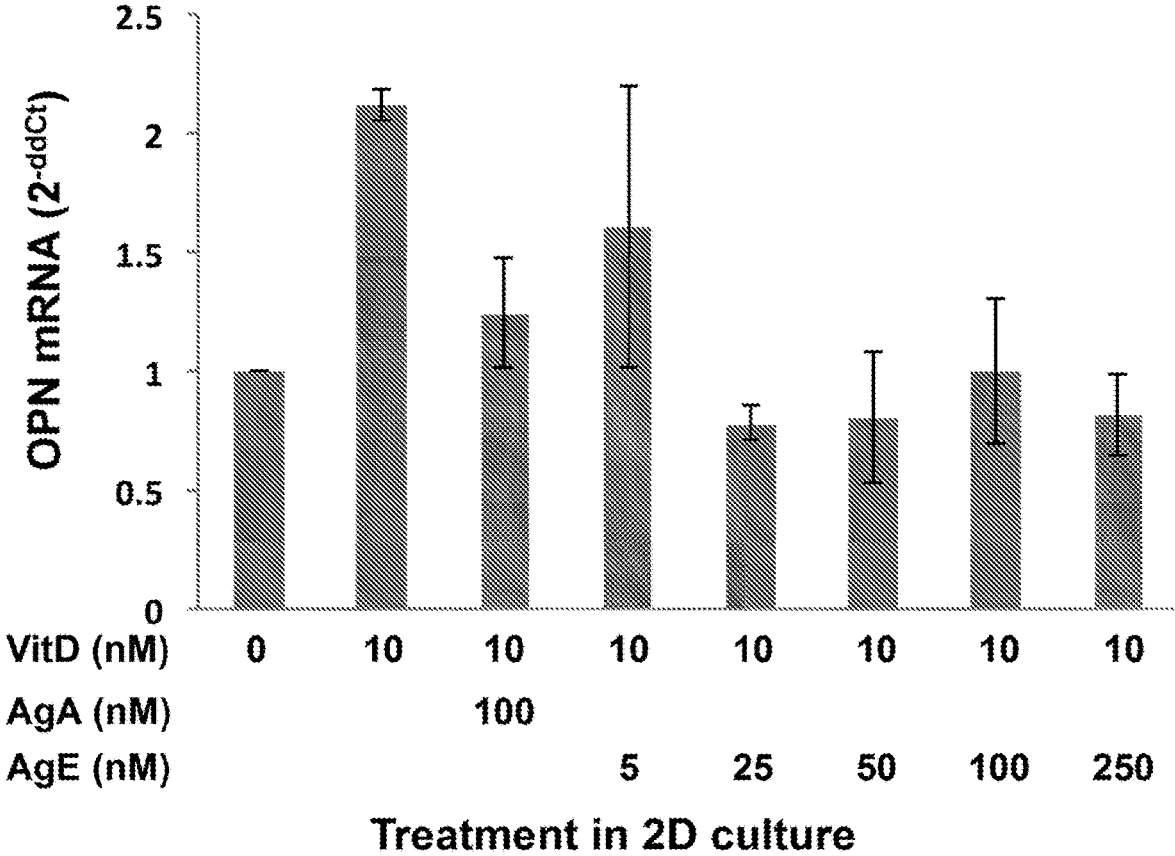


FIG. 4

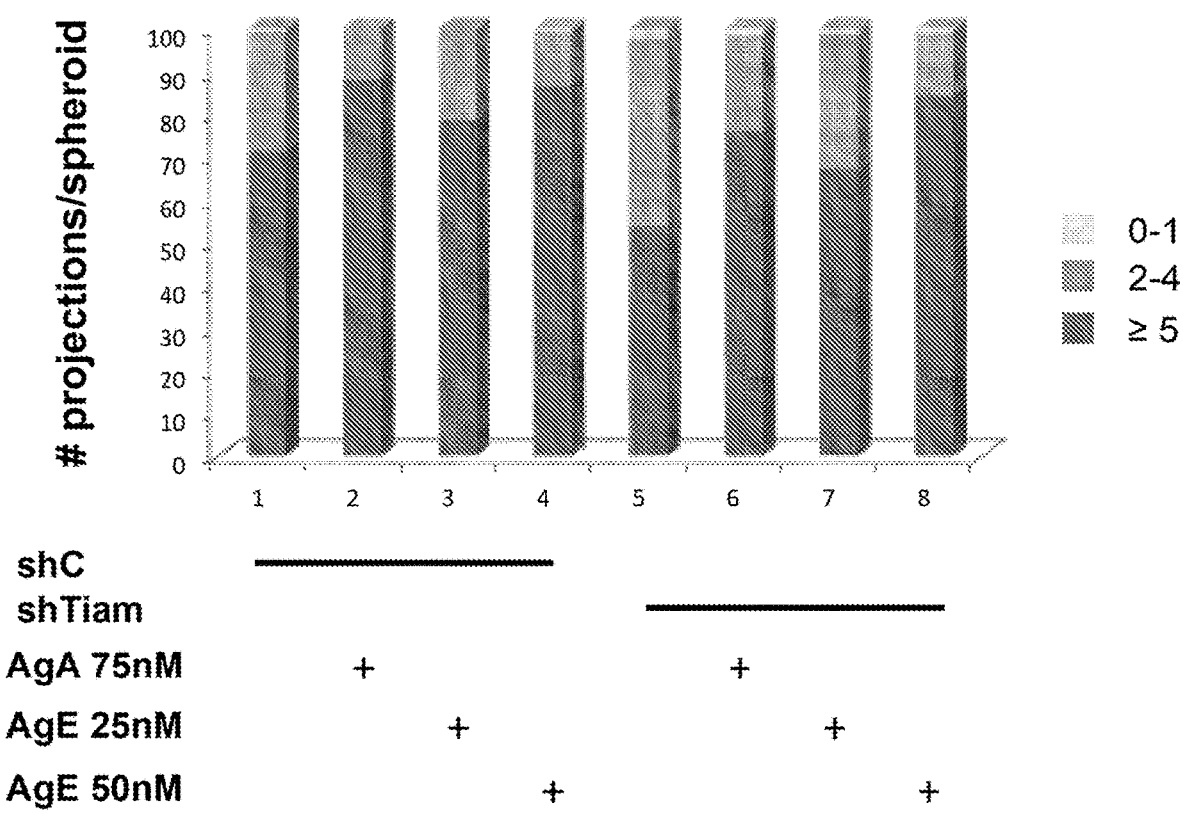


FIG. 5

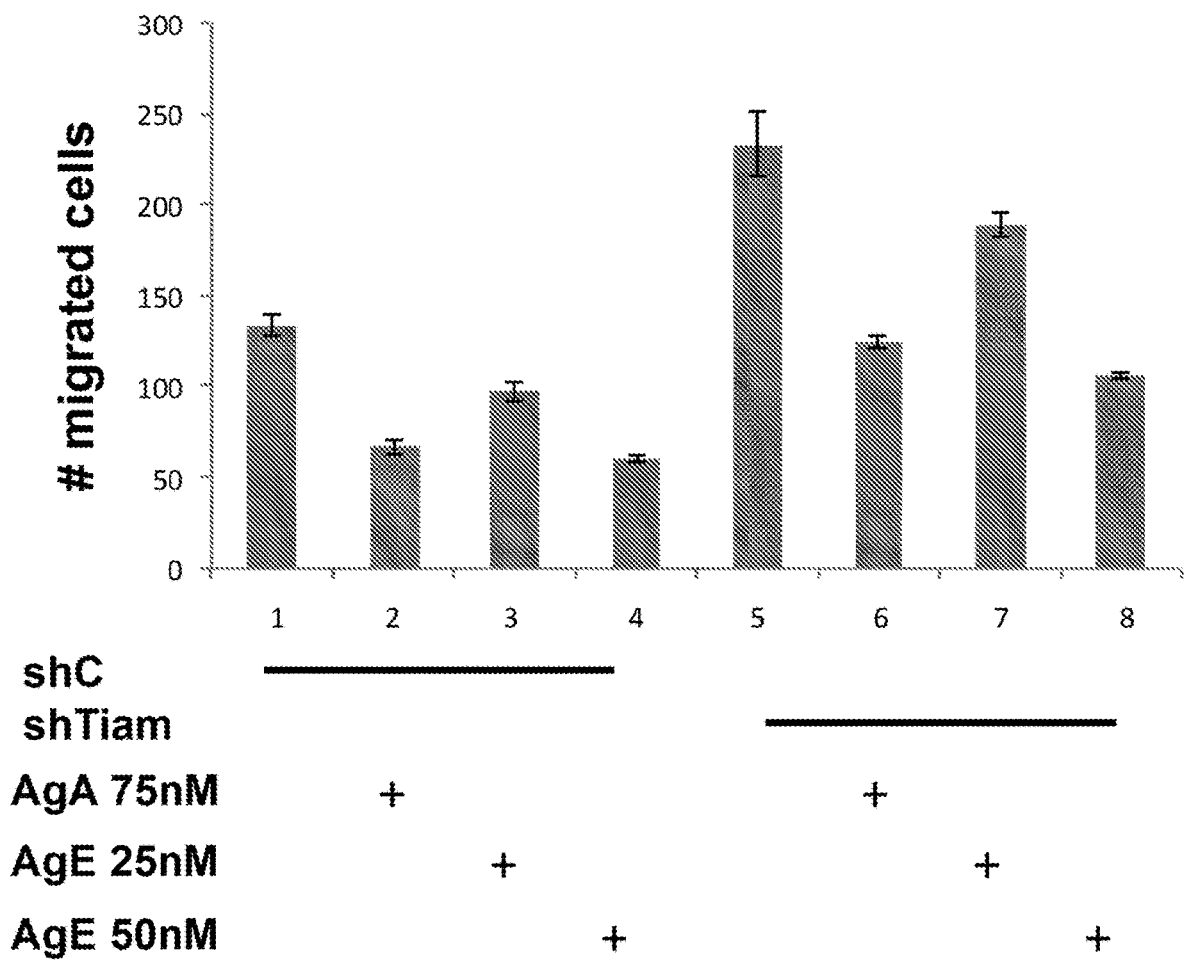


FIG. 6

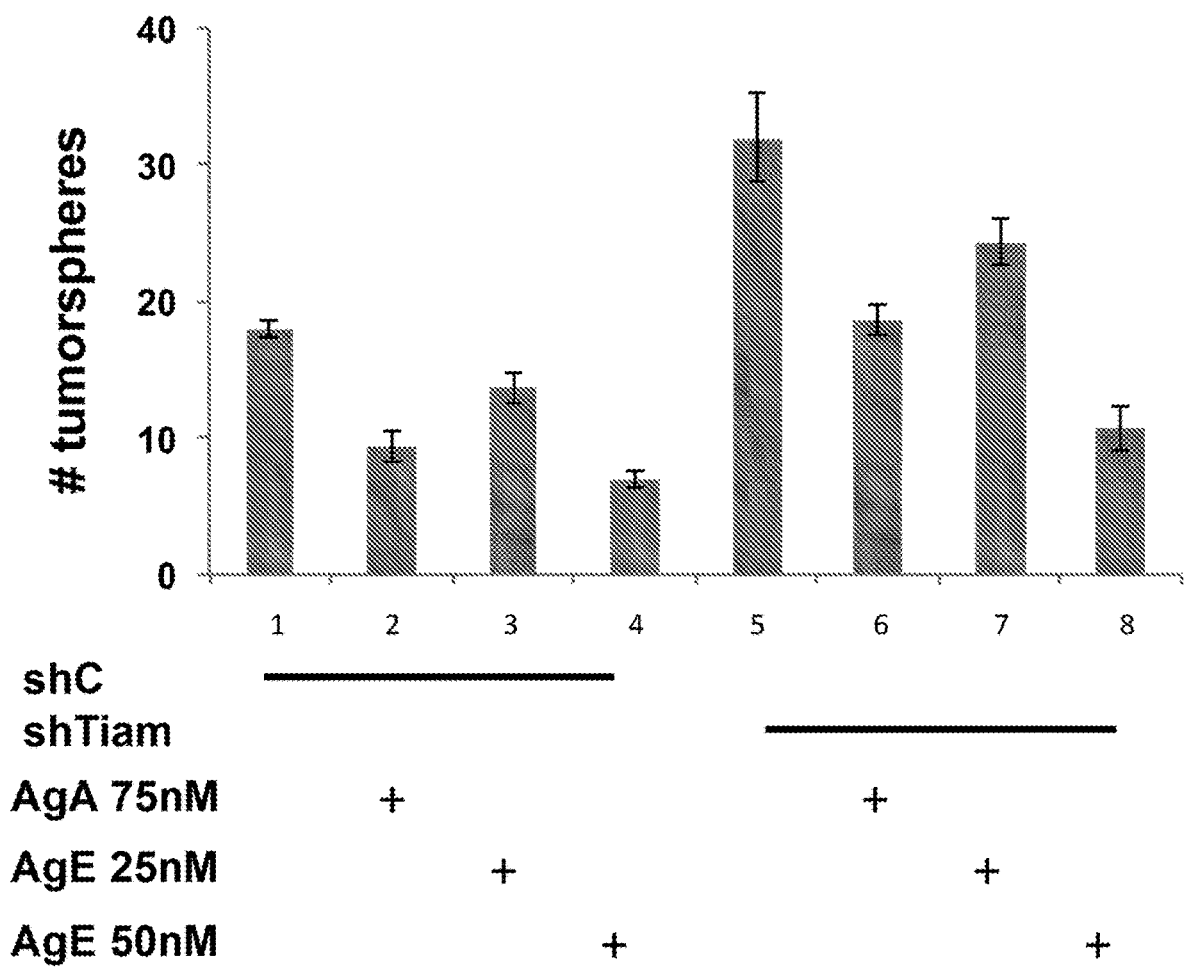


FIG. 7

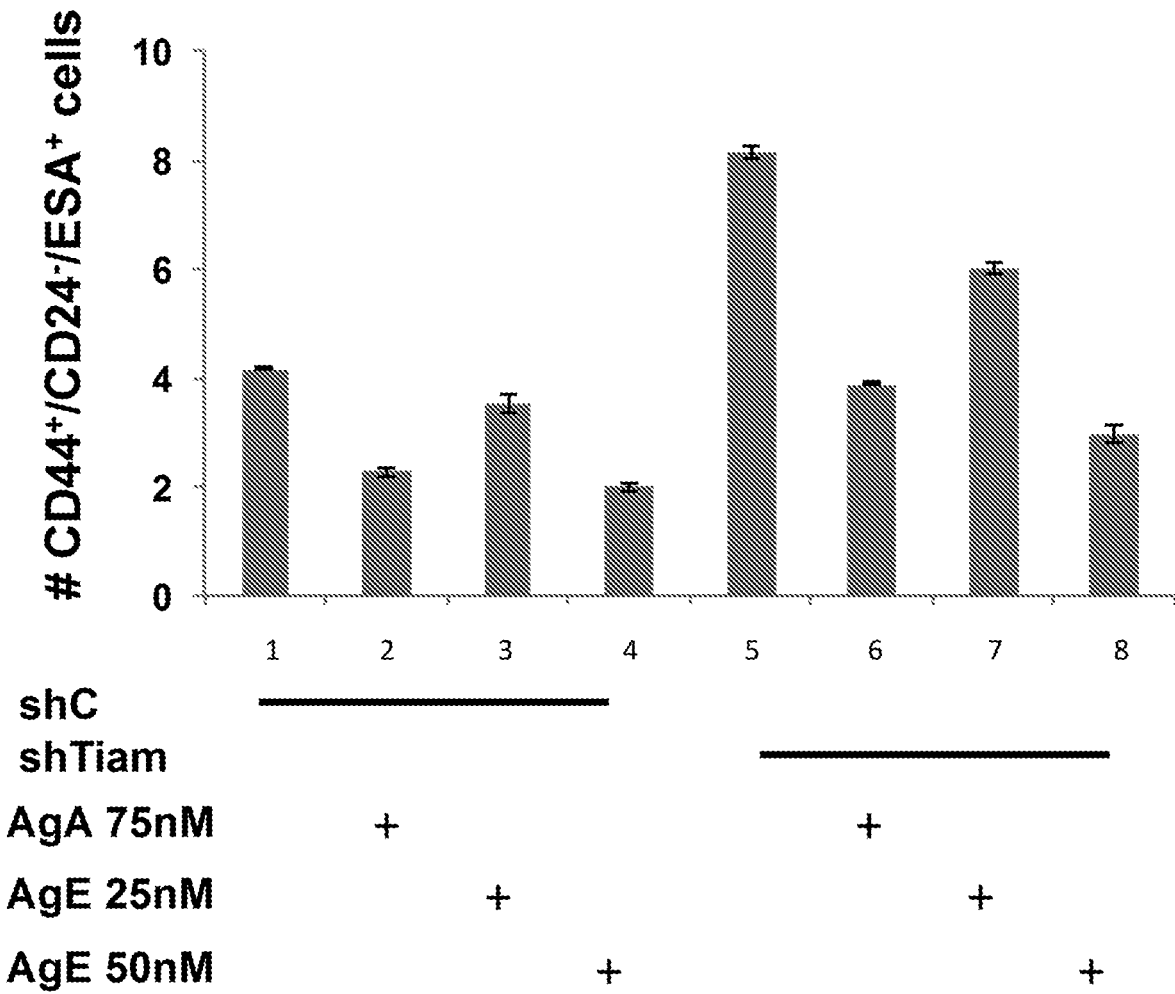




FIG. 8

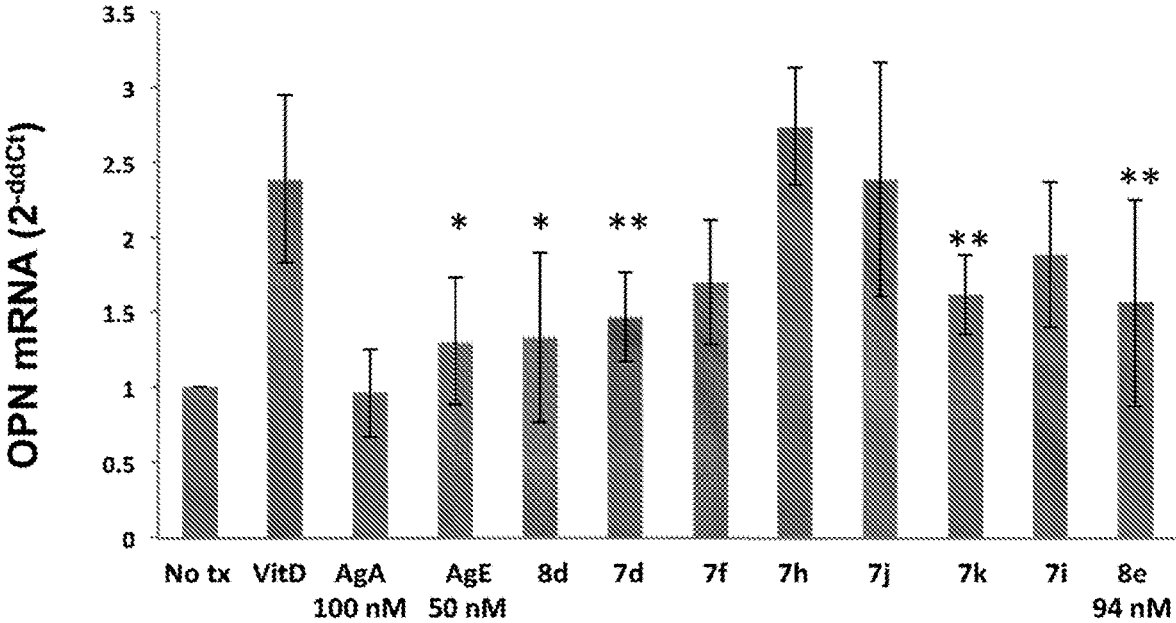


FIG. 9

### Serial AgA dosing in mice: dose toxicity experiment

Serial mouse weights

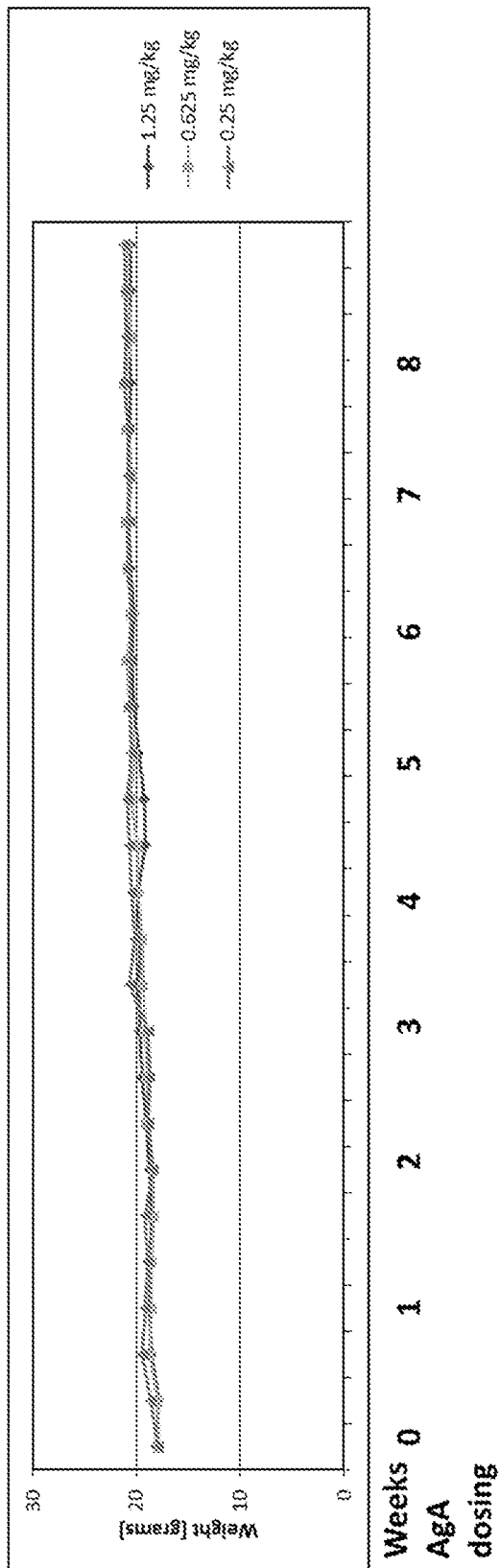
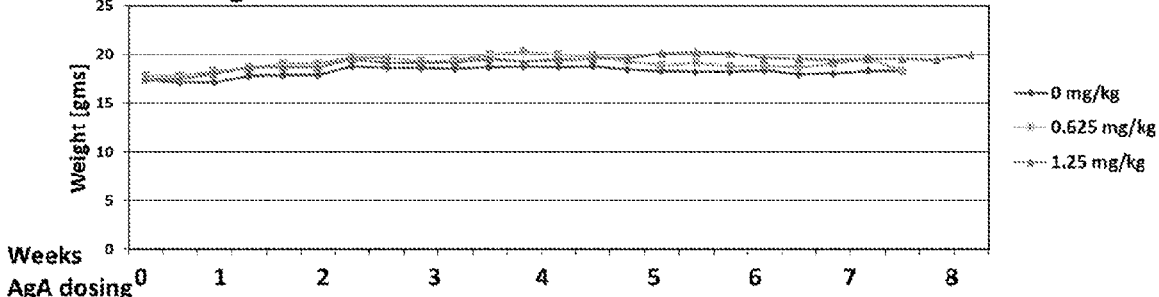


FIG. 10

A. Serial mouse weights



B. Final tumor weights

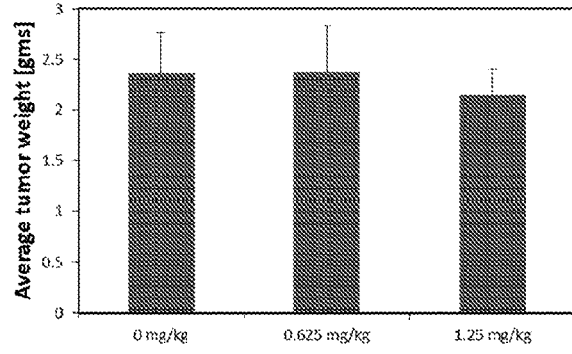


FIG. 11

Lung metastases in AgA-treated mice after mixed cell xenograft co-implantation (SUM1315 / Tiam1deficient mammary fibroblast xenograft)

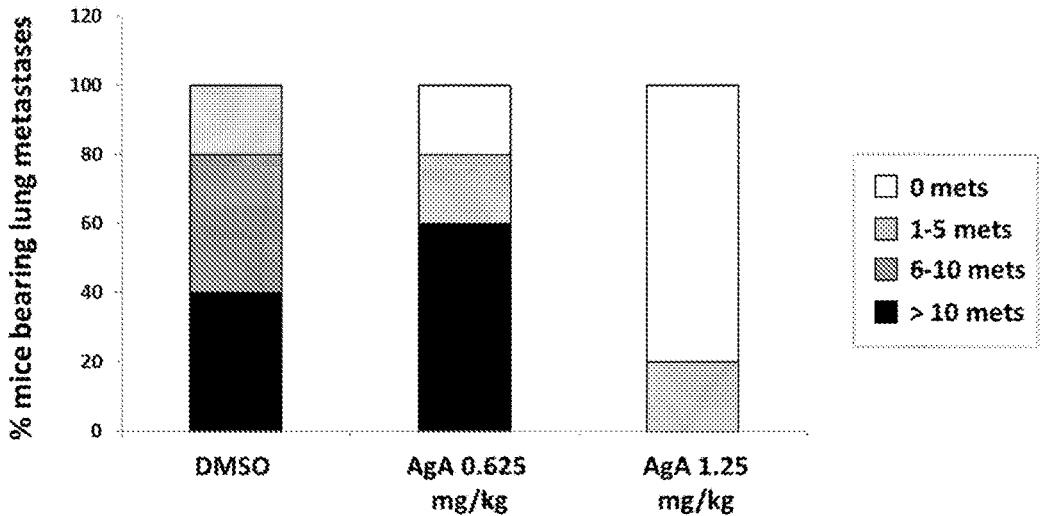


FIG. 12

**Serial AgE treatment post mixed cell xenograft co-implantation  
(AgE starting 12 days post implantation)**

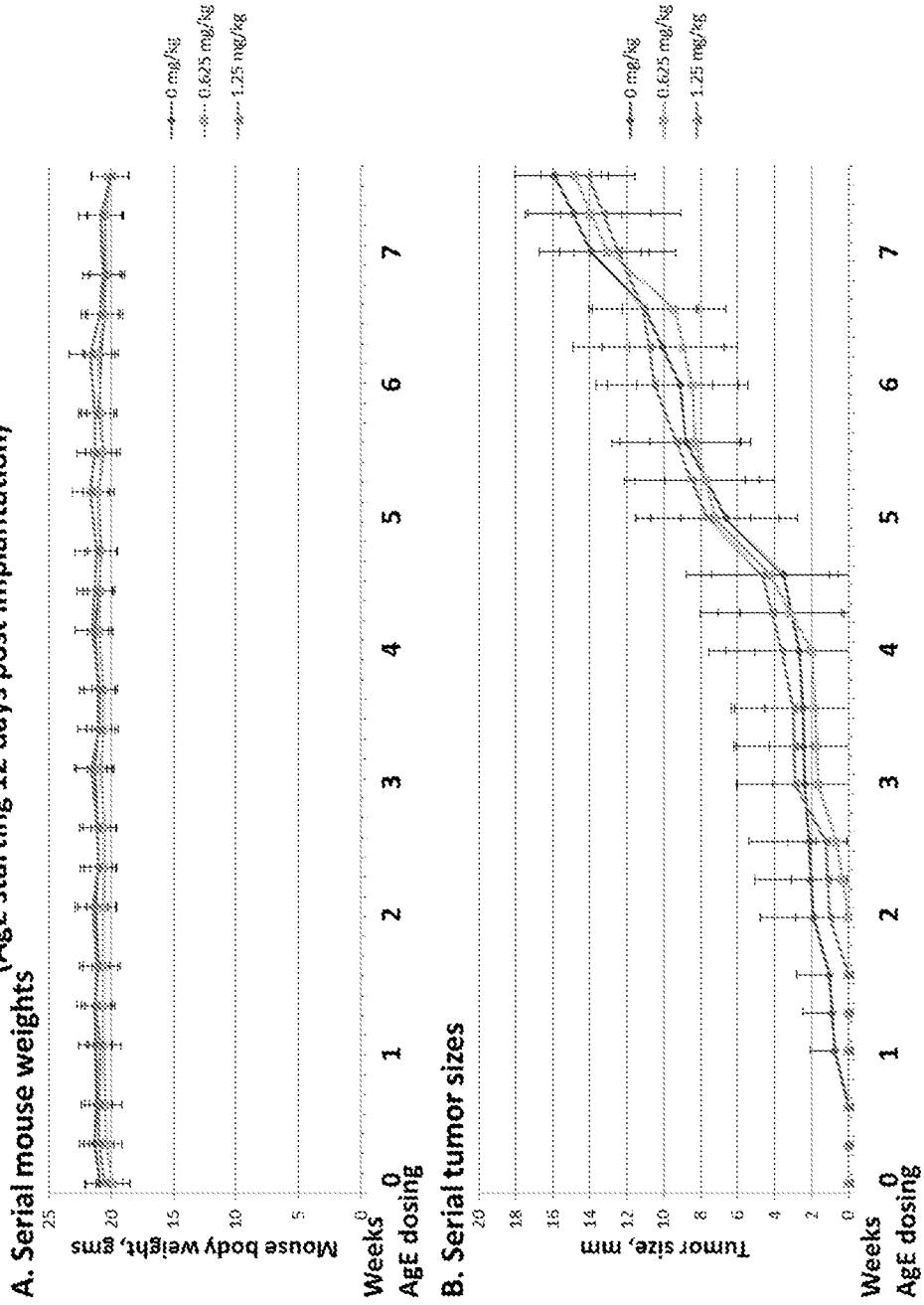


FIG. 13

Lung metastases in AgE-treated mice after mixed cell xenograft co-implantation (SUM1315 / Tiam1deficient mammary fibroblast xenograft)

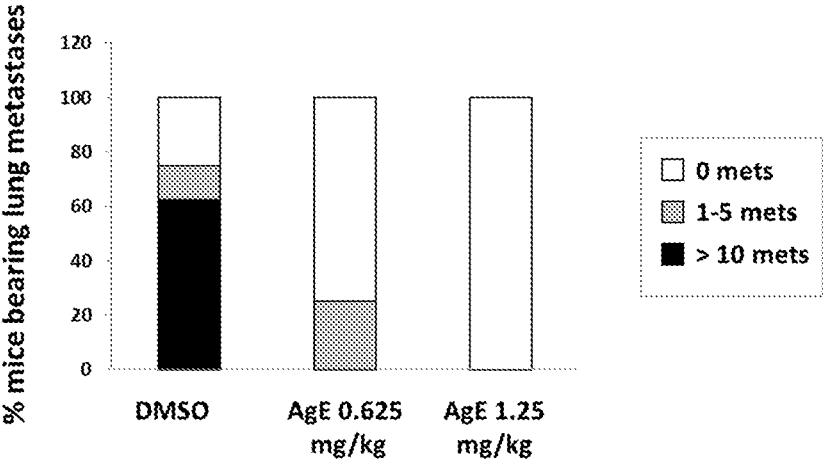


FIG. 14

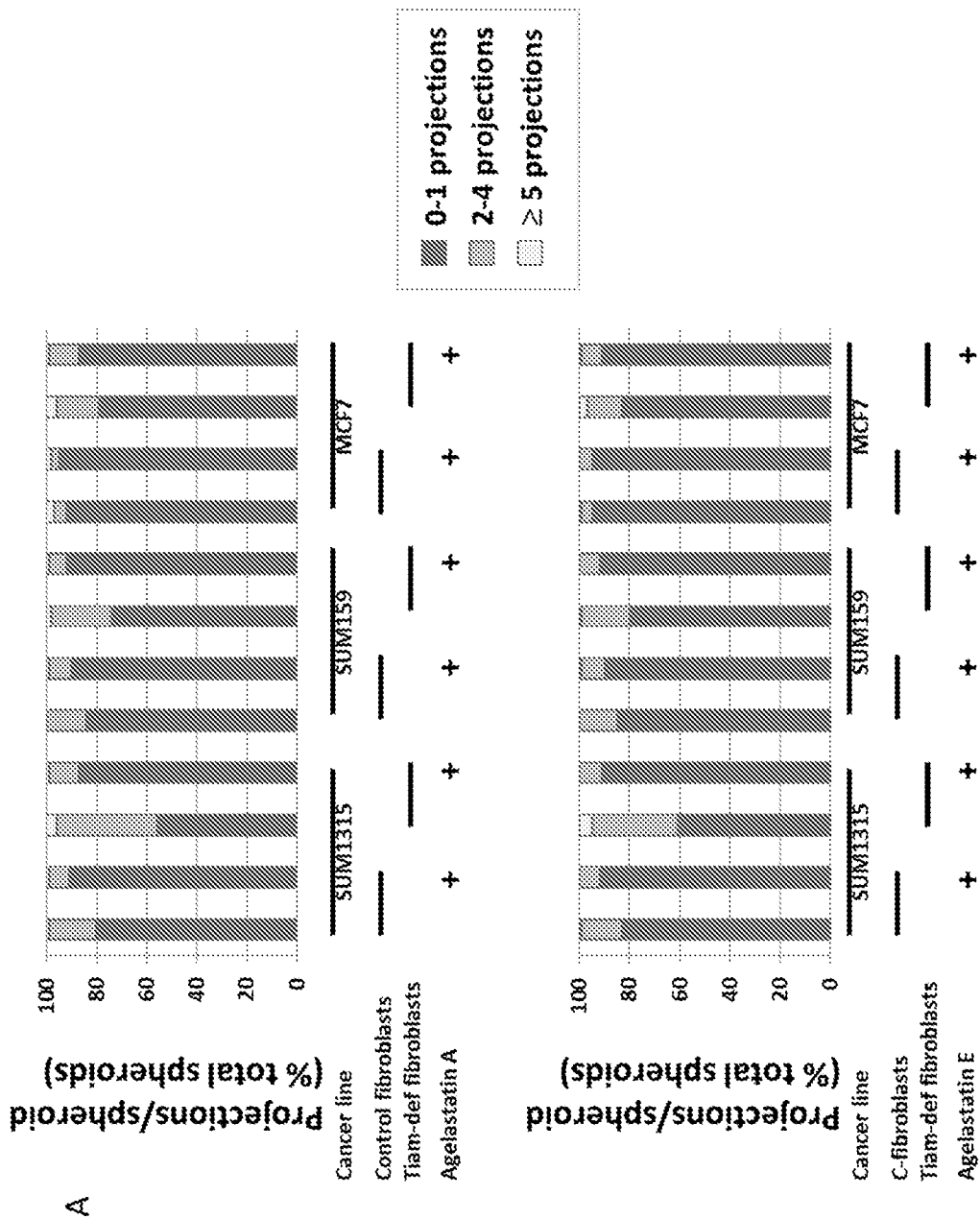
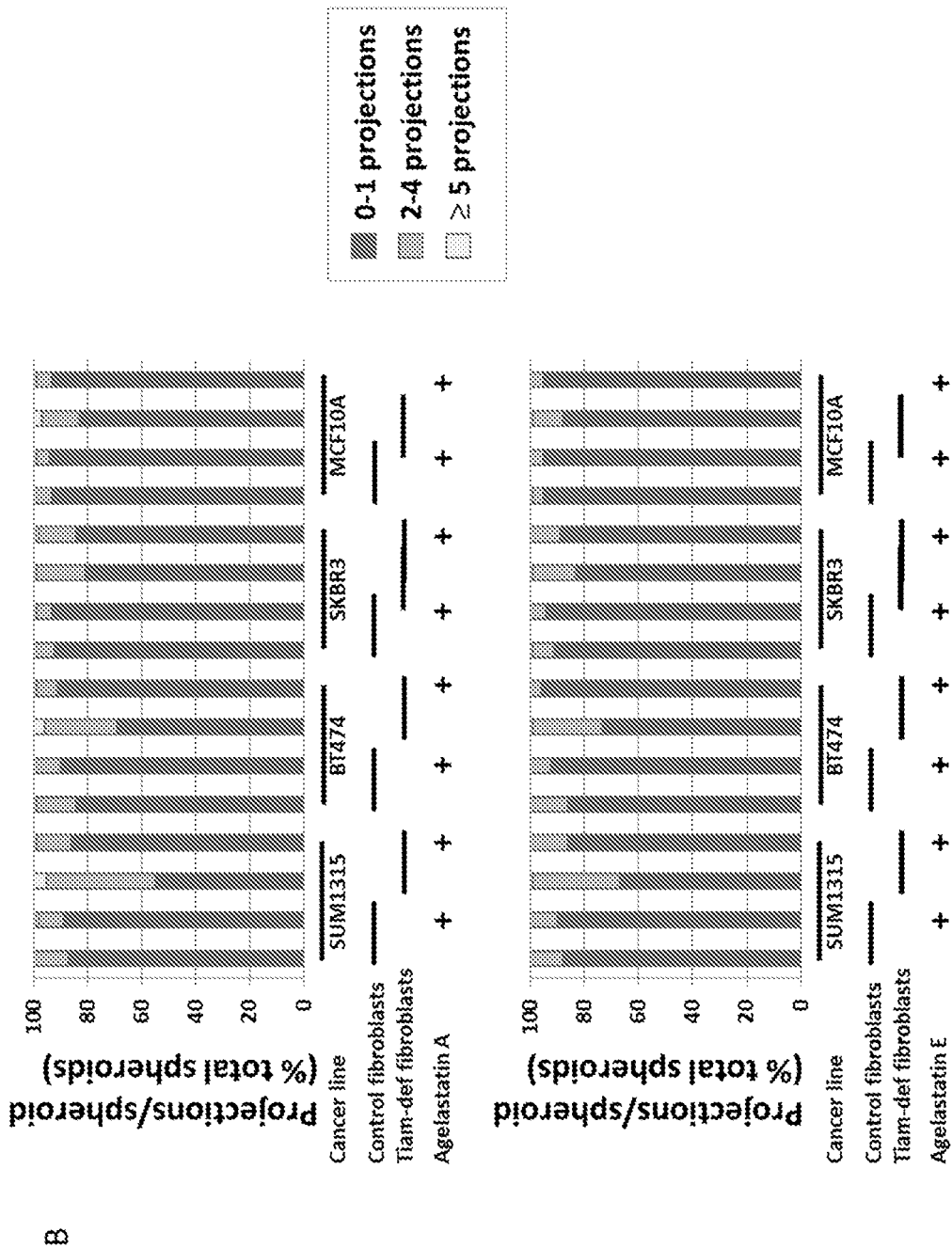


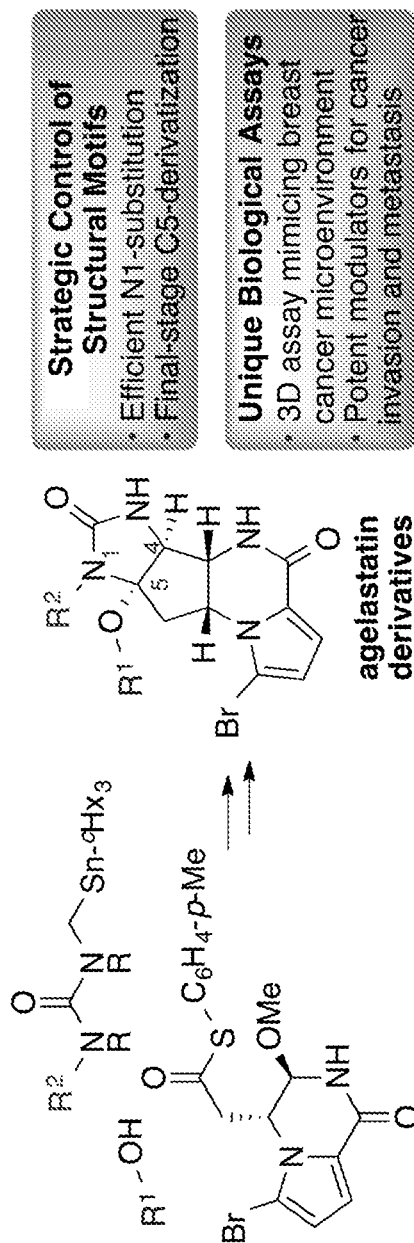
FIG. 14 (cont.)



B



FIG. 15



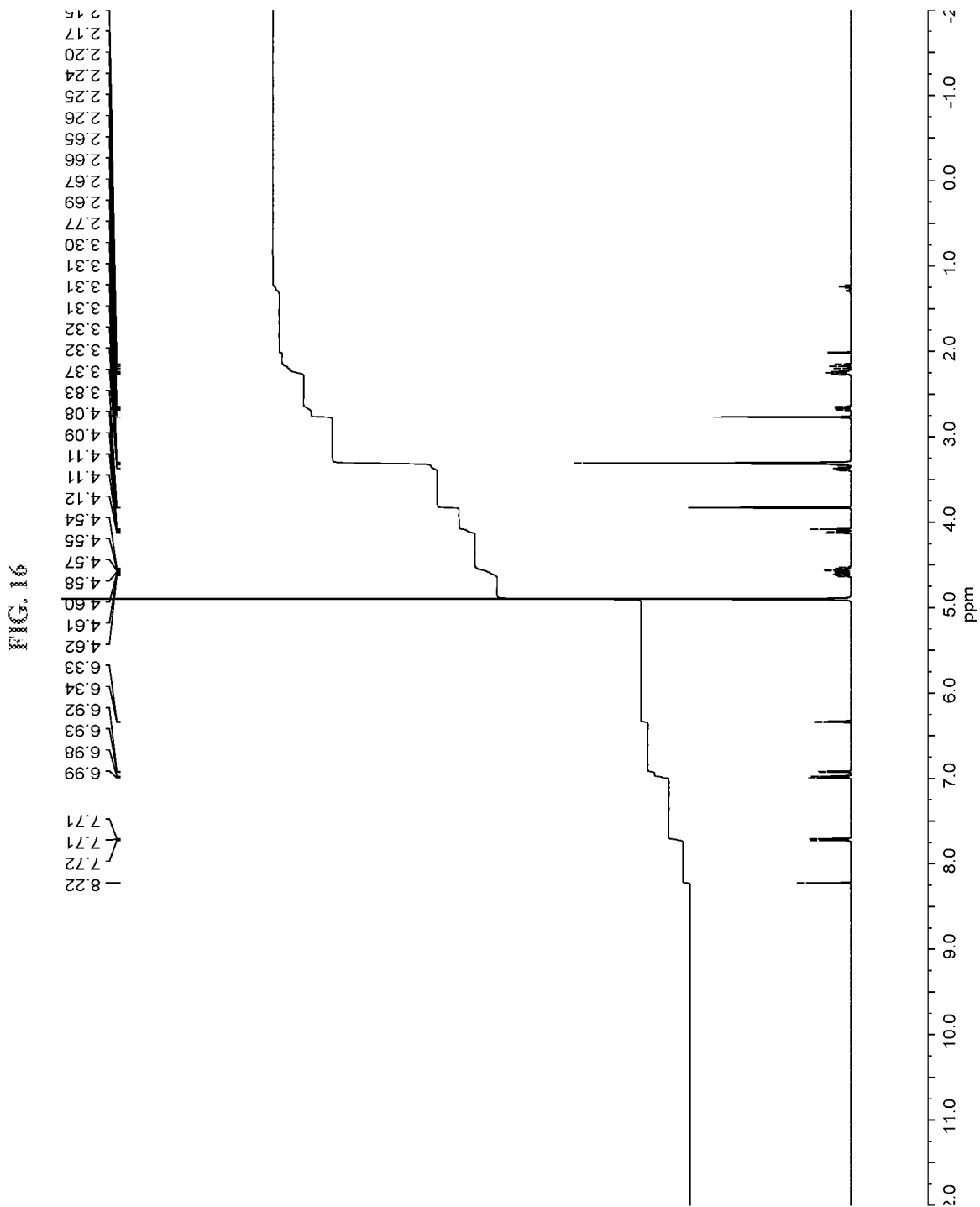


FIG. 17

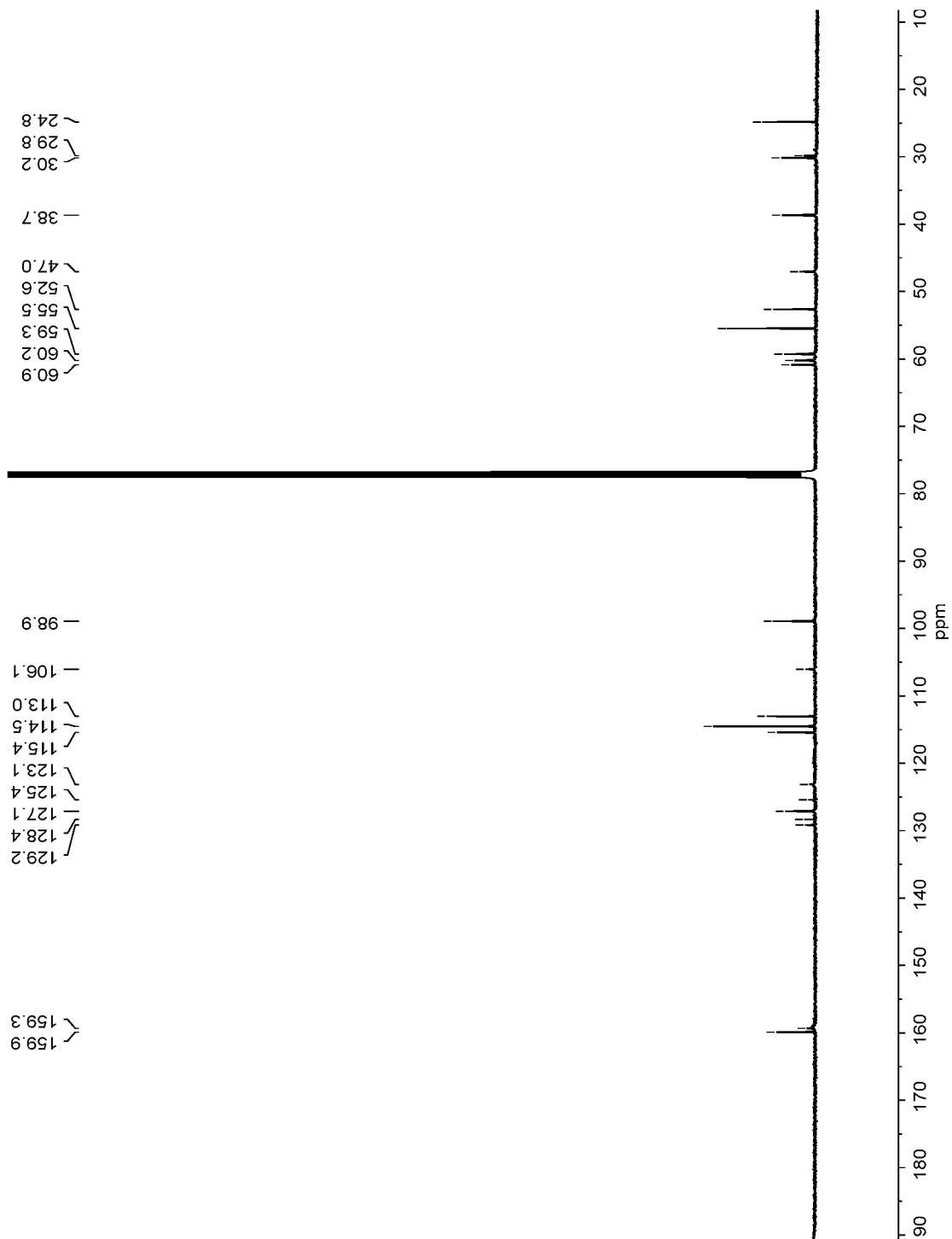


FIG. 18

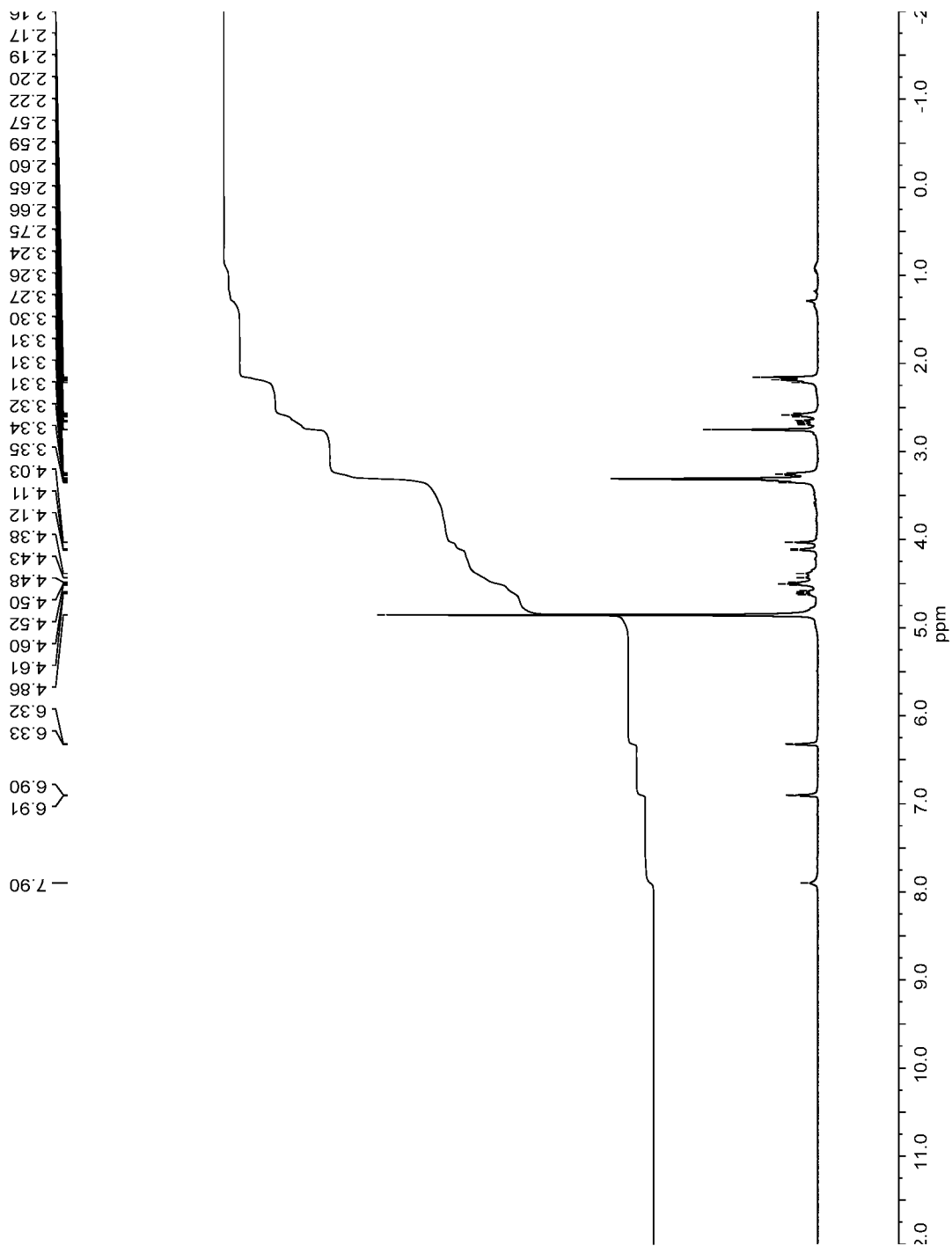
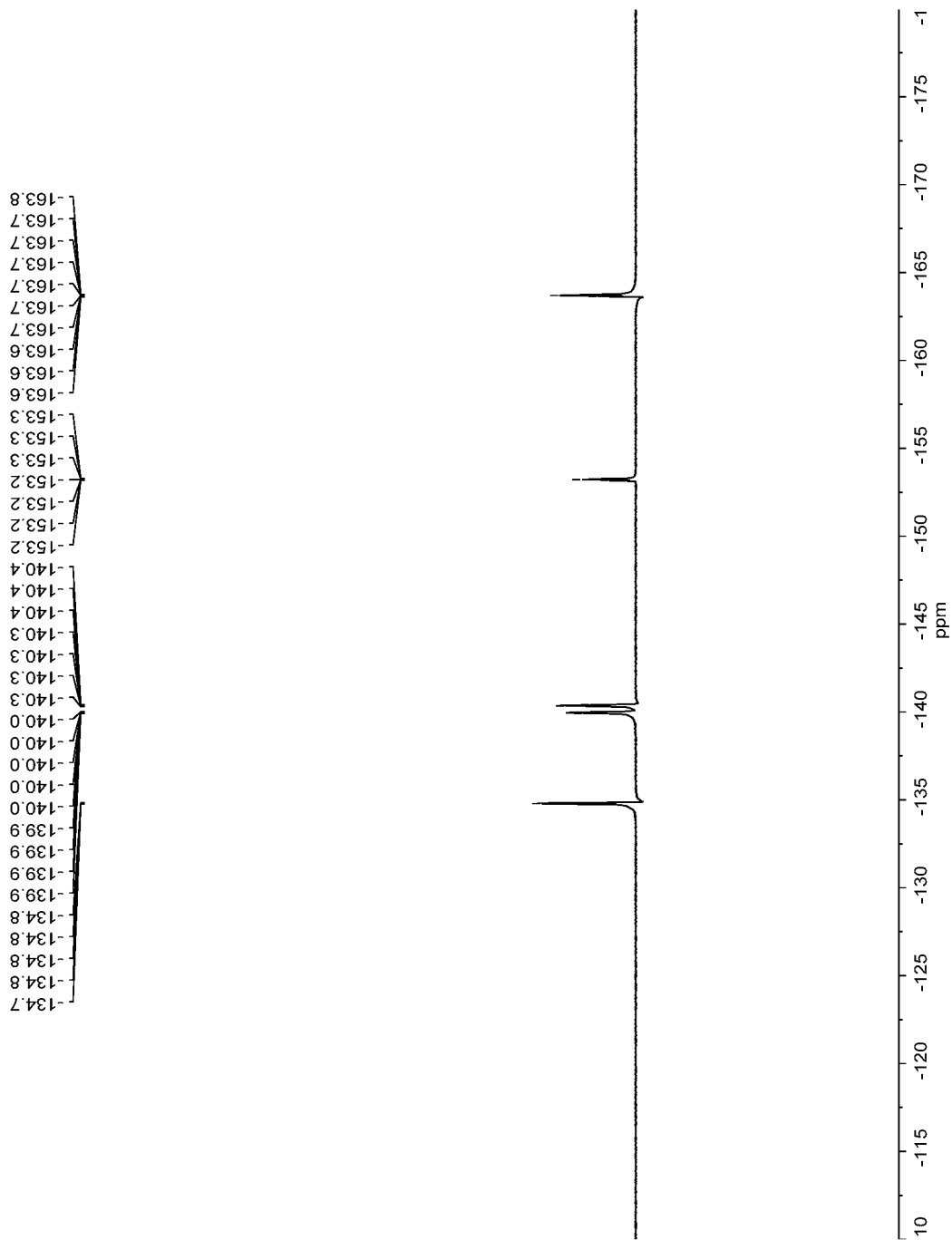


FIG. 19



**POTENT AGELASTATIN DERIVATIVES AS  
MODULATORS FOR CANCER INVASION  
AND METASTASIS**

CROSS REFERENCE TO RELATED  
APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/504,877, filed May 11, 2017, the contents of which are incorporated herein by reference in their entireties.

STATEMENT REGARDING FEDERALLY  
SPONSORED RESEARCH

[0002] This invention was made with government support awarded by Grant No. R01 GM074825, awarded by the National Institutes of Health and under Grant No. W81XWH-11-1-0814, awarded by the U.S Army Medical Research and Materiel Command. The U.S. Government has certain rights in the invention.

FIELD OF DISCLOSURE

[0003] The present disclosure relates to potent agelastatin derivatives as modulators for cancer invasion and metastasis.

BACKGROUND

[0004] The agelastatin alkaloids' have been of interest to the scientific community for many years due to their interesting molecular structure as well as potent biological activities.<sup>2</sup> A general strategy for the total synthesis of all known agelastatins (FIG. 1) has been previously reported, enabling the comprehensive, comparative anticancer study of these naturally occurring alkaloids along with many synthetic derivatives.<sup>3-5</sup> Recent work in this area has offered new synthetic strategies<sup>6</sup> as well as biological study of the natural alkaloids and synthetic derivatives.<sup>7</sup>

[0005] AgA (1, FIG. 1) has been shown to inhibit vitamin D-induced transcription of osteopontin (OPN) in mammary fibroblasts at concentrations well below the cytotoxic range.<sup>9</sup> In this context, OPN transcription and secretion is induced by down-regulation of the Rac GTPase exchange factor Tiam1.<sup>11</sup> Down-regulation of fibroblast Tiam1 and up-regulation of fibroblast OPN in the tumor microenvironment are associated with increased invasiveness in human breast cancers.<sup>9</sup>

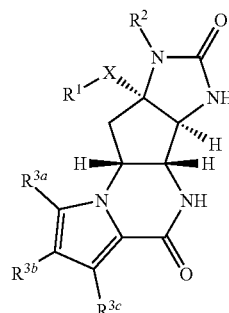
[0006] A method of 3D co-culture was developed which enables assessment of the effects of mammary fibroblasts on associated breast cancer cells.<sup>9</sup> This led to the discovery that the mammary fibroblast Tiam1-OPN pathway modulates breast cancer invasion and metastasis through regulating epithelial-mesenchymal transition (EMT) and cancer stem cell populations in associated breast cancer cells.<sup>9,12</sup> The concentrations of AgA (1) in the aforementioned assays (75-100 nM) are far below cytotoxic range in cell culture. However, direct dosing of mice with AgA (1) at the previously published *in vivo* dose used in one to four day studies<sup>1c,2b,d</sup> (2.5 mg/kg/day) led to toxicity in the animals within three to four weeks that precluded further dosing.<sup>13</sup>

[0007] There is a need to provide compounds with improved safety profiles which can provide a useful alternative to known agelastatin compounds.

SUMMARY

[0008] The present disclosure provides compounds that are effective in treating, preventing, and/or delaying cancer (e.g., breast cancer).

[0009] The present disclosure provides compounds and compositions useful in the treatment of cancer (e.g., breast cancer). In various embodiments, a compound is provided having the structure of Formula (I):



(I)

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

[0010] X is —O—, —S—, or —N(R<sup>6</sup>)—;

[0011] R<sup>1</sup> is H, C<sub>1-10</sub> alkyl, C<sub>2-10</sub> alkenyl, or C<sub>2-8</sub> alkynyl, optionally substituted with one or more R<sup>4</sup>;

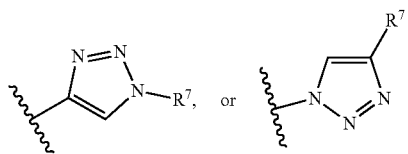
[0012] or alternatively, X and combine to form —N<sub>3</sub>.

[0013] R<sup>2</sup> is C<sub>1-10</sub> alkyl, C<sub>2-10</sub> alkenyl, or C<sub>2-8</sub> alkynyl, optionally substituted with one or more R<sup>5</sup>, wherein up to 3 —CH<sub>2</sub>— units of R<sup>2</sup> are optionally replaced by an —O—, —S—, or —NR<sup>6</sup>—, provided that no adjacent —CH<sub>2</sub>— is replaced;

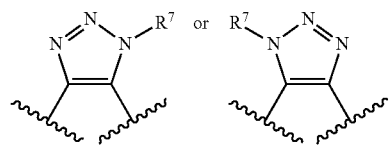
[0014] or alternatively, R<sup>1</sup> and R<sup>2</sup> taken together with the atoms to which they are attached form a C<sub>5-10</sub> heterocycloalkyl, C<sub>5-10</sub> heterocycloalkenyl, or C<sub>5-10</sub> heterocycloalkynyl ring, optionally substituted with one or more R<sup>4</sup>;

[0015] R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are each independently H or halogen;

[0016] R<sup>4</sup> is halogen, —C<sub>1-5</sub> alkyl, oxo, —OH, —OR<sup>6</sup>, —N<sub>3</sub>, —N(R<sup>6</sup>)<sub>2</sub>, —NH(C=O)R<sup>6</sup>, —NH(C=O)OR<sup>6</sup>, —NH(C=O)N(R<sup>6</sup>)<sub>2</sub>, —(C=O)R<sup>6</sup>, heteroaryl,

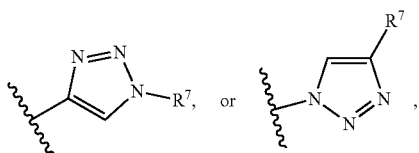


and two adjacent R<sup>4</sup> groups may take the form

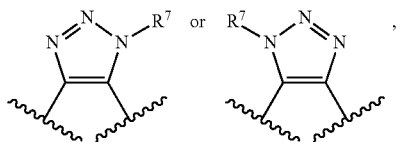


wherein the C=C double bond of the triazole is part of R<sup>1</sup>;

[0017] R<sup>5</sup> is halogen, oxo, —OH, —OR<sup>6</sup>, —N<sub>3</sub>, —N(R<sup>6</sup>)<sub>2</sub>,



and two adjacent  $R^5$  groups may take the form

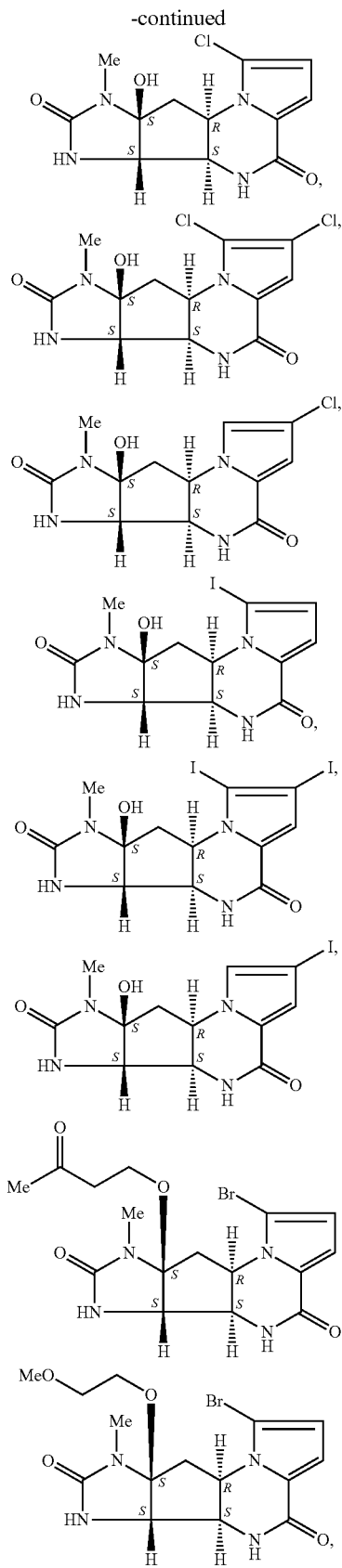
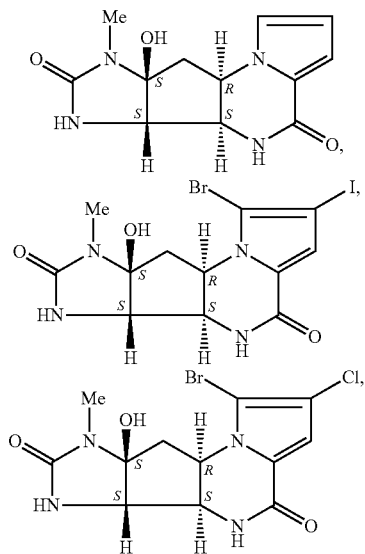


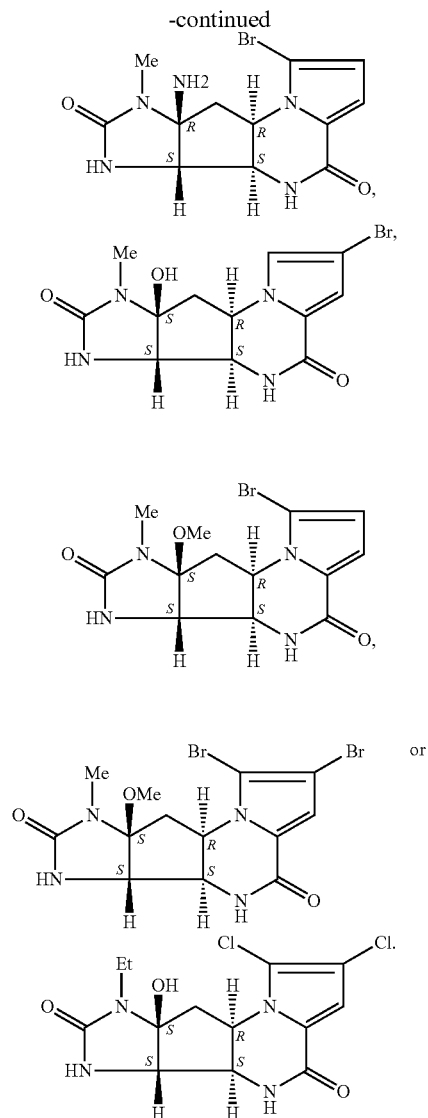
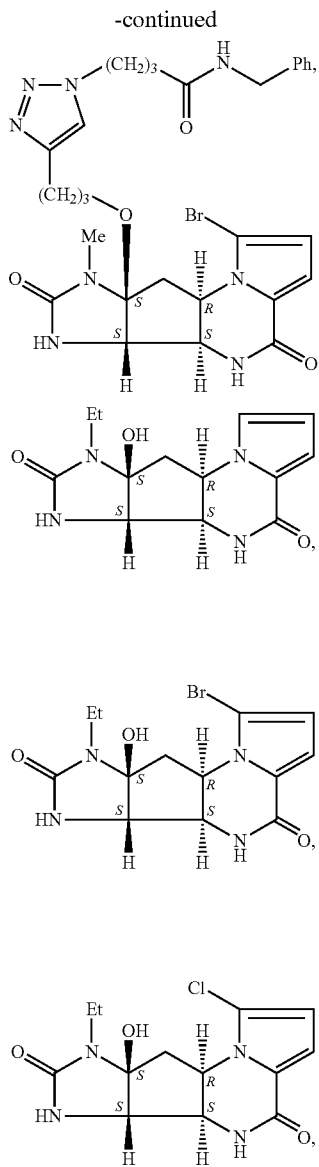
wherein the  $C=C$  double bond of the triazole is part of  $R^2$ ; and

**[0018]**  $R^6$  is independently H,  $-C_{1-5}$  alkyl,  $-C_{2-5}$  alkenyl,  $-C_{1-5}$  alkynyl,  $-C_{1-5}$  alkyl-SiMe<sub>3</sub>, aryl, or heteroaryl;

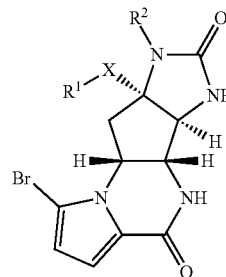
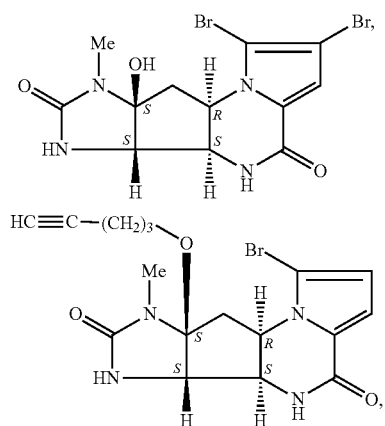
**[0019]**  $R^7$  is selected from the group consisting of hydrogen, optionally substituted aliphatic, optionally substituted heteroaliphatic, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted acyl, resin, protein, reporter molecule, and label molecule;

**[0020]** wherein,  $R^7$  is optionally joined to the core by a linker L, wherein the linker L is selected from the group consisting of optionally substituted alkylene, optionally substituted alkenylene, optionally substituted alkynylene, optionally substituted heteroalkylene, optionally substituted heteroalkynylene, optionally substituted arylene, optionally substituted heteroarylene, and optionally substituted acylene, with the proviso that the compound is not agelastatin A, agelastatin B, agelastatin E,





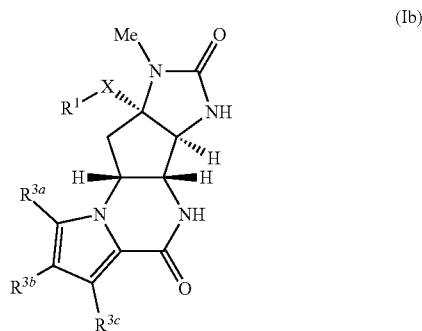
**[0021]** In some embodiments, the compound is a compound of Formula (Ia):



or a pharmaceutically acceptable salt, tautomer, or stereoisomer thereof, wherein  $\text{R}^1$ ,  $\text{R}^2$ , and  $\text{X}$  are as described above.

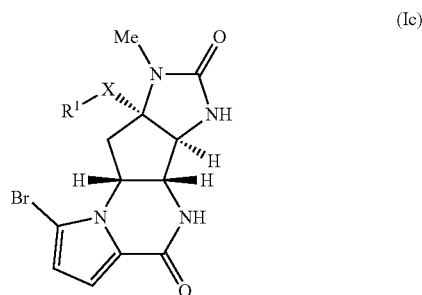


[0022] In some embodiments, the compound is a compound of Formula (Ib):



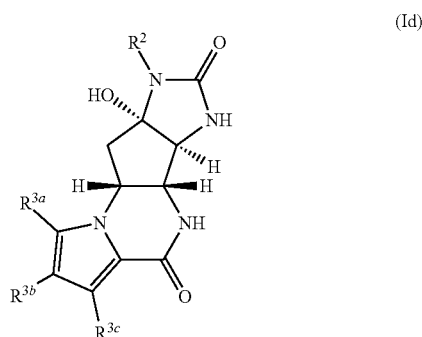
or a pharmaceutically acceptable salt, tautomer, or stereoisomer thereof, wherein X, R<sup>1</sup>, and R<sup>3a-3c</sup> are as defined above.

[0023] In some embodiments, the compound is a compound of Formula (Ic):



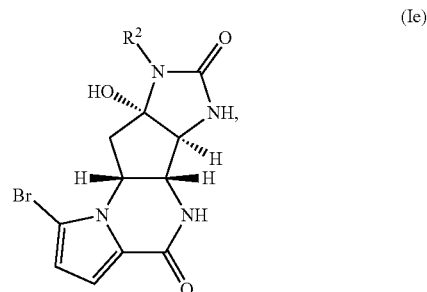
or a pharmaceutically acceptable salt, tautomer, or stereoisomer thereof, wherein X and R<sup>1</sup> are as described above.

[0024] In some embodiments, the compound is a compound of Formula (Id):



or a pharmaceutically acceptable salt, tautomer, or stereoisomer thereof, wherein R<sup>2</sup> and R<sup>3a-3c</sup> are as defined above.

[0025] In some embodiments, the compound is a compound of Formula (Ie):



or a pharmaceutically acceptable salt, tautomer, or stereoisomer thereof, wherein R<sup>2</sup> is as defined above.

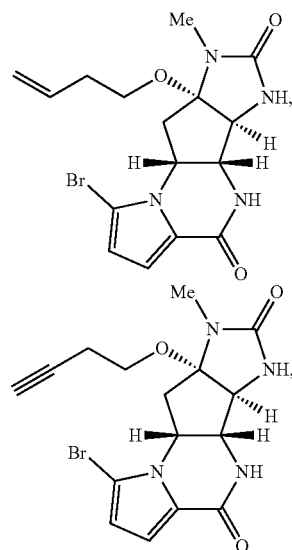
[0026] In some embodiments of Formula (I)-(Ic), R<sup>1</sup> is C<sub>1-10</sub> alkyl, C<sub>2-10</sub> alkenyl, or C<sub>2-8</sub> alkynyl, optionally substituted with one or more R<sup>4</sup>, or alternatively, X and R<sup>1</sup> are combined to form —N<sub>3</sub>.

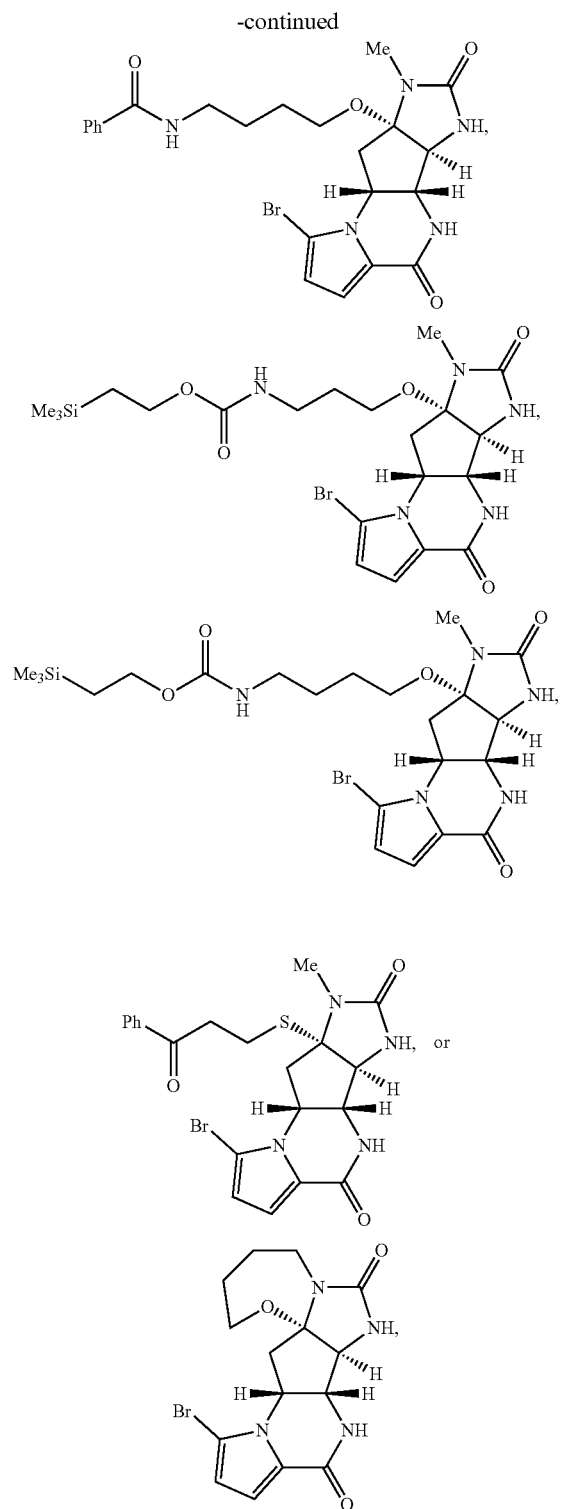
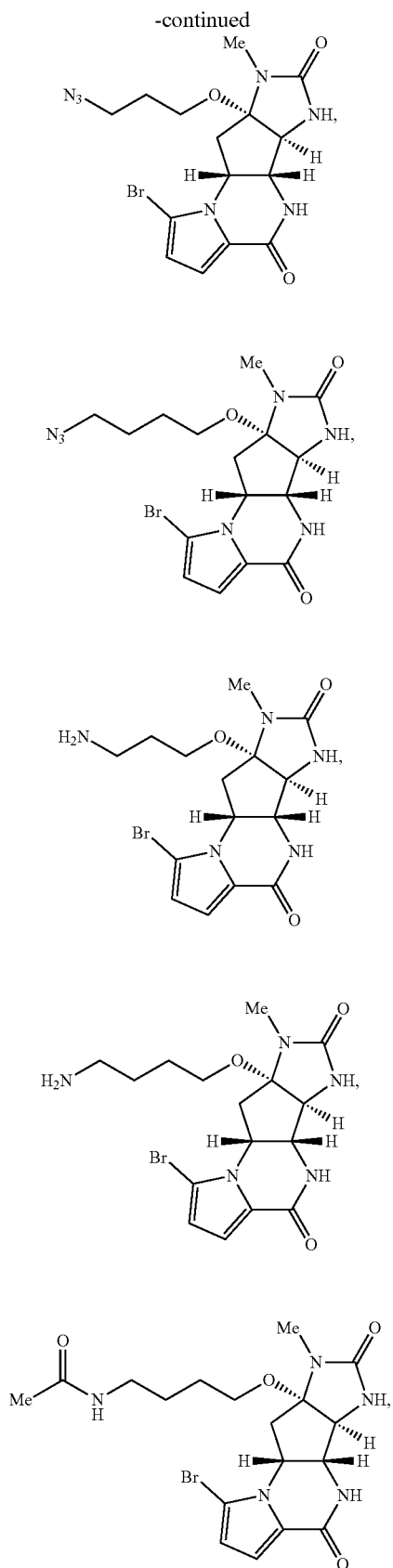
[0027] In some embodiments of the present disclosure, compounds of Formula (I)-(Ic) are provided, wherein X is —O— or —S—. In certain embodiments, X is —O—.

[0028] In some embodiments of Formula (I), (Ia), (Id), or (Ie), R<sup>2</sup> is C<sub>1-10</sub> alkyl, optionally substituted with one or more R<sup>5</sup>, wherein up to 3 —CH<sub>2</sub>— units of R<sup>2</sup> are optionally replaced by an —O—, —S—, or —NR<sup>6</sup>—, provided that no adjacent —CH<sub>2</sub>— is replaced. In some embodiments, up to 3 —CH<sub>2</sub>— units of R<sup>2</sup> are optionally replaced by an O. In other embodiments, R<sup>2</sup> is methyl.

[0029] In some embodiments, compounds of Formula (I), (Ib), and (Id) are provided, wherein R<sup>3a</sup> is Br or Cl, R<sup>3b</sup> is H, and R<sup>3c</sup> is H. In other embodiments, R<sup>3a</sup> is Br, R<sup>3b</sup> is H, and R<sup>3c</sup> is H. In some embodiments, R<sup>3a</sup> is Br or Cl, R<sup>3b</sup> is Br or Cl, and R<sup>3c</sup> is H. In other embodiments, R<sup>3a</sup> is Br, R<sup>3b</sup> is Br, and R<sup>3c</sup> is H.

[0030] In various embodiments of the present disclosure, a compound of Formula (I) is provided having a structure selected from:

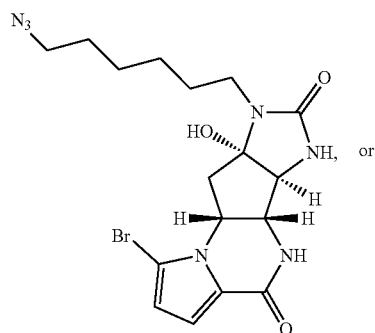
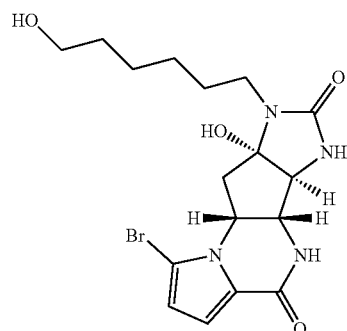
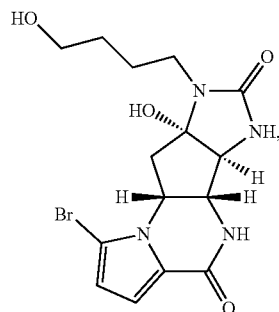
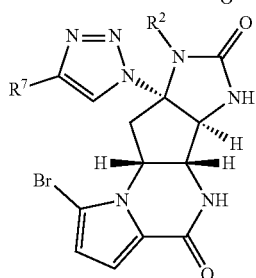
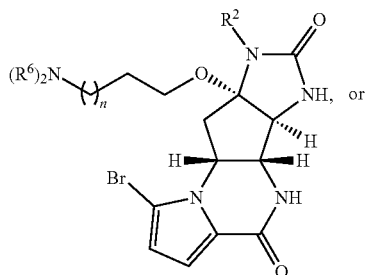
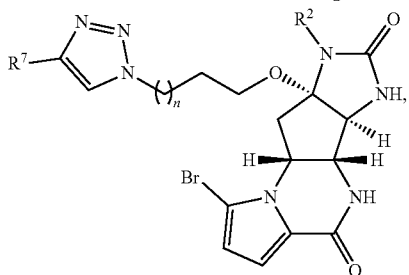
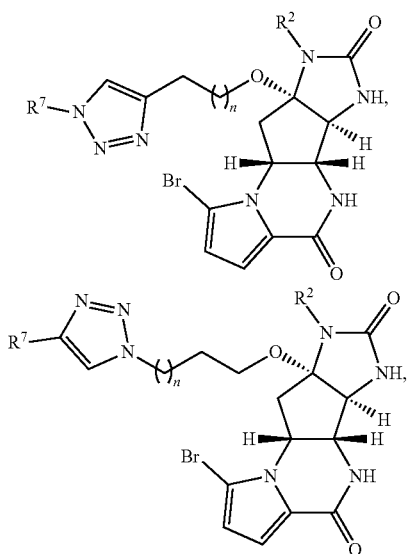




or a pharmaceutically acceptable salt, tautomer, or stereoisomer thereof.

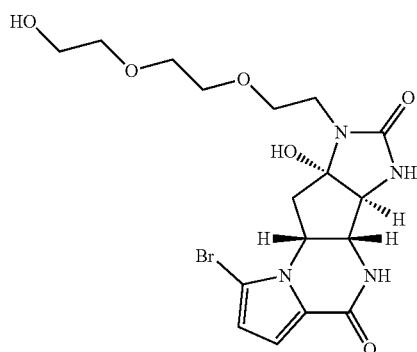
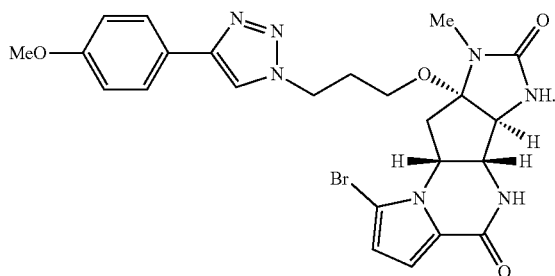
[0031] In various embodiments of the present disclosure, a compound of Formula (I) is provided having a structure selected from:

**[0033]** In some embodiments of the present disclosure, the compound of Formula (I) is selected from:

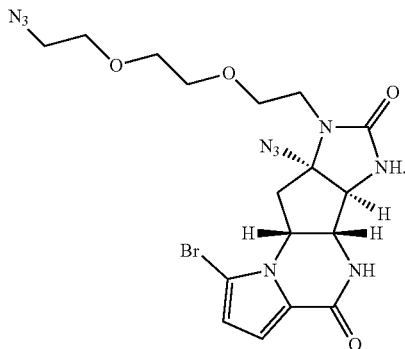


wherein  $R^2$  is H or Me.

**[0032]** In some embodiments of the present disclosure, the compound of Formula (I) is:



**[0034]** In some embodiments, the compound of Formula (I) is:



**[0035]** In various embodiments of the present disclosure, a pharmaceutical composition is provided comprising any of the compounds of Formula (I)-(Ie) disclosed herein and a pharmaceutically acceptable excipient.

**[0036]** The present disclosure also provides a method for the treatment, prevention, or delay of cancer, comprising administering a therapeutically effective amount of a compound of Formula (I)-(Ie), a pharmaceutically acceptable salt thereof, or a composition thereof to a subject in need thereof.

**[0037]** In some embodiments, the cancer is prevented or delayed. In some embodiments, the cancer is recurrent cancer at the primary site, metastatic cancer, or recurrent metastatic cancer.

**[0038]** In some embodiments, the cancer is breast cancer, lung cancer, colorectal cancer, stomach cancer, ovarian cancer, papillary thyroid carcinoma, melanoma, prostate cancer, esophageal cancer, liver cancer, bladder cancer, renal cancer, head and neck cancers, salivary gland cancer, endometrial cancer, cervical cancer, pancreatic cancer, sarcoma, glioblastoma and glioma, or pleural mesothelioma.

**[0039]** In certain embodiments, the cancer is breast cancer. In specific embodiments, the breast cancer is metastatic breast cancer or recurrent metastatic breast cancer.

**[0040]** In some embodiments, the cancer is characterized by a tumor microenvironment exhibiting down regulation of fibroblast Tiam1 and upregulation of fibroblast OPN. In other embodiments, the cancer is characterized by a tumor microenvironment exhibiting upregulation of fibroblast OPN.

**[0041]** In some embodiments, the administering of a compound of Formula (I)-(Ie) is before surgery and/or radiotherapy and/or systemic medical therapy. In other embodiments, the administering is after surgery and/or radiotherapy and/or systemic medical therapy. In still other embodiments, the administering is concurrent with systemic medical therapy. In related embodiments, systemic medical therapy includes chemotherapies, hormonal therapies, targeted biologic therapies, and/or immunotherapies.

**[0042]** In various embodiments, treatment with compounds of the present disclosure results in inhibition of induced transcription of osteopontin (OPN) in fibroblasts, inflammatory cells, and immune cells of the tumor microenvironment. In some embodiments, the inhibition occurs at or below the cytotoxic range determined for the cancer cells being treated. In some embodiments, the inhibition results in

interference with cancer cell adhesion, cancer cell invasion, and cancer stem cell populations.

**[0043]** In some embodiments, the transcription is of splice variants of OPN. In certain embodiments, the splice variants are osteopontin-a, osteopontin-b, or osteopontin-c.

**[0044]** In certain embodiments, the fibroblasts are mammary fibroblasts.

**[0045]** In some embodiments of the present disclosure, the method further comprising coadministration to the subject one of the following:

**[0046]** an antitumor agent selected from the group consisting of paclitaxel, doxorubicin, daunorubicin, cyclophosphamide, methotrexate, 5-fluorouracil, thiotepa, busulfan, improsulfan, piposulfan, benzodopa, carboquone, meturedopa, uredopa, altretamine, triethylenemelamine, triethyl-nephosphoramidate, triethylenethiophosphoramidate, trimethylolomelamine, bullatacin, bullatacinone, camptothecin, bryostatin, callistatin, cryptophycin 1, cryptophycin 8, dolastatin, duocarmycin, eleutherobin, pancratistatin, sarcodicty-in, spongistatin, chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard, carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine, calicheamicin, dynemicin, clodronate, esperamicin, aclacinomycin, actinomycin, anthramycin, azaserine, bleomycin, cactinomycin, carabacin, carminomycin, carzinophilin, chromomycin, dactinomycin, detorbicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycin C, mycophenolic acid, nogalamycin, olivomycin, pep-lomycin, potfiromycin, puromycin, quelamycin, rodorubi-cin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin, denopterin, pteropterin, trimetrexate, fludarabine, 6-mercaptopurine, thiamiprine, thioguanine, ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, androgens such as calusterone, dromostanolone propionate, epitostanol, mepitiostane, testolactone, aminoglutethimide, mitotane, trilostane, frolic acid, aceglatone, aldophosph-amide glycoside, aminolevulinic acid, eniluracil, amsacrine, bestrabucil, bisantrene, edatraxate, defofamine, demecol-cine, diaziquone, elformithine, elliptinium acetate, epoth-ilone, etoglucid, lentinan, lonidamine, maytansine, ansami-tocine, mitoguazone, mitoxantrone, mopidanmol, nitraerine, pentostatin, phenamet, pirarubicin, losoxantrone, podoph-yllic acid, 2-ethylhydrazide, procarbazine, razoxane, rhizoxin, schizophyllan, spirogermanium, tenuazonic acid, triaziquone, roridine A, anguidine, urethane, vindesine, dac-arbazine, mannomustine, mitobronitol, mitolactol, pibobro-man, gacytosine, docetaxel, chlorambucil, gemcitabine, 6-thioguanine, mercaptopurine, cisplatin, oxaliplatin, carbo-platin, vinblastine, etoposide, ifosfamide, mitoxantrone, vin-cristine, vinorelbine, novantrone, teniposide, edatrexate, daunomycin, aminopterin, xeloda, ibandronate, irinotecan, topoisomerase inhibitor, difluoromethylornithine (DMFO), retinoic acid, capecitabine, and pharmacologically accept-able salts or derivatives thereof;

**[0047]** an anti-metabolite agent selected from the group consisting of, 5-fluorouracil, 5-fluorouracil prodrugs (e.g., capecitabine), 5-fluorodeoxyuridine monophosphate, cytarabine, 5-azacytidine, gemcitabine, mercaptopurine, thio-guanine, azathioprine, adenosine, pentostatin, erythrohy-

droxynonyladenine, cladribine and pharmacologically acceptable salts or derivatives thereof;

**[0048]** an anti-estrogen agent selected from the group consisting of selective estrogen receptor modulators, pure receptor antagonists, aromatase inhibitors, and anti-gonadotropins and pharmacologically acceptable salts or derivatives thereof; wherein

**[0049]** the selective estrogen receptor modulator (SERM) is selected from the group consisting of anandrin, bazedoxifene, broparexol, clomifene, cyclofenil, lasofoxifene, ormeloxifene, ospemifene, raloxifene, tamoxifen citrate, toremifene citrate, and pharmacologically acceptable salts or derivatives thereof;

**[0050]** the pure receptor antagonist is selected from the group consisting of fulvestrant, brilanestrant, elacestrant, and pharmacologically acceptable salts or derivatives thereof;

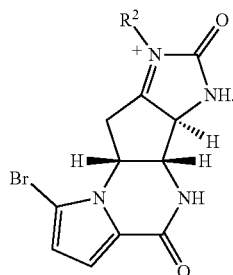
**[0051]** the aromatase inhibitor is selected from the group consisting of anastrozole, letrozole, vorozole, exemestane, formestane and pharmacologically acceptable salts or derivatives thereof;

**[0052]** the anti-gonadotropin is selected from the group consisting of triptorelin, leuprolide acetate, and pharmacologically acceptable salts or derivatives thereof;

**[0053]** a tyrosine kinase inhibitor selected from the group consisting of trastuzumab, pertuzumab, imatinib, gefitinib, erlotinib, sunitinib, adavoseritib, lapatinib and pharmacologically acceptable salts or derivatives thereof; and

**[0054]** an immune checkpoint inhibitor selected from the group consisting of ipilimumab, pembrolizumab, nivolumab, avelumab, durvalumab, atezolizumab and pharmacologically acceptable salts or derivatives thereof.

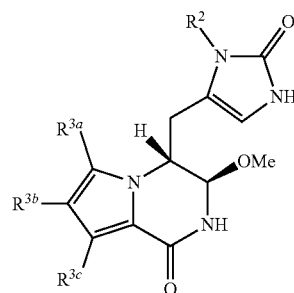
**[0055]** In various embodiments, the present disclosure provides a method of making a compound of claim 1, comprising addition of a nucleophile to an iminium intermediate of Formula (II).



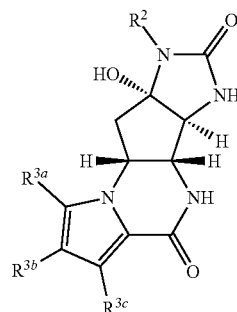
(II)

**[0056]** In various embodiments, the nucleophile added to Formula (II) is  $R^1-XH$ , wherein X is  $-O-$ ,  $-S-$ , or  $-N(R^4)-$ .

**[0057]** In various embodiments, the present disclosure provides a method of making a compound of Formula (I), comprising acid-promoted cyclization of a compound of Formula (III) to afford a compound of Formula (Id):



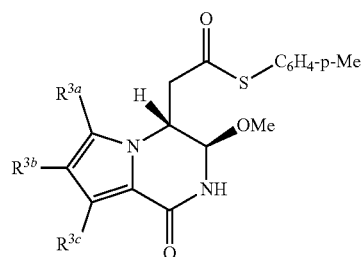
(III)



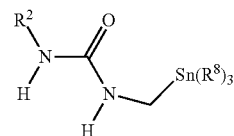
(Id)

**[0058]** In various embodiments, the acid used to promote the acid-promoted cyclization is methanesulfonic acid, p-toluenesulfonic acid, sulfuric acid, hydrochloric acid, trifluoroacetic acid, trifluoromethanesulfonic acid, and nitric acid.

**[0059]** In various embodiments, the present disclosure provides a method of making compound (III), comprising copper-mediated coupling between a compound of Formula (IV) and a compound of Formula (V):



(IV)



(V)

wherein  $R^8$  is alkyl or cycloalkyl.

**[0060]** In various embodiments, the copper-mediated coupling is carried out with copper (I)-thiophene-2-carboxylate (CuTC) or copper(I) diphenylphosphinate (CuDPP).

**[0061]** In some embodiments, the copper-mediated coupling further comprises treatment with an acid.

## BRIEF DESCRIPTION OF THE DRAWINGS

[0062] FIG. 1 shows the structures of (-)-agelastatins A-F (1-6).

[0063] FIG. 2 shows a retrosynthetic analysis of agelastatin alkaloid derivatives.

[0064] FIG. 3 shows that AgE (5) blocks stimulated transcription of osteopontin in fibroblasts.

[0065] FIG. 4 shows the effect of AgE (5) on breast cancer cell invasion in co-cultures with mammary fibroblasts. Number of projections/spheroid for SUM1315 breast cancer cells and indicated mammary fibroblasts in 3D mixed cell spheroid co-culture, shown as percent of total spheroids. shC=control silencing retroviral hairpin vector. shTiam=Tiam-1 silencing hairpin vector.

[0066] FIG. 5 shows the effect of AgE (5) on migration potential of breast cancer cells isolated from co-culture with mammary fibroblasts.

[0067] FIG. 6 shows the effect of AgE (5) on tumorsphere formation by breast cancer cells isolated from co-culture with mammary fibroblasts.

[0068] FIG. 7 shows the effect of AgE (5) on CD44<sup>+</sup>/CD24<sup>-</sup>/ESA<sup>+</sup> populations in breast cancer cells isolated from co-culture with mammary fibroblasts.

[0069] FIG. 8 shows the varying effects of agelastatin derivatives in blocking stimulated fibroblast expression of osteopontin. All agelastatin derivatives were tested at 100-nM concentration unless noted otherwise. \* indicates statistical equivalence with AgA (1) at 100-nM concentration; \*\* indicates equivalence with AgE (5) at 50-nM concentration, but not AgA (1) at 100-nM concentration by T-test.

[0070] FIG. 9 shows a dose-toxicity experiment for AgA.

[0071] FIG. 10 shows the direct treatment of mixed cell xenograft-bearing mice with AgA. Mixed cell tumor-fibroblast xenografts were established in all mice.

[0072] FIG. 11 shows AgA effects on lung metastasis in mixed cell xenograft-bearing mice.

[0073] FIG. 12 shows the direct treatment of mixed cell xenograft-bearing mice with AgE.

[0074] FIG. 13 shows AgE effects on lung metastasis in mixed cell xenograft-bearing mice.

[0075] FIG. 14 shows the effect of Tiam1-deficient fibroblasts and agelastatin inhibition in breast cancer co-cultures. Human breast cancers consist of a group of sub-types that are distinguished clinically by cellular expression of estrogen receptor (ER), progesterone receptor (PR), and HER2.

[0076] FIG. 15 shows an overview of the functionalization of the agelastatin core in accordance with certain embodiments of the present disclosure.

[0077] FIG. 16 is a <sup>1</sup>H NMR spectrum confirming the structure of triazole 17.

[0078] FIG. 17 is a <sup>13</sup>C NMR spectrum confirming the structure of triazole 17.

[0079] FIG. 18 is <sup>1</sup>H NMR spectrum confirming the structure of triazole 19.

[0080] FIG. 19 is <sup>19</sup>F NMR spectrum confirming the structure of triazole 19.

## DETAILED DESCRIPTION

[0081] Described herein is the synthesis of new agelastatin alkaloid derivatives and their anticancer evaluation in the context of the breast cancer microenvironment. A variety of N1-alkyl and C5-ether agelastatin derivatives were accessed via a convergent imidazolone synthesis from a common

thioester along with appropriately substituted components (e.g., urea and alcohol). These agelastatin derivatives were evaluated in a variety of ways, including in a three-dimensional co-culture assay for determining the effects of mammary fibroblasts on associated breast cancer cells. It was discovered that agelastatin alkaloids are potent modulators for cancer invasion and metastasis at non-cytotoxic doses. Herein the increased potency of (-)-agelastatin E as compared to (-)-agelastatin A is described, in addition to identification of new agelastatin derivatives with activity that is equivalent to (-)-agelastatin E. The chemistry described here provides a platform for the rapid synthesis and facile derivitization of agelastatin with excellent potency (e.g., 50-100 nM) as modulators for cancer invasion and metastasis.

[0082] Developing a method for the direct conversion of a common thioester intermediate to the corresponding imidazolone that served as a nucleophile in a biogenetically inspired cyclization event to secure the CD-ring portion of these alkaloids was important to the synthesis of agelastatin derivatives. In addition to streamlining the synthesis of all known agelastatin alkaloids, this strategy provides the foundation for access to various derivatives.<sup>3,8</sup> Late-stage diversification allows for agelastatin derivatives with variations (for example at the N1- and C5-positions) on the agelastatin core. The synthesis of agelastatin derivatives is described herein, including their evaluation along with natural agelastatins in a three-dimensional (3D) co-culture assay probing their impact on fibroblasts in the tumor microenvironment, 9-10 and the discovery of the increased potency of (-)-agelastatin E (5, AgE, FIG. 1) and distinct new derivatives as compared to (-)-agelastatin A (1, AgA, FIG. 1) as modulators for cancer invasion and metastasis.

[0083] As used above, and throughout this disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings. If a term is missing, the conventional term as known to one skilled in the art controls.

[0084] As used herein, the terms “including,” “containing,” and “comprising” are used in their open, non-limiting sense.

[0085] The indefinite articles “a” and “an,” as used herein, unless clearly indicated to the contrary, should be understood to mean “at least one” The phrase “and/or,” as used herein, should be understood to mean “either or both” of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with “and/or” should be construed in the same fashion, i.e., “one or more” of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the “and/or” clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to “A and/or B”, when used in conjunction with open-ended language such as “comprising” can refer, in one embodiment, to A only (optionally including elements other than B), in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements), etc.

[0086] As used herein, “or” should be understood to have the same meaning as “and/or” as defined above. For example, when separating items in a list, “or” or “and/or” shall be interpreted as being inclusive, i.e., the inclusion of

at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as “only one of” or “exactly one of,” or, when used in claims, “consisting of,” will refer to the inclusion of exactly one element of a number or list of elements. In general, the term “or” as used herein shall only be interpreted as indicating exclusive alternatives (i.e. “one or the other but not both”) when preceded by terms of exclusivity, such as “either,” “one of,” “only one of,” or “exactly one of.” “Consisting essentially of,” when used in claims, shall have its ordinary meaning as used in the field of patent law.

**[0087]** As used herein, the phrase “at least one,” in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase “at least one” refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, “at least one of A and B” (or, equivalently, “at least one of A or B,” or, equivalently “at least one of A and/or B”) can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

**[0088]** To provide a more concise description, some of the quantitative expressions given herein are not qualified with the term “about” It is understood that, whether the term “about” is used explicitly or not, every quantity given herein is meant to refer to the actual given value, and it is also meant to refer to the approximation to such given value that would reasonably be inferred based on the ordinary skill in the art, including equivalents and approximations due to the experimental and/or measurement conditions for such given value. Whenever a yield is given as a percentage, such yield refers to a mass of the entity for which the yield is given with respect to the maximum amount of the same entity that could be obtained under the particular stoichiometric conditions. Concentrations that are given as percentages refer to mass ratios, unless indicated differently.

**[0089]** A “patient” is a mammal, e.g., a human, mouse, rat, guinea pig, dog, cat, horse, cow, pig, or non-human primate, such as a monkey, chimpanzee, baboon or rhesus. “Patient” includes both human and animals.

**[0090]** The terms “effective amount” or “therapeutically effective amount” when used in connection with a compound refer to a sufficient amount of the compound to provide the desired biological result. That result can be reduction and/or alleviation of the signs, symptoms, or causes of a disease, or any other desired alteration of a biological system. For example, an “effective amount” for therapeutic use is the amount of the composition comprising a compound as disclosed herein required to provide a clinically significant decrease in a disease. An appropriate “effective amount” in any individual case may be deter-

mined by one of ordinary skill in the art using routine experimentation. Thus, the expression “effective amount” generally refers to the quantity for which the active substance has therapeutic effects. In the present case the active substance is the inhibitor of the inflammasome.

**[0091]** As used herein, the terms “treat” or “treatment” are synonymous with the term “prevent” and are meant to indicate a postponement of development of diseases, preventing the development of diseases, and/or reducing severity of such symptoms that will or are expected to develop. Thus, these terms include ameliorating existing disease symptoms, preventing additional symptoms, ameliorating or preventing the underlying causes of symptoms, inhibiting the disorder or disease, e.g., arresting the development of the disorder or disease, relieving the disorder or disease, causing regression of the disorder or disease, relieving a condition caused by the disease or disorder, or stopping or alleviating the symptoms of the disease or disorder.

**[0092]** The term “disorder” is used in this disclosure to mean, and is used interchangeably with, the terms disease, condition, or illness, unless otherwise indicated.

**[0093]** “Adjuvant setting” refers to a clinical setting in which an individual has had a history of cancer, and generally (but not necessarily) been responsive to therapy, which includes, but is not limited to, surgery (e.g., surgery resection), radiotherapy, and chemotherapy. However, because of their history of cancer, these individuals are considered at risk of development of the disease. Treatment or administration in the “adjuvant setting” (also referred to herein as adjuvant therapy) refers to a prior or subsequent mode of treatment. The degree of risk (e.g., when an individual in the adjuvant setting is considered as “high risk” or “low risk”) depends upon several factors, most usually the extent of disease when first treated. In some embodiments, “adjuvant therapy” herein refers to therapy given after definitive surgery, after which no evidence of residual disease can be detected, so as to reduce the risk of disease recurrence, either local or metastatic. The goal of adjuvant therapy is to prevent or delay recurrence of the cancer, and therefore to reduce the chance of cancer-related death.

**[0094]** “Neoadjuvant setting” refers to a clinical setting in which the method is carried out before the primary/definitive therapy.

**[0095]** As used herein, “delaying” the development of cancer means to defer, hinder, slow, retard, stabilize, and/or postpone development of the disease. This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease. A method that “delays” development of cancer is a method that reduces probability of disease development in a given time frame and/or reduces the extent of the disease in a given time frame, when compared to not using the method. Such comparisons are typically based on clinical studies, using a statistically significant number of subjects. Cancer development can be detectable using standard methods, including, but not limited to, computerized axial tomography (CAT Scan), Magnetic Resonance Imaging (MRI), abdominal ultrasound, clotting tests, arteriography, or biopsy. Development may also refer to cancer progression that may be initially undetectable and includes occurrence, recurrence, and onset.

**[0096]** As used herein, by “combination therapy” is meant that a first agent be administered in conjunction with another agent. “In conjunction with” refers to administration of one treatment modality in addition to another treatment modality, such as administration of an primary cancer therapy (e.g., anti-tumor agent) described herein in addition to administration of the other agent to the same individual. As such, “in conjunction with” refers to administration of one treatment modality before, during, or after delivery of the other treatment modality to the individual. Such combinations are considered to be part of a single treatment regimen.

**[0097]** By using the terms “pharmaceutically acceptable” or “pharmacologically acceptable” it is intended to mean a material which is not biologically, or otherwise, undesirable—the material may be administered to an individual without causing any substantially undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained.

**[0098]** The term “carrier”, as used in this disclosure, encompasses carriers, excipients, and diluents and means a material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting a pharmaceutical agent from one organ, or portion of the body, to another organ, or portion of the body of a subject. Excipients should be selected on the basis of compatibility and the release profile properties of the desired dosage form. Exemplary carrier materials include, e.g., binders, suspending agents, disintegration agents, filling agents, surfactants, solubilizers, stabilizers, lubricants, wetting agents, diluents, spray-dried dispersions, and the like.

**[0099]** The term “pharmaceutically compatible carrier materials” may comprise, e.g., acacia, gelatin, colloidal silicon dioxide, calcium glycerophosphate, calcium lactate, maltodextrin, glycerine, magnesium silicate, sodium caseinate, soy lecithin, sodium chloride, tricalcium phosphate, dipotassium phosphate, sodium stearyl lactylate, carrageenan, monoglyceride, diglyceride, pregelatinized starch, and the like. See, e.g., Hoover, John E, *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pa. 1975.

**[0100]** As used herein, the term “subject” encompasses mammals and non-mammals. Examples of mammals include, but are not limited to, any member of the class Mammalia: humans, non-human primates such as chimpanzees, and other apes and monkey species; farm animals such as cattle, horses, sheep, goats, swine; domestic animals such as rabbits, dogs, and cats; laboratory animals including rodents, such as rats, mice and guinea pigs, and the like. Examples of non-mammals include, but are not limited to, birds, fish and the like. In one embodiment of the present disclosure, the mammal is a human.

**[0101]** The present disclosure also includes “prodrugs” of compounds. The term “prodrug” means a compound which is convertible in vivo by metabolic means (e.g., by hydrolysis) to a disclosed compound or active ingredient. Prodrugs can be prepared by techniques known to one skilled in the art. These techniques generally modify appropriate functional, e.g., a hydroxy, amino, carboxylic, etc., groups in a given compound. These modified functional groups, however, regenerate original functional groups by routine manipulation or in vivo. Examples of prodrugs include, but are not limited to esters (e.g., acetate, formate, and benzoate derivatives), carbamates (e.g., N,N-dimethylaminocarbo-

nyl) of hydroxy or amino functional groups in compounds of the present disclosure, amides (e.g., trifluoroacetylamino, acetylamino, and the like), and the like. Since prodrugs are known to enhance numerous desirable qualities of pharmaceuticals (e.g., solubility, bioavailability, manufacturing, transport, pharmacodynamics, etc.), the compounds of the present disclosure may be delivered in prodrug form. Prodrugs, for instance, may be bioavailable by oral administration even when the parent drug is not. Thus, the present disclosure is intended to cover prodrugs of the presently claimed compounds, methods of delivering the same, and compositions containing the same. Generally speaking, prodrugs are derivatives of per se drugs that after administration undergo conversion or metabolism to the physiologically active species. The conversion may be spontaneous, such as hydrolysis in the physiological environment, or may be enzyme-catalyzed. Prodrugs include compounds that can be oxidized, reduced, aminated, deaminated, hydroxylated, dehydroxylated, hydrolyzed, esterified, alkylated, dealkylated, acylated, deacylated, phosphorylated, and/or dephosphorylated to produce the active compound.

**[0102]** The terms “administered”, “administration”, or “administering” as used in this disclosure refers to either directly administering a disclosed compound or pharmaceutically acceptable salt of the disclosed compound or a composition to a subject, or administering a prodrug derivative or analog of the compound or pharmaceutically acceptable salt of the compound or composition to the subject, which can form an equivalent amount of active compound within the subject's body, including an animal, in need of treatment by bringing such individual in contact with, or otherwise exposing such individual to, such compound.

**[0103]** As used herein, “alkyl” means a straight chain or branched saturated chain having from 1 to 10 carbon atoms. Representative saturated alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, 2-methyl-1-propyl, 2-methyl-2-propyl, 2-methyl-1-butyl, 3-methyl-1-butyl, 2-methyl-3-butyl, 2,2-dimethyl-1-propyl, 2-methyl-1-pentyl, 3-methyl-1-pentyl, 4-methyl-1-pentyl, 2-methyl-2-pentyl, 3-methyl-2-pentyl, 4-methyl-2-pentyl, 2,2-dimethyl-1-butyl, 3,3-dimethyl-1-butyl, 2-ethyl-1-butyl, butyl, isobutyl, t-butyl, n-pentyl, isopentyl, neopentyl, n-hexyl and the like, and longer alkyl groups, such as heptyl, and octyl and the like. An alkyl group can be unsubstituted or substituted. Alkyl groups containing three or more carbon atoms may be straight, or branched. As used herein, “lower alkyl” means an alkyl having from 1 to 6 carbon atoms.

**[0104]** As used herein, an “alkenyl” includes an unbranched or branched hydrocarbon chain containing 2-10 carbon atoms. The “alkenyl” group contains at least one double bond. The double bond of an alkenyl group can be unconjugated or conjugated to another unsaturated group. Examples of alkenyl groups include, but are not limited to, ethylenyl, vinyl, allyl, butenyl, pentenyl, hexenyl, butadienyl, pentadienyl, hexadienyl, 2-ethylhexenyl, 2-propyl-2-butenyl, 4-(2-methyl-3-butene)-pentenyl and the like. An alkenyl group can be unsubstituted or substituted. Alkenyl, as defined herein, may also be branched or straight.

**[0105]** As used herein, “alkynyl” includes an unbranched or branched unsaturated hydrocarbon chain containing 2-10 carbon atoms. The “alkynyl” group contains at least one triple bond. The triple bond of an alkynyl group can be unconjugated or conjugated to another unsaturated group. Examples of alkynyl groups include, but are not limited to,



ethynyl, propynyl, butynyl, pentynyl, hexynyl, methylpropynyl, 4-methyl-1-butynyl, 4-propyl-2-pentynyl, 4-butyl-2-hexynyl and the like. An alkynyl group can be unsubstituted or substituted.

**[0106]** The term “hydroxyl” or “hydroxy” means an OH group:

**[0107]** The term “alkoxy” as used herein refers to a straight or branched chain saturated hydrocarbon containing 1-12 carbon atoms containing a terminal “O” in the chain, i.e., —O(alkyl). Examples of alkoxy groups include, without limitation, methoxy, ethoxy, propoxy, butoxy, t-butoxy, or pentoxy groups.

**[0108]** It should also be noted that any carbon as well as heteroatom with unsatisfied valences in the text, schemes, examples and Tables herein is assumed to have the sufficient number of hydrogen atom(s) to satisfy the valences.

**[0109]** As used herein, references to hydrogen may also refer to a deuterium substitution if desired. The term “deuterium” as used herein means a stable isotope of hydrogen having odd numbers of protons and neutrons.

**[0110]** The term “halo” or “halogen” refers to fluorine, chlorine, bromine, or iodine.

**[0111]** The term “haloalkyl” as used herein refers to an alkyl group, as defined herein, which is substituted one or more halogen. Examples of haloalkyl groups include, but are not limited to, trifluoromethyl, difluoromethyl, pentafluoroethyl, trichloromethyl, etc.

**[0112]** The term “haloalkoxy” as used herein refers to an alkoxy group, as defined herein, which is substituted one or more halogen. Examples of haloalkoxy groups include, but are not limited to, trifluoromethoxy, difluoromethoxy, pentafluoroethoxy, trichloromethoxy, etc.

**[0113]** The term “cyano” as used herein means a substituent having a carbon atom joined to a nitrogen atom by a triple bond, i.e., =.

**[0114]** The term “amino” as used herein means a substituent containing at least one nitrogen atom. Specifically, NH<sub>2</sub>, —NH(alkyl) or alkylamino, —N(alkyl)<sub>2</sub> or dialkylamino, amide, carboxamide, urea, and sulfamide substituents are included in the term “amino”.

**[0115]** Unless otherwise specifically defined, the term “aryl” refers to cyclic, aromatic hydrocarbon groups that have 1 to 3 aromatic rings, including monocyclic or bicyclic groups such as phenyl, biphenyl or naphthyl. Where containing two aromatic rings (bicyclic, etc), the aromatic rings of the aryl group may be joined at a single point (e.g., biphenyl), or fused (e.g., naphthyl). The aryl group may be optionally substituted by one or more substituents, e.g., 1 to 5 substituents, at any point of attachment. The substituents can themselves be optionally substituted. Furthermore when containing two fused rings the aryl groups herein defined may have an unsaturated or partially saturated ring fused with a fully saturated ring. Exemplary ring systems of these aryl groups include, but are not limited to, phenyl, biphenyl, naphthyl, anthracenyl, phenalenyl, phenanthrenyl, indanyl, indenyl, tetrahydronaphthalenyl, tetrahydrobenzoannulenyl, and the like.

**[0116]** Unless otherwise specifically defined, “heteroaryl” means a monovalent monocyclic or polycyclic aromatic radical of 5 to 18 ring atoms or a polycyclic aromatic radical, containing one or more ring heteroatoms selected from N, O, or S, the remaining ring atoms being C. Heteroaryl as herein defined also means a bicyclic heteroaromatic group wherein the heteroatom is selected from N, O, or S. The aromatic

radical is optionally substituted independently with one or more substituents described herein. The substituents can themselves be optionally substituted. Examples include, but are not limited to, benzothiophene, furyl, thienyl, pyrrolyl, pyridyl, pyrazinyl, pyrazolyl, pyridazinyl, pyrimidinyl, imidazolyl, isoxazolyl, oxazolyl, oxadiazolyl, pyrazinyl, indolyl, thiophen-2-yl, quinolyl, benzopyranyl, isothiazolyl, thiazolyl, thiadiazolyl, thieno[3,2-b]thiophene, triazolyl, triazinyl, imidazo[1,2-b]pyrazolyl, furo[2,3-c]pyridinyl, imidazo[1,2-a]pyridinyl, indazolyl, pyrrolo[2,3-c]pyridinyl, pyrrolo[3,2-c]pyridinyl, pyrazolo[3,4-c]pyridinyl, benzoimidazolyl, thieno[3,2-c]pyridinyl, thieno[2,3-c]pyridinyl, thieno[2,3-b]pyridinyl, benzothiazolyl, indolyl, indolinyl, indolinonyl, dihydrobenzothiophenyl, dihydrobenzofuranyl, benzofuran, chromanyl, thiochromanyl, tetrahydroquinolinyl, dihydrobenzothiazine, dihydrobenzoxanlyl, quinolinyl, isoquinolinyl, 1,6-naphthyridinyl, benzo[de]isoquinolinyl, pyrido[4,3-b][1,6]naphthyridinyl, thieno[2,3-b]pyrazinyl, quinazoliny, tetrazolo[1,5-a]pyridinyl, [1,2,4]triazolo[4,3-a]pyridinyl, isoindolyl, pyrrolo[2,3-b]pyridinyl, pyrrolo[3,4-b]pyridinyl, pyrrolo[3,2-b]pyridinyl, imidazo[5,4-b]pyridinyl, pyrrolo[1,2-a]pyrimidinyl, tetrahydropyrrolo[1,2-a]pyrimidinyl, 3,4-dihydro-2H-1λ<sup>2</sup>-pyrrolo[2,1-b]pyrimidine, dibenzo[b,d]thiophene, pyridin-2-one, furo[3,2-c]pyridinyl, furo[2,3-c]pyridinyl, 1H-pyrido[3,4-b][1,4]thiazinyl, benzooxazolyl, benzoisoxazolyl, furo[2,3-b]pyridinyl, benzothiophenyl, 1,5-naphthyridinyl, furo[3,2-b]pyridine, [1,2,4]triazolo[1,5-a]pyridinyl, benzo [1,2,3]triazolyl, imidazo[1,2-a]pyrimidinyl, [1,2,4]triazolo[4,3-b]pyridazinyl, benzo[c][1,2,5]thiadiazolyl, benzo[c][1,2,5]oxadiazole, 1,3-dihydro-2H-benzo[d]imidazol-2-one, 3,4-dihydro-2H-pyrazolo[1,5-b][1,2]oxazinyl, 4,5,6,7-tetrahydropyrazolo[1,5-a]pyridinyl, thiazolo[5,4-d]thiazolyl, imidazo[2,1-b][1,3,4]thiadiazolyl, thieno[2,3-b]pyrrolyl, 3-H-indolyl, and derivatives thereof. Furthermore when containing two fused rings the heteroaryl groups herein defined may have an unsaturated or partially saturated ring fused with a fully saturated ring.

**[0117]** As used herein, the term “cycloalkyl” refers to a saturated or partially saturated, monocyclic, fused or spiro polycyclic, carbocycle having from 3 to 18 carbon atoms per ring. The cycloalkyl ring or carbocycle may be optionally substituted by one or more substituents, e.g., 1 to 5 substituents, at any point of attachment. The substituents can themselves be optionally substituted. Examples of cycloalkyl groups include, without limitations, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptanyl, cyclooctanyl, norboranyl, norborenyl, bicyclo[2.2.2]octanyl, bicyclo[2.2.2]octenyl, decahydronaphthalenyl, octahydro-1H-indenyl, cyclopentenyl, cyclohexenyl, cyclohexa-1,4-dienyl, cyclohexa-1,3-dienyl, 1,2,3,4-tetrahydronaphthalenyl, octahydropentalenyl, 3a,4,5,6,7,7a-hexahydro-1H-indenyl, 1,2,3,3a-tetrahydropentalenyl, bicyclo[3.1.0]hexanyl, bicyclo[2.1.0]pentanyl, spiro[3.3]heptanyl, bicyclo[2.2.1]heptanyl, bicyclo[2.2.1]hept-2-enyl, bicyclo[2.2.2]octanyl, 6-methylbicyclo[3.1.1]heptanyl, 2,6,6-trimethylbicyclo[3.1.1]heptanyl, and derivatives thereof.

**[0118]** As used herein, the term “cycloalkenyl” refers to a partially saturated, monocyclic, fused or spiro polycyclic, carbocycle having from 3 to 18 carbon atoms per ring and contains at least one double bond. The cycloalkenyl ring may be optionally substituted by one or more substituents, e.g., 1 to 5 substituents, at any point of attachment. The substituents can themselves be optionally substituted.

**[0119]** As used herein, the term “heterocycloalkyl” or “heterocyclyl” refers to a saturated or partially unsaturated and non-aromatic monocyclic, or fused or spiro, polycyclic, ring structure of 4- to 18 atoms containing carbon and heteroatoms taken from oxygen, nitrogen, or sulfur and wherein there is not delocalized  $\pi$ -electrons (aromaticity) shared among the ring carbon or heteroatoms. The heterocycloalkyl or heterocyclyl ring structure may be substituted by one or more substituents. The substituents can themselves be optionally substituted. Examples of heterocycloalkyl or heterocyclyl rings include, but are not limited to, oxetanyl, azetidiny, tetrahydrofuranyl, pyrrolidinyl, oxazoliny, oxazolidiny, thiazoliny, thiazolidiny, pyranyl, thiopyranyl, tetrahydropyranyl, dioxaliny, piperidinyl, morpholinyl, thiomorpholinyl, thiomorpholinyl S-oxide, thiomorpholinyl S-dioxide, piperazinyl, azepiny, oxepiny, diazepiny, tropanyl, homotropanyl, dihydrothiophen-2(3H)-onyl, tetrahydrothiophene 1,1-dioxide, 2,5-dihydro-1H-pyrrolyl, imidazolidin-2-one, pyrrolidin-2-one, dihydrofuran-2(3H)-one, 1,3-dioxolan-2-one, isothiazolidine 1,1-dioxide, 4,5-dihydro-1H-imidazolyl, 4,5-dihydrooxazolyl, oxiranyl, pyrazolidiny, 4H-1,4-thiaziny, thiomorpholinyl, 1,2,3,4-tetrahydropyridiny, 1,2,3,4-tetrahydropyraziny, 1,3-oxazinan-2-one, tetrahydro-2H-thiopyran 1,1-dioxide, 7-oxabicyclo[2.2.1]heptanyl, 1,2-thiazepane 1,1-dioxide, octahydro-2H-quinoliziny, 1,3-diazabicyclo[2.2.2]octanyl, 2,3-dihydrobenzo[b][1,4]dioxine, 3-azabicyclo[3.2.1]octanyl, 8-azaspiro[4.5]decane, 8-oxa-3-azabicyclo[3.2.1]octanyl, 2-azabicyclo[2.2.1]heptane, 2,8-diazaspiro[5.5]undecanyl, 2-azaspiro[5.5]undecanyl, 3-azaspiro[5.5]undecanyl, decahydroisoquinoliny, 1-oxa-8-azaspiro[4.5]decanyl, 8-azabicyclo[3.2.1]octanyl, 1,4'-bipiperidinyl, azepanyl, 8-oxa-3-azabicyclo[3.2.1]octanyl, 3,4-dihydro-2H-benzo[b][1,4]oxaziny, 5,6,7,8-tetrahydroimidazo[1,2-a]pyridiny, 1,4-diazepanyl, phenoxathiiny, benzo[d][1,3]dioxolyl, 2,3-dihydrobenzofuranyl, 2,3-dihydrobenzo[b][1,4]dioxiny, 4-(piperidin-4-yl)morpholinyl, 3-azaspiro[5.5]undecanyl, decahydroquinoliny, piperazin-2-one, 1-(pyrrolidin-2-yl)methylpyrrolidinyl, 1,3'-bipyrrolidinyl, and 6,7,8,9-tetrahydro-1H,5H-pyrazolo[1,2-a][1.0.2]diazepiny.

**[0120]** Numerical ranges, as used herein, are intended to include sequential integers. For example, a range expressed as “from 0 to 5” would include 0, 1, 2, 3, 4 and 5.

**[0121]** As used herein, the term “substituted” means that the specified group or moiety bears one or more suitable substituents wherein the substituents may connect to the specified group or moiety at one or more positions. For example, an aryl substituted with a cycloalkyl may indicate that the cycloalkyl connects to one atom of the aryl with a bond or by fusing with the aryl and sharing two or more common atoms.

**[0122]** As used herein, the term “unsubstituted” means that the specified group bears no substituents.

**[0123]** The term “optionally substituted” is understood to mean that a given chemical moiety (e.g., an alkyl group) can (but is not required to) be bonded other substituents (e.g., heteroatoms). For instance, an alkyl group that is optionally substituted can be a fully saturated alkyl chain (i.e., a pure hydrocarbon). Alternatively, the same optionally substituted alkyl group can have substituents different from hydrogen. For instance, it can, at any point along the chain be bonded to a halogen atom, a hydroxyl group, or any other substituent described herein. Thus the term “optionally substituted” means that a given chemical moiety has the potential to

contain other functional groups, but does not necessarily have any further functional groups. Suitable substituents used in the optional substitution of the described groups include, without limitation, oxo, -halogen,  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  alkoxy,  $C_1$ - $C_6$  haloalkyl,  $C_1$ - $C_6$  haloalkoxy,  $-OC_1$ - $C_6$  alkenyl,  $-OC_1$ - $C_6$  alkynyl,  $-C_1$ - $C_6$  alkenyl,  $-C_1$ - $C_6$  alkynyl,  $-OH$ ,  $CN$  (cyano),  $-CH_2CN$ ,  $-OP(O)(OH)_2$ ,  $-C(O)OH$ ,  $-OC(O)C_1$ - $C_6$  alkyl,  $-C(O)C_1$ - $C_6$  alkyl,  $-C(O)-C_0$ - $C_6$  alkylenyl-cycloalkyl,  $-C(O)-C_0$ - $C_6$  alkylenyl-heterocycloalkyl,  $-C(O)-C_0$ - $C_6$  alkylenyl-aryl,  $-C(O)-C_0$ - $C_6$  alkylenyl-heteroaryl,  $-OC(O)OC_1$ - $C_6$  alkyl,  $NH_2$ ,  $NH(C_1$ - $C_6$  alkyl),  $N(C_1$ - $C_6$  alkyl) $_2$ ,  $-C(O)NH_2$ ,  $-C(O)NH(C_1$ - $C_6$  alkyl),  $-C(O)N(C_1$ - $C_6$  alkyl) $_2$ ,  $-C(O)NH$  cycloalkyl,  $-C(O)N(C_1$ - $C_6$  alkyl)cycloalkyl,  $-C(O)NH$ heterocycloalkyl,  $-C(O)N(C_1$ - $C_6$  alkyl)heterocycloalkyl,  $-C(O)NH$ aryl,  $-C(O)N(C_1$ - $C_6$  alkyl)aryl,  $-C(O)NH$ heteroaryl,  $-C(O)N(C_1$ - $C_6$  alkyl)heteroaryl,  $-S(O)_2-C_1$ - $C_6$  alkyl,  $-S(O)_2-C_1$ - $C_6$  haloalkyl,  $-S(O)_2$ -cycloalkyl,  $-S(O)_2$ -heterocycloalkyl,  $-S(O)_2$ -aryl,  $-S(O)_2$ -heteroaryl  $-C_0$ - $C_6$  alkylenyl- $S(O)_2NH_2$ ,  $-S(O)_2NHC_1$ - $C_6$  alkyl,  $-S(O)_2N(C_1$ - $C_6$  alkyl) $_2$ ,  $-S(O)_2NH$ cycloalkyl,  $-S(O)_2NH$ heterocycloalkyl,  $-S(O)_2NH$ aryl,  $-S(O)_2NH$ heteroaryl,  $-NHS(O)_2C_1$ - $C_6$  alkyl,  $-N(C_1$ - $C_6$  alkyl) $S(O)_2(C_1$ - $C_6$  alkyl),  $-NHS(O)_2$ aryl,  $-N(C_1$ - $C_6$  alkyl) $S(O)_2$ aryl,  $-NHS(O)_2$ heteroaryl,  $-N(C_1$ - $C_6$  alkyl) $S(O)_2$ heteroaryl,  $-NHS(O)_2$ cycloalkyl,  $-N(C_1$ - $C_6$  alkyl) $S(O)_2$ cycloalkyl,  $-NHS(O)_2$ heterocycloalkyl,  $-N(C_1$ - $C_6$  alkyl) $S(O)_2$ heterocycloalkyl,  $-N(C_1$ - $C_6$  alkyl) $S(O)_2$ aryl,  $-C_0$ - $C_6$  alkylenyl-aryl,  $-C_0$ - $C_6$  alkylenyl-heteroaryl,  $-C_0$ - $C_6$  alkylenyl-cycloalkyl,  $-C_0$ - $C_6$  alkylenyl-heterocycloalkyl,  $-O$ -aryl,  $-NH$ -aryl, and  $N(C_1$ - $C_6$  alkyl)aryl. The substituents can themselves be optionally substituted. When a multifunctional moiety is shown, the point of attachment to the core is indicated by a line, e.g., (cycloalkyloxy)alkyl refers to alkyl being the point of attachment to the core while cycloalkyl is attached to alkyl via the oxy group. “Optionally substituted” also refers to “substituted” or “unsubstituted”, with the meanings described above.

**[0124]** The term “oxa” as used herein refers to an “ $-O-$ ” group.

**[0125]** The term “oxo” as used herein refers to an “ $=O$ ” group.

**[0126]** The term “solvate” refers to a complex of variable stoichiometry formed by a solute and solvent. Such solvents for the purpose of the present disclosure may not interfere with the biological activity of the solute. Examples of suitable solvents include, but are not limited to, water, methanol, ethanol, and acetic acid. Solvates wherein water is the solvent molecule are typically referred to as hydrates. Hydrates include compositions containing stoichiometric amounts of water, as well as compositions containing variable amounts of water.

**[0127]** The term “salt(s)”, as employed herein, denotes acidic salts formed with inorganic and/or organic acids, as well as basic salts formed with inorganic and/or organic bases. In addition, when a compound of the Formula contains both a basic moiety, such as, but not limited to a pyridine or imidazole, and an acidic moiety, such as, but not limited to a carboxylic acid, zwitterions (“inner salts”) may be formed and are included within the term “salt(s)” as used herein. Pharmaceutically acceptable (i.e., non-toxic, physiologically acceptable) salts are preferred, although other salts are also useful. Salts of the compounds of the Formula may be formed, for example, by reacting a compound of

Formula with an amount of acid or base, such as an equivalent amount, in a medium such as one in which the salt precipitates or in an aqueous medium followed by lyophilization.

**[0128]** In another embodiment of the present disclosure, the compounds of Formula (I) are enantiomers. In some embodiments the compounds are the (S)-enantiomer. In other embodiments the compounds are the (R)-enantiomer. In yet other embodiments, the compounds of Formula (I) may be (+) or (-) enantiomers.

**[0129]** It should be understood that all isomeric forms are included within the present disclosure, including mixtures thereof. If the compound contains a double bond, the substituent may be in the E or Z configuration. If the compound contains a disubstituted cycloalkyl, the cycloalkyl substituent may have a cis- or trans-configuration. All tautomeric forms are also intended to be included.

**[0130]** Compounds of the various Formulae, and salts, solvates, esters and prodrugs thereof, may exist in their tautomeric form (for example, as an amide or imino ether). All such tautomeric forms are contemplated herein as part of the present disclosure.

**[0131]** The compounds of the various Formulae may contain asymmetric or chiral centers, and, therefore, exist in different stereoisomeric forms. It is intended that all stereoisomeric forms of the compounds of the various Formulae as well as mixtures thereof, including racemic mixtures, form part of the present disclosure. In addition, the present disclosure embraces all geometric and positional isomers. For example, if a compound of the various Formulae incorporates a double bond or a fused ring, both the cis- and trans-forms, as well as mixtures, are embraced within the scope of the present disclosure. Each compound herein disclosed includes all the enantiomers that conform to the general structure of the compound. The compounds may be in a racemic or enantiomerically pure form, or any other form in terms of stereochemistry. The assay results may reflect the data collected for the racemic form, the enantiomerically pure form, or any other form in terms of stereochemistry.

**[0132]** Diastereomeric mixtures can be separated into their individual diastereomers on the basis of their physical chemical differences by methods well known to those skilled in the art, such as, for example, by chromatography and/or fractional crystallization. Enantiomers can be separated by converting the enantiomeric mixture into a diastereomeric mixture by reaction with an appropriate optically active compound (e.g., chiral auxiliary such as a chiral alcohol or Mosher's acid chloride), separating the diastereomers and converting (e.g., hydrolyzing) the individual diastereomers to the corresponding pure enantiomers. Also, some of the compounds of the various Formulae may be atropisomers (e.g., substituted biaryls) and are considered as part of the present disclosure. Enantiomers can also be separated by use of a chiral HPLC column.

**[0133]** It is also possible that the compounds of the present disclosure may exist in different tautomeric forms, and all such forms are embraced within the scope of the present disclosure. Also, for example, all keto-enol and imine-enamine forms of the compounds are included in the present disclosure.

**[0134]** All stereoisomers (for example, geometric isomers, optical isomers and the like) of the present compounds (including those of the salts, solvates, esters and prodrugs of the compounds as well as the salts, solvates and esters of the prodrugs), such as those which may exist due to asymmetric carbons on various substituents, including enantiomeric forms (which may exist even in the absence of asymmetric carbons), rotameric forms, atropisomers, and diastereomeric forms, are contemplated within the scope of the present disclosure, as are positional isomers (such as, for example, 4-pyridyl and 3-pyridyl). (For example, if a compound of the various Formulae incorporates a double bond or a fused ring, both the cis- and trans-forms, as well as mixtures, are embraced within the scope of the present disclosure. Also, for example, all keto-enol and imine-enamine forms of the compounds are included in the present disclosure.) Individual stereoisomers of the compounds of the present disclosure may, for example, be substantially free of other isomers, or may be admixed, for example, as racemates or with all other, or other selected, stereoisomers. The chiral centers of the present disclosure can have the S or R configuration as defined by the IUPAC 1974 Recommendations.

**[0135]** The present disclosure also embraces isotopically-labelled compounds of the present disclosure which are identical to those recited herein, but for the fact that one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into compounds of the present disclosure include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorus, fluorine and chlorine, such as  $^2\text{H}$  (or D),  $^3\text{H}$ ,  $^{13}\text{C}$ ,  $^{14}\text{C}$ ,  $^{15}\text{N}$ ,  $^{18}\text{O}$ ,  $^{17}\text{O}$ ,  $^{31}\text{P}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$ ,  $^{18}\text{F}$ , and  $^{36}\text{Cl}$ , respectively.

**[0136]** Certain isotopically-labelled compounds of the various Formulae (e.g., those labeled with  $^3\text{H}$  and  $^{14}\text{C}$ ) are useful in compound and/or substrate tissue distribution assays. Tritiated (i.e.,  $^3\text{H}$ ) and carbon-14 (i.e.,  $^{14}\text{C}$ ) isotopes are particularly preferred for their ease of preparation and detectability. Further, substitution with heavier isotopes such as deuterium (i.e.,  $^2\text{H}$ ) may afford certain therapeutic advantages resulting from greater metabolic stability (e.g., increased in vivo half-life or reduced dosage requirements) and hence may be preferred in some circumstances. Isotopically labelled compounds of the various Formulae can generally be prepared by following procedures analogous to those disclosed in the Schemes and/or in the Examples herein below, by substituting an appropriate isotopically labelled reagent for a non-isotopically labelled reagent.

**[0137]** In some embodiments, the compound comprises at least one deuterium atom. For example, one or more hydrogen atoms in a compound of the present disclosure can be replaced or substituted by deuterium. In some embodiments, the compound comprises two or more deuterium atoms. In some embodiments, the compound comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 deuterium atoms.

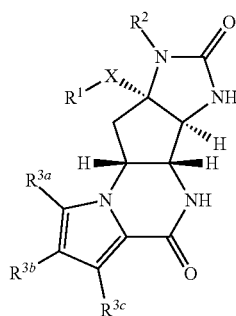
**[0138]** The compounds of the present disclosure may form salts which are also within the scope of the present disclosure. Reference to a compound of the Formula herein is understood to include reference to salts thereof, unless otherwise indicated.

**[0139]** The present disclosure is directed to compounds as described herein and pharmaceutically acceptable salts, enantiomers, hydrates, solvates, prodrugs, isomers, or tau-

tomers thereof, and pharmaceutical compositions comprising one or more compounds as described herein, or pharmaceutically acceptable salts, enantiomers, hydrates, solvates, prodrugs, isomers, or tautomers thereof.

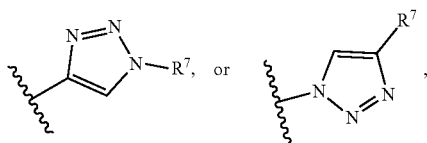
### Compounds

**[0140]** The present disclosure provides compounds having the structure of Formula (I):

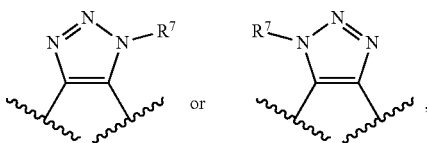


(I)

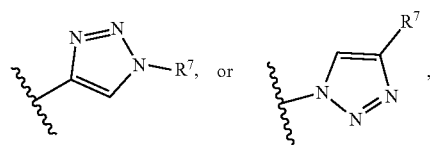
or a pharmaceutically acceptable salt, tautomer, or stereoisomer thereof, wherein: X is —O—, —S—, or —N(R<sup>6</sup>)—; R<sup>1</sup> is H, C<sub>1-10</sub> alkyl, C<sub>2-10</sub> alkenyl, or C<sub>2-8</sub> alkynyl, optionally substituted with one or more R<sup>4</sup>; or alternatively, X and combine to form —N<sub>3</sub>; R<sup>2</sup> is C<sub>1-10</sub> alkyl, C<sub>2-10</sub> alkenyl, or C<sub>2-8</sub> alkynyl, optionally substituted with one or more R<sup>5</sup>, wherein up to 3 —CH<sub>2</sub>— units of R<sup>2</sup> are optionally replaced by an —O—, —S—, or —NR<sup>6</sup>—, provided that no adjacent —CH<sub>2</sub>— is replaced; or alternatively, R<sup>1</sup> and R<sup>2</sup> taken together with the atoms to which they are attached form a C<sub>5-10</sub> heterocycloalkyl, C<sub>5-10</sub> heterocycloalkenyl, or C<sub>5-10</sub> heterocycloalkynyl ring, optionally substituted with one or more R<sup>4</sup>; R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are each independently H or halogen; R<sup>4</sup> is halogen, —C<sub>1-5</sub> alkyl, oxo, —OH, —OR<sup>6</sup>, —N<sub>3</sub>, —N(R<sup>6</sup>)<sub>2</sub>, —NH(C=O)R<sup>6</sup>, —NH(C=O)OR<sup>6</sup>, —NH(C=O)N(R<sup>6</sup>)<sub>2</sub>, —(C=O)R<sup>6</sup>, heteroaryl,



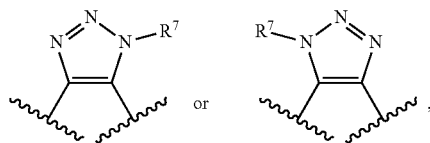
and two adjacent R<sup>4</sup> groups may take the form



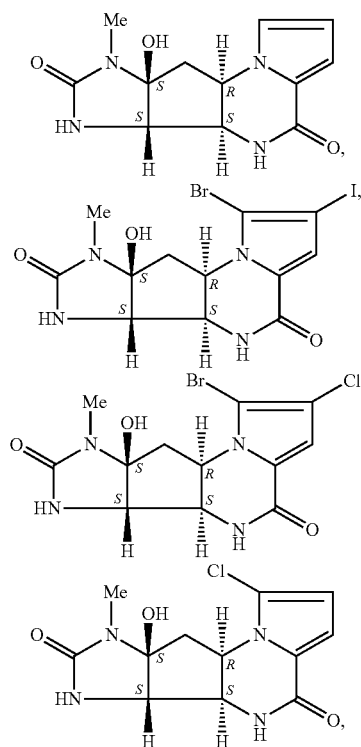
wherein the C=C double bond of the triazole is part of R<sup>1</sup>; R<sup>5</sup> is halogen, oxo, —OH, —OR<sup>6</sup>, —N<sub>3</sub>, —N(R<sup>6</sup>)<sub>2</sub>,



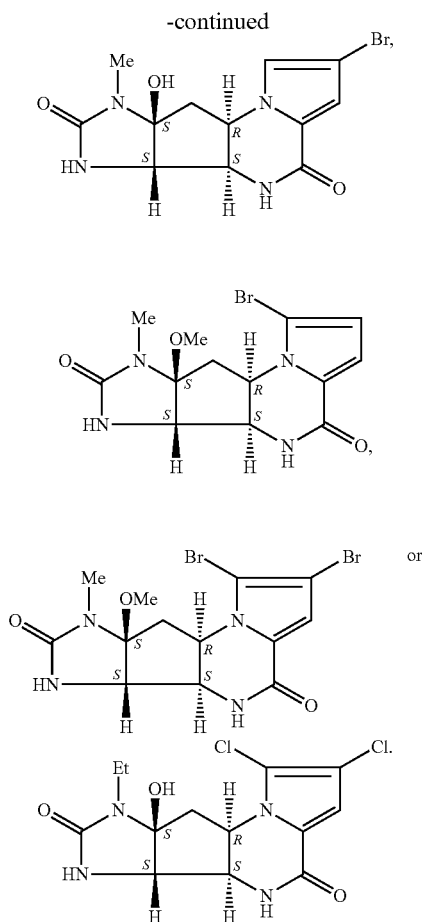
and two adjacent R<sup>5</sup> groups may take the form



wherein the C=C double bond of the triazole is part of R<sup>2</sup>; and R<sup>6</sup> is independently H, —C<sub>1-5</sub> alkyl, —C<sub>2-5</sub> alkenyl, —C<sub>1-5</sub> alkynyl, —C<sub>1-5</sub> alkyl-SiMe<sub>3</sub>, aryl, or heteroaryl; R<sup>7</sup> is selected from the group consisting of hydrogen, optionally substituted aliphatic, optionally substituted heteroaliphatic, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted acyl, resin, protein, reporter molecule, and label molecule, wherein R<sup>7</sup> is optionally joined to the core by a linker L, wherein the linker L is selected from the group consisting of optionally substituted alkylene, optionally substituted alkenylene, optionally substituted heteroalkylene, optionally substituted heteroalkenylene, optionally substituted heteroalkynylene, optionally substituted arylene, optionally substituted heteroarylene, and optionally substituted acylene. In some embodiments, the compound of Formula (I) is not is not agelastatin A, agelastatin B, agelastatin E,

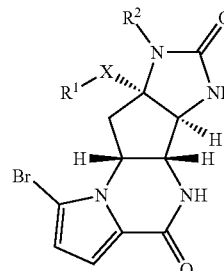






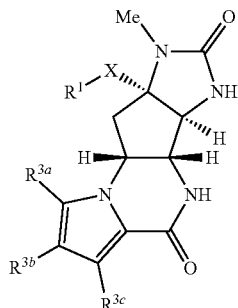
In some embodiments, X is —O—. In some embodiments, X is —S—. In some embodiments, X is —N(R<sup>6</sup>)—. In some embodiments, R<sup>1</sup> is H. In some embodiments, R<sup>1</sup> is C<sub>1-10</sub> alkyl. In some embodiments, R<sup>1</sup> is C<sub>2-10</sub> alkenyl. In some embodiments, R<sup>1</sup> is C<sub>2-8</sub> alkynyl. In some embodiments, R<sup>1</sup> is optionally substituted with one or more R<sup>4</sup>, where R<sup>4</sup> is halogen, —C<sub>1-5</sub> alkyl, oxo, —OH, —OR<sup>6</sup>, —N<sub>3</sub>, —N(R<sup>6</sup>)<sub>2</sub>, —NH(C=O)R<sup>6</sup>, —NH(C=O)OR<sup>6</sup>, —NH(C=O)N(R<sup>6</sup>)<sub>2</sub>, —(C=O)R<sup>6</sup>, or heteroaryl. In some embodiments, X and R<sup>1</sup> combine to form —N<sub>3</sub>. In some embodiments, R<sup>2</sup> is C<sub>1-10</sub> alkyl. In some embodiments, R<sup>2</sup> is C<sub>2-10</sub> alkenyl. In some embodiments, R<sup>2</sup> is C<sub>2-8</sub> alkynyl. In some embodiments, R<sup>2</sup> is optionally substituted with one or more R<sup>5</sup>, where R<sup>5</sup> is halogen. In some embodiments, R<sup>2</sup> is optionally substituted with one or more R<sup>5</sup>, where R<sup>5</sup> is oxo. In some embodiments, R<sup>2</sup> is optionally substituted with one or more R<sup>5</sup>, where R<sup>5</sup> is —OH. In some embodiments, R<sup>2</sup> is optionally substituted with one or more R<sup>5</sup>, where R<sup>5</sup> is halogen. In some embodiments, R<sup>2</sup> is optionally substituted with one or more R<sup>5</sup>, where R<sup>5</sup> is —OR<sup>6</sup>. In some embodiments, R<sup>2</sup> is optionally substituted with one or more R<sup>5</sup>, where R<sup>5</sup> is —N<sub>3</sub>. In some embodiments, R<sup>2</sup> is optionally substituted with one or more R<sup>5</sup>, where R<sup>5</sup> is —N(R<sup>6</sup>)<sub>2</sub>. In some embodiments, up to 3 —CH<sub>2</sub>— units of R<sup>2</sup> are optionally replaced by an —O—, —S—, or —NR<sup>6</sup>—, provided that no adjacent —CH<sub>2</sub>— is replaced. In some embodiments, each R<sup>6</sup> is independently H, —C<sub>1-5</sub> alkyl, —C<sub>2-5</sub> alkenyl, —C<sub>1-5</sub> alkynyl, —C<sub>1-5</sub> alkyl-SiMe<sub>3</sub>, aryl, or heteroaryl. R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are each independently H or halogen (i.e., iodo, bromo, chloro, fluoro). Thus in some embodiments, R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are each H. In some embodiments, R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are each halogen. In some embodiments, R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are a combination of H and halogen. In some embodiments, R<sup>3a</sup> is bromo and R<sup>3b</sup> and R<sup>3c</sup> are H. In some embodiments, R<sup>3a</sup> and R<sup>3b</sup> are each bromo and R<sup>3c</sup> is H. In some embodiments, R<sup>3a</sup> and R<sup>3b</sup> are each H and R<sup>3c</sup> is bromo. In some embodiments, R<sup>3a</sup> and R<sup>3c</sup> are each H and R<sup>3b</sup> is bromo. In some embodiments, R<sup>3a</sup> and R<sup>3c</sup> are each bromo and R<sup>3b</sup> is H. In some embodiments, R<sup>3a</sup> is H and R<sup>3b</sup> and R<sup>3c</sup> are each bromo. In some embodiments, R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are each bromo. In any of the preceding embodiments for R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup>, any bromo or H may be independently replaced with a fluoro, iodo, or chloro moiety allowing for all possible permutations.

**[0141]** In some embodiments, the present disclosure provides a compound of Formula (Ia):



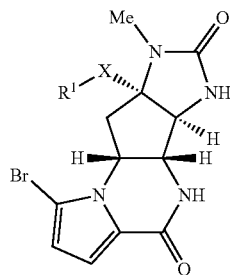
or a pharmaceutically acceptable salt, tautomer, or stereoisomer thereof. In some embodiments, X is —O—. In some embodiments, X is —S—. In some embodiments, X is —N(R<sup>6</sup>)—. In some embodiments, R<sup>1</sup> is H. In some embodiments, R<sup>1</sup> is C<sub>1-10</sub> alkyl. In some embodiments, R<sup>1</sup> is C<sub>2-10</sub> alkenyl. In some embodiments, R<sup>1</sup> is C<sub>2-8</sub> alkynyl. In some embodiments, R<sup>1</sup> is optionally substituted with one or more R<sup>4</sup>, where R<sup>4</sup> is halogen, —C<sub>1-5</sub> alkyl, oxo, —OH, —OR<sup>6</sup>, —N<sub>3</sub>, —N(R<sup>6</sup>)<sub>2</sub>, —NH(C=O)R<sup>6</sup>, —NH(C=O)OR<sup>6</sup>, —NH(C=O)N(R<sup>6</sup>)<sub>2</sub>, —(C=O)R<sup>6</sup>, or heteroaryl. In some embodiments, X and R<sup>1</sup> combine to form —N<sub>3</sub>. In some embodiments, R<sup>2</sup> is C<sub>1-10</sub> alkyl. In some embodiments, R<sup>2</sup> is C<sub>2-10</sub> alkenyl. In some embodiments, R<sup>2</sup> is C<sub>2-8</sub> alkynyl. In some embodiments, R<sup>2</sup> is optionally substituted with one or more R<sup>5</sup>, where R<sup>5</sup> is halogen. In some embodiments, R<sup>2</sup> is optionally substituted with one or more R<sup>5</sup>, where R<sup>5</sup> is oxo. In some embodiments, R<sup>2</sup> is optionally substituted with one or more R<sup>5</sup>, where R<sup>5</sup> is —OH. In some embodiments, R<sup>2</sup> is optionally substituted with one or more R<sup>5</sup>, where R<sup>5</sup> is halogen. In some embodiments, R<sup>2</sup> is optionally substituted with one or more R<sup>5</sup>, where R<sup>5</sup> is —OR<sup>6</sup>. In some embodiments, R<sup>2</sup> is optionally substituted with one or more R<sup>5</sup>, where R<sup>5</sup> is —N<sub>3</sub>. In some embodiments, R<sup>2</sup> is optionally substituted with one or more R<sup>5</sup>, where R<sup>5</sup> is —N(R<sup>6</sup>)<sub>2</sub>. In some embodiments, up to 3 —CH<sub>2</sub>— units of R<sup>2</sup> are optionally replaced by an —O—, —S—, or —NR<sup>6</sup>—, provided that no adjacent —CH<sub>2</sub>— is replaced. In some embodiments, each R<sup>6</sup> is independently H, —C<sub>1-5</sub> alkyl, —C<sub>2-5</sub> alkenyl, —C<sub>1-5</sub> alkynyl, —C<sub>1-5</sub> alkyl-SiMe<sub>3</sub>, aryl, or heteroaryl. R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are each independently H or halogen (i.e., iodo, bromo, chloro, fluoro). Thus in some embodiments, R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are each H. In some embodiments, R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are each halogen. In some embodiments, R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are a combination of H and halogen. In some embodiments, R<sup>3a</sup> is bromo and R<sup>3b</sup> and R<sup>3c</sup> are H. In some embodiments, R<sup>3a</sup> and R<sup>3b</sup> are each bromo and R<sup>3c</sup> is H. In some embodiments, R<sup>3a</sup> and R<sup>3b</sup> are each H and R<sup>3c</sup> is bromo. In some embodiments, R<sup>3a</sup> and R<sup>3c</sup> are each H and R<sup>3b</sup> is bromo. In some embodiments, R<sup>3a</sup> and R<sup>3c</sup> are each bromo and R<sup>3b</sup> is H. In some embodiments, R<sup>3a</sup> is H and R<sup>3b</sup> and R<sup>3c</sup> are each bromo. In some embodiments, R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are each bromo. In any of the preceding embodiments for R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup>, any bromo or H may be independently replaced with a fluoro, iodo, or chloro moiety allowing for all possible permutations.

[0142] In some embodiments, the present disclosure provides a compound of Formula (Ib):



or a pharmaceutically acceptable salt, tautomer, or stereoisomer thereof. In some embodiments, X is —O—. In some embodiments, X is —S—. In some embodiments, X is —N(R<sup>6</sup>)—. In some embodiments, R<sup>1</sup> is H. In some embodiments, R<sup>1</sup> is C<sub>1-10</sub> alkyl. In some embodiments, R<sup>1</sup> is C<sub>2-10</sub> alkenyl. In some embodiments, R<sup>1</sup> is C<sub>2-8</sub> alkynyl. In some embodiments, R<sup>1</sup> is optionally substituted with one or more R<sup>4</sup>, where R<sup>4</sup> is halogen, —C<sub>1-5</sub> alkyl, oxo, —OH, —OR<sup>6</sup>, —N<sub>3</sub>, —N(R<sup>6</sup>)<sub>2</sub>, —NH(C=O)R<sup>6</sup>, —NH(C=O)OR<sup>6</sup>, —NH(C=O)N(R<sup>6</sup>)<sub>2</sub>, —(C=O)R<sup>6</sup>, or heteroaryl. In some embodiments, X and R<sup>1</sup> combine to form —N<sub>3</sub>. R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are each independently H or halogen (i.e., iodo, bromo, chloro, fluoro). Thus in some embodiments, R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are each H. In some embodiments, R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are each halogen. In some embodiments, R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are a combination of H and halogen. In some embodiments, R<sup>3a</sup> is bromo and R<sup>3b</sup> and R<sup>3c</sup> are H. In some embodiments, R<sup>3a</sup> and R<sup>3b</sup> are each bromo and R<sup>3c</sup> is H. In some embodiments, R<sup>3a</sup> and R<sup>3b</sup> are each H and R<sup>3c</sup> is bromo. In some embodiments, R<sup>3a</sup> and R<sup>3c</sup> are each H and R<sup>3b</sup> is bromo. In some embodiments, R<sup>3a</sup> and R<sup>3c</sup> are each bromo and R<sup>3b</sup> is H. In some embodiments, R<sup>3a</sup> is H and R<sup>3b</sup> and R<sup>3c</sup> are each bromo. In some embodiments, R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are each bromo. In any of the preceding embodiments for R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup>, any bromo or H may be independently replaced with a fluororo, iodo, or chloro moiety allowing for all possible permutations.

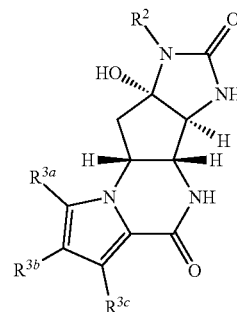
[0143] In some embodiments, the present disclosure provides a compound of Formula (Ic):



or a pharmaceutically acceptable salt, tautomer, or stereoisomer thereof. In some embodiments, X is —O—. In some embodiments, X is —S—. In some embodiments, X is

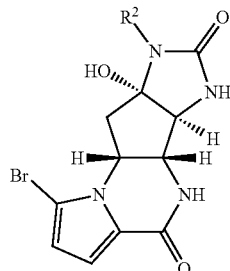
—N(R<sup>6</sup>)—. In some embodiments, R<sup>1</sup> is H. In some embodiments, R<sup>1</sup> is C<sub>1-10</sub> alkyl. In some embodiments, R<sup>1</sup> is C<sub>2-10</sub> alkenyl. In some embodiments, R<sup>1</sup> is C<sub>2-8</sub> alkynyl. In some embodiments, R<sup>1</sup> is optionally substituted with one or more R<sup>4</sup>, where R<sup>4</sup> is halogen, —C<sub>1-5</sub> alkyl, oxo, —OH, —OR<sup>6</sup>, —N<sub>3</sub>, —N(R<sup>6</sup>)<sub>2</sub>, —NH(C=O)R<sup>6</sup>, —NH(C=O)OR<sup>6</sup>, —NH(C=O)N(R<sup>6</sup>)<sub>2</sub>, —(C=O)R<sup>6</sup>, or heteroaryl. In some embodiments, X and R<sup>1</sup> combine to form —N<sub>3</sub>.

[0144] In some embodiments, the present disclosure provides a compound of Formula (Id):



or a pharmaceutically acceptable salt, tautomer, or stereoisomer thereof. In some embodiments, R<sup>2</sup> is C<sub>1-10</sub> alkyl. In some embodiments, R<sup>2</sup> is C<sub>2-10</sub> alkenyl. In some embodiments, R<sup>2</sup> is C<sub>2-8</sub> alkynyl. In some embodiments, R<sup>2</sup> is optionally substituted with one or more R<sup>5</sup>, where R<sup>5</sup> is halogen. In some embodiments, R<sup>2</sup> is optionally substituted with one or more R<sup>5</sup>, where R<sup>5</sup> is oxo. In some embodiments, R<sup>2</sup> is optionally substituted with one or more R<sup>5</sup>, where R<sup>5</sup> is —OH. In some embodiments, R<sup>2</sup> is optionally substituted with one or more R<sup>5</sup>, where R<sup>5</sup> is halogen. In some embodiments, R<sup>2</sup> is optionally substituted with one or more R<sup>5</sup>, where R<sup>5</sup> is —OR<sup>6</sup>. In some embodiments, R<sup>2</sup> is optionally substituted with one or more R<sup>5</sup>, where R<sup>5</sup> is —N<sub>3</sub>. In some embodiments, R<sup>2</sup> is optionally substituted with one or more R<sup>5</sup>, where R<sup>5</sup> is —N(R<sup>6</sup>)<sub>2</sub>. In some embodiments, up to 3 —CH<sub>2</sub>— units of R<sup>2</sup> are optionally replaced by an —O—, —S—, or —NR<sup>6</sup>—, provided that no adjacent —CH<sub>2</sub>— is replaced. In some embodiments, each R<sup>6</sup> is independently H, —C<sub>1-5</sub> alkyl, —C<sub>2-5</sub> alkenyl, —C<sub>1-5</sub> alkynyl, —C<sub>1-5</sub> alkyl-SiMe<sub>3</sub>, aryl, or heteroaryl. R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are each independently H or halogen (i.e., iodo, bromo, chloro, fluoro). Thus in some embodiments, R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are each H. In some embodiments, R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are each halogen. In some embodiments, R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are a combination of H and halogen. In some embodiments, R<sup>3a</sup> is bromo and R<sup>3b</sup> and R<sup>3c</sup> are H. In some embodiments, R<sup>3a</sup> and R<sup>3b</sup> are each bromo and R<sup>3c</sup> is H. In some embodiments, R<sup>3a</sup> and R<sup>3b</sup> are each H and R<sup>3c</sup> is bromo. In some embodiments, R<sup>3a</sup> and R<sup>3c</sup> are each H and R<sup>3b</sup> is bromo. In some embodiments, R<sup>3a</sup> and R<sup>3c</sup> are each bromo and R<sup>3b</sup> is H. In some embodiments, R<sup>3a</sup> is H and R<sup>3b</sup> and R<sup>3c</sup> are each bromo. In some embodiments, R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are each bromo. In any of the preceding embodiments for R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup>, any bromo or H may be independently replaced with a fluororo, iodo, or chloro moiety allowing for all possible permutations.

[0145] In some embodiments, the present disclosure provides a compound of Formula (Ie):



or a pharmaceutically acceptable salt, tautomer, or stereoisomer thereof. In some embodiments, R<sup>2</sup> is C<sub>1-10</sub> alkyl. In some embodiments, R<sup>2</sup> is C<sub>2-10</sub> alkenyl. In some embodiments, R<sup>2</sup> is C<sub>2-8</sub> alkynyl. In some embodiments, R<sup>2</sup> is optionally substituted with one or more R<sup>5</sup>, where R<sup>5</sup> is halogen. In some embodiments, R<sup>2</sup> is optionally substituted with one or more R<sup>5</sup>, where R<sup>5</sup> is oxo. In some embodiments, R<sup>2</sup> is optionally substituted with one or more R<sup>5</sup>, where R<sup>5</sup> is —OH. In some embodiments, R<sup>2</sup> is optionally substituted with one or more R<sup>5</sup>, where R<sup>5</sup> is halogen. In some embodiments, R<sup>2</sup> is optionally substituted with one or more R<sup>5</sup>, where R<sup>5</sup> is —OR<sup>6</sup>. In some embodiments, R<sup>2</sup> is optionally substituted with one or more R<sup>5</sup>, where R<sup>5</sup> is —N<sub>3</sub>. In some embodiments, R<sup>2</sup> is optionally substituted with one or more R<sup>5</sup>, where R<sup>5</sup> is —N(R<sup>6</sup>)<sub>2</sub>. In some embodiments, up to 3 —CH<sub>2</sub>— units of R<sup>2</sup> are optionally replaced by an —O—, —S— or —NR<sup>6</sup>—, provided that no adjacent —CH<sub>2</sub>— is replaced. In some embodiments, each R<sup>6</sup> is independently H, —C<sub>1-5</sub> alkyl, —C<sub>2-5</sub> alkenyl, —C<sub>1-5</sub> alkynyl, —C<sub>1-5</sub> alkyl-SiMe<sub>3</sub>, aryl, or heteroaryl.

[0146] In some embodiments, R<sup>2</sup> is C<sub>1-10</sub> alkyl, optionally substituted with one or more R<sup>5</sup>, wherein up to 3 —CH<sub>2</sub>— units of R<sup>2</sup> are optionally replaced by an —O—, —S—, or —NR<sup>6</sup>—, provided that no adjacent —CH<sub>2</sub>— is replaced. In some embodiments, R<sup>2</sup> is C<sub>1-10</sub> alkyl, substituted with one or more R<sup>5</sup>, wherein up to 3 —CH<sub>2</sub>— units of R<sup>2</sup> are optionally replaced by an —O—, —S—, or —NR<sup>6</sup>—, provided that no adjacent —CH<sub>2</sub>— is replaced. In some embodiments, R<sup>2</sup> is an unsubstituted C<sub>1-10</sub> alkyl, wherein up to 3 —CH<sub>2</sub>— units of R<sup>2</sup> are optionally replaced by an —O—, —S—, or —NR<sup>6</sup>—, provided that no adjacent —CH<sub>2</sub>— is replaced. In some embodiments, R<sup>2</sup> is C<sub>1-10</sub> alkyl, optionally substituted with one or more R<sup>5</sup>, wherein no —CH<sub>2</sub>— units of R<sup>2</sup> are optionally replaced by an —O—, —S—, or —NR<sup>6</sup>—. In some embodiments, R<sup>2</sup> is C<sub>1-10</sub> alkyl, substituted with one or more R<sup>5</sup>, wherein no —CH<sub>2</sub>— units of R<sup>2</sup> are optionally replaced by an —O—, —S—, or —NR<sup>6</sup>—. In some embodiments, R<sup>2</sup> is an unsubstituted C<sub>1-10</sub> alkyl, wherein no 3 —CH<sub>2</sub>— units of R<sup>2</sup> are optionally replaced by an —O—, —S—, or —NR<sup>6</sup>—. In some embodiments, up to 3 —CH<sub>2</sub>— units of R<sup>2</sup> are optionally replaced by an O.

[0147] In some embodiments, R<sup>2</sup> is methyl.

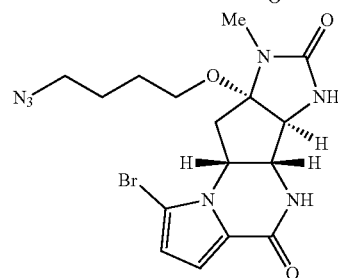
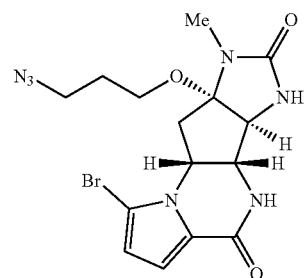
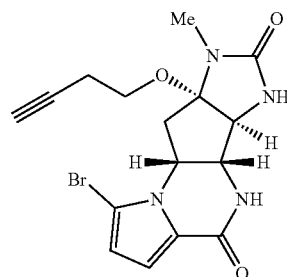
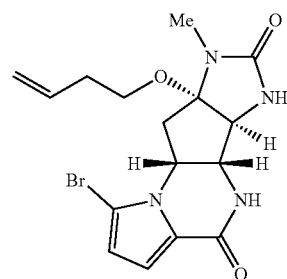
[0148] In some embodiments, R<sup>1</sup> is C<sub>1-10</sub> alkyl, C<sub>2-10</sub> alkenyl, or C<sub>2-8</sub> alkynyl, optionally substituted with one or more R<sup>4</sup>, or alternatively, X and R<sup>1</sup> are combined to form —N<sub>3</sub>. In some embodiments, R<sup>1</sup> is substituted with one or more R<sup>4</sup>. In some embodiments, R<sup>1</sup> is not substituted with one or more R<sup>4</sup>. In some embodiments, R<sup>1</sup> is optionally

substituted with one or more R<sup>4</sup>. In some embodiments, X and R<sup>1</sup> are combined to form —N<sub>3</sub>.

[0149] In some embodiments, X is —O— or —S—. In some embodiments, X is —O—.

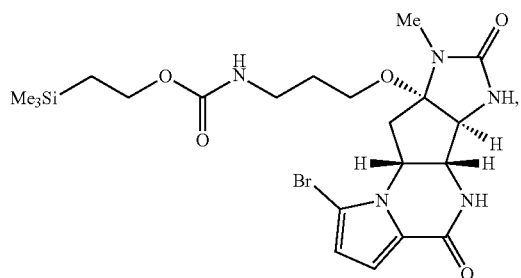
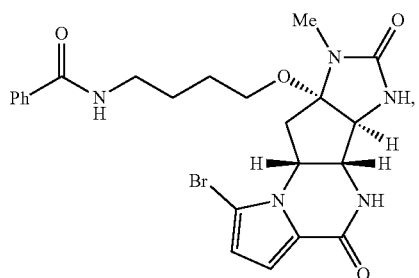
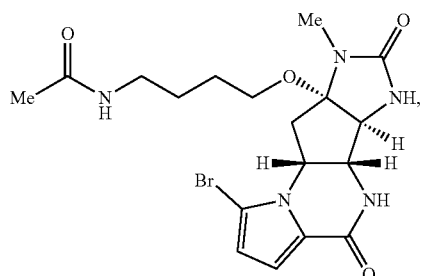
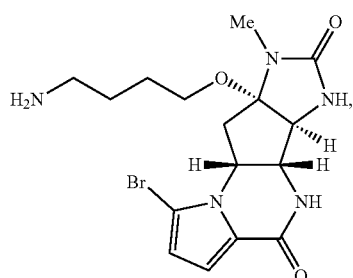
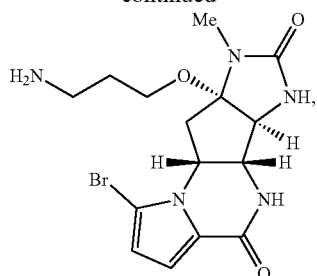
[0150] In some embodiments, R<sup>3a</sup> is Br or Cl, R<sup>3b</sup> is H, and R<sup>3c</sup> is H. In some embodiments, R<sup>3a</sup> is Br, R<sup>3b</sup> is H, and R<sup>3c</sup> is H. In some embodiments, R<sup>3a</sup> is Br or Cl, R<sup>3b</sup> is Br or Cl, and R<sup>3c</sup> is H. In some embodiments, R<sup>3a</sup> is Br, R<sup>3b</sup> is Br, and R<sup>3c</sup> is H.

[0151] In certain embodiments, the present disclosure provides for a compound, and pharmaceutically acceptable salts, solvates, hydrates, isomers, and tautomers thereof, selected from the group consisting of

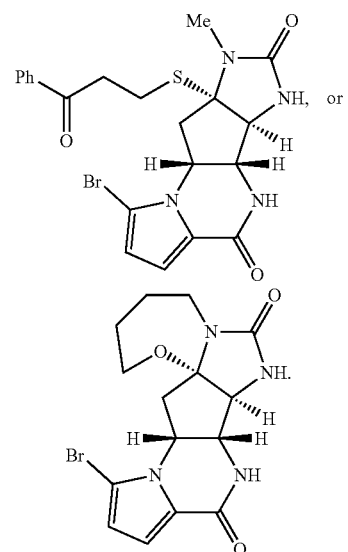
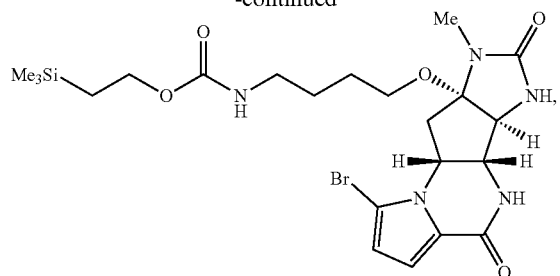




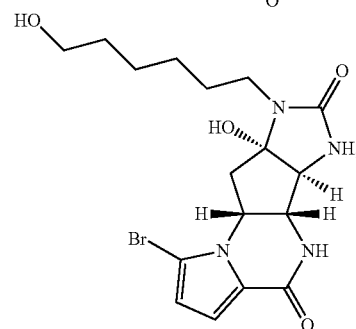
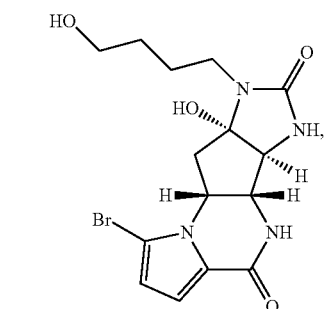
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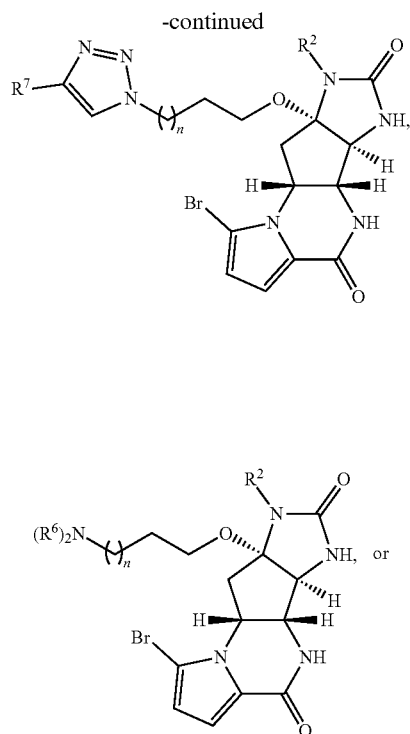
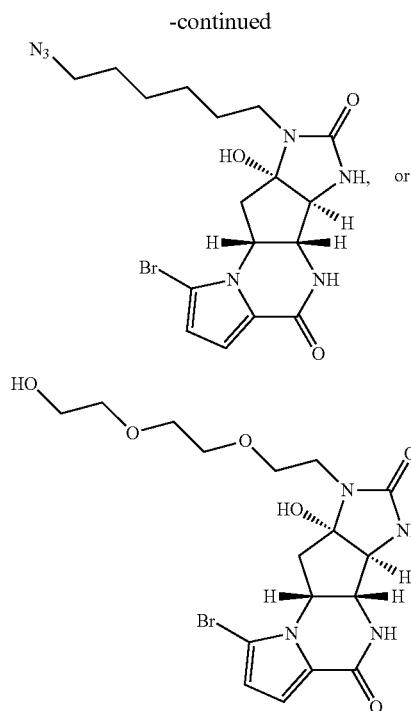


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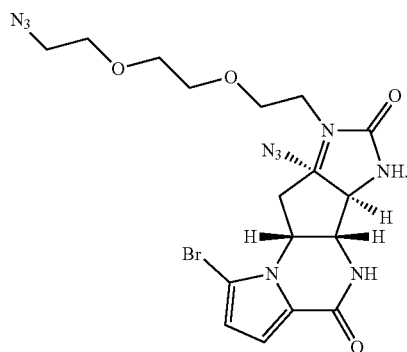


In certain embodiments, the present disclosure provides for a compound, and pharmaceutically acceptable salts, solvates, hydrates, isomers, and tautomers thereof, selected from the group consisting of

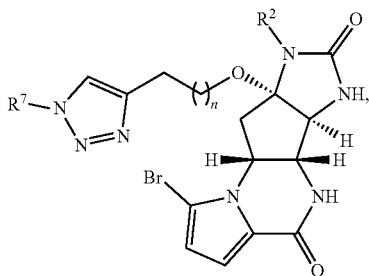




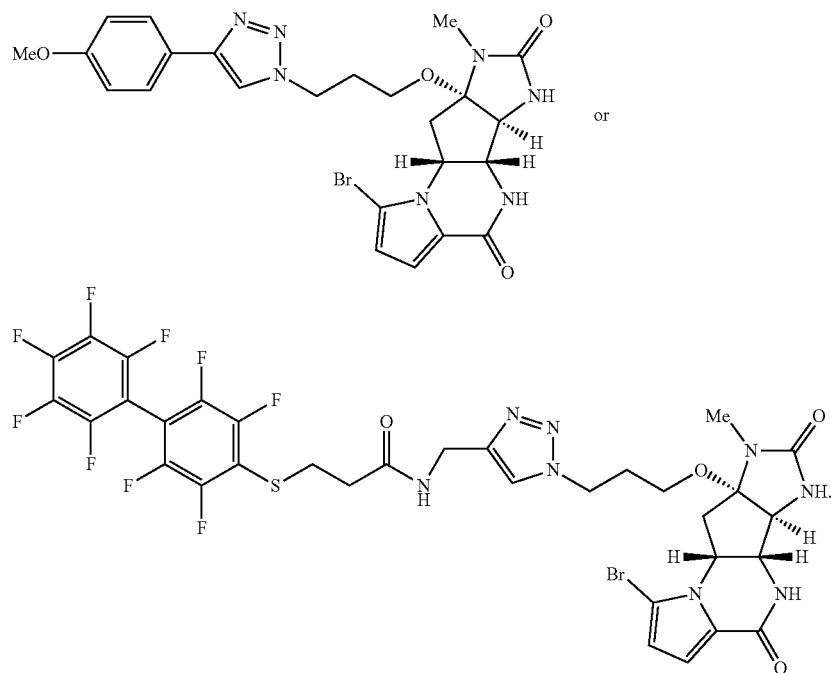
In certain embodiments, the present disclosure provides for a compound, and pharmaceutically acceptable salts, solvates, hydrates, isomers, and tautomers thereof, where the compound is



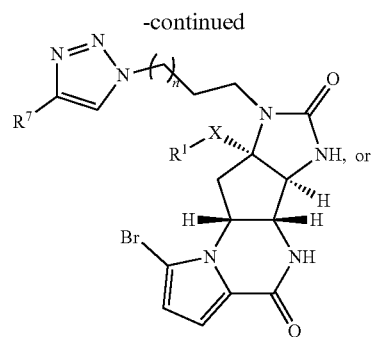
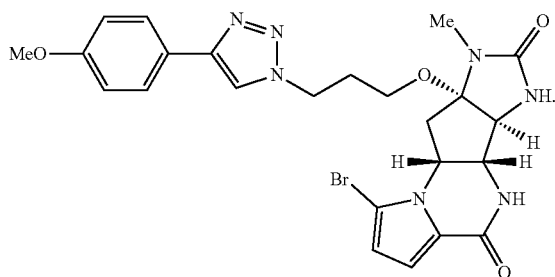
**[0152]** In certain embodiments, the present disclosure provides for a compound, and pharmaceutically acceptable salts, solvates, hydrates, isomers, and tautomers thereof, where the compound is



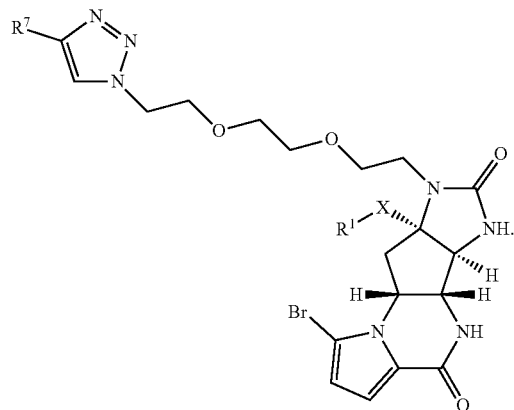
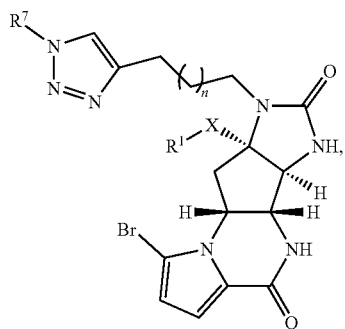
**[0153]** wherein R<sup>2</sup> is H or Me; R<sup>7</sup> is selected from the group consisting of hydrogen, optionally substituted aliphatic, optionally substituted heteroaliphatic, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted acyl, resin, protein, reporter molecule, and label molecule; wherein, R<sup>7</sup> is optionally joined to the core by a linker L, wherein the linker L is selected from the group consisting of optionally substituted alkylene, optionally substituted alkenylene, optionally substituted alkynylene, optionally substituted heteroalkylene, optionally substituted heteroalkynylene, optionally substituted arylene, optionally substituted heteroarylene, and optionally substituted acylene. In some embodiments, R<sup>2</sup> is H. In other embodiments, R<sup>2</sup> is Me. In certain embodiments, the present disclosure provides for a compound, and pharmaceutically acceptable salts, solvates, hydrates, isomers, and tautomers thereof, where the compound is



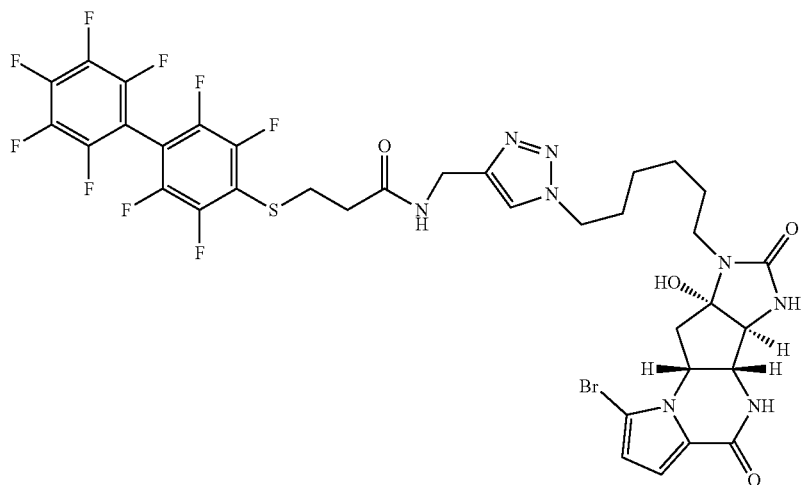
In specific embodiments, the compound of Formula (I) is



**[0154]** In certain embodiments, the present disclosure provides for a compound, and pharmaceutically acceptable salts, solvates, hydrates, isomers, and tautomers thereof, where the compound is

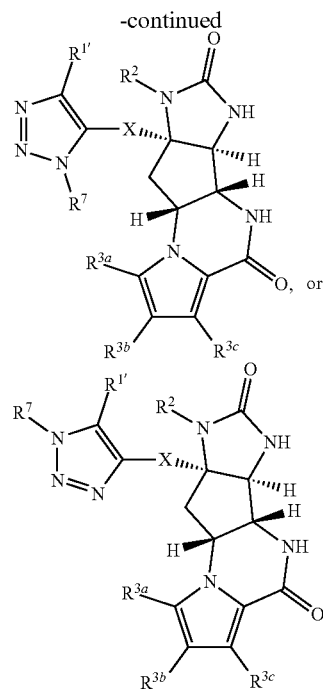
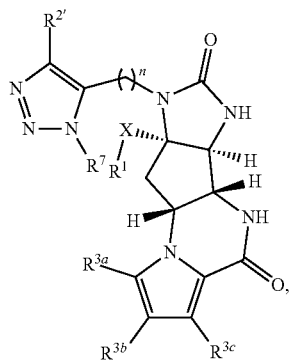
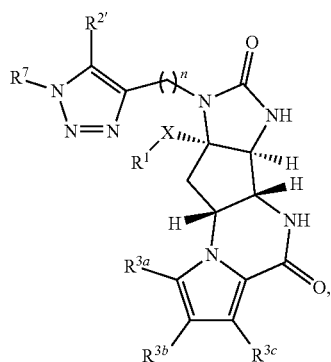


In specific embodiments, the compound of Formula (I) is



wherein X, R<sup>1</sup>, R<sup>7</sup>, and n are as defined above.

**[0155]** In various embodiments, the present disclosure also provides for internal triazole compounds of Formula (I). As an example meant only for illustrative purposes, by internal triazole compounds of Formula (I), triazoles of this type may have a general structure such as the following examples:



where R<sup>1'</sup> and R<sup>2'</sup> represent the rest of the R<sup>1</sup> or R<sup>2</sup> moiety.

**[0156]** In some embodiments, X is —O—, R<sup>1</sup> is H, R<sup>2</sup> is C<sub>1-10</sub> alkyl and R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are each independently H or halogen. For example, R<sup>2</sup> may be methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, and all isomers and branched derivatives thereof. In some embodiments, R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are each H. In some embodiments, R<sup>3a</sup> is bromo and R<sup>3b</sup> and R<sup>3c</sup> are H. In some embodiments, R<sup>3a</sup> and R<sup>3b</sup> are each bromo and R<sup>3c</sup> is H. In some embodiments, R<sup>3a</sup> and R<sup>3b</sup> are each H and R<sup>3c</sup> is bromo. In some embodiments R<sup>3a</sup> and R<sup>3c</sup> are each H and R<sup>3b</sup> is bromo. In some embodiments, R<sup>3a</sup> and R<sup>3c</sup> are each bromo and R<sup>3b</sup> is H. In some embodiments, R<sup>3a</sup> is H and R<sup>3b</sup> and R<sup>3c</sup> are each bromo. In some embodiments, R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are each











$R^{3c}$  are H. In some embodiments,  $R^{3a}$  and  $R^{3b}$  are each bromo and  $R^{3c}$  is H. In some embodiments,  $R^{3a}$  and  $R^{3b}$  are each H and  $R^{3c}$  is bromo. In some embodiments,  $R^{3a}$  and  $R^{3c}$  are each H and  $R^{3b}$  is bromo. In some embodiments,  $R^{3a}$  and  $R^{3c}$  are each bromo and  $R^{3b}$  is H. In some embodiments,  $R^{3a}$  is H and  $R^{3b}$  and  $R^{3c}$  are each bromo. In some embodiments,  $R^{3a}$ ,  $R^{3b}$ , and  $R^{3c}$  are each bromo. In some embodiments,  $R^2$  is substituted with one or more  $R^5$ . For example, in some embodiments,  $R^2$  is substituted with halogen, oxo, —OH, —OR<sup>6</sup>, —N<sub>3</sub>, or —N(R<sup>6</sup>)<sub>2</sub>. In some embodiments, up to 3 —CH<sub>2</sub>— units of  $R^2$  are replaced by an —O—S—, or —NR<sup>6</sup>—, provided that no adjacent —CH<sub>2</sub>— is replaced. In some embodiments,  $R^1$  is substituted with one or more  $R^4$  moieties. For example, in some embodiments,  $R^1$  is substituted with halogen, —C<sub>1-5</sub> alkyl, oxo, —OH, —OR<sup>6</sup>, —N<sub>3</sub>, —N(R<sup>6</sup>)<sub>2</sub>, —NH(C=O)R<sup>6</sup>, —NH(C=O)OR<sup>6</sup>, —NH(C=O)N(R<sup>6</sup>)<sub>2</sub>, —(C=O)R<sup>6</sup>, or heteroaryl.

**[0177]** In some embodiments, X is —S—,  $R^1$  is C<sub>2-10</sub> alkynyl,  $R^2$  is C<sub>1-10</sub> alkyl and  $R^{3a}$ ,  $R^{3b}$ , and  $R^{3c}$  are each independently H or halogen. For example,  $R^1$  may be acetylnyl, propylene, butylene, pentylene, hexylene, heptylene, octylene, nonylene, decylene, and all isomers and branched derivatives thereof. In some embodiments,  $R^{3a}$ ,  $R^{3b}$ , and  $R^{3c}$  are each H. In some embodiments,  $R^{3a}$  is bromo and  $R^{3b}$  and  $R^{3c}$  are H. In some embodiments,  $R^{3a}$  and  $R^{3b}$  are each bromo and  $R^{3c}$  is H. In some embodiments,  $R^{3a}$  and  $R^{3b}$  are each H and  $R^{3c}$  is bromo. In some embodiments,  $R^{3a}$  and  $R^{3c}$  are each H and  $R^{3b}$  is bromo. In some embodiments,  $R^{3a}$  and  $R^{3c}$  are each bromo and  $R^{3b}$  is H. In some embodiments,  $R^{3a}$  is H and  $R^{3b}$  and  $R^{3c}$  are each bromo. In some embodiments,  $R^{3a}$ ,  $R^{3b}$ , and  $R^{3c}$  are each bromo. In some embodiments,  $R^2$  is substituted with one or more  $R^5$ . For example, in some embodiments,  $R^2$  is substituted with halogen, oxo, —OH, —OR<sup>6</sup>, —N<sub>3</sub>, or —N(R<sup>6</sup>)<sub>2</sub>. In some embodiments, up to 3 —CH<sub>2</sub>— units of  $R^2$  are replaced by an —O—, —S—, or —NR<sup>6</sup>—, provided that no adjacent —CH<sub>2</sub>— is replaced. In some embodiments,  $R^1$  is substituted with one or more  $R^4$  moieties. For example, in some embodiments,  $R^1$  is substituted with halogen, —C<sub>1-5</sub> alkyl, oxo, —OH, —OR<sup>6</sup>, —N<sub>3</sub>, —N(R<sup>6</sup>)<sub>2</sub>, —NH(C=O)R<sup>6</sup>, —NH(C=O)OR<sup>6</sup>, —NH(C=O)N(R<sup>6</sup>)<sub>2</sub>, —(C=O)R<sup>6</sup>, or heteroaryl.

**[0178]** In some embodiments, X is —S—,  $R^1$  is C<sub>2-10</sub> alkynyl,  $R^2$  is C<sub>2-10</sub> alkenyl and  $R^{3a}$ ,  $R^{3b}$ , and  $R^{3c}$  are each independently H or halogen. For example,  $R^1$  may be ethylene, propylene, butylene, pentylene, hexylene, heptylene, octylene, nonylene, decylene, and all isomers and branched derivatives thereof. For example,  $R^2$  may be ethylene, propylene, butylene, pentylene, hexylene, heptylene, octylene, nonylene, decylene, and all isomers and branched derivatives thereof. In some embodiments,  $R^{3a}$ ,  $R^{3b}$ , and  $R^{3c}$  are each H. In some embodiments,  $R^{3a}$  is bromo and  $R^{3b}$  and  $R^{3c}$  are H. In some embodiments,  $R^{3a}$  and  $R^{3b}$  are each bromo and  $R^{3c}$  is H. In some embodiments,  $R^{3a}$  and  $R^{3b}$  are each H and  $R^{3c}$  is bromo. In some embodiments,  $R^{3a}$  and  $R^{3c}$  are each H and  $R^{3b}$  is bromo. In some embodiments,  $R^{3a}$  and  $R^{3c}$  are each bromo and  $R^{3b}$  is H. In some embodiments,  $R^{3a}$  is H and  $R^{3b}$  and  $R^{3c}$  are each bromo. In some embodiments,  $R^{3a}$ ,  $R^{3b}$ , and  $R^{3c}$  are each bromo. In some embodiments,  $R^2$  is substituted with one or more  $R^5$ . For example, in some embodiments,  $R^2$  is substituted with halogen, oxo, —OH, —OR<sup>6</sup>, —N<sub>3</sub>, or —N(R<sup>6</sup>)<sub>2</sub>. In some embodiments, up to 3

—CH<sub>2</sub>— units of  $R^2$  are replaced by an —O—, —S—, or —NR<sup>6</sup>—, provided that no adjacent —CH<sub>2</sub>— is replaced. In some embodiments,  $R^1$  is substituted with one or more  $R^4$  moieties. For example, in some embodiments,  $R^2$  is substituted with halogen, —C<sub>1-5</sub> alkyl, oxo, —OH, —OR<sup>6</sup>, —N<sub>3</sub>, —N(R<sup>6</sup>)<sub>2</sub>, —NH(C=O)R<sup>6</sup>, —NH(C=O)OR<sup>6</sup>, —NH(C=O)N(R<sup>6</sup>)<sub>2</sub>, —(C=O)R<sup>6</sup>, or heteroaryl.

**[0179]** In some embodiments, X is —S—,  $R^1$  is C<sub>2-10</sub> alkynyl,  $R^2$  is C<sub>2-10</sub> alkynyl and  $R^{3a}$ ,  $R^{3b}$ , and  $R^{3c}$  are each independently H or halogen. For example,  $R^1$  may be ethylene, propylene, butylene, pentylene, hexylene, heptylene, octylene, nonylene, decylene, and all isomers and branched derivatives thereof. For example,  $R^2$  may be acetylnyl, propylene, butylene, pentylene, hexylene, heptylene, octylene, nonylene, decylene, and all isomers and branched derivatives thereof. In some embodiments,  $R^{3a}$ ,  $R^{3b}$ , and  $R^{3c}$  are each H. In some embodiments,  $R^{3a}$  is bromo and  $R^{3b}$  and  $R^{3c}$  are H. In some embodiments,  $R^{3a}$  and  $R^{3b}$  are each bromo and  $R^{3c}$  is H. In some embodiments,  $R^{3a}$  and  $R^{3b}$  are each H and  $R^{3c}$  is bromo. In some embodiments,  $R^{3a}$  and  $R^{3c}$  are each H and  $R^{3b}$  is bromo. In some embodiments,  $R^{3a}$  and  $R^{3b}$  are each bromo and  $R^{3c}$  is H. In some embodiments,  $R^{3a}$  is H and  $R^{3b}$  and  $R^{3c}$  are each bromo. In some embodiments,  $R^{3a}$ ,  $R^{3b}$ , and  $R^{3c}$  are each bromo. In some embodiments,  $R^2$  is substituted with one or more  $R^5$ . For example, in some embodiments,  $R^2$  is substituted with halogen, oxo, —OH, —OR<sup>6</sup>, —N<sub>3</sub>, or —N(R<sup>6</sup>)<sub>2</sub>. In some embodiments, up to 3 —CH<sub>2</sub>— units of  $R^2$  are replaced by an —O—, —S—, or —NR<sup>6</sup>—, provided that no adjacent —CH<sub>2</sub>— is replaced. In some embodiments,  $R^1$  is substituted with one or more  $R^4$  moieties. For example, in some embodiments,  $R^1$  is substituted with halogen, —C<sub>1-5</sub> alkyl, oxo, —OH, —OR<sup>6</sup>, —N<sub>3</sub>, —N(R<sup>6</sup>)<sub>2</sub>, —NH(C=O)R<sup>6</sup>, —NH(C=O)OR<sup>6</sup>, —NH(C=O)N(R<sup>6</sup>)<sub>2</sub>, —(C=O)R<sup>6</sup>, or heteroaryl.

**[0180]** In some embodiments, X is —N(R<sup>6</sup>)—,  $R^1$  is H,  $R^2$  is C<sub>1-10</sub> alkyl and  $R^{3a}$ ,  $R^{3b}$ , and  $R^{3c}$  are each independently H or halogen. For example,  $R^2$  may be methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, and all isomers and branched derivatives thereof. In some embodiments,  $R^{3a}$ ,  $R^{3b}$ , and  $R^{3c}$  are each H. In some embodiments,  $R^{3a}$  is bromo and  $R^{3b}$  and  $R^{3c}$  are H. In some embodiments,  $R^{3a}$  and  $R^{3b}$  are each bromo and  $R^{3c}$  is H. In some embodiments,  $R^{3a}$  and  $R^{3b}$  are each H and  $R^{3c}$  is bromo. In some embodiments,  $R^{3a}$  and  $R^{3c}$  are each H and  $R^{3b}$  is bromo. In some embodiments,  $R^{3a}$  and  $R^{3c}$  are each bromo and  $R^{3b}$  is H. In some embodiments,  $R^{3a}$  is H and  $R^{3b}$  and  $R^{3c}$  are each bromo. In some embodiments,  $R^{3a}$ ,  $R^{3b}$ , and  $R^{3c}$  are each bromo. In any of the preceding embodiments for  $R^{3a}$ ,  $R^{3b}$ , and  $R^{3c}$ , any bromo or H may be independently replaced with a fluororo, iodo, or chloro moiety allowing for all possible permutations. In some embodiments,  $R^2$  is substituted with one or more  $R^5$ . For example, in some embodiments,  $R^2$  is substituted with halogen, oxo, —OH, —OR<sup>6</sup>, —N<sub>3</sub>, or —N(R<sup>6</sup>)<sub>2</sub>. In some embodiments, up to 3 —CH<sub>2</sub>— units of  $R^2$  are replaced by an —O—, —S—, or —NR<sup>6</sup>—, provided that no adjacent —CH<sub>2</sub>— is replaced. Each R<sup>6</sup> may independently be H, —C<sub>1-5</sub> alkyl, —C<sub>2-5</sub> alkenyl, —C<sub>1-5</sub> alkynyl, —C<sub>1-5</sub> alkyl-SiMe<sub>3</sub>, aryl, or heteroaryl.

**[0181]** In some embodiments, X is —N(R<sup>6</sup>)—,  $R^1$  is H,  $R^2$  is C<sub>2-10</sub> alkenyl and  $R^{3a}$ ,  $R^{3b}$ , and  $R^{3c}$  are each independently H or halogen. For example,  $R^2$  may be ethylene, propylene, butylene, pentylene, hexylene, heptylene, octylene, nonylene, decylene, and all isomers and branched derivatives thereof. In some embodiments,  $R^{3a}$ ,  $R^{3b}$ , and  $R^{3c}$





are each H. In some embodiments, R<sup>3a</sup> is bromo and R<sup>3b</sup> and R<sup>3c</sup> are H. In some embodiments, R<sup>3a</sup> and R<sup>3b</sup> are each bromo and R<sup>3c</sup> is H. In some embodiments, R<sup>3a</sup> and R<sup>3b</sup> are each H and R<sup>3c</sup> is bromo. In some embodiments, R<sup>3a</sup> and R<sup>3c</sup> are each H and R<sup>3b</sup> is bromo. In some embodiments, R<sup>3a</sup> and R<sup>3c</sup> are each bromo and R<sup>3b</sup> is H. In some embodiments, R<sup>3a</sup> is H and R<sup>3b</sup> and R<sup>3c</sup> are each bromo. In some embodiments, R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are each bromo. In some embodiments, R<sup>2</sup> is substituted with one or more R<sup>5</sup>. For example, in some embodiments, R<sup>2</sup> is substituted with halogen, oxo, —OH, —OR<sup>6</sup>, —N<sub>3</sub>, or —N(R<sup>6</sup>)<sub>2</sub>. In some embodiments, up to 3 —CH<sub>2</sub>— units of R<sup>2</sup> are replaced by an —O—, —S—, or —NR<sup>6</sup>—, provided that no adjacent —CH<sub>2</sub>— is replaced. In some embodiments, R<sup>1</sup> is substituted with one or more R<sup>4</sup> moieties. For example, in some embodiments, R<sup>1</sup> is substituted with halogen, —C<sub>1-5</sub> alkyl, oxo, —OH, —OR<sup>6</sup>, —N<sub>3</sub>, —N(R<sup>6</sup>)<sub>2</sub>, —NH(C=O)R<sup>6</sup>, —NH(C=O)OR<sup>6</sup>, —NH(C=O)N(R<sup>6</sup>)<sub>2</sub>, —(C=O)R<sup>6</sup>, or heteroaryl. Each R<sup>6</sup> may independently be H, —C<sub>1-5</sub> alkyl, —C<sub>1-5</sub> alkenyl, —C<sub>1-5</sub> alkynyl, —C<sub>1-5</sub> alkyl-SiMe<sub>3</sub>, aryl, or heteroaryl.

**[0191]** In some embodiments, X is —N(R<sup>6</sup>)—, R<sup>1</sup> is C<sub>2-10</sub> alkynyl, R<sup>2</sup> is C<sub>2-10</sub> to alkynyl and R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are each independently H or halogen. For example, R<sup>1</sup> may be ethylene, propylene, butylene, pentylene, hexylene, heptylene, octylene, nonylene, decylene, and all isomers and branched derivatives thereof. For example, R<sup>2</sup> may be acetylenyl, propylene, butylene, pentylyne, hexylene, heptylyne, octylene, nonylene, decylene, and all isomers and branched derivatives thereof. In some embodiments, R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are each H. In some embodiments, R<sup>3a</sup> is bromo and R<sup>3b</sup> and R<sup>3c</sup> are H. In some embodiments, R<sup>3a</sup> and R<sup>3b</sup> are each bromo and R<sup>3c</sup> is H. In some embodiments, R<sup>3a</sup> and R<sup>3b</sup> are each H and R<sup>3c</sup> is bromo. In some embodiments, R<sup>3a</sup> and R<sup>3c</sup> are each H and R<sup>3b</sup> is bromo. In some embodiments, R<sup>3a</sup> and R<sup>3c</sup> are each bromo and R<sup>3b</sup> is H. In some embodiments, R<sup>3a</sup> is H and R<sup>3b</sup> and R<sup>3c</sup> are each bromo. In some embodiments, R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are each bromo. In some embodiments, R<sup>2</sup> is substituted with one or more R<sup>5</sup>. For example, in some embodiments, R<sup>2</sup> is substituted with halogen, oxo, —OH, —OR<sup>6</sup>, —N<sub>3</sub>, or —N(R<sup>6</sup>)<sub>2</sub>. In some embodiments, up to 3 —CH<sub>2</sub>— units of R<sup>2</sup> are replaced by an —O—, —S—, or —NR<sup>6</sup>—, provided that no adjacent —CH<sub>2</sub>— is replaced. In some embodiments, R<sup>1</sup> is substituted with one or more R<sup>4</sup> moieties. For example, in some embodiments, R<sup>1</sup> is substituted with halogen, —C<sub>1-5</sub> alkyl, oxo, —OH, —OR<sup>6</sup>, —N<sub>3</sub>, —N(R<sup>6</sup>)<sub>2</sub>, —NH(C=O)R<sup>6</sup>, —N(C=O)OR<sup>6</sup>, —NH(C=O)N(R<sup>6</sup>)<sub>2</sub>, —(C=O)R<sup>6</sup>, or heteroaryl. Each R<sup>6</sup> may independently be H, —C<sub>1-5</sub> alkyl, —C<sub>2-8</sub> alkenyl, —C<sub>1-5</sub> alkynyl, —C<sub>1-5</sub> alkyl-SiMe<sub>3</sub>, aryl, or heteroaryl.

**[0192]** Unless otherwise stated, structures depicted herein are also meant to include compounds which differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structure except for the replacement of a hydrogen atom by deuterium or tritium, or the replacement of a carbon atom by <sup>13</sup>C or <sup>14</sup>C, or the replacement of a nitrogen atom by <sup>15</sup>N, or the replacement of an oxygen atom with <sup>17</sup>O or <sup>18</sup>O are within the scope of the present disclosure. Such isotopically labeled compounds are useful as research or diagnostic tools.

#### Methods of Treatment

**[0193]** The disclosed compounds (e.g., compounds of Formula I-Ie (i.e., compounds of Formula I, Ia, Ib, Ic, Id, and

Ie), and their pharmaceutically acceptable salts have activity as pharmaceuticals, as discussed herein.

**[0194]** The present disclosure provides a method of treatment or prevention of a disease, disorder or condition including the step of administering an effective amount of a compound of the present disclosure, and pharmaceutically acceptable salts, prodrugs, solvates, hydrates, isomers, and tautomers thereof to thereby treat or prevent the disease, disorder or condition in a subject in need thereof.

**[0195]** The present disclosure provides a compound of the present disclosure, and pharmaceutically acceptable salts, prodrugs, solvates, hydrates, isomers, and tautomers thereof, or the pharmaceutical composition of the present disclosure for use in the treatment or prevention of a disease, disorder or condition in a subject in need thereof.

**[0196]** The present disclosure provides for use of a compound of the present disclosure, and pharmaceutically acceptable salts, prodrugs, solvates, hydrates, isomers, and tautomers thereof, for the treatment or prevention of a disease, disorder or condition in a subject in need thereof.

**[0197]** The present disclosure provides for use of a compound of the present disclosure, and pharmaceutically acceptable salts, prodrugs, solvates, hydrates, isomers, and tautomers thereof, in the manufacture of a medicament for the treatment or prevention of a disease, disorder or condition.

**[0198]** It was surprisingly discovered AgE (5) is more potent than AgA (1) in blocking the effects of fibroblast OPN on the invasiveness, migration potential, and cancer stem cell populations in associated cancer cells. As will be appreciated by a skilled artisan, this has wide ranging applications. For example, because AgE (5) and AgA (1) both block the effects of fibroblast OPN on the invasiveness, migration potential, and cancer stem cell populations in associated cancer cells, such as breast cancer, this implies that natural and derivatized agelastatin compounds are useful at treating, preventing, and/or delaying a variety of cancers associated with OPN overexpression. Thus, in some embodiments, the present disclosure provides a method for the treatment, prevention, or delay of cancer, comprising administering a therapeutically effective amount of a compound of the present disclosure, a pharmaceutically acceptable salt thereof, or a composition thereof to a subject in need thereof.

**[0199]** In one embodiment, the disease, disorder or condition is a cancer, tumor or other malignancy. As used herein, cancers tumors and malignancies, refer to diseases, disorders or conditions, or to cells or tissues associated with the diseases, disorders or conditions, characterized by aberrant or abnormal cell proliferation, differentiation and/or migration often accompanied by an aberrant or abnormal molecular phenotype that includes one or more genetic mutations or other genetic changes associated with oncogenesis, expression of tumor markers, loss of tumor suppressor expression or activity and/or aberrant or abnormal cell surface marker expression. In general embodiments, cancers, tumors and malignancies may include sarcomas, lymphomas, leukemias, solid tumors, blastomas, gliomas, carcinomas, melanomas and metastatic cancers, although without limitation thereto. A more comprehensive listing of cancers tumors and malignancies may be found at the National Cancer Institutes website <http://www.cancer.gov/cancertopics/types/alphabet>, which is hereby incorporated by reference in its entirety.

**[0200]** The cancer may be any cancer associated with OPN overexpression. For example, in some embodiments, the cancer is breast cancer, lung cancer, colorectal cancer, stomach cancer, ovarian cancer, papillary thyroid carcinoma, melanoma, prostate cancer, esophageal cancer, liver cancer, bladder cancer, renal cancer, head and neck cancers, salivary gland cancer, endometrial cancer, cervical cancer, pancreatic cancer, sarcoma, glioblastoma and glioma, or pleural mesothelioma. In some embodiments, the cancer is breast cancer. A skilled artisan will appreciate that a variety of breast cancers may be linked to OPN overexpression. In some embodiments, the breast cancer is selected from the group consisting of ductal carcinoma in situ (DCIS), invasive ductal carcinoma (IDC), tubular carcinoma, medullary carcinoma, mucinous carcinoma, papillary carcinoma, cribriform carcinoma, invasive lobular carcinoma (ILC), inflammatory breast cancer, lobular carcinoma in situ (LCIS), luminal A, luminal B, triple-negative/basal-like, and HER2-enriched, normal-like breast cancer. In some embodiments, the breast cancer is metastatic breast cancer or recurrent metastatic breast cancer.

**[0201]** In some embodiments, the cancer (e.g., breast cancer) is recurrent cancer at the primary site, metastatic cancer, or recurrent metastatic cancer. In some embodiments, the cancer (e.g., breast cancer) is prevented or delayed. In some embodiments, the cancer (e.g., breast cancer) is prevented. In some embodiments, the cancer (e.g., breast cancer) is delayed.

**[0202]** In some embodiments, the compounds of the present disclosure are administered before surgery and/or radiotherapy and/or systemic medical therapy. In some embodiments, the compounds of the present disclosure are administered before surgery. In some embodiments, the compounds of the present disclosure are administered before radiotherapy. In some embodiments, the compounds of the present disclosure are administered before systemic medical therapy. In various embodiments, systemic medical therapy includes chemotherapies, hormonal therapies, targeted biologic therapies, and/or immunotherapies.

**[0203]** Systemic therapies of the present disclosure are treatments that can spread throughout the body to treat cancer cells wherever they may be located. As noted, such drugs can be chemotherapies, hormonal therapies, targeted drugs, and/or immunotherapies. In various embodiments, systemic therapies reach cells throughout the body by traveling through the bloodstream.

**[0204]** In some embodiments, the compounds of the present disclosure are administered after surgery and/or radiotherapy and/or systemic medical therapy. In some embodiments, the compounds of the present disclosure are administered after surgery. In some embodiments, the compounds of the present disclosure are administered after radiotherapy. In some embodiments, the compounds of the present disclosure are administered after systemic medical therapy. In various embodiments, systemic medical therapy includes chemotherapies, hormonal therapies, targeted biologic therapies, and/or immunotherapies.

**[0205]** In some embodiments, the compounds of the present disclosure are administered concurrently with radiotherapy and/or systemic medical therapy. In some embodiments, the compounds of the present disclosure are administered concurrently with radiotherapy. In some embodiments, the compounds of the present disclosure are administered concurrently with systemic medical treatment.

In various embodiments, systemic medical therapy includes chemotherapies, hormonal therapies, targeted biologic therapies, and/or immunotherapies.

**[0206]** In some embodiments, the compounds of the present disclosure are administered before and after surgery and/or radiotherapy and/or systemic medical therapy. In some embodiments, the compounds of the present disclosure are administered before and after surgery. In some embodiments, the compounds of the present disclosure are administered before and after radiotherapy. In some embodiments, the compounds of the present disclosure are administered before and after systemic medical therapy. In various embodiments, systemic medical therapy includes chemotherapies, hormonal therapies, targeted biologic therapies, and/or immunotherapies.

**[0207]** In some embodiments, the treatment results in inhibition of induced transcription of osteopontin (OPN) in fibroblasts, inflammatory cells, and immune cells of the tumor microenvironment. In some embodiments, the treatment results in inhibition of induced transcription of osteopontin (OPN) in fibroblasts. In some embodiments, the treatment results in inhibition of induced transcription of osteopontin (OPN) in inflammatory cells. In some embodiments, the treatment results in inhibition of induced transcription of osteopontin (OPN) in immune cells of the tumor microenvironment. In some embodiments, the fibroblasts are mammary fibroblasts. In some embodiments, the cancer is characterized by a tumor microenvironment exhibiting down regulation of fibroblast Tiam1 and upregulation of fibroblast OPN. In other embodiments, the cancer is characterized by a tumor microenvironment exhibiting upregulation of fibroblast OPN.

**[0208]** Osteopontin transcription can be stimulated by multiple factors, including but not limited to, stimulation by vitamin D. Without being bound by any particular theory, it has been discovered that agelastatin A can inhibit the induction of osteopontin transcription by cancer cell secreted factors as well as vitamin D. Thus, in various embodiments, such inhibition by agelastatin A can occur in any tissue, e.g., breast tissue. Multiple cell types in the tumor stroma, including macrophages and lymphocytes, also secrete osteopontin. Since agelastatin A can block metastasis of breast cancer cells to lung in vivo, which is a complex process involving various tumor microenvironments, it is understood that in various embodiments, agelastatin A can inhibit osteopontin transcription in macrophages and lymphocytes as well as fibroblasts.

**[0209]** In some embodiments, the inhibition occurs at or below the cytotoxic range determined for the cancer cells being treated. In some embodiments, the inhibition occurs at the cytotoxic range determined for the cancer cells being treated. In other embodiments, the inhibition occurs below the cytotoxic range determined for the cancer cells being treated. In some embodiments, the transcription is of splice variants of OPN. In some embodiments, the splice variants are osteopontin-a, osteopontin-b, or osteopontin-c. In some embodiments, the inhibition results in interference with cancer cell adhesion, cancer cell invasion, and cancer stem cell populations.

**[0210]** For the above-mentioned therapeutic uses the dosage administered will, of course, vary with the compound employed, the mode of administration, the treatment desired and the disorder indicated. For example, the daily dosage of the compound of the present disclosure, if inhaled, may be

in the range from about 0.05 micrograms per kilogram body weight ( $\mu\text{g}/\text{kg}$ ) to about 100 micrograms per kilogram body weight ( $\mu\text{g}/\text{kg}$ ). Alternatively, if the compound is administered orally, then the daily dosage of the compound of the present disclosure may be in the range from about 0.01 micrograms per kilogram body weight ( $\mu\text{g}/\text{kg}$ ) to about 100 milligrams per kilogram body weight ( $\text{mg}/\text{kg}$ ).

#### Pharmaceutical Compositions

**[0211]** The disclosed compounds (e.g., compounds of Formula I-Ie), and pharmaceutically acceptable salts, tautomers, prodrugs or stereoisomers thereof may be used on their own but will generally be administered in the form of a pharmaceutical composition in which the disclosed compound/salt (e.g., compounds of Formula I-Ie and salts thereof) (active ingredient) is in association with a pharmaceutically acceptable adjuvant, diluent and/or carrier. Thus in some embodiments, the present disclosure provides a pharmaceutical composition comprising compounds of Formula I-Ie and a pharmaceutically acceptable adjuvant, diluent, carrier and/or excipient. In some embodiments, the present disclosure provides a pharmaceutical composition comprising compounds of Formula I-Ie and a pharmaceutically acceptable adjuvant. In some embodiments, the present disclosure provides a pharmaceutical composition comprising compounds of Formula I-Ie and a pharmaceutically acceptable diluent. In some embodiments, the present disclosure provides a pharmaceutical composition comprising compounds of Formula I-Ie and a pharmaceutically acceptable carrier. In some embodiments, the present disclosure provides a pharmaceutical composition comprising compounds of Formula I-Ie and a pharmaceutically acceptable excipient. Conventional procedures for the selection and preparation of suitable pharmaceutical formulations are described in, for example, "Pharmaceuticals—The Science of Dosage Form Designs", M. E. Aulton, Churchill Livingstone, 1988, which is hereby incorporated by reference in its entirety.

**[0212]** Depending on the mode of administration, the pharmaceutical composition will comprise from about 0.05 to about 99% w (percent by weight), more particularly from about 0.05 to about 80% w, still more particularly from about 0.10 to about 70% w, and even more particularly from about 0.10 to about 50% w, of active ingredient, all percentages by weight being based on total composition.

**[0213]** The present disclosure also provides a pharmaceutical composition comprising a disclosed compound (e.g., compound of Formula I-Ie), or a pharmaceutically acceptable salt thereof as hereinbefore defined, in association with a pharmaceutically acceptable adjuvant, diluent, carrier, and/or excipient.

**[0214]** The present disclosure further provides a process for the preparation of a pharmaceutical composition of the present disclosure which comprises mixing a disclosed compound (e.g., compound of Formula I-Ie), or a pharmaceutically acceptable salt thereof as hereinbefore defined with a pharmaceutically acceptable adjuvant, diluent, carrier, and/or excipient.

**[0215]** The pharmaceutical compositions may be administered topically (e.g. to the skin or to the lung and/or airways) in the form, e.g., of creams, solutions, suspensions, heptafluoroalkane (HFA) aerosols and dry powder formulations, for example, formulations in the inhaler device known as the Turbuhaler®; or systemically, e.g. by oral administration in the form of tablets, capsules, syrups, powders or

granules; or by parenteral administration in the form of a sterile solution, suspension or emulsion for injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion); or by rectal administration in the form of suppositories.

**[0216]** Dry powder formulations and pressurized HFA aerosols of the compounds of the present disclosure (including pharmaceutically acceptable salts) may be administered by oral or nasal inhalation. For inhalation, the compound is desirably finely divided. The finely divided compound preferably has a mass median diameter of less than 10 micrometres ( $\mu\text{m}$ ), and may be suspended in a propellant mixture with the assistance of a dispersant, such as a  $\text{C}_8$ - $\text{C}_{20}$  fatty acid or salt thereof, (for example, oleic acid), a bile salt, a phospholipid, an alkyl saccharide, a perfluorinated or polyethoxylated surfactant, or other pharmaceutically acceptable dispersant.

**[0217]** The compounds of the present disclosure may also be administered by means of a dry powder inhaler. The inhaler may be a single or a multi dose inhaler, and may be a breath actuated dry powder inhaler.

**[0218]** One possibility is to mix the finely divided compound of the present disclosure with a carrier substance, for example, a mono-, di- or polysaccharide, a sugar alcohol, or another polyol. Suitable carriers are sugars, for example, lactose, glucose, raffinose, melezitose, lactitol, maltitol, trehalose, sucrose, mannitol; and starch. Alternatively the finely divided compound may be coated by another substance. The powder mixture may also be dispensed into hard gelatin capsules, each containing the desired dose of the active compound.

**[0219]** Another possibility is to process the finely divided powder into spheres which break up during the inhalation procedure. This spheronized powder may be filled into the drug reservoir of a multidose inhaler, for example, that known as the Turbuhaler® in which a dosing unit meters the desired dose which is then inhaled by the patient. With this system the active ingredient, with or without a carrier substance, is delivered to the patient.

**[0220]** Another possibility is to process the compound as an amorphous dispersion in a polymer matrix such as hydroxypropyl methylcellulose (HPMC) or hydroxypropyl methylcellulose acetate succinate (HPMCAS). As the name suggests, spray-dried dispersions (SDDs) are obtained by dissolving drug and polymer in an organic solvent, atomizing the resulting solution into droplets, and evaporation to dried solid particles. SDDs are usually amenable for use a variety of final oral dosage forms, including capsules and tablets.

**[0221]** For oral administration the compound of the present disclosure may be admixed with an adjuvant or a carrier, for example, lactose, saccharose, sorbitol, mannitol; a starch, for example, potato starch, corn starch or amylopectin; a cellulose derivative; a binder, for example, gelatin or polyvinylpyrrolidone; and/or a lubricant, for example, magnesium stearate, calcium stearate, polyethylene glycol, a wax, paraffin, and the like, and then compressed into tablets. If coated tablets are required, the cores, prepared as described above, may be coated with a concentrated sugar solution which may contain, for example, gum arabic, gelatin, talcum and titanium dioxide. Alternatively, the tablet may be coated with a suitable polymer dissolved in a readily volatile organic solvent.

**[0222]** Suitable excipients include, but are not limited to, polymers, absorption enhancers, solubility enhancing agents, dissolution rate enhancing agents, bioadhesive agents, and controlled release agents. More particularly, suitable excipients include cellulose ethers, acrylic acid polymers, and bile salts. Other suitable excipients are described in detail in the Handbook of Pharmaceutical Excipients, published jointly by the American Pharmaceutical Association and The Pharmaceutical Society of Great Britain, the Pharmaceutical Press, 1986, which is incorporated by reference herein. Such excipients are commercially available and/or can be prepared by techniques known in the art.

**[0223]** For the preparation of soft gelatin capsules, the compound of the present disclosure may be admixed with, for example, a vegetable oil or polyethylene glycol. Hard gelatin capsules may contain granules of the compound using either the above-mentioned excipients for tablets. Also liquid or semisolid formulations of the compound of the present disclosure may be filled into hard gelatin capsules.

**[0224]** Liquid preparations for oral application may be in the form of syrups or suspensions, for example, solutions containing the compound of the present disclosure, the balance being sugar and a mixture of ethanol, water, glycerol and propylene glycol. Optionally such liquid preparations may contain colouring agents, flavouring agents, saccharine and/or carboxymethylcellulose as a thickening agent or other excipients known to those skilled in art.

#### Combination Therapy

**[0225]** The compounds of the present disclosure (that is, compounds of Formula I-Ie, and pharmaceutically acceptable salts thereof) may also be administered in conjunction with other compounds used for the treatment of the above conditions.

**[0226]** The present disclosure therefore further relates to combination therapies wherein a compound of the present disclosure or a pharmaceutical composition or formulation comprising a compound of the present disclosure is administered concurrently or sequentially or as a combined preparation with another therapeutic agent or agents, for the treatment of one or more of the conditions listed.

**[0227]** In some embodiments, the present disclosure provides a method for the treatment, prevention, or delay of cancer, comprising administering a therapeutically effect amount of a compound of the present disclosure, a pharmaceutically acceptable salt thereof, or a composition thereof to a subject in need thereof comprising coadministration to the subject of an additional therapeutic agent selected from the group consisting of antitumor agents, anti-metabolites, anti-estrogens, aromatase inhibitors, estrogen receptor antagonists, targeted therapies, tyrosine kinase inhibitors, and immune checkpoint inhibitors.

**[0228]** In some embodiments, the present disclosure provides a method for the treatment, prevention, or delay of cancer, comprising administering a therapeutically effect amount of a compound of the present disclosure, a pharmaceutically acceptable salt thereof, or a composition thereof to a subject in need thereof comprising coadministering an antitumor agent. A variety of antitumor agents can be used in the context of the present disclosure and will be readily apparent to a skilled artisan, including, but not limited to paclitaxel, doxorubicin, daunorubicin, cyclophosphamide, methotrexate, 5-fluorouracil, thiotepa, busulfan, impropul-

fan, pipsulfan, benzodopa, carboquone, meturedopa, uredopa, altretamine, triethylenemelamine, triethylenephosphoramidate, triethylenethiophosphoramidate, trimethylolomelamine, bullatacin, bullatacinone, camptothecin, bryostatins, callystatin, cryptophycin 1, cryptophycin 8, dolastatin, duocarmycin, eleutherobin, pancratistatin, sarcodictyin, spongistatin, chlorambucil, chlornaphazine, chlophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard, carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine, calicheamicin, dynemicin, clodronate, esperamicin, aclacinomycin, actinomycin, anthracycline, azaserine, bleomycin, cactinomycin, carabycin, carminomycin, carzinophilin, chromomycin, dactinomycin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycin C, mycophenolic acid, nogalamycin, olivomycin, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin, denopterin, pteropterin, trimetrexate, fludarabine, 6-mercaptopurine, thiampirine, thioguanine, ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, androgens such as calusterone, dromostanolone propionate, epitio stanol, mepitio stanone, testolactone, aminoglutethimide, mitotane, trilostane, frolic acid, aceglatone, aldophosphamide glycoside, aminolevulinic acid, eniluracil, amsacrine, bestrabucil, bisantrene, edatraxate, defofamine, demecolcine, diaziquone, elformithine, elliptinium acetate, epothilone, etoglucid, lentinan, lonidamine, maytansine, ansamitocine, mitoguazone, mitoxantrone, mopidanmol, nitraerine, pentostatin, phenamet, pirarubicin, losoxantrone, podophyllinic acid, 2-ethylhydrazide, procarbazine, razoxane, rhizoxin, schizophyllan, spirogermanium, tenuazonic acid, triaziquone, roridine A, anguidine, urethane, vindesine, dacarbazine, mannomustine, mitobronitol, mitolactol, pipobroman, gacytosine, docetaxel, chlorambucil, gemcitabine, 6-thioguanine, mercaptopurine, cisplatin, oxaliplatin, carboplatin, vinblastine, etoposide, ifosfamide, mitoxantrone, vincristine, vinorelbine, novantrone, teniposide, edatrexate, daunomycin, aminopterin, xeloda, ibandronate, irinotecan, topoisomerase inhibitor, difluoromethylornithine (DMFO), retinoic acid, capecitabine, and pharmacologically acceptable salts or derivatives thereof.

**[0229]** In some embodiments, the present disclosure provides a method for the treatment, prevention, or delay of cancer, comprising administering a therapeutically effect amount of a compound of the present disclosure, a pharmaceutically acceptable salt thereof, or a composition thereof to a subject in need thereof comprising coadministering an anti-metabolite agent. A variety of anti-metabolite agents can be used in the context of the present disclosure and will be readily apparent to a skilled artisan, including, but not limited to methotrexate, 5-fluorouracil, 5-fluorouracil prodrugs (e.g., capecitabine), 5-fluorodeoxyuridine monophosphate, cytarabine, 5-azacytidine, gemcitabine, mercaptopurine, thioguanine, azathioprine, adenosine, pentostatin, erythrohydroxynonyladenine, and cladribine.

**[0230]** In some embodiments, the present disclosure provides a method for the treatment, prevention, or delay of cancer, comprising administering a therapeutically effect amount of a compound of the present disclosure, a pharmaceutically acceptable salt thereof, or a composition thereof to

a subject in need thereof comprising coadministering of an anti-estrogen agent selected from the group consisting of selective estrogen receptor modulators, pure receptor antagonists, aromatase inhibitors, and anti-gonadotropins and pharmacologically acceptable salts or derivatives thereof.

**[0231]** In various embodiments, the anti-estrogen agent is a selective estrogen receptor modulator (SERM). A variety of SERMs can be used in the context of the present disclosure and will be readily apparent to a skilled artisan, including, but not limited to anordrin, bazedoxifene, broparestrol, clomifene, cyclofenil, lasofoxifene, ormeloxifene, ospemifene, raloxifene, tamoxifen citrate, toremifene citrate, and pharmacologically acceptable salts or derivatives thereof.

**[0232]** In various embodiments, the anti-estrogen agent is a pure receptor antagonist. A variety of pure receptor antagonists can be used in the context of the present disclosure and will be readily apparent to a skilled artisan, including, but not limited to fulvestrant, brilanestrant, elacestrant, and pharmacologically acceptable salts or derivatives thereof.

**[0233]** In various embodiments, the anti-estrogen agent is an aromatase inhibitor. A variety of aromatase inhibitors can be used in the context of the present disclosure and will be readily apparent to a skilled artisan, including, but not limited to anastrozole, letrozole, vorozole, exemestane, formestane and pharmacologically acceptable salts or derivatives thereof.

**[0234]** In various embodiments, the anti-estrogen agent is an anti-gonadotropin. A variety of anti-gonadotropins can be used in the context of the present disclosure and will be readily apparent to a skilled artisan, including, but not limited to triptorelin, leuprolide acetate, and pharmacologically acceptable salts or derivatives thereof.

**[0235]** In some embodiments, the present disclosure provides a method for the treatment, prevention, or delay of cancer, comprising administering a therapeutically effect amount of a compound of the present disclosure, a pharmaceutically acceptable salt thereof, or a composition thereof to a subject in need thereof comprising coadministering an tyrosine kinase inhibitor. A variety of tyrosine kinase inhibitors can be used in the context of the present disclosure and will be readily apparent to a skilled artisan, but not limited to trastuzumab, pertuzumab, imatinib, gefitinib, erlotinib, sunitinib, adavosertib, and lapatinib.

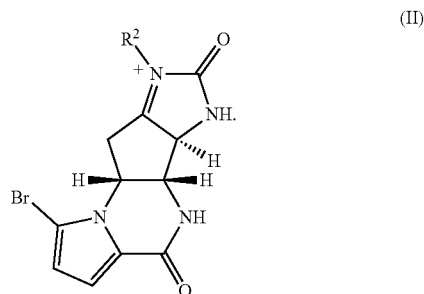
**[0236]** In some embodiments, the present disclosure provides a method for the treatment, prevention, or delay of cancer, comprising administering a therapeutically effect amount of a compound of the present disclosure, a pharmaceutically acceptable salt thereof, or a composition thereof to a subject in need thereof comprising coadministering an immune checkpoint inhibitor. A variety of immune checkpoint inhibitors can be used in the context of the present disclosure and will be readily apparent to a skilled artisan, including, but not limited to ipilimumab, pembrolizumab, nivolumab, avelumab, durvalumab, and atezolizumab

#### Methods of Synthesizing the Compounds

**[0237]** The compounds of the present disclosure can be made according to the following methods. A skilled artisan will understand that such methods can be implemented in any of numerous ways. While a certain order may be implied, the methods may be arranged according to any

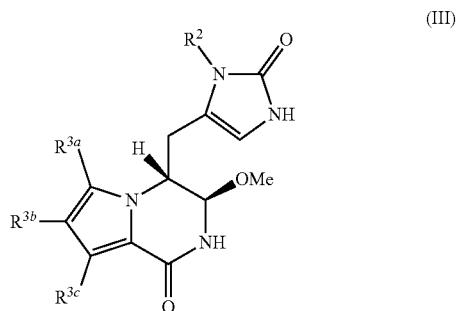
suitable sequence. Embodiments, ordered in a manner different from those explicitly described are encompassed by the present disclosure.

**[0238]** In various embodiments, the present disclosure provides a method of making a compound of claim 1, comprising addition of a nucleophile to an iminium intermediate of Formula (II):

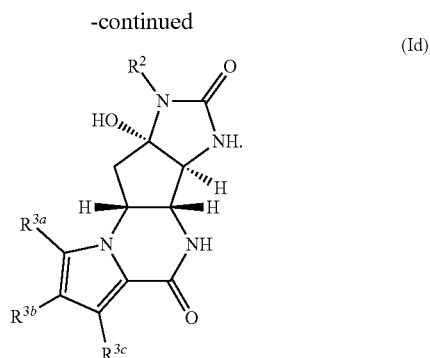


**[0239]** In various embodiments, the nucleophile added to Formula (II) is  $R^1-XH$ , wherein X is  $-O-$ ,  $-S-$ , or  $-N(R^4)-$ . In some embodiments, X is  $-O-$ . In other embodiments, X is  $-S-$ . In still other embodiments, X is  $-N(R^4)-$ . In some embodiments, the nucleophile is the reaction solvent. In other embodiments, the nucleophile is used as a volume fraction ranging from about 1:1 to about 1:200 with reaction solvent, e.g., about 1:1, about 1:10, about 1:20, about 1:30, about 1:40, about 1:50, about 1:60, about 1:70, about 1:80, about 1:90, about 1:100, about 1:110, about 1:120, about 1:130, about 1:140, about 1:150, about 1:160, about 1:170, about 1:180, about 1:190, or about 1:200, including all ranges and subvalues therebetween. In other embodiments, the nucleophile is used in from about 1 equivalent (eq.) to about 50 equivalents compared to the reagents, e.g.; about 1 eq., about 5 eq., about 10 eq., about 15 eq., about 20 eq., about 25 eq., about 30 eq., about 35 eq., about 40 eq., about 45 eq., or about 50 eq., including all ranges and subvalues therebetween.

**[0240]** In various embodiments, the present disclosure provides a method of making a compound of Formula (I), comprising acid-promoted cyclization of a compound of Formula (II) to afford a compound of Formula (Id):

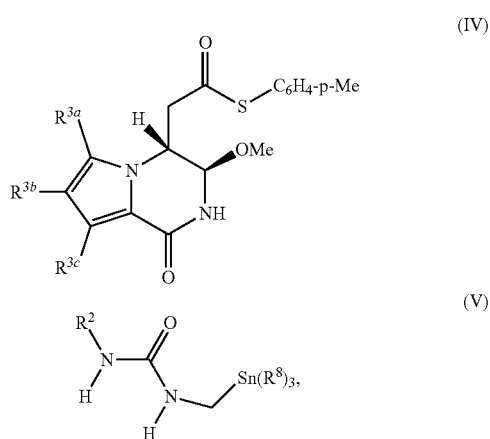






**[0241]** In various embodiments, the acid used to promote the acid-promoted cyclization is methanesulfonic acid, p-toluenesulfonic acid, sulfuric acid, hydrochloric acid, trifluoroacetic acid, trifluoromethanesulfonic acid, and nitric acid. In some embodiments, the acid is trifluoroacetic acid. In other embodiments, the acid is methanesulfonic acid. In still other embodiments, the acid is hydrochloric acid. In yet other embodiments, the acid is trifluoromethanesulfonic acid.

**[0242]** In various embodiments, the present disclosure provides a method of making compound (III), comprising copper-mediated coupling between a compound of Formula (IV) and a compound of Formula (V):



wherein  $R^8$  is alkyl or cycloalkyl. In some embodiments,  $R^8$  is alkyl. In other embodiments,  $R^8$  is cycloalkyl. In specific embodiments, the alkyl is n-butyl. In other specific embodiments, the cycloalkyl is cyclohexyl.

**[0243]** In various embodiments, the copper-mediated coupling is carried out with copper (I)-thiophene-2-carboxylate (CuTC) or copper(I) diphenylphosphinate (CuDPP). In specific embodiments, the copper-mediated coupling is carried out with CuTC.

**[0244]** In some embodiments, the copper-mediated coupling further comprises treatment with an acid.

**[0245]** The compounds of the present disclosure (e.g., compound of Formula I-Ie), or a pharmaceutically acceptable salt, enantiomer, hydrate, solvate, prodrug, isomer, or tautomer thereof, may be prepared as described herein and modified by methods known in the art of organic synthesis

as set forth in part by the following synthetic schemes. In the schemes described below, it is well understood that protecting groups for sensitive or reactive groups are employed where necessary in accordance with general principles or chemistry. Protecting groups are manipulated according to standard methods of organic synthesis (T. W. Greene and P. G. M. Wuts, "Protective Groups in Organic Synthesis", Third edition, Wiley, New York 1999, which is hereby incorporated by reference in its entirety). These groups are removed at a convenient stage of the compound synthesis using methods that are readily apparent to those skilled in the art. The selection processes, as well as the reaction conditions and order of their execution, shall be consistent with the preparation of disclosed compounds (e.g., compounds of Formula I-Ie).

**[0246]** Those skilled in the art will recognize if a stereocenter exists in the compounds of Formula I-Ie. Accordingly, the present disclosure includes both possible stereoisomers (unless specified in the synthesis) and includes not only racemic compounds but the individual enantiomers and/or diastereomers as well. When a compound is desired as a single enantiomer or diastereomer, it may be obtained by stereospecific synthesis or by resolution of the final product or any convenient intermediate. Resolution of the final product, an intermediate, or a starting material may be affected by any suitable method known in the art. See, for example, "Stereochemistry of Organic Compounds" by E. L. Eliel, S. H. Wilen, and L. N. Mander (Wiley-Interscience, 1994), which is hereby incorporated by reference in its entirety.

**[0247]** The compounds described herein may be made from commercially available starting materials or synthesized using known organic, inorganic, and/or enzymatic processes.

## EXAMPLES

**[0248]** The following examples are provided to illustrate the present disclosure, and should not be construed as limiting thereof. In these examples, all parts and percentages are by weight, unless otherwise noted. Abbreviations in the examples are noted below.

### Abbreviations

**[0249]** HPLC high performance liquid chromatography  
 mL milliliter  
 mmol millimole  
 MeOH methanol  
 NMR nuclear magnetic resonance  
 THF tetrahydrofuran  
 TLC thin layer chromatography

### Example 1: Synthesis and Biological Examination of New Agelastatin a and E Derivatives

#### **[0250]** Results and Discussion

**[0251]** In addition to studying other natural members of the agelastatin alkaloid family, the examination of new agelastatin derivatives in a 3D co-culture assay modeling the breast cancer microenvironment was desired.<sup>9</sup> Prompted by preliminary biological data (vide infra), it was of particular interest to examine derivatives which were accessible via late-stage modifications in the synthesis of agelastatins.<sup>3</sup> As illustrated in FIG. 2, the AgE derivative series 7 includes C5-ether variants that were envisioned to be prepared by

nucleophilic trapping of iminium ion 9.<sup>3a</sup> Complementarily, the AgA derivative series 8 (FIG. 2) includes N1-substituted analogs that were planned to be accessed via application of imidazolone D-ring synthesis methodology. Starting with the common thioester (+)-12 and urea derivative 13, rapid access to N-substituted pre-agelastatin 11 as the substrate for C-ring cyclization reaction was envisioned.<sup>3</sup>

[0252] Numerous agelastatin alkaloid derivatives were prepared (Table 1) through application of the synthesis of pre-agelastatin 11, which is subject to C-ring cyclization followed by C5-substitution. Under optimal conditions (vide infra), C5-ionization of AgA (1) followed by nucleophilic trapping afforded the C5-substituted AgE derivative series 7 (Table 1, entries 1-11). A versatile imidazolone synthesis methodology using a common thioester (+)-12 allowed introduction of the N1-substituent in pre-agelastatin 11, which after cyclization afforded the desired N1-substituted AgA derivative series 8 (Table 1, entries 12-16). These new derivatives offer diverse functional groups enabling further derivatization with potential for future studies concerning agelastatin alkaloids. Importantly, the synthesis of agelastatin derivatives 7 and 8 (vide infra) was informed and guided by concurrent biological studies of these alkaloids and their derivatives. Specifically, the biological evaluation of AgA (1) and AgE (5) disclosed herein has been critical to the design and synthesis of agelastatin derivatives including those illustrated in Table 1.

TABLE 1

Representative agelastatin derivatives prepared for this study.

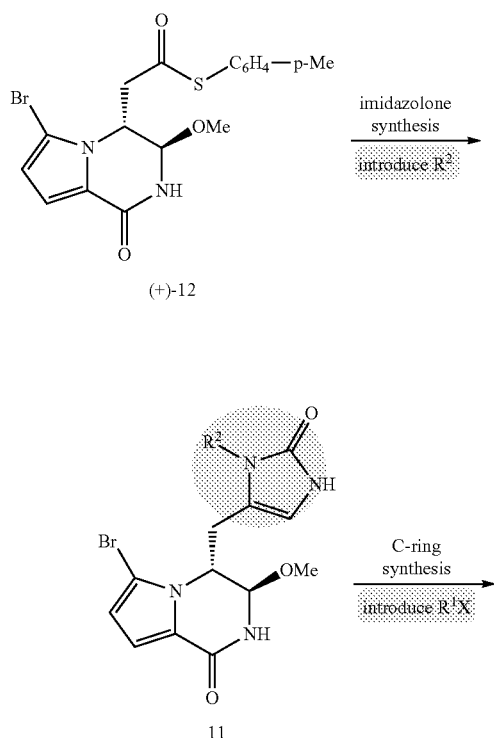
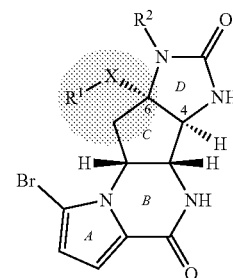


TABLE 1-continued

entry	substituent	agelastatin derivative
agelastatin E derivatives R <sup>2</sup> = Me		
1		7a
2		7b
3		7c
4		7d, n = 2
5		7e, n = 1
6		7f, n = 2
7		7g, n = 1
8		7h, n = 2
9		7i, n = 2
10		7j, R = Me
11		7k, R = Ph
agelastatin A derivatives R <sup>1</sup> X = OH		
12		8a, n = 1
13		8b, n = 3



7, 8, 14

TABLE 1-continued

14		8c
15		8d
16		8e, R <sup>1</sup> X = N <sub>3</sub>
agelastatin derivative		
17		14

**[0253]** Studies of AgE in Breast Cancer Microenvironments.

**[0254]** In the aforementioned 3D assay system, AgA (1) at low concentrations abrogates the effects of up-regulated fibroblast OPN on cancer cell adhesion, invasion, and cancer stem cell populations. Cancer cells exposed in co-culture to fibroblasts with up-regulated OPN demonstrated increased levels of lung metastasis in murine xenograft models, which were completely blocked by AgA (1) treatment of the co-cultures.<sup>9</sup> Given the interest in examining other agelastatins for activity in blocking vitamin D-induced OPN transcription in fibroblasts, the other natural members of the agelastatin alkaloid family, (-)-agelastatins B-F (2-6, FIG. 1) were first examined, prepared as described in earlier synthetic studies.<sup>3</sup> It was found that AgA (1) and AgE (5) had the desired activity, suggesting a possible negative influence by N<sub>1</sub>-dealkylation (AgD and AgF), C4-hydroxylation (AgC), or C14-bromination (AgB and AgF) of the agelastatin core.<sup>14</sup> Interestingly, it was found that AgE (5) consistently blocked stimulated OPN transcription and demonstrated increased potency (FIG. 3) in comparison to AgA (1) in two dimensional (2D) cultures, prompting future examination of new C5-substituted agelastatins in blocking OPN transcription in fibroblasts.

**[0255]** Furthermore, the efficacy of AgE (5) was tested in blocking the effects of up-regulated fibroblast OPN in 3D co-cultures with the breast cancer cell line SUM1315. Cancer cells were co-cultured in 3D media mixture with reduction mammary fibroblasts (RMF) with either wild-type retroviral hairpin control vector (C-RMF) or Tiam1 silencing hairpin vector (shTiam-RMF). While both control and Tiam1-deficient fibroblasts secrete OPN to some degree, fibroblasts with Tiam1 silencing have up-regulated OPN.<sup>9,11</sup> Co-cultures were treated with dimethylsulfoxide (DMSO), AgA (1) at 75-nM concentration, or AgE (5) at 25-nM or 50-nM concentration.

**[0256]** In the co-cultures, fibroblasts and breast cancer cells aggregate together to form spheres, with the fibroblasts forming the interior core and the cancer cells on the exterior.<sup>12</sup> SUM1315 is an aggressive breast cancer cell line, and under these conditions the cancer cells form multicellular projections extending out into the 3D matrix, with the number and/or length of the projections indicating degree of invasiveness. Co-culture with Tiam1-deficient fibroblasts promotes increased invasiveness, increased cancer stem cell populations, and metastasis of breast cancer cells after isolation from co-culture and implantation into mice. This is consistent with increased OPN secretion, seen as increased numbers of projections per sphere (FIG. 4, column 5) as compared to control fibroblasts (FIG. 4, column 1). Incorporation of AgA (1) at 75-nM concentration with Tiam1-deficient fibroblasts (FIG. 4, column 6) decreases the number of projections to the baseline number seen with control fibroblast co-culture. Furthermore, including AgE (5) at 25-nM concentration (FIG. 4, column 7) partially restores the number of projections toward baseline, while AgE (5) at 50-nM concentration (FIG. 4, column 8) decreases the number of projections to below the baseline condition. Treatment of co-cultures of SUM1315/Tiam1-deficient human mammary fibroblasts with AgA prevents subsequent lung metastasis of breast cancer cells when implanted into immune deficient mice. Excitingly, these results suggest that AgE (5) is more potent than AgA (1) in decreasing the invasiveness induced by Tiam1-deficient fibroblasts.

**[0257]** Breast cancer cells from the 3D co-cultures were isolated to greater than 990/o purity as described previously<sup>11,9</sup> for further assessment. Adhesion of these post-co-culture (PCC) cells was assessed through transwell migration assay (FIG. 5). As with the invasion assay results, migration was notably increased in PCC cells exposed to Tiam1 I-deficient fibroblasts (FIG. 5, column 5) compared with PCC cells exposed to control fibroblasts (FIG. 5, column 1), and this was blocked by incorporation of AgA (1) at 75-nM concentration in the co-cultures (FIG. 5, column 6). Significantly, including AgE (5) at 25-nM and 50-nM concentrations (FIG. 5, columns 7-8) also decreased the migration, with the 50-nM treatment decreasing the number of migrating cells below the baseline condition.

**[0258]** Two assays for breast cancer stem cell populations include tumorsphere formation in low adherence culture conditions and flow cytometry for specific cell surface markers (CD44<sup>+</sup>/CD24<sup>-</sup>/ESA<sup>+</sup>). Results of both assays on the PCC cells showed analogous findings to the aforementioned invasion and migration assays. Incorporation of AgA (1) at 75-nM concentration completely blocked the increased numbers of tumorspheres (FIG. 6, column 6) or cancer stem cells (FIG. 7, column 6) induced by Tiam1-deficient fibroblasts, compared with control fibroblasts (FIGS. 6-7, column 1). Incorporation of AgE (5) at 25-nM concentration had a partial effect (FIGS. 6-7, column 7), while AgE (5) at 50-nM concentration decreased tumorsphere (FIG. 6, column 8) and cancer stem cell (FIG. 7, column 8) numbers below baseline. Significantly, these results consistently suggest that AgE (5) is more potent than AgA (1) in blocking the effects of fibroblast OPN on the invasiveness, migration potential, and cancer stem cell populations in associated breast cancer cells.

**[0259]** The next tests were directed to whether direct treatment of mice with agelastatins could block the lung

metastasis seen with the mixed cell xenograft model (co-implantation of breast cancer cell line SUM1315 and Tiam1-deficient human).

**[0260]** The initial experiments with serial-treated mice bearing breast cancer xenografts with AgA at 2.5 mg/kg (the only published dose in the literature) induced toxicity in the mice within 2 weeks of treatment initiation. In the next studies, mice were treated with 3 lower doses of AgA (1.25 mg/kg, 0.625 mg/kg, and 0.25 mg/kg) in DMSO using 5×/week IP injection (5 mice/cohort) and observed for toxicity (FIG. 9). All mice gained weight as expected and none exhibited physical signs of toxicity, such as fur ruffling, hunching, decreased movement. Next, the direct treatment of mixed cell xenograft-bearing mice with AgA was examined. Mixed cell tumor-fibroblast xenografts were established in all mice and after healing from implantation surgery, treatment of 3 cohorts with AgA or DMSO vehicle was initiated (5 mice/cohort) (FIG. 10). All mice gained weight as expected (FIG. 10A) and final tumor weights at the implantation site were the same in all cohorts at necropsy, indicating no effect on primary tumorigenesis (FIG. 10B). The effects of AgA on lung metastasis in mixed cell xenograft-bearing mice was examined next (FIG. 11). Lung sections (FFPE) from mice shown in FIG. 10 were examined using hematoxylin & eosin and vimentin staining (SUM1315 exhibits strong vimentin expression) and clusters of metastatic cells counted. Two lung sections/mouse were examined; data represent numbers/individual lung section. As can be seen from FIG. 11, at 1.25 mg/kg, almost 800 of mice had 0 metastases and the remaining mice all had between 1 and 5 (compared to about 40 of mice having >10 metastases in the control group).

**[0261]** The direct treatment of mixed cell xenograft-bearing mice with AgE was next tested (FIG. 12). Mixed cell tumor-fibroblast xenografts were established in all mice and after healing from implantation surgery, treatment of 3 cohorts with AgE or DMSO vehicle was initiated as indicated (8 mice per cohort). All mice gained weight as expected (FIG. 12A) and growth kinetics of tumor growth at the implantation site were the same in all cohorts, again indicating no effect on primary tumorigenesis (FIG. 12B).

**[0262]** The effects of AgE on lung metastasis in mixed cell xenograft-bearing mice was examined next (FIG. 13). Lung sections (FFPE) from mice shown in FIG. 10 were examined using hematoxylin & eosin and vimentin staining (SUM1315 exhibits strong vimentin expression) and clusters of metastatic cells counted. Two lung sections/mouse were examined; data represent numbers/individual lung section. As can be seen from FIG. 13, at 1.25 mg/kg, all mice had 0 metastases and about 80% of mice at 0.625 mg/kg had 0 metastases (compared to about 60% of mice having >10 metastases in the control group).

**[0263]** The above data indicate that both AgA and AgE are effective in suppressing/preventing lung metastasis at tolerable doses in the mixed cell xenograft model. At the doses used, AgA and AgE do not significantly impair primary tumorigenesis. Metastatic spread of cancer is primary cause of cancer death and there are no drugs currently available directed against cancer metastasis. This is a significant unmet clinical need. The data suggest that AgA, AgE, and derivatives may therefore have important uses in the adjuvant setting—i.e. prevention of metastatic spreading—in conjunction with other treatment(s) directed at the primary tumor.

**[0264]** Lastly, the effect of Tiam1-deficient fibroblasts and agelastatin inhibition in breast cancer co-cultures was examined (FIG. 14). Human breast cancers consist of a group of sub-types that are distinguished clinically by cellular expression of estrogen receptor (ER), progesterone receptor (PR), and HER2. This is often recapitulated in experimental models with a panel of breast cancer cell lines expressing different immunophenotypes, and are often classified as luminal A or B sub-types (ER and/or PR+), HER2+, unclassified triple-negative (ER-PR- HER2-, EGFR or CK5/6-) and basal-like (ER-PR-HER2-, EGFR or CK5/6+). The 3D co-culture model has been used to test representative lines of each sub-type for responsiveness to the fibroblast Tiam1-osteopontin pathway and agelastatin inhibition:

SUM 1315	triple negative
SUM159	triple negative
MCF7	luminal A
BT474	luminal B
SKBR3	HER2
MCF10A	basal

**[0265]** Each cancer cell line was established in co-culture with either control or Tiam1-deficient fibroblasts to form the mixed cell spheroids, and treated with DMSO or agelastatin A or E as indicated. Invasiveness is assessed by the formation of multi-cellular projections emanating from the spheroid into the 3D media, and is scored by assessing proportions of spheroids exhibiting increasing numbers of projections. It was previously shown that changes in invasiveness correlate with changes in migration, cancer stem cells, and metastatic potential. For each cell line, co-culture with Tiam-deficient fibroblasts increases the invasiveness of the line, and treatment with agelastatin A or E blocks the increased invasiveness. The degree of the effects vary across the lines, with SUM1315 and BT474 being most sensitive, and MCF7 and MCF10A being least sensitive. This is not unexpected, as the co-culture conditions were optimized for SUM1315. It is likely that varying the co-culture conditions would affect the behavior of each cell line in the system and potentially augment degree of response. Nevertheless, all cell lines responded to the fibroblast co-culture in the same way, suggesting that treatment with agelastatins A, E, and their respective derivatives will be effective across the range of breast cancer sub-types.

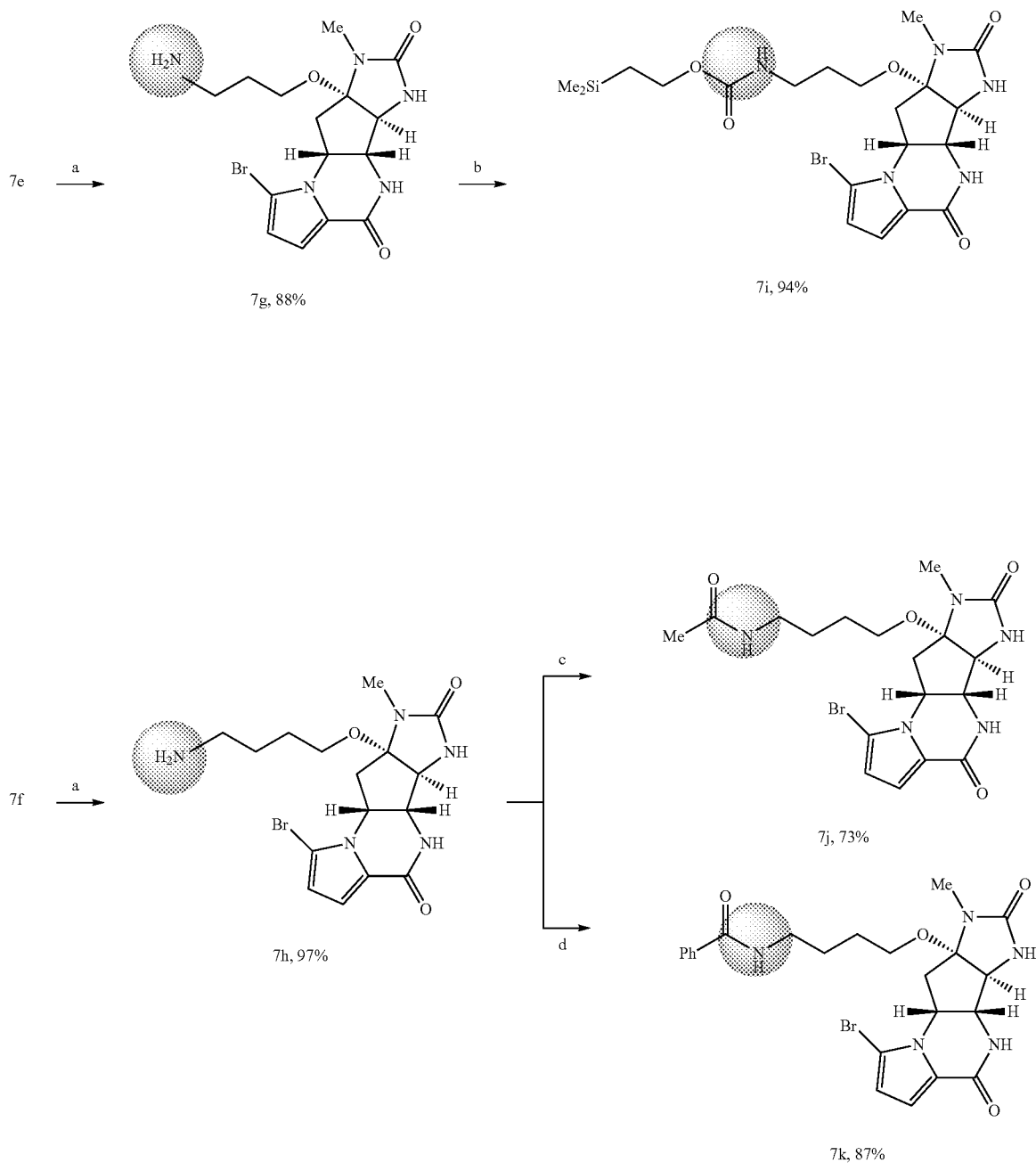
**[0266]** With increased understanding of the effects of the agelastatin alkaloids in breast cancer microenvironments and on preventing and delaying the spread of metastasis, particularly the observation that AgE (5) showed increased potency as compared to AgA (1), a series of agelastatin derivatives was prepared (Table 1) and their biological effects on breast cancer invasiveness were studied.

**[0267]** Development of Agelastatin E Derivatives.

**[0268]** By treatment of AgA (1) with methanesulfonic acid to promote the formation of C5-iminium ion 9 (FIG. 2), followed by in situ trapping with a series of nucleophiles, the agelastatin derivatives 7a-7f (Table 2) were synthesized. Condensation of commercially available 3-butyln-1-ol and 3-buten-1-ol with AgA (1) provided the desired derivatives 7a and 7b, respectively (Table 2, entries 1-2). The use of 3-mercaptopropiophenone<sup>15</sup> as the nucleophile afforded the C5-sulfide derivative 7c (Table 2, entry 3). The carbamate derivative 7d was prepared using the corresponding trimethylsilyl ethoxy carbamate-protected 4-aminobutan-1-ol as



Scheme 1 Chemical diversification of AgE derivatives 7e-7h.



Conditions: (a) PPh<sub>3</sub>, THF — H<sub>2</sub>O (9:1). (b) 4-nitrophenyl 2-(trimethylsilyl)ethyl carbonate, NEt<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, (c) Ac<sub>2</sub>O, NEt<sub>3</sub>, DMAP, THF. (d) BzCl, NEt<sub>3</sub>, THF.

**[0270]** Development of Agelastatin A Derivatives.

**[0271]** The preparation of the urea-based organostannane reagent 13 (Table 3) was necessary for the introduction of substituents at the N1-position of agelastatin. The use of substituted urea 13 enabled access to the corresponding

N1-substituted pre-agelastatins en route to the desired N1-substituted agelastatins. Through the use of 1,1'-carbonyldiimidazole<sup>17</sup> as a phosgene equivalent, the versatile intermediate 16 was accessed and converted to substituted ureas 13a-13c upon treatment with the desired primary amine (Table 3).<sup>3,18</sup>

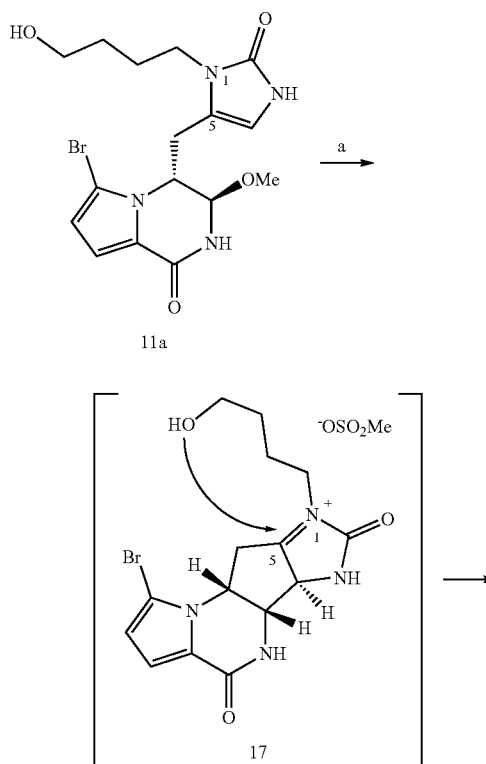
TABLE 3

Synthesis of substituted ureas 13a-13c. Conditions: (a) 1,1'-carbonyldiimidazole, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 85%. (b) DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 40° C.

entry		derivative	yield
1		13a, n = 1	91%
2		13b, n = 3	95%
3		13c	84%

**[0272]** The copper-mediated coupling of substituted urea 13 with versatile thioester (+)-12 directly afforded the N1-substituted imidazolone 11 that served as the substrate for the C-ring cyclization chemistry to afford the N1-substituted AgA derivative series 8 (Table 4).<sup>3</sup> The use of N1-substituents with a primary alcohol functional group was envisioned to enable post-cyclization diversification of the agelastatin core in analogy with the C5-ether series. Pre-agelastatins 11b and 11c provided modest yield of the corresponding N1-substituted agelastatin derivatives 8b and 8c, respectively (Table 4, entries 2-3). Interestingly, the shorter 4-methylene spacer pre-agelastatin 11a provided the N1-substituted AgA derivative 8a (Table 4, entry 1) along with the pentacyclic agelastatin derivative 14 (Scheme 2). The inefficient formation of N1-substituted agelastatin derivative 8a is likely due to competitive intramolecular trapping of the C5-iminium ion 17 to afford the pentacyclic derivative 14 (Scheme 2). Notably, ether 14 serves as a link between AgE and AgA derivatives, including both N1- and C5-modifications. An anticipated slower rate of intramolecular cyclization using the longer N1-substituents in pre-agelastatins 11b and 11c is consistent with the observed greater, albeit modest, yield of the corresponding derivatives 8b and 8c, respectively (Table 4).

Scheme 2 Observed double cyclization of tricycle 11a to pentacycle 14. Conditions: (a) MeSO<sub>3</sub>H, H<sub>2</sub>O, 100° C.

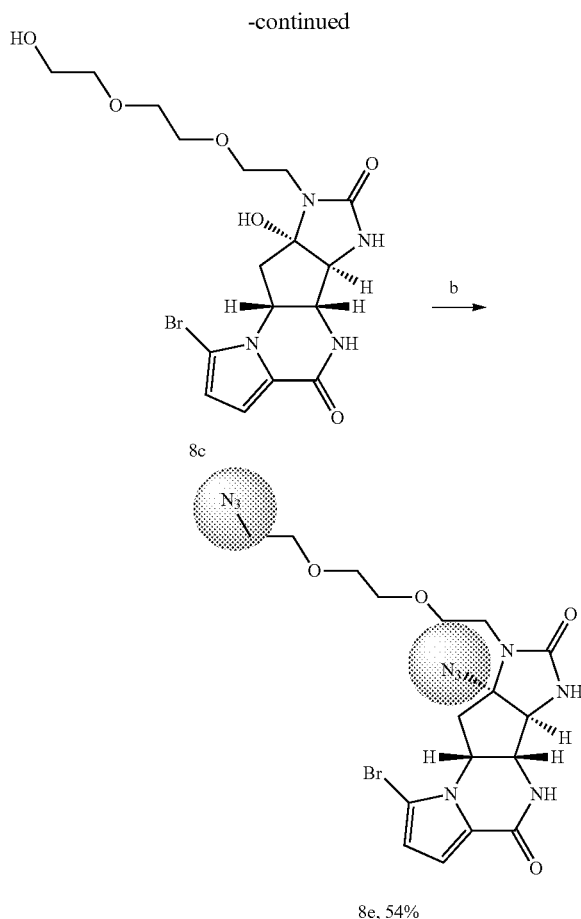
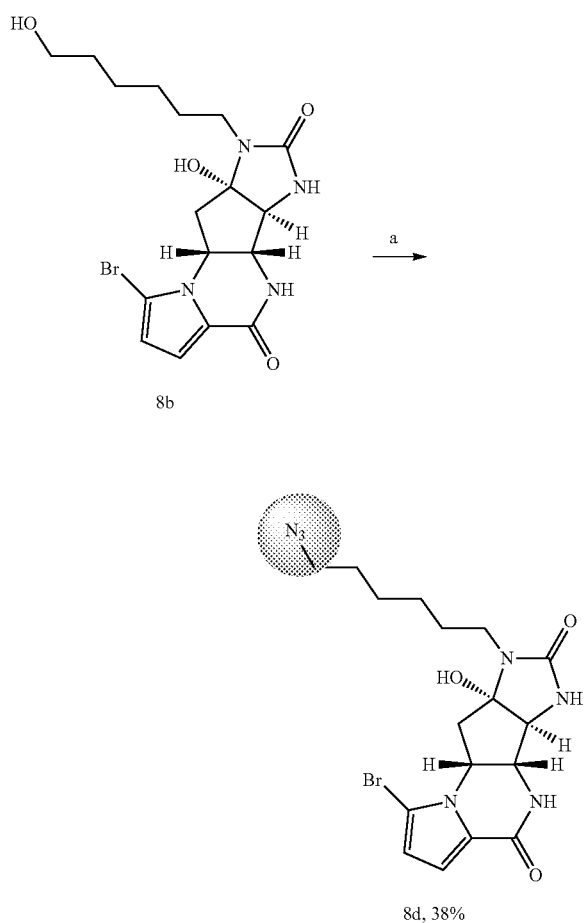






[0273] The primary alcohol of the N1-substituent of AgA derivatives 8a-8c presents an opportunity for introduction of an azide functional group that may be used in the future for further modifications similar to those accomplished in the AgE derivative series 7. Indeed, using the more readily accessible agelastatin derivatives 8b and 8c the corresponding azide derivative was prepared in a single step (Scheme 3). The primary alcohol 8b was converted to the agelastatin azide 8d in 38% yield, along with 34% recovery of the starting material (72% BRSM). When using more forcing conditions required for complete conversion of the more recalcitrant triethyleneglycol derivative 8c to the corresponding azide, the formation of the bis-azide 8e consistent with an additional C5-azidation was observed. Interestingly, mass spectrometric analysis of bis-azide 8e shows consistency with other AgA derivatives in formation of its corresponding C5-iminium ion as a major observed molecular ion. This was the first example of azide substitution at the C5-position of the agelastatin alkaloids. The covalent linkage of the alcohol and azide functional groups offered in the AgA derivatives 8a-8e was designed to be complementary to the ionizable linkage present in the AgE derivative series 7.

Scheme 3 Chemical diversification of AgA derivatives 8b and 8c to the corresponding azides.



Conditions:  $\text{PPh}_3$ , (X equiv), diisopropylazodicarboxylate (X equiv), diphenyl phosphoryl azide (X equiv), THF. (a) X = 2, 38% (isolated along with 34% recovered starting material). (b) X = 10, 54%.

#### [0274] Biological Study of Agelastatin Alkaloid Derivatives.

[0275] The synthesized Agelastatin derivatives were compared to AgA (1) and AgE (5) for efficacy in blocking vitamin D-induced OPN transcription. The results of these investigations with selected and most informative derivatives are summarized in FIG. 8. Of particular interest in these screens were the AgE derivative carbamate 7d and the AgA derivative azide 8d. In further studies of AgA derivatives the triethyleneglycol linked bis-azide 8e showed improved efficacy in the assays. Indeed, bis-azide 8e at 94-nM concentration demonstrates statistical equivalence to AgE (5) at 50-nM concentration in blocking vitamin D-induced OPN transcription. The AgE derivatives 7i-7k (Scheme 1) were designed in an effort to better understand the promising potency of carbamate 7d in preliminary assays. The aim was to differentiate between the different aspects of the linker that led to increased potency, such as the electronic properties, steric bulk, and linker length. Interestingly, the 4-methylene linked carbamate 7d maintained slightly improved potency compared to the related 3-methylene linked carbamate 7i, consistent with the notion that the linker length is important to maintaining the desired inhibitory activity in such AgE derivatives. While the acetamide derivative 7j maintains the substituent chain length of car-

bamate 7d, its comparatively decreased activity suggests that the acetamide group is not as effective as the larger trimethylsilylethoxy substituent of carbamate 7d. Simple benzamide derivative 7k excitingly shows the desired potency comparable to carbamate 7d by preserving the substituent chain length of carbamate 7d while offering a larger amide group as compared to acetamide 7j. Notably, these results highlight the notion that agelastatin derivitization (such as N1- and C5-substitution) are not only tolerated in agelastatins with potency in modulation of breast cancer invasiveness, but also have already offered ample opportunities to access compounds that begin to approach the newly discovered potency of AgE (5) in this context. Importantly, the chemistry described herein can easily be modified (for example by using different nucleophile classes) to enable access to a wide range of new synthetic agelastatin derivatives (e.g., AgA and AgE derivatives) as potential modulators of breast cancer invasion and metastasis.

### CONCLUSIONS

**[0276]** It was demonstrated that (-)-AgE (5) is more potent than (-)-AgA (1) in blocking fibroblast-mediated effects on cancer cell invasion, migration, and cancer stem cell populations. Importantly, non-cytotoxic doses were established for delivery of agelastatin alkaloids to breast cancer microenvironments in order to study their activity in blocking the induced OPN transcription in fibroblasts that can modulate these cancer cell behaviors. Based on the exciting recognition of the potent activity of AgE (5) and AgA (1) in this context, the synthesis of a variety of C5- and N1-substituted agelastatin derivatives were prepared, culminating in the AgE and AgA derivative series 7 and 8, respectively (Table 1). Highlights of the synthetic strategy include: 1) efficient C5-derivatization of AgA (1) using functional nucleophiles based on the conversion of AgA (1) to AgE (5),<sup>3</sup> 2) selective N1-functionalization using imidazolone synthesis methodology,<sup>3</sup> 3) diversification of complex agelastatin derivatives and establishment of precedence for access to more complex synthetic derivatives. Furthermore, it is demonstrated that new synthetic derivatives 7d and 7k (100-nM concentration) as well as derivative 8e (94-nM concentration) are statistically equivalent to AgE (5) at 50-nM concentration. The chemistry described here provides a foundation for rapid access to agelastatin derivatives with high potency (50-100 nM) as modulators for cancer invasion and metastasis. These findings highlight the outstanding potential for development of potent agelastatin derivatives with functional handles for further chemical derivatization and biological applications.

### Example 2. Experimental

**[0277]** General Methods.

**[0278]** All reactions were performed in oven-dried or flame-dried round-bottom flasks. The flasks were fitted with rubber septa, and reactions were conducted under a positive pressure of argon. Cannulae or gas-tight syringes with stainless steel needles were used to transfer air- or moisture-sensitive liquids. Where necessary (so noted) solutions were deoxygenated by argon purging for a minimum of 10 min. Flash column chromatography was performed as described by Still et al.<sup>19</sup> using granular silica gel (60-Å pore size, 40-63 μm, 4-6% H<sub>2</sub>O content). Analytical thin layer chromatography (TLC) was performed using glass plates pre-

coated with 0.25 mm 230-400 mesh silica gel impregnated with a fluorescent indicator (254 nm). Thin layer chromatography plates were visualized by exposure to short wave ultraviolet light (254 nm) and irreversibly stained by treatment with an aqueous solution of ceric ammonium molybdate (CAM) or an aqueous solution of potassium permanganate (KMnO<sub>4</sub>) or an alcoholic solution of ninhydrin, followed by heating (~1 min) on a hot plate (~250° C.). Organic solutions were concentrated at 29-30° C. on rotary evaporators capable of achieving a minimum pressure of ~2 Torr, then at ~0.5 Torr (vacuum pump) unless otherwise indicated. Proton (<sup>1</sup>H) and carbon (<sup>13</sup>C) nuclear magnetic resonance spectra were recorded with 600 MHz, 500 MHz and 400 MHz spectrometers. Proton nuclear magnetic resonance (<sup>1</sup>H NMR) spectra are reported in parts per million on the δ scale and are referenced from the residual proton in the NMR solvent [CDCl<sub>3</sub>: δ 7.26 (CDCl<sub>3</sub>), CD<sub>3</sub>OD: δ 3.31 (CD<sub>2</sub>HOD), DMSO-d<sub>6</sub>: δ 2.50 (DMSO-d<sub>5</sub>)]. Data are reported as follows: chemical shift [multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet), coupling constant(s) in Hertz, integration]. Carbon-13 nuclear magnetic resonance (<sup>13</sup>C NMR) spectra are recorded in parts per million on the δ scale and are referenced from the carbon signals of the solvent (CDCl<sub>3</sub>: δ 77.16, CD<sub>3</sub>OD: δ 49.15, DMSO-d<sub>6</sub>: δ 39.52). Data are reported as follows: chemical shift. Infrared data (IR) were obtained with a FTIR and are reported as follows: [frequency of absorption (cm<sup>-1</sup>), intensity of absorption (s=strong, m=medium, w=weak, br=broad)]. High-resolution mass spectrometric data (HRMS) were recorded on a FT-ICR-MS spectrometer using electrospray ionization (ESI) source or direct analysis in real time (DART) ionization source.

**[0279]** General Procedure for Synthesis of AgE Derivatives 7a-7f.

**[0280]** Methanesulfonic acid (10 equiv) was added slowly to a solution of (-)-agelastatin A (1 equiv) and nucleophile in acetonitrile or dichloromethane. Upon consumption of starting material as shown by thin layer chromatography, the reaction mixture was diluted with ethyl acetate or dichloromethane as indicated (10 mL). Reactions conducted over molecular sieves were filtered through a plug of cotton and further diluted with the indicated solvent (10 mL). The crude organic mixture was washed sequentially with saturated aqueous sodium bicarbonate (2×15 mL) and saturated aqueous sodium chloride (1×10 mL). The combined aqueous layers were extracted with organic solvent (2×10 mL). The combined organic layers were dried over anhydrous sodium sulfate, were filtered, and were concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel to afford agelastatin derivatives 7a-7f.

**[0281]** Alkyne Derivative 7a was synthesized according to the general procedure for synthesis of AgE derivatives using 3-butyne-1-ol (0.5 mL) and acetonitrile (2.5 mL) over 4 Å molecular sieves (30 mg). After 17.5 h, the crude residue after work-up using ethyl acetate was purified by flash column chromatography on silica gel (eluent: 10% acetone in dichloromethane, then 5%→10% methanol in dichloromethane) to afford alkyne 7a (10.0 mg, 87%) as a white solid. <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD, 23° C.): δ 6.92 (d, J=4.1 Hz, 1H), 6.34 (d, J=4.1 Hz, 1H), 4.62 (dt, J=12.1, 6.0 Hz, 1H), 4.15-4.07 (m, 2H), 3.53-3.44 (m, 1H), 3.38-3.31 (m, 1H), 2.82 (s, 3H), 2.73-2.65 (m, 1H), 2.47 (ddt, J=6.8, 4.3, 2.4 Hz, 2H), 2.32 (t, J=2.7 Hz, 1H), 2.23-2.14 (m, 1H). <sup>13</sup>C

NMR (126 MHz, CD<sub>3</sub>OD, 23° C.): δ 161.8, 161.1, 124.2, 116.2, 114.0, 107.5, 99.9, 82.0, 71.0, 62.8, 62.3, 61.8, 53.8, 39.2, 24.9, 20.5. FTIR (thin film) cm<sup>-1</sup>: 2930 (w), 1667 (s), 1551 (w), 1425 (m), 1098 (w), 747 (w). HRMS (ESI) (m/z): calc'd for C<sub>16</sub>H<sub>18</sub>BrN<sub>4</sub>O<sub>3</sub>, [M+H]<sup>+</sup>: 393.0557, found: 393.0552. TLC (10% methanol in dichloromethane), Rf: 0.69 (UV, CAM).

**[0282]** Alkene Derivative 7b was synthesized according to the general procedure for synthesis of AgE derivatives using 3-buten-1-ol (0.5 mL) and acetonitrile (2.5 mL) over 4 Å molecular sieves (30 mg). After 18 h, the crude residue after work-up using ethyl acetate was purified by flash column chromatography on silica gel (eluent: 10% acetone in dichloromethane, then 5%→10% methanol in dichloromethane) to afford alkene 7b (8.1 mg, 68%) as a white solid. <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD, 23° C.): δ 6.91 (d, J=4.1 Hz, 1H), 6.33 (d, J=4.2 Hz, 1H), 5.84 (td, J=15.4, 14.5, 6.5 Hz, 1H), 5.17-5.00 (m, 2H), 4.61 (dt, J=12.2, 5.9 Hz, 1H), 4.18-4.02 (m, 2H), 3.42 (q, J=7.6 Hz, 1H), 3.31-3.21 (m, 1H), 2.78 (s, 3H), 2.66 (dd, J=13.4, 6.5 Hz, 1H), 2.35 (q, J=6.8 Hz, 2H), 2.16 (t, J=12.8 Hz, 1H). <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD, 23° C.): δ 161.9, 161.1, 136.3, 124.2, 117.5, 116.2, 114.0, 107.5, 99.8, 63.8, 62.3, 61.7, 53.8, 39.3, 35.1, 24.9. FTIR (thin film) cm<sup>-1</sup>: 2926 (w), 1668 (s), 1551 (w), 1425 (m), 1091 (w), 747 (w). HRMS (ESI) (m/z): calc'd for C<sub>16</sub>H<sub>20</sub>BrN<sub>4</sub>O<sub>3</sub>, [M+H]<sup>+</sup>: 395.0713, found: 395.0692. TLC (10% methanol in dichloromethane), Rf: 0.43 (UV, CAM).

**[0283]** Sulfide Derivative 7c was synthesized according to the general procedure for synthesis of AgE derivatives using 3-mercaptopropiophenone<sup>15</sup> (99.7 mg, 6.00×10<sup>2</sup> μmol, 20.5 equiv) in acetonitrile (3.0 mL) over 4 Å molecular sieves (20 mg). After 1 h, the crude residue after work-up using ethyl acetate was purified by flash column chromatography on silica gel (eluent: 0%→10% methanol in dichloromethane) to afford sulfide 7c (13.6 mg, 93%) as a white solid. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD, 23° C.): δ 8.04-7.94 (m, 2H), 7.61 (d, J=7.4 Hz, 1H), 7.51 (dd, J=8.3, 7.0 Hz, 2H), 6.91 (d, J=4.1 Hz, 1H), 6.32 (d, J=4.1 Hz, 1H), 4.78 (dt, J=11.8, 6.1 Hz, 1H), 4.47 (s, 1H), 4.19 (d, J=5.4 Hz, 1H), 3.35 (t, J=6.9 Hz, 2H), 2.91 (dd, J=12.5, 6.9 Hz, 1H), 2.87 (s, 3H), 2.81-2.67 (m, 2H), 1.95 (dd, J=13.5, 11.7 Hz, 1H). <sup>13</sup>C NMR (101 MHz, CD<sub>3</sub>OD, 23° C.): δ 199.7, 161.6, 161.0, 138.0, 134.7, 130.0, 129.3, 124.3, 116.3, 114.1, 107.5, 77.9, 67.5, 63.4, 54.8, 41.0, 38.8, 25.2, 24.1. FTIR (thin film) cm<sup>-1</sup>: 2920 (w), 2361 (w), 1667 (s), 1551 (w), 1423 (m), 1195 (w). HRMS (ESI) (m/z): calc'd for C<sub>21</sub>H<sub>22</sub>BrN<sub>4</sub>O<sub>3</sub>S, [M+H]<sup>+</sup>: 489.0591, found: 489.0595. TLC (5% methanol in dichloromethane), Rf: 0.46 (UV, CAM).

**[0284]** 4-Methylene Carbamate Derivative 7d was synthesized according to the general procedure for synthesis of AgE derivatives using 2-(trimethylsilyl)ethyl (4-hydroxybutyl)carbamate<sup>20</sup> (1.40×10<sup>2</sup> mg, 6.00×10<sup>2</sup> μmol, 20.5 equiv) in acetonitrile (3.0 mL) over 4 Å molecular sieves (25 mg). After 3 h, the crude residue after work-up using ethyl acetate was purified by flash column chromatography on silica gel (eluent: 20%→30% acetone in dichloromethane, then 5%→10% methanol in dichloromethane) to afford 4-methylene carbamate 7d (10.5 mg, 63%) as a white solid. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD, 23° C.): δ 6.89 (d, J=4.1 Hz, 1H), 6.31 (d, J=4.1 Hz, 1H), 4.59 (dt, J=12.1, 6.0 Hz, 1H), 4.17-4.05 (m, 4H), 3.36 (dt, J=9.0, 5.8 Hz, 1H), 3.24 (dt, J=9.1, 5.8 Hz, 1H), 3.08 (td, J=6.8, 4.3 Hz, 2H), 2.76 (s, 3H), 2.68-2.60 (m, 1H), 2.20-2.10 (m, 1H), 1.65-1.49 (m, 4H), 0.99-0.92 (m, 2H), 0.02 (s, 9H). <sup>13</sup>C NMR (101 MHz,

CD<sub>3</sub>OD, 23° C.): δ 161.9, 161.1, 159.5, 124.2, 116.2, 114.0, 107.5, 99.9, 63.9, 63.7, 62.3, 61.7, 53.8, 41.4, 39.4, 27.9, 27.8, 24.9, 18.8, -1.3. FTIR (thin film) cm<sup>-1</sup>: 2949 (w), 1669 (s), 1489 (w), 1423 (m), 1249 (m), 1106 (w), 835 (m), 747 (m). HRMS (ESI) (m/z): calc'd for C<sub>22</sub>H<sub>35</sub>BrN<sub>5</sub>O<sub>5</sub>Si, [M+H]<sup>+</sup>: 556.1585, found: 556.1567. TLC (10% methanol in dichloromethane), Rf: 0.59 (UV, CAM).

**[0285]** 3-Methylene Azide Derivative 7e was synthesized according to the general procedure for synthesis of AgE derivatives using 3-azidopropan-1-ol<sup>16</sup> (415 mg, 4.10 mmol, 20.0 equiv) in dichloromethane (13 mL). The reaction mixture became homogeneous upon addition of methane-sulfonic acid. After 14 h, the reaction mixture was diluted with dichloromethane and quenched with aqueous sodium hydroxide (0.5 N, 20 mL) before general work-up procedure. The crude residue was purified by flash column chromatography on silica gel (eluent: 20% acetone in dichloromethane, then 5%→10% methanol in dichloromethane) to afford 3-methylene azide 7e (64.4 mg, 74%) as a white solid. <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>, 23° C.): δ 7.96 (s, 1H), 7.34 (s, 1H), 6.74 (d, J=4.0 Hz, 1H), 6.35 (d, J=4.0 Hz, 1H), 4.41 (dt, J=12.0, 5.9 Hz, 1H), 4.02 (d, J=5.4 Hz, 1H), 3.97 (d, J=1.8 Hz, 1H), 3.41 (td, J=6.6, 2.6 Hz, 2H), 3.28 (dt, J=9.3, 6.2 Hz, 1H), 3.24-3.17 (m, 1H), 2.65 (s, 3H), 2.56-2.51 (m, 1H), 1.99 (t, J=12.6 Hz, 1H), 1.79 (p, J=6.5 Hz, 2H). <sup>13</sup>C NMR (126 MHz, DMSO-d<sub>6</sub>, 23° C.): δ 158.7, 157.6, 123.6, 113.5, 112.0, 104.7, 97.5, 60.1, 59.5, 59.3, 51.9, 47.8, 37.7, 28.3, 23.9. FTIR (thin film) cm<sup>-1</sup>: 2928 (w), 2097 (w), 1666 (s), 1549 (w), 1423 (m), 1348 (w), 1107 (w), 746 (m). HRMS (DART) (m/z): calc'd for C<sub>15</sub>H<sub>19</sub>BrN<sub>7</sub>O<sub>3</sub>, [M+H]<sup>+</sup>: 424.0727, found: 424.0717. TLC (10% methanol in dichloromethane), Rf: 0.53 (UV, CAM).

**[0286]** 4-Methylene Azide Derivative 7f was synthesized according to the general procedure for synthesis of AgE derivatives using 4-azidobutan-1-ol<sup>16</sup> (276 mg, 2.40 mmol, 20.0 equiv) in dichloromethane (8.0 mL). The reaction mixture became homogeneous upon addition of methane-sulfonic acid. After 26 h, the reaction mixture was diluted with dichloromethane and quenched with aqueous sodium hydroxide (0.5 N, 20 mL) before general work-up procedure. The crude residue was purified by flash column chromatography on silica gel (eluent: 20% acetone in dichloromethane, then 5%→10% methanol in dichloromethane) to afford 4-methylene azide 7f (39.8 mg, 76%) as a white solid. <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>, 23° C.): δ 7.97 (s, 1H), 7.31 (d, J=2.0 Hz, 1H), 6.74 (d, J=3.9 Hz, 1H), 6.35 (d, J=4.0 Hz, 1H), 4.41 (dt, J=11.9, 6.0 Hz, 1H), 4.01 (d, J=5.4 Hz, 1H), 3.97 (d, J=2.2 Hz, 1H), 3.37-3.34 (m, 2H), 3.28-3.19 (m, 1H), 3.19-3.11 (m, 1H), 2.64 (s, 3H), 2.58-2.51 (m, 1H), 1.98 (t, J=12.5 Hz, 1H), 1.58 (s, 4H). <sup>13</sup>C NMR (126 MHz, DMSO-d<sub>6</sub>, 23° C.): δ 158.7, 157.6, 123.6, 113.5, 112.0, 104.7, 97.5, 61.6, 60.1, 59.4, 51.9, 50.5, 37.8, 26.3, 25.4, 24.0. FTIR (thin film) cm<sup>-1</sup>: 2925 (w), 2097 (w), 1668 (s), 1549 (w), 1424 (m), 1107 (w), 745 (w). HRMS (DART) (m/z): calc'd for C<sub>16</sub>H<sub>21</sub>BrN<sub>7</sub>O<sub>3</sub>, [M+H]<sup>+</sup>: 438.0884, found: 438.0875. TLC (18% methanol, 2% ammonium hydroxide in chloroform), Rf: 0.43 (UV, CAM).

**[0287]** General Procedure for Staudinger Reduction.

**[0288]** Triphenylphosphine (2.40 equiv) was added to a suspension of azide derivative (1 equiv) in tetrahydrofuran-water (9:1, 0.1 M). Upon consumption of starting material as shown by thin layer chromatography, reaction mixture was diluted with dichloromethane and concentrated under reduced pressure. Crude residue was purified by flash col-

umn chromatography on silica gel (eluent: 0→10% methanol in dichloromethane, then 18% methanol and 2% ammonium hydroxide in chloroform) to afford amines 7g-7h.

**[0289]** 3-Methylene Amine Derivative 7g was synthesized according to the general procedure for Staudinger reduction of azide derivatives using 3-methylene azide 7e (26.5 mg, 62.5  $\mu\text{mol}$ , 1 equiv). After 3.5 days, the crude residue was purified by flash column chromatography on silica gel to afford amine 7g (21.9 mg, 88%) as a white solid  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ , 23° C.):  $\delta$  6.91 (d, J=4.1 Hz, 1H), 6.33 (d, J=4.1 Hz, 1H), 4.62 (dt, J=12.0, 6.0 Hz, 1H), 4.16-4.04 (m, 2H), 3.50-3.37 (m, 1H), 3.30-3.27 (m, 1H), 2.79 (s, 3H) 2.74 (t, J=7.0 Hz, 2H), 2.71-2.63 (m, 1H), 2.16 (t, J=12.7 Hz, 1H), 1.76 (p, J=6.5 Hz, 2H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CD}_3\text{OD}$ , 23° C.):  $\delta$  161.9, 161.1, 124.2, 116.2, 114.0, 107.5, 99.9, 62.2 (2C), 61.7, 53.8, 39.9, 39.4, 33.3, 24.9. FTIR (thin film)  $\text{cm}^{-1}$ : 2925 (w), 2359 (w), 1695 (m), 1652 (s), 1550 (m), 1424 (m), 1096 (w), 745 (w). HRMS (DART) (m/z): calc'd for  $\text{C}_{16}\text{H}_{21}\text{BrN}_5\text{O}_3$ ,  $[\text{M}+\text{H}]^+$ : 398.0822, found: 398.0823. TLC (18% methanol, 2% ammonium hydroxide in chloroform), Rf: 0.07 (UV, CAM, ninhydrin).

**[0290]** 4-Methylene Amine Derivative 7h was synthesized according to the general procedure for Staudinger reduction of azide derivatives using 4-methylene azide 7f (29.0 mg, 66.2  $\mu\text{mol}$ , 1 equiv). After 3 days, the crude residue was purified by flash column chromatography on silica gel to afford 4-methylene amine 7h (26.6 mg, 97%) as a white solid.  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ , 23° C.):  $\delta$  6.91 (d, J=4.1 Hz, 1H), 6.33 (d, J=3.9 Hz, 1H), 4.61 (dt, J=12.1, 6.0 Hz, 1H), 4.12 (d, J=5.4 Hz, 1H), 4.08 (s, 1H), 3.38 (dt, J=9.1, 5.9 Hz, 1H), 3.26 (dt, J=9.1, 6.1 Hz, 1H), 2.78 (s, 3H), 2.71-2.63 (m, 3H), 2.15 (t, J=12.7 Hz, 1H), 1.69-1.50 (m, 4H).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CD}_3\text{OD}$ , 23° C.):  $\delta$  161.8, 161.1, 124.2, 116.2, 114.0, 107.5, 99.8, 63.9, 62.2, 61.7, 53.8, 42.3, 39.4, 30.3, 28.1, 24.9. FTIR (thin film)  $\text{cm}^{-1}$ : 2926 (w), 2359 (w), 1652 (s), 1550 (m), 1423 (s), 1303 (w), 1096 (m), 745 (m). HRMS (ESI) (m/z): calc'd for  $\text{C}_{16}\text{H}_{23}\text{BrN}_5\text{O}_3$ ,  $[\text{M}+\text{H}]^+$ : 412.0979, found: 412.0994. TLC (18% methanol, 2% ammonium hydroxide in chloroform), Rf: 0.08 (UV, CAM, ninhydrin).

**[0291]** 3-Methylene Carbamate Derivative 7i.

**[0292]** A solution of 4-nitrophenyl 2-(trimethylsilyl)ethyl carbonate<sup>21</sup> (5.8 mg, 21  $\mu\text{mol}$ , 1.2 equiv) in dichloromethane (20  $\mu\text{L}$ ) was added to a solution of 3-methylene amine 7g (6.8 mg, 17  $\mu\text{mol}$ , 1 equiv), triethylamine (3.6  $\mu\text{L}$ , 26  $\mu\text{mol}$ , 1.5 equiv), and 4-dimethylaminopyridine (0.4 mg, 3  $\mu\text{mol}$ , 0.2 equiv) in dichloromethane (170  $\mu\text{L}$ ). After 26 h. reaction mixture was diluted with dichloromethane (1 mL) and purified by flash column chromatography on silica gel (eluent: 20% acetone in dichloromethane, then 0→18% methanol and 2% ammonium hydroxide in chloroform) to afford 3-methylene carbamate 7i (8.7 mg, 94%) as a white solid.  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ , 23° C.):  $\delta$  6.92 (d, J=4.2 Hz, 1H), 6.34 (d, J=3.9 Hz, 1H), 4.61 (dt, J=12.1, 6.0 Hz, 1H), 4.15-4.10 (m, 3H), 4.08 (s, 1H), 3.43-3.34 (m, 1H), 3.30-3.11 (m, 3H), 2.78 (s, 3H), 2.71-2.63 (m, 1H), 2.17 (t, J=12.7 Hz, 1H), 1.77 (p, J=6.3 Hz, 2H), 1.03-0.93 (m, 2H), 0.05 (s, 9H).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CD}_3\text{OD}$ , 23° C.):  $\delta$  161.8, 161.1, 159.5, 124.2, 116.2, 114.0, 107.5, 99.9, 64.0, 62.2, 61.6 (2C), 53.8, 39.4, 39.0, 31.0, 24.9, 18.8, -1.3. FTIR (thin film)  $\text{cm}^{-1}$ : 2950 (w), 1698 (s), 1661 (s), 1552 (w), 1424 (m), 1250 (w), 838 (w). HRMS (DART) (m/z): calc'd for

$\text{C}_{21}\text{H}_{33}\text{BrN}_5\text{O}_5\text{Si}$ ,  $[\text{M}+\text{H}]^+$ : 542.1429, found: 542.1429. TLC (18% methanol, 2% ammonium hydroxide in chloroform), Rf: 0.63 (UV, CAM).

**[0293]** General Procedure for Acylation of Amines Acylating reagent (2.0 equiv) was added to a solution of amine derivative (1 equiv) and triethylamine (2.0 equiv) in tetrahydrofuran (400  $\mu\text{L}$ ). Upon complete conversion of starting material as shown by thin layer chromatography, reaction was diluted with dichloromethane (3 mL) and quenched with saturated aqueous sodium bicarbonate (3 mL). Layers were separated and aqueous was extracted with dichloromethane (3×5 mL). Combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure. Crude residue was purified by flash column chromatography on silica gel (eluent: 0→7% methanol in dichloromethane, then 9% methanol and 1% ammonium hydroxide in chloroform→18% methanol and 2% ammonium hydroxide in chloroform) to afford amides 7j-7k.

**[0294]** Acetamide Derivative 7j was synthesized according to the general procedure for acylation of amine derivatives using acetic anhydride and amine 7h (6.8 mg, 17  $\mu\text{mol}$ , 1 equiv) with 4-dimethylaminopyridine (0.4 mg, 3  $\mu\text{mol}$ , 0.2 equiv) additive. After 2.5 hours, the crude residue after work-up was purified by flash column chromatography on silica gel to afford acetamide 7j (5.5 mg, 73%) as a white solid.  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ , 23° C.):  $\delta$  6.91 (d, J=4.1 Hz, 1H), 6.33 (d, J=4.0 Hz, 1H), 4.61 (dt, J=12.1, 6.1 Hz, 1H), 4.12 (d, J=5.5 Hz, 1H), 4.10 (s, 1H), 3.42-3.36 (m, 1H), 3.29-3.12 (m, 3H), 2.79 (s, 3H), 2.70-2.63 (m, 1H), 2.15 (t, J=12.7 Hz, 1H), 1.93 (s, 3H), 1.66-1.53 (m, 4H).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CD}_3\text{OD}$ , 23° C.):  $\delta$  173.4, 161.9, 161.1, 124.2, 116.2, 114.0, 107.5, 99.9, 63.7, 62.2, 61.7, 53.8, 40.2, 39.4, 27.9, 27.4, 24.9, 22.7. FTIR (thin film)  $\text{cm}^{-1}$ : 2929 (w), 2359 (w), 1652 (s), 1550 (m), 1423 (m), 1373 (w), 1096 (w), 747 (w). HRMS (ESI) (m/z): calc'd for  $\text{C}_{18}\text{H}_{25}\text{BrN}_5\text{O}_4$ ,  $[\text{M}+\text{H}]^+$ : 454.1084, found: 454.1082. TLC (18% methanol, 2% ammonium hydroxide in chloroform), Rf: 0.34 (UV, CAM).

**[0295]** Benzamide Derivative 7k was synthesized according to the general procedure for acylation of amine derivatives using benzoyl chloride and amine 7h (7.1 mg, 17  $\mu\text{mol}$ , 1 equiv). After 3.5 hours, the crude residue after work-up was purified by flash column chromatography on silica gel to afford benzamide 7k (7.7 mg, 87%) as a white solid.  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ , 23° C.):  $\delta$  7.85-7.78 (m, 2H), 7.56-7.49 (m, 1H), 7.4-7.40 (m, 2H), 6.91 (d, J=4.1 Hz, 1H), 6.33 (d, J=3.9 Hz, 1H), 4.61 (dt, J=12.0, 6.0 Hz, 1H), 4.14 (s, 1H), 4.12 (d, J=5.4 Hz, 1H), 3.47-3.32 (m, 4H), 2.79 (s, 3H), 2.70-2.63 (m, 1H), 2.16 (t, J=12.7 Hz, 1H), 1.77-1.65 (m, 4H).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CD}_3\text{OD}$ , 23° C.):  $\delta$  170.5, 161.9, 161.1, 135.9, 132.7, 129.7, 128.4, 124.2, 116.2, 114.0, 107.5, 99.9, 63.7, 62.3, 61.7, 53.8, 40.8, 39.4, 28.0, 27.5, 24.9. FTIR. (thin film)  $\text{cm}^{-1}$ : 2933 (w), 2359 (w), 1700 (s), 1652 (s), 1550 (m), 1424 (m), 1096 (w), 712 (w). HRMS (ESI) (m/z): calc'd for  $\text{C}_{23}\text{H}_{27}\text{BrN}_5\text{O}_4$ ,  $[\text{M}+\text{H}]^+$ : 516.1241, found: 516.1225. TLC (18% methanol, 2% ammonium hydroxide in chloroform), Rf: 0.50 (UV, CAM).

**[0296]** Urea Intermediate 16.

**[0297]** 1,1'-Carbonyldiimidazole (362 mg, 2.23 mmol, 1.10 equiv) and 4-dimethylaminopyridine (37.3 mg, 305  $\mu\text{mol}$ , 0.150 equiv) were added sequentially to a solution of 1-(tricyclohexylstannyl)-methanamine (15) (theoretical: 808 mg, 2.03 mmol, 1 equiv)<sup>22</sup> in dichloromethane (34 mL). After 45 min, the reaction mixture was concentrated and the

crude residue was purified by flash column chromatography on silica gel (eluent: 30→75% ethyl acetate in hexanes) to afford urea intermediate 16 (847 mg, 85% over two steps) as a white crystalline solid. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>, 23° C.): δ 8.07 (s, 1H), 7.31 (s, 1H), 7.07 (s, 1H), 6.32 (t, J=5.5 Hz, 1H), 3.18-3.04 (m, 2H), 1.95-1.81 (m, 6H), 1.72-1.50 (m, 18H), 1.37-1.19 (m, 9H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>, 23° C.): δ 149.2, 135.7, 129.9, 116.3, 32.4, 29.3, 27.6, 27.2, 23.7. FTIR (thin film) cm<sup>-1</sup>: 3223 (w), 3036 (w), 2913 (s), 2843 (m), 1710 (s), 1288 (m), 1075 (m), 842 (m). HRMS (ESI) (m/z): calc'd for C<sub>23</sub>H<sub>40</sub>N<sub>3</sub>O<sub>3</sub>Sn, [M+H]<sup>+</sup>: 494.2188, found: 494.2223. TLC (50% ethyl acetate in hexanes), Rf: 0.27 (UV, CAM).

**[0298]** General Procedure for the Synthesis of Substituted Ureas.

**[0299]** Amine (1.00 equiv) and 4-dimethylaminopyridine (0.150 equiv) were added to a solution of urea intermediate 16 (1 equiv) in dichloromethane (0.2 M). The reaction flask was sealed with a Teflon wrapped glass stopper and heated to 40° C. Upon consumption of the starting materials as determined by thin layer chromatography, the reaction mixture was cooled to 23° C. and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel to afford substituted ureas 13a-13c.

**[0300]** 4-Methylene Alcohol Urea 13a was prepared according to the general procedure for synthesis of substituted ureas using 4-aminobutanol (156 μL, 1.69 mmol, 1.00 equiv). After 24 h, the crude residue was purified by flash column chromatography on silica gel (eluent: 30→100% ethyl acetate in hexanes) to afford 4-methylene alcohol urea 13a (790 mg, 91%) as a white solid. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>, 23° C.): δ 4.66 (s, 1H), 4.23 (t, J=4.8 Hz, 1H), 3.69 (q, J=5.6 Hz, 2H), 3.26 (q, J=6.3 Hz, 2H), 2.85-2.64 (m, 2H), 1.94-1.77 (m, 6H), 1.72-1.46 (m, 20H), 1.38-1.21 (m, 9H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>, 23° C.): δ 160.2, 62.3, 40.3, 32.4, 29.7, 29.2, 27.2, 27.1, 26.9, 22.1. FTIR (thin film) cm<sup>-1</sup>: 3307 (m), 2911 (s), 2841 (m), 1616 (m), 1569 (s), 1443 (m), 990 (m). HRMS (ESI) (m/z): calc'd for C<sub>24</sub>H<sub>46</sub>N<sub>2</sub>NaO<sub>2</sub>Sn [M+Na]<sup>+</sup>: 537.2473, found: 537.2488. TLC (75% ethyl acetate in hexanes), Rf: 0.28 (UV, CAM).

**[0301]** 6-Methylene Alcohol Urea 13b was prepared according to the general procedure for synthesis of substituted ureas using 6-aminohexanol (357 mg, 3.05 mmol, 1.00 equiv). After 25 h, the crude residue was purified by flash column chromatography on silica gel (eluent: 40→80% ethyl acetate in hexanes) to afford 6-methylene alcohol urea 13b (1.56 g, 95%) as a white solid. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>, 23° C.): δ 4.97 (t, J=5.7 Hz, 1H), 4.62 (s, 1H), 3.56 (q, J=6.1 Hz, 2H), 3.12 (q, J=6.7 Hz, 2H), 2.96 (d, J=5.1 Hz, 1H), 2.78-2.68 (m, 2H), 1.88-1.75 (m, 6H), 1.66-1.44 (m, 22H), 1.38-1.16 (m, 13H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>, 23° C.): δ 160.0, 62.6, 40.5, 32.7, 32.4, 30.5, 29.3, 27.2, 26.9, 26.6, 25.4, 22.2. FTIR (thin film) cm<sup>-1</sup>: 3336 (w), 2915 (s), 2845 (s), 1729 (w), 1585 (m), 1444 (m), 991 (m). HRMS (DART) (m/z): calc'd for C<sub>26</sub>H<sub>51</sub>BrN<sub>2</sub>O<sub>2</sub>Sn, [M+H]<sup>+</sup>: 543.2967, found: 543.2966. TLC (75% ethyl acetate in hexanes), Rf: 0.57 (UV, CAM).

**[0302]** Triethyleneglycol Urea 13c was prepared according to the general procedure for synthesis of substituted ureas using 2-(2-(2-aminoethoxy)ethoxy)ethan-1-ol<sup>23</sup> (455 mg, 3.05 mmol, 1.00 equiv). After 24 h, the crude residue was purified by flash column chromatography on silica gel (eluent: 40→100% ethyl acetate in hexanes) to afford tri-

ethyleneglycol urea 13c (1.47 g, 84%) as a white solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, 23° C.): δ 5.13 (t, J=4.9 Hz, 1H), 4.47 (s, 1H), 3.81-3.75 (m, 2H), 3.73-3.59 (m, 8H), 3.43 (q, J=5.3 Hz, 2H), 2.88-2.77 (m, 2H), 2.72 (t, J=6.1 Hz, 1H), 1.96-1.81 (m, 6H), 1.73-1.54 (m, 18H), 1.42-1.21 (m, 9H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>, 23° C.): δ 159.9, 72.6, 70.9, 70.5 (2C), 61.9, 40.5, 32.4, 29.3, 27.2, 26.9, 22.2. FTIR (thin film) cm<sup>-1</sup>: 3339 (w), 2914 (s), 2845 (s), 1729 (w), 1553 (m), 1445 (m), 1070 (s), 991 (m). HRMS (ESI) (m/z): calc'd for C<sub>26</sub>H<sub>50</sub>N<sub>2</sub>NaO<sub>4</sub>Sn, [M+Na]<sup>+</sup>: 597.2685, found: 597.2714. TLC (75% ethyl acetate in hexanes), Rf: 0.18 (UV, CAM).

**[0303]** 4-Methylene Alcohol Pre-Agelastatin 11a was prepared according to the published procedure for synthesis of pre-agelastatins<sup>3a</sup> using urea 13a (659 mg, 1.28 mmol, 3.00 equiv). The crude residue adsorbed onto silica gel was purified by flash column chromatography on silica gel (eluent: 9% methanol and 1% ammonium hydroxide in chloroform→18% methanol and 2% ammonium hydroxide in chloroform) to afford 4-methylene alcohol pre-agelastatin 11a (94 mg, 53% over two steps) as an off-white solid. <sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD, 23° C.): δ 6.91 (d, J=4.0 Hz, 1H), 6.28 (d, J=4.0 Hz, 1H), 6.00 (s, 1H), 4.77 (s, 1H), 4.55 (t, J=7.3 Hz, 1H), 3.68-3.60 (m, 1H), 3.58-3.55 (m, 2H), 3.52-3.46 (m, 1H), 3.34 (s, 3H), 2.93 (dd, J=15.5, 6.8 Hz, 1H), 2.78 (dd, J=15.5, 7.9 Hz, 1H), 1.71-1.64 (m, 2H), 1.54-1.51 (m, 2H). <sup>13</sup>C NMR (126 MHz, CD<sub>3</sub>OD, 23° C.): δ 161.1, 155.9, 124.5, 119.7, 116.1, 113.5, 108.8, 108.7, 84.7, 62.5, 58.2, 55.2, 41.9, 30.8, 29.5, 27.5. δ FTIR (thin film) cm<sup>-1</sup>: 3207 (br-m), 2932 (w), 2871 (w), 1652 (s), 1550 (m), 1418 (m), 1076 (s). HRMS (DART) (m/z): calc'd for C<sub>16</sub>H<sub>22</sub>BrN<sub>4</sub>O<sub>4</sub>, [M+H]<sup>+</sup>: 413.0819, found: 413.0816. TLC (18% methanol, 2% ammonium hydroxide in chloroform), Rf: 0.31 (UV, CAM).

**[0304]** 6-Methylene Alcohol Pre-Agelastatin 11 b was prepared according to the published procedure for synthesis of pre-agelastatins<sup>3a</sup> using urea 13b (975 mg, 1.80 mmol, 3.00 equiv). The crude residue adsorbed onto silica gel was purified by flash column chromatography on silica gel (eluent: 6% methanol and 0.6% ammonium hydroxide in chloroform→14% methanol and 1.6% ammonium hydroxide in chloroform) to afford 6-methylene alcohol pre-agelastatin 11b (178 mg, 67% over two steps) as an off-white solid. <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD, 23° C.): δ 6.91 (d, J=4.1 Hz, 1H), 6.28 (d, J=4.1 Hz, 1H), 6.02 (s, 1H), 4.76 (d, J=1.4 Hz, 1H), 4.55 (ddd, J=7.7, 6.5, 1.3 Hz, 1H), 3.63-3.51 (m, 3H), 3.44-3.38 (m, 1H), 3.34 (s, 3H), 2.92 (dd, J=15.4, 6.7 Hz, 1H), 2.77 (ddd, J=15.4, 7.9, 0.9 Hz, 1H), 1.65-1.49 (m, 4H), 1.42-1.30 (m, 4H). <sup>13</sup>C NMR (126 MHz, CD<sub>3</sub>OD, 23° C.): δ 161.1, 155.9, 124.5, 119.8, 116.1, 113.6, 108.7 (2C), 84.7, 62.9, 58.3, 55.2, 42.0, 33.6, 30.9, 29.6, 27.7, 26.7. FTIR (thin film) cm<sup>-1</sup>: 3212 (br-m), 2931 (m), 2857 (w), 1658 (s), 1551 (w), 1419 (m), 1084 (m). HRMS (DART) (m/z): calc'd for C<sub>18</sub>H<sub>26</sub>BrN<sub>4</sub>O<sub>4</sub>, [M+H]<sup>+</sup>: 441.1132, found: 441.1132. TLC (18% methanol, 2% ammonium hydroxide in chloroform), Rf: 0.56 (UV, CAM).

**[0305]** Triethyleneglycol Pre-Agelastatin 11c was prepared according to the published procedure for synthesis of pre-agelastatins<sup>3a</sup> using urea 13c (1.29 g, 2.25 mmol, 3.00 equiv). The crude residue adsorbed onto silica gel was purified by flash column chromatography on silica gel (eluent: 9% methanol and 1% ammonium hydroxide in chloroform→18% methanol and 2% ammonium hydroxide in chloroform) to afford triethyleneglycol pre-agelastatin

11c (218 mg, 61% over two steps) as an off-white solid.  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ ,  $23^\circ\text{C}$ .):  $\delta$  6.91 (d,  $J=4.1$  Hz, 1H), 6.27 (d,  $J=4.0$  Hz, 1H), 5.97 (s, 1H), 4.76 (d,  $J=1.5$  Hz, 1H), 4.69 (td,  $J=7.4, 1.5$  Hz, 1H), 3.70-3.65 (m, 2H), 3.65-3.61 (m, 4H), 3.60-3.55 (m, 4H), 3.54-3.47 (m, 2H), 3.34 (s, 3H), 2.99 (dd,  $J=15.5, 6.9$  Hz, 1H), 2.83 (dd,  $J=15.5, 7.8$  Hz, 1H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CD}_3\text{OD}$ ,  $23^\circ\text{C}$ .):  $\delta$  161.2, 155.9, 124.5, 120.9, 116.0, 113.4, 108.8, 108.2, 84.7, 73.8, 71.8, 71.5, 70.6, 62.3, 57.9, 55.2, 42.7, 29.7. FTIR (thin film)  $\text{cm}^{-1}$ : 3226 (br-m), 2921 (m), 2870 (m), 1652 (s), 1419 (m), 1086 (m). HRMS (DART) (m/z): calc'd for  $\text{C}_{18}\text{H}_{26}\text{BrN}_4\text{O}_6$ ,  $[\text{M}+\text{H}]^+$ : 473.1030, found: 473.1021. TLC (18% methanol, 2% ammonium hydroxide in chloroform). Rf: 0.47 (UV, CAM).

**[0306]** 4-Methylene Alcohol Derivative 8a and Pentacyclic Derivative 14 were prepared according to the published procedure for synthesis of (-)-agelastatin  $\text{A}^{3a}$  using 4-methylene alcohol pre-agelastatin 11a (26.0 mg, 63.0  $\mu\text{mol}$ , 1 equiv). The crude residue adsorbed onto silica gel was purified by flash column chromatography on silica gel (chloroform $\rightarrow$ 18% methanol and 2% ammonium hydroxide in chloroform) to afford 4-methylene alcohol derivative 8a (0.8 mg, 3%) as an off-white solid along with the pentacyclic derivative 14 (2.6 mg, 11%) as an off-white solid. 4-Methylene Alcohol Derivative 8a:  $^1\text{H}$  NMR (600 MHz,  $\text{CD}_3\text{OD}$ ,  $23^\circ\text{C}$ .):  $\delta$  6.91 (d,  $J=4.1$  Hz, 1H), 6.33 (d,  $J=4.1$  Hz, 1H), 4.65 (dt,  $J=12.0, 5.9$  Hz, 1H), 4.09 (d,  $J=5.5$  Hz, 1H), 3.86 (s, 1H), 3.61 (td,  $J=6.6, 2.8$  Hz, 2H), 3.30-3.18 (m, 2H), 2.65 (dd,  $J=13.1, 6.3$  Hz, 1H), 2.17 (t,  $J=12.7$  Hz, 1H), 1.84-1.75 (m, 2H), 1.62 (p,  $J=6.8$  Hz, 2H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CD}_3\text{OD}$ ,  $23^\circ\text{C}$ .):  $\delta$  161.9, 161.2, 124.3, 116.2, 113.9, 107.4, 96.1, 67.7, 62.8, 62.4, 54.5, 41.3, 40.3, 31.4, 28.0. FTIR (thin film)  $\text{cm}^{-1}$ : 3276 (br-s), 2933 (w), 1653 (s), 1552 (w), 1424 (m), 1375 (w). HRMS (DART) (m/z): calc'd for  $\text{C}_{15}\text{H}_{20}\text{BrN}_4\text{O}_4$ ,  $[\text{M}+\text{H}]^+$ : 399.0662, found: 399.0655. TLC (18% methanol, 2% ammonium hydroxide in chloroform). Rf: 0.19 (UV, CAM).

**[0307]** Pentacyclic Derivative 14:

**[0308]**  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ ,  $23^\circ\text{C}$ .):  $\delta$  6.91 (d,  $J=4.1$  Hz, 1H), 6.33 (d,  $J=4.1$  Hz, 1H), 4.64 (dt,  $J=12.0, 6.0$  Hz, 1H), 4.14 (d,  $J=5.3$  Hz, 1H), 3.95 (s, 1H), 3.87 (d,  $J=12.9$  Hz, 1H), 3.79 (d,  $J=14.4$  Hz, 1H), 3.41-3.32 (m, 2H), 2.97-2.89 (m, 1H), 2.50 (dd,  $J=13.1, 6.7$  Hz, 1H), 2.14 (t,  $J=12.6$  Hz, 1H), 1.77-1.59 (m, 4H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CD}_3\text{OD}$ ,  $23^\circ\text{C}$ .):  $\delta$  161.9, 161.2, 124.3, 116.3, 114.0, 107.5, 100.7, 66.2, 64.8, 62.0, 54.2, 41.9, 41.2, 31.3, 27.4. FTIR (thin film)  $\text{cm}^{-1}$ : 3247 (br-m), 2940 (m), 1696 (s), 1659 (s), 1552 (m), 1422 (s), 1091 (m). HRMS (DART) (m/z): calc'd for  $\text{C}_{15}\text{H}_{18}\text{BrN}_4\text{O}_3$ ,  $[\text{M}+\text{H}]^+$ : 381.0557, found: 381.0552. TLC (18% methanol, 2% ammonium hydroxide in chloroform). Rf: 0.59 (UV, CAM).

**[0309]** 6-Methylene Alcohol Derivative 8b was prepared according to the published procedure for synthesis of (-)-agelastatin  $\text{A}^{3a}$  using 6-methylene alcohol pre-agelastatin 11b (1.00 $\times 10^2$  mg, 227  $\mu\text{mol}$ , 1 equiv). The crude residue adsorbed onto silica gel was purified by flash column chromatography on silica gel (6% methanol and 0.6% ammonium hydroxide in chloroform $\rightarrow$ 18% methanol and 2% ammonium hydroxide in chloroform) to afford 6-methylene alcohol derivative 8b (35 mg, 36%) as an off-white solid.  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ ,  $23^\circ\text{C}$ .):  $\delta$  6.91 (d,  $J=4.0$  Hz, 1H), 6.33 (d,  $J=4.1$  Hz, 1H), 4.63 (dt,  $J=12.0, 5.9$  Hz, 1H), 4.09 (d,  $J=5.5$  Hz, 1H), 3.85 (s, 1H), 3.56 (t,  $J=6.5$  Hz, 2H), 3.23 (dq,  $J=14.5, 8.4$  Hz, 2H), 2.63 (dd,  $J=13.0, 6.3$  Hz,

1H), 2.17 (t,  $J=12.6$  Hz, 1H), 1.84-1.67 (nm, 2H), 1.56 (dd,  $J=8.8, 5.2$  Hz, 2H), 1.47-1.39 (m, 4H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CD}_3\text{OD}$ ,  $23^\circ\text{C}$ .):  $\delta$  161.9, 161.2, 124.3, 116.2, 114.0, 107.3, 96.1, 67.7, 63.0, 62.4, 54.5, 41.4, 40.4, 33.8, 31.5, 28.3, 26.9. FTIR (thin film)  $\text{cm}^{-1}$ : 3254 (br-s), 2929 (m), 2856 (m), 1652 (s), 1551 (m), 1422 (s), 1027 (w). HRMS (DART) (m/z): calc'd for  $\text{C}_{17}\text{H}_{24}\text{BrN}_4\text{O}_4$ ,  $[\text{M}+\text{H}]^+$ : 427.0975, found: 427.0970. TLC (18% methanol, 2% ammonium hydroxide in chloroform). Rf: 0.27 (UV, CAM).

**[0310]** Triethyleneglycol Derivative 8c was prepared according to the published procedure for synthesis of (-)-agelastatin  $\text{A}^{3a}$  using triethylene glycol pre-agelastatin 11c (1.80 $\times 10^2$  mg, 3.80 $\times 10^2$   $\mu\text{mol}$ , 1 equiv). The crude residue adsorbed onto silica gel was purified by flash column chromatography on silica gel (6% methanol and 0.6% ammonium hydroxide in chloroform $\rightarrow$ 18% methanol and 2% ammonium hydroxide in chloroform) to afford triethyleneglycol derivative 8c (52 mg, 30%) as an off-white solid.  $^1\text{H}$  NMR (600 MHz,  $\text{CD}_3\text{OD}$ ,  $23^\circ\text{C}$ .):  $\delta$  6.92 (d,  $J=3.9$  Hz, 1H), 6.33 (d,  $J=3.9$  Hz, 1H), 4.68-4.59 (m, 1H), 4.09 (d,  $J=5.7$  Hz, 1H), 3.89 (s, 1H), 3.73-3.62 (m, 9H), 3.55 (h,  $J=7.3, 6.5$  Hz, 2H), 3.42-3.33 (m, 1H), 2.79 (dd,  $J=12.9, 6.4$  Hz, 1H), 2.19-2.09 (m, 1H).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CD}_3\text{OD}$ ,  $23^\circ\text{C}$ .):  $\delta$  161.7, 161.2, 124.3, 116.2, 113.9, 107.4, 95.9, 73.8, 71.6, 71.5, 70.6, 67.8, 62.3, 62.2, 54.6, 41.5, 40.1. FTIR (thin film)  $\text{cm}^{-1}$ : 3264 (br-s), 2921 (w), 1645 (s), 1551 (m), 1093 (m), 1024 (m). HRMS (DART) (m/z): calc'd for  $\text{C}_{17}\text{H}_{24}\text{BrN}_4\text{O}_6$ ,  $[\text{M}+\text{H}]^+$ : 459.0874, found: 459.0895. TLC (18% methanol, 2% ammonium hydroxide in chloroform). Rf: 0.35 (UV, CAM).

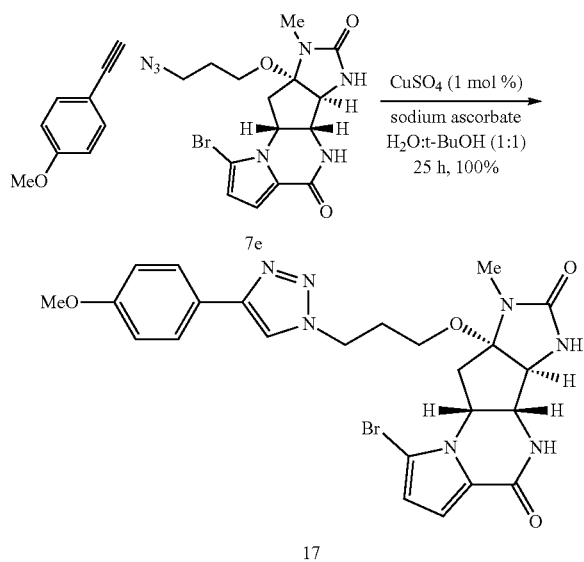
**[0311]** General Procedure for the Synthesis of Azide Derivatives.

**[0312]** Alcohol derivative (1 equiv) and triphenylphosphine were dissolved in tetrahydrofuran. After 5 min, diisopropylazodicarboxylate and diphenylphosphorylazide were added sequentially. Upon consumption of the alcohol derivative by thin layer chromatography, the reaction mixture was concentrated under reduced pressure. The crude residue was purified by flash column chromatography to afford azide derivatives 8d-e.

**[0313]** 6-Methylene Azide Derivative 8d was synthesized from 6-methylene alcohol derivative 8b according to the general procedure for the synthesis of azide derivatives using triphenylphosphine (12.6 mg, 48.0  $\mu\text{mol}$ , 2.00 equiv), diisopropylazodicarboxylate (9.4  $\mu\text{L}$ , 48  $\mu\text{mol}$ , 2.0 equiv), and diphenylphosphorylazide (10.6  $\mu\text{L}$ , 48.0  $\mu\text{mol}$ , 2.00 equiv) in tetrahydrofuran (350  $\mu\text{L}$ ). After 21 h, the crude residue was purified by flash column chromatography on silica gel (chloroform $\rightarrow$ 18% methanol and 2% ammonium hydroxide in chloroform) to afford 6-methylene azide derivative 8d (4.2 mg, 38%) as a white solid along with recovered 6-methylene alcohol derivative 8b (3.5 mg, 34%). Reaction yield was 72% based on recovered starting material.  $^1\text{H}$  NMR (600 MHz,  $\text{CD}_3\text{OD}$ ,  $23^\circ\text{C}$ .):  $\delta$  6.91 (d,  $J=4.1$  Hz, 1H), 6.34 (d,  $J=4.1$  Hz, 1H), 4.64 (dt,  $J=12.1, 5.9$  Hz, 1H), 4.09 (d,  $J=5.5$  Hz, 1H), 3.86 (s, 1H), 3.28-3.16 (m, 2H), 2.63 (dd,  $J=13.1, 6.3$  Hz, 1H), 2.17 (t,  $J=12.6$  Hz, 1H), 1.83-1.67 (m, 2H), 1.66-1.37 (m, 6H), 1.32-1.22 (m, 2H).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CD}_3\text{OD}$ ,  $23^\circ\text{C}$ .):  $\delta$  161.9, 161.2, 124.3, 116.2, 114.0, 107.2, 96.1, 67.7, 62.4, 54.5, 52.5, 41.4, 40.3, 31.4, 30.0, 27.9, 27.8. FTIR (thin film)  $\text{cm}^{-1}$ : 3222 (br-m), 2930 (m), 2856 (s), 2095 (s), 1669 (s), 1118 (m). HRMS (DART) (m/z) calc'd for  $\text{C}_{17}\text{H}_{23}\text{BrN}_7\text{O}_3$ ,  $[\text{M}+\text{H}]^+$ : 452.

1040, found: 452.1058 TLC (9% methanol, 10% ammonium hydroxide in chloroform), Rf: 0.28 (UV, CAM).

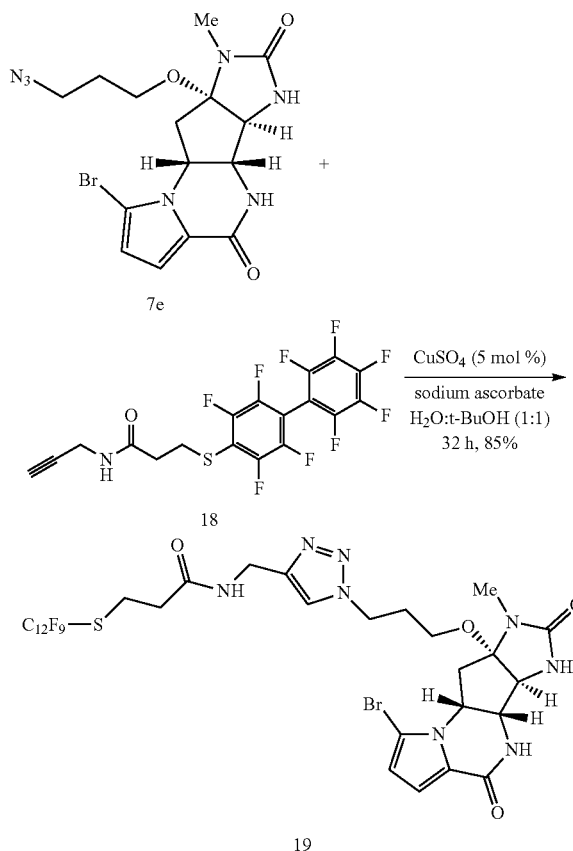
**[0314]** Triethyleneglycol Bis-Azide Derivative Se was synthesized from triethyleneglycol derivative 8c according to the general procedure for the synthesis of azide derivatives using triphenylphosphine (36.0 mg, 137  $\mu\text{mol}$ , 10.0 equiv), diisopropylazodicarboxylate (27.0  $\mu\text{L}$ , 137  $\mu\text{mol}$ , 10.0 equiv), and diphenylphosphorylazide (31.0  $\mu\text{L}$ , 137  $\mu\text{mol}$ , 10.0 equiv) in tetrahydrofuran (274  $\mu\text{L}$ ). After 6 h, the crude residue was purified by flash column chromatography on silica gel (chloroform $\rightarrow$ 18% methanol and 2% ammonium hydroxide in chloroform) to afford triethyleneglycol bis-azide derivative 8e (3.7 mg, 54%) as a white solid.  $^1\text{H}$  NMR. (500 MHz,  $\text{CD}_3\text{OD}$ , 23 $^\circ$  C.):  $\delta$  6.93 (d, J=4.1 Hz, 1H), 6.35 (d, J=4.0 Hz, 1H), 4.79 (dt, J=12.1, 6.2 Hz, 1H), 4.23 (s, 1H), 4.18 (d, J=5.5 Hz, 1H), 3.82-3.56 (m, 6H), 3.53 (t, J=5.7 Hz, 2H), 3.33 (dd, J=5.2, 1.8 Hz, 2H), 2.98 (dd, J=13.5, 6.5 Hz, 1H), 2.13-2.06 (m, 1H), 1.35-1.26 (m, 2H).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CD}_3\text{OD}$ , 23 $^\circ$  C.): S 161.3, 161.0, 124.2, 116.4, 114.1, 107.7, 88.5, 71.7 (2C), 71.3, 70.4, 66.1, 62.1, 54.6, 51.9, 41.5, 39.9. FTIR (thin film)  $\text{cm}^{-1}$ . 3255 (br-w), 2920 (m), 2852 (w), 2105 (s), 1700 (m), 1662 (s), 1424 (m), 1102 (br-m). HRMS (DART) (m/z): calc'd for  $\text{C}_{17}\text{H}_{21}\text{BrN}_7\text{O}_4$ ,  $[\text{M}-\text{N}]^+$ : 466.0833, found: 466.0922 TOF MS (ESI) (m/z): calc'd for  $\text{C}_{17}\text{H}_{21}\text{BrN}_{10}\text{NaO}_4$ ,  $[\text{M}+\text{Na}]^+$ : 531.0823, found: 531.12. TLC (18% methanol, 2% ammonium hydroxide in chloroform), Rf: 0.50 (UV, CAM).



**[0315]** Procedure for the Synthesis of Triazole 17 from Azide 7e.

**[0316]** Azide 7e (10.6 mg, 25.0  $\mu\text{mol}$ , 1 equiv) and 4-ethynylanisole (3.3  $\mu\text{L}$ , 25.0  $\mu\text{mol}$ , 1.0 equiv) were suspended in t-BuOH (75  $\mu\text{L}$ ) and  $\text{H}_2\text{O}$  (75  $\mu\text{L}$ ). A freshly prepared solution of sodium ascorbate (1.0 M in  $\text{H}_2\text{O}$ , 2.5  $\mu\text{L}$ , 0.1 equiv) was added, followed by a freshly prepared solution of copper (II) sulfate pentahydrate (0.3 M in  $\text{H}_2\text{O}$ , 0.8  $\mu\text{L}$ , 0.01 equiv). The reaction mixture was sealed under an atmosphere of argon and stirred vigorously. After 25 h, thin layer chromatography indicated full consumption of starting material. The reaction solution was diluted with dichloromethane (2 mL) and methanol (2 mL) and concentrated

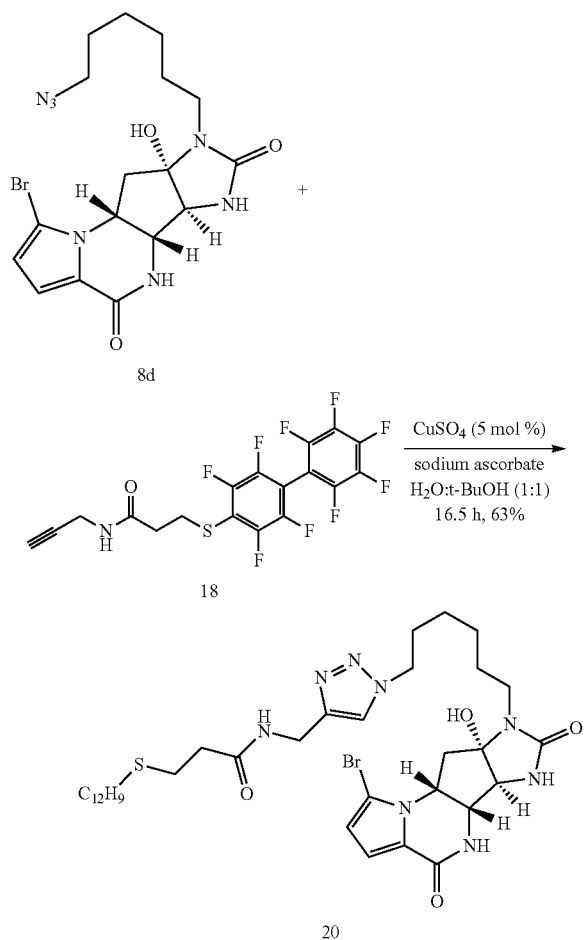
under reduced pressure. The crude residue was purified flash column chromatography on silica gel (eluent: 5 $\rightarrow$ 10% methanol in dichloromethane with 2% toluene) to afford 1,2,3-triazole 17 (13.9 mg, 100%) as a white solid. Spectral data is shown in FIGS. 16 and 17.  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ , 25 $^\circ$  C.):  $\delta$  8.22 (s, 1H), 7.72 (d, J=8.8 Hz, 1H), 6.98 (d, J=8.8 Hz, 2H), 6.92 (d, J=4.1 Hz, 1H), 6.34 (d, J=4.1 Hz, 1H), 4.64-4.50 (m, 3H), 4.12 (d, J=5.5 Hz, 1H), 4.08 (s, 1H), 3.83 (s, 3H), 3.41-3.35 (m, 1H), 3.36-3.30 (m, 1H), 2.77 (s, 3H), 2.67 (dd, J=13.3, 6.4 Hz, 1H), 2.25 (p, J=6.3 Hz, 2H), 2.17 (t, J=12.8 Hz, 1H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , 25 $^\circ$  C.):  $\delta$  159.9, 159.3, 129.2, 128.4, 127.1, 125.4, 123.1, 115.4, 114.5, 113.0, 106.1, 98.9, 60.9, 60.2, 59.3, 55.5, 52.6, 47.0, 38.7, 30.2, 29.8, 24.8. TLC (7% methanol in dichloromethane with 2% toluene). Rf: 0.28 (UV, CAM).



**[0317]** Procedure for the Synthesis of Triazole 19 from Azide 7e.

**[0318]** Azide 7e (10.6 mg, 25.0  $\mu\text{mol}$ , 1.0 equiv) and compound 18 (11.4 mg, 25.0  $\mu\text{mol}$ , 1.0 equiv) were suspended in t-BuOH (125  $\mu\text{L}$ ) and  $\text{H}_2\text{O}$  (125  $\mu\text{L}$ ). Sodium ascorbate (2.5 mg, 12.5  $\mu\text{mol}$ , 0.5 equiv) was added, followed by copper (II) sulfate pentahydrate (0.3 mg, 1.25  $\mu\text{mol}$ , 0.05 equiv). The reaction mixture was sealed under an atmosphere of argon and stirred vigorously. After 32 h, thin layer chromatography indicated full consumption of starting material. The reaction solution was diluted with dichloromethane (2 mL) and methanol (2 mL) and concentrated under reduced pressure. The crude residue was purified flash column chromatography on silica gel (eluent: 0 $\rightarrow$ 10%

methanol in dichloromethane) to afford 1,2,3-triazole 19 (18.7 mg, 85%) as a white solid. Spectral data is shown in FIGS. 18 and 19. <sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD, 25° C.): δ 7.86 (s, 1H), 6.90 (d, J=4.2 Hz, 1H), 6.32 (d, J=4.3 Hz, 1H), 4.63-4.59 (m, 1H), 4.40-4.38 (m, 2H), 4.24-4.20 (m, 1H), 4.12-4.04 (m, 1H), 3.35-3.32 (m, 2H), 3.31-3.26 (m, 2H), 2.58-2.53 (m, 1H), 2.15 (t, J=12.2 Hz, 1H), 1.94-1.91 (m, 2H), 1.62-1.55 (m, 2H), 1.48-1.38 (m, 6H), 1.28-1.24 (m, 2H). <sup>19</sup>F NMR (376 MHz, CD<sub>3</sub>OD, 25° C.): δ -134.7 (m, 2F), -140.0 (m, 2F), -140.4 (m, 2F), -153.2 (m, 1F), -163.7 (m, 2F). TLC (8% methanol in dichloromethane), Rf: 0.08 (UV, CAM).



**[0319]** Procedure for the Synthesis of Triazole 20 from Azide 8d.

**[0320]** Azide 8d (2.0 mg, 4.4 μmol, 1 equiv) and compound 18 (2.0 mg, 4.4 μmol, 1.0 equiv) were suspended in t-BuOH (40 μL) and H<sub>2</sub>O (40 μL). Sodium ascorbate (0.44 mg, 2.2 μmol, 0.50 equiv) was added, followed by copper (II) sulfate pentahydrate (0.05 mg, 0.2 μmol, 0.05 equiv). The reaction mixture was sealed under an atmosphere of argon and stirred vigorously. After 16.5 h, thin layer chromatography indicated full consumption of starting material. The reaction solution was diluted with dichloromethane (2 mL) and methanol (2 mL) and concentrated under reduced pressure. The crude residue was purified flash column chro-

matography on silica gel (eluent: 0→10% methanol in dichloromethane) to afford 1,2,3-triazole 20 (18.7 mg, 85%) as a white solid. <sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD, 25° C.): δ 7.86 (s, 1H), 6.90 (d, J=4.2 Hz, 1H), 6.32 (d, J=4.3 Hz, 1H), 4.63-4.59 (m, 1H), 4.40-4.38 (m, 2H), 4.24-4.20 (m, 1H), 4.12-4.04 (m, 1H), 3.35-3.32 (m, 2H), 3.31-3.26 (m, 2H), 2.58-2.53 (m, 1H), 2.15 (t, J=12.2 Hz, 1H), 1.94-1.91 (m, 2H), 1.62-1.55 (m, 2H), 1.48-1.38 (m, 6H), 1.28-1.24 (m, 2H). <sup>19</sup>F NMR (376 MHz, CD<sub>3</sub>OD, 25° C.): δ -134.7 (m, 2F), -140.0 (m, 2F), -140.4 (m, 2F), -153.2 (m, 1F), -163.7 (m, 2F). TLC (8% methanol in dichloromethane), Rf: 0.08 (UV, CAM).

**[0321]** Cell Culture Methods.

**[0322]** All RMF cell lines were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% bovine calf serum. The human breast cancer line, SUM1315, was grown in Ham's F12 nutrient mixture with 5% fetal bovine serum, 10 ng/mL epidermal growth factor (EGF), and 5 μg/mL insulin. All culture media contained 100 Units/mL penicillin and 100 μg/mL streptomycin and 0.1% fungizone. The parental RMF cell line expressing green fluorescent protein (GFP) and the human breast cancer cell line SUM1315 were kind gifts from Dr. Charlotte Kuperwasser, Tufts University.<sup>24</sup> Derivation of the stable fibroblast sub-lines shTiam-RMF and C-RMF has been described previously.<sup>12</sup>

**[0323]** Quantitative Reverse Transcription Polymerase Chain Reaction (RT-PCR) for OPN (FIGS. 3 and 8).

**[0324]** Total RNA synthesis was carried out using the acid guanidinium thiocyanate-phenol-chloroform extraction method<sup>25</sup> per manufacturer protocol. First strand cDNA synthesis was performed using a version of Moloney murine leukemia virus reverse transcriptase per manufacturer protocol. Some mammary fibroblasts were treated with 48 hours of 10 nM vitamin D and AgA, AgE, or agelastatin derivative as indicated prior to assay. Quantitative PCR was performed in triplicate reactions in 20-μL volumes containing cDNA with cyanine dye and DNA polymerase master mix, using the following primer sets: glyceraldehyde 3-phosphate dehydrogenase (GAPDH), 5'-CTGCACCACCACTGCTTAG-3' (sense), 5'-TTCAGCTCAGGGATGACCTT-3' (antisense); OPN, 5'-GCCATACCAGTTAAACAGGC-3' (sense), 5'-GACCTCAGAAGATGCACTAT-3' (antisense). Real-time PCR parameters: 95° C. for 10 min; 95° C. for 30 s, 60° C. for 60 s, 72° C. for 60 s for 40 cycles. Data analysis was done using a continuous fluorescence detector. The comparative threshold cycle (2<sup>-ΔΔCT</sup>) value<sup>26</sup> was calculated following GAPDH normalization. Results indicate mean±SD and represent a composite of at least duplicate experiments, each with triplicate conditions.

**[0325]** Spheroid Co-Culture Methods (FIG. 4).

**[0326]** Three-dimensional co-cultures with SUM1315 cells and fibroblast lines were established in phenol-red free basement membrane matrix from the Engelbreth-Holm-Swarm mouse sarcoma (medium mixture as previously described)<sup>9</sup> Cultures had equal volumes of DMSO, AgA, or AgE incorporated into the matrix and culture medium at the indicated concentrations. Stock solutions (1 mM) were stored in DMSO at -20° C. and diluted in culture medium to indicated final concentrations.

**[0327]** Number of projections per spheroid was quantified using an Eclipse TS100 inverted tissue culture microscope and depicted as number of spheroids with indicated number of projections as percent of total spheroids. At least 100



spheroids were counted for each experimental condition. Results are representative of duplicate experiments. Cells were isolated from 3D co-cultures and cancer cells were separated from fibroblasts as described previously. Flow cytometry was used to demonstrate purity of cells isolated post-co-culture to greater than 99% cancer cells using GFP markers to identify RMFs.

**[0328]** Transwell Migration Assays (FIG. 5).

**[0329]** Transwell migration of SUM1315 after isolation from mixed cell spheroid co-cultures with indicated mammary fibroblasts, with or without indicated agelastatin compound. Cultured cells were deprived of serum overnight, trypsinized, and plated at a density of  $1 \times 10^5$ /mL. ( $2 \times (10^4$  cells/basket) in the upper basket of transwell chambers with a filter pore size of 8  $\mu$ m. Cells were allowed to migrate for 5 h at 37° C. toward lower chambers containing either DMEM alone or supplemented with 25% filter-sterilized conditioned medium harvested from NIH3T3 cells. Non-migrated cells were then removed from the upper side of the filter using a cotton swab. Filters were fixed and stained with a three-step hematology stain protocol comparable to the Wright-Giemsa method. Filters were cut out and mounted on glass slides under coverslips using microscope immersion oil. Migrated cells were counted in nine random fields for each replicate of biologic triplicates using an Eclipse TS100 microscope and 20 $\times$  objective. Results are representative of duplicate experiments.

**[0330]** Tumorsphere Assays (FIG. 6).

**[0331]** The SUM1315 breast cancer cells isolated from 3D co-cultures with indicated mammary fibroblasts and indicated concentrations of AgA or AgE were trypsinized and cell clumps were broken up by gentle pipetting several times. After low-speed centrifugation (1200 rpm), cell pellets were re-suspended in corresponding culture medium, and passed through a 40- $\mu$ m filter to obtain single cell suspensions. Five thousand cells in 4 mL culture medium were plated per well in ultra-low attachment 6-well plates. Tumorspheres were quantitated within 10 to 14 days in culture and sphere images were obtained on an Eclipse TS100 inverted microscope. Results are representative of duplicate experiments, each with biologic triplicates.

**[0332]** Flow Cytometry Assays (FIG. 7).

**[0333]** Populations of SUM1315 breast cancer cells were quantified by flow cytometry for expression of indicated cell surface markers using fluorophore-conjugated antibodies after isolation from 3D co-cultures with indicated fibroblasts. Aliquots of  $0.5 \times 10^6$  cells in 100  $\mu$ L of fluorescence-activated cell sorting buffer (phosphate-buffered saline with 2% bovine serum albumin) were stained with epithelial cell adhesion molecule epithelial specific antigen (ESA) fluorescein isothiocyanate (FITC) [clone VU-ID9, 1:10], CD24-phycoerythrin (PE) [clone ML5, 1:400], and CD44-allophycocyanin (APC) [clone G44-26, 1:50] antibodies in the dark at room temperature for 30 min. At the end of incubation, 900  $\mu$ L of fluorescence-activated cell sorting buffer was added to the cell/antibody mixture, and cells were then analyzed using an advanced digital processing analyzer. To set background gating, other cell aliquots were stained with isotype control antibodies conjugated with corresponding fluorophore (APC mouse immunoglobulin G (IgG) subclass 2b kappa ( $\kappa$ ); PE mouse IgG2a  $\kappa$ ; FITC mouse IgG) so that the nonspecific CD44<sup>+</sup>/CD24<sup>-</sup>/ESA<sup>+</sup> staining represented less than 0.5% of the cell population. Cells were first gated using APC and PE to identify the CD44<sup>+</sup>/CD24<sup>-</sup> population,

followed by secondary gating on FITC to identify the CD44<sup>+</sup>/CD24<sup>-</sup>/ESA<sup>+</sup> cells. Results are representative of duplicate experiments, each with biologic triplicates.

**[0334]** Effects of Agelastatin Derivatives (FIG. 8).

**[0335]** Quantitative RT-PCR for OPN mRNA relative to GAPDH control from mammary fibroblasts treated with 48 hours of vitamin D, and AgA, AgE, or agelastatin derivatives as indicated. Results indicate mean $\pm$ -SD and represent a composite of at least duplicate experiments, each with triplicate conditions. Detailed experimental methods are described above for FIG. 3.

#### EQUIVALENTS

**[0336]** While the present invention has been described in conjunction with the specific embodiments set forth above, many alternatives, modifications and other variations thereof will be apparent to those of ordinary skill in the art. All such alternatives, modifications and variations are intended to fall within the spirit and scope of the present invention.

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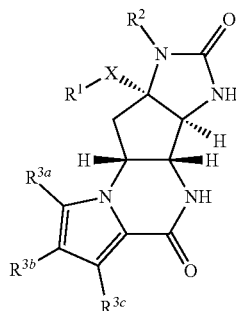
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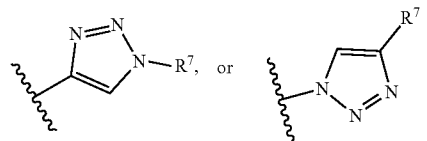
### 1. A compound of Formula (I):



(I)

heterocycloalkyl, C<sub>5-10</sub> heterocycloalkenyl, or C<sub>5-10</sub> heterocycloalkynyl ring, optionally substituted with one or more R<sup>4</sup>;

R<sup>3a</sup>, R<sup>3b</sup>, and R<sup>3c</sup> are each independently H or halogen;  
 R<sup>4</sup> is halogen, —C<sub>1-5</sub> alkyl, oxo, —OH, —OR<sup>6</sup>, —N<sub>3</sub>, —N(R<sup>6</sup>)<sub>2</sub>, —NH(C=O)R<sup>6</sup>, —NH(C=O)OR<sup>6</sup>, —NH(C=O)N(R<sup>6</sup>)<sub>2</sub>, —(C=O)R<sup>6</sup>, heteroaryl,



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

X is —O—, —S—, or —N(R<sup>6</sup>)—;

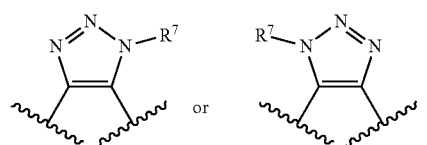
R<sup>1</sup> is H, C<sub>1-10</sub> alkyl, C<sub>2-10</sub> alkenyl, or C<sub>2-8</sub> alkynyl, optionally substituted with one or more R<sup>4</sup>;

or alternatively, X and combine to form —N<sub>3</sub>,

R<sup>2</sup> is C<sub>1-10</sub> alkyl, C<sub>2-10</sub> alkenyl, or C<sub>2-8</sub> alkynyl, optionally substituted with one or more R<sup>5</sup>, wherein up to 3 —CH<sub>2</sub>— units of R<sup>2</sup> are optionally replaced by an —O—, —S—, or —NR<sup>6</sup>—, provided that no adjacent —CH<sub>2</sub>— is replaced;

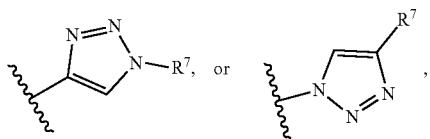
or alternatively, R<sup>1</sup> and R<sup>2</sup> taken together with the atoms to which they are attached form a C<sub>5-10</sub>

and two adjacent R<sup>4</sup> groups may take the form

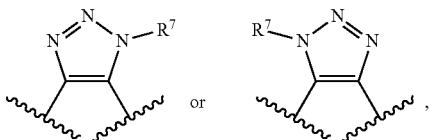


wherein the C=C double bond of the triazole is part of R<sup>1</sup>;

R<sup>5</sup> is halogen, oxo, —OH, —OR<sup>6</sup>, —N<sub>3</sub>, —N(R<sup>6</sup>)<sub>2</sub>,



and two adjacent  $R^5$  groups may take the form



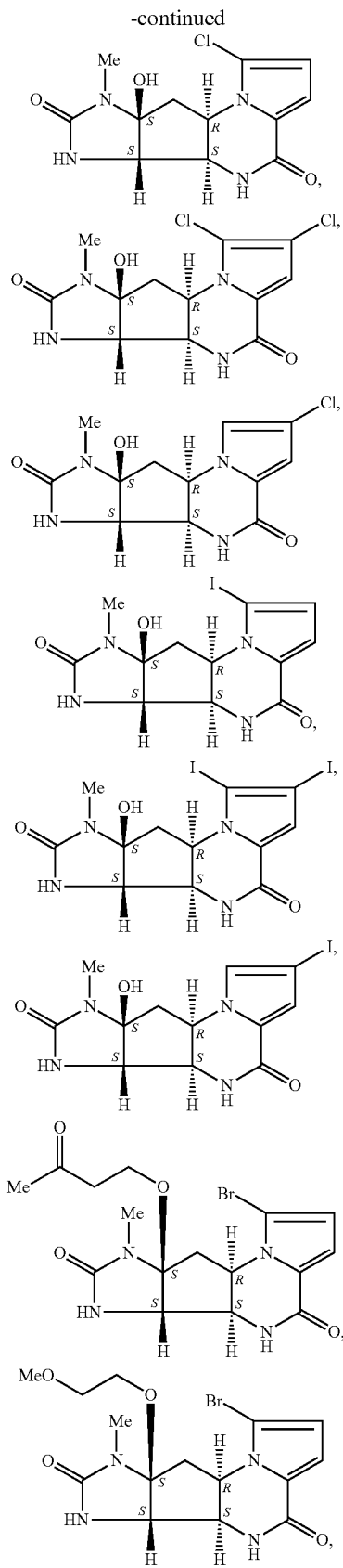
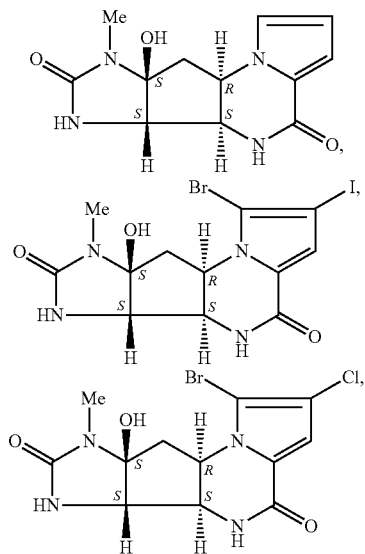
wherein the C=C double bond of the triazole is part of  $R^2$ ; and

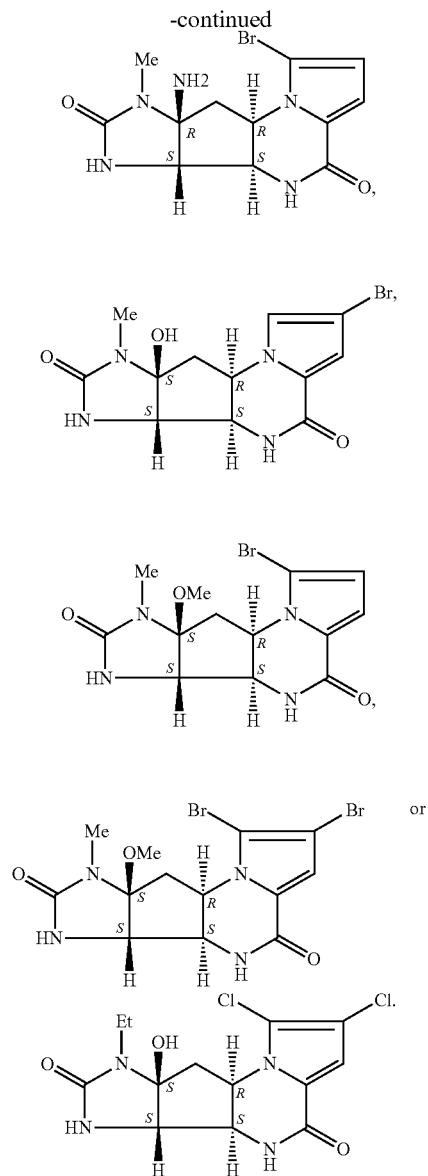
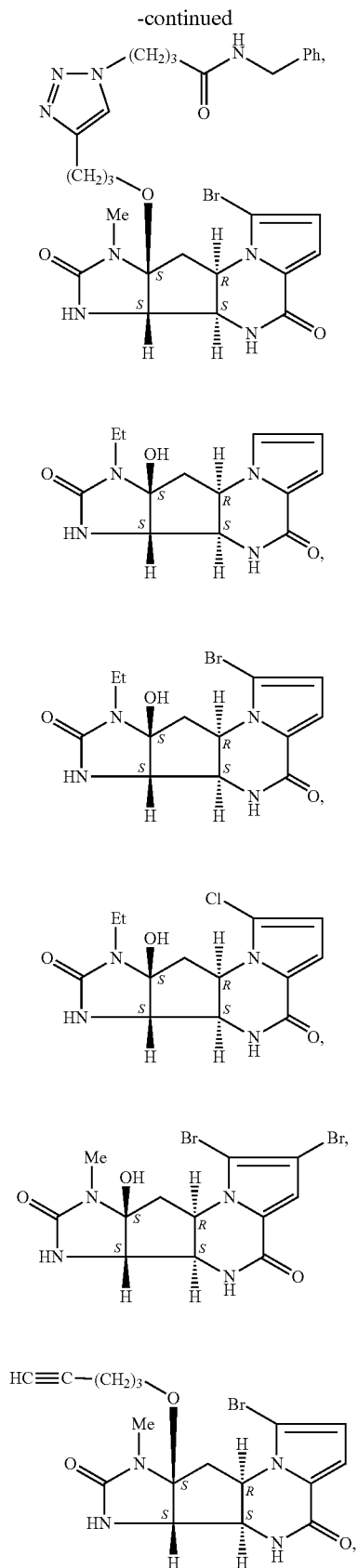
$R^6$  is independently H,  $-C_{1-5}$  alkyl,  $-C_{2-5}$  alkenyl,  $-C_{1-5}$  alkynyl,  $-C_{1-5}$  alkyl-SiMe<sub>3</sub>, aryl, or heteroaryl;

$R^7$  is selected from the group consisting of hydrogen, optionally substituted aliphatic, optionally substituted heteroaliphatic, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted acyl, resin, protein, reporter molecule, and label molecule;

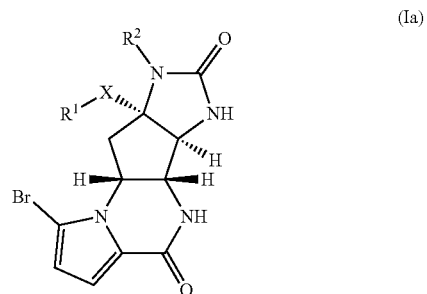
wherein,  $R^7$  is optionally joined to the core by a linker L, wherein the linker L is selected from the group consisting of optionally substituted alkylene, optionally substituted alkenylene, optionally substituted alkynylene, optionally substituted heteroalkylene, optionally substituted heteroalkenylene, optionally substituted heteroalkynylene, optionally substituted arylene, optionally substituted heteroarylene, and optionally substituted acylene;

with the proviso that the compound is not agelastatin A, agelastatin B, agelastatin E,



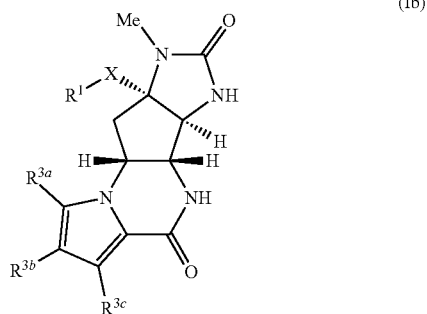


2. The compound of claim 1, wherein the compound is a compound of Formula (1a):



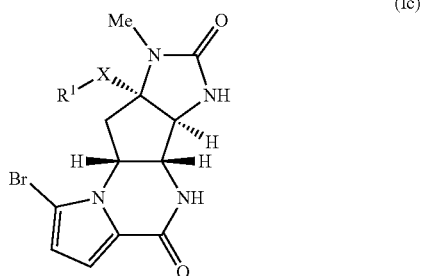
or a pharmaceutically acceptable salt, tautomer, or stereoisomer thereof.

3. The compound of claim 1, wherein the compound is a compound of Formula (Ib):



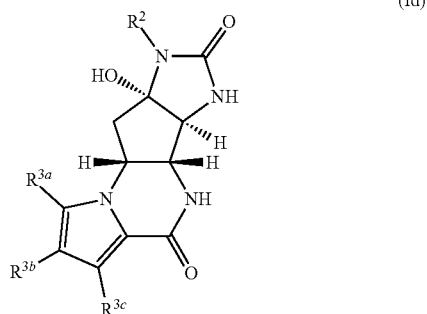
or a pharmaceutically acceptable salt, tautomer, or stereoisomer thereof.

4. The compound of claim 3, wherein the compound is a compound of Formula (Ic):



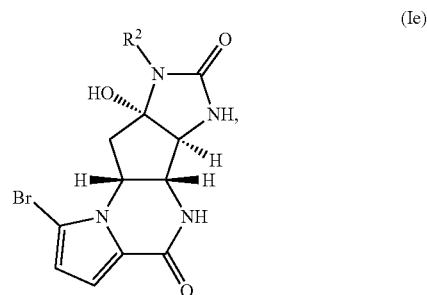
or a pharmaceutically acceptable salt, tautomer, or stereoisomer thereof.

5. The compound of claim 1, wherein the compound is a compound of Formula (Id):



or a pharmaceutically acceptable salt, tautomer, or stereoisomer thereof.

6. The compound of claim 5, wherein the compound is a compound of Formula (Ie):



or a pharmaceutically acceptable salt, tautomer, or stereoisomer thereof.

7. The compound of any of claims 1, 2, 5, or 6, wherein  $R^2$  is  $C_{1-10}$  alkyl, optionally substituted with one or more  $R^5$ , wherein up to 3  $-CH_2-$  units of  $R^2$  are optionally replaced by an  $-O-$ ,  $-S-$ , or  $-NR^6-$ , provided that no adjacent  $-CH_2-$  is replaced.

8. The compound of claim 7, wherein up to 3  $-CH_2-$  units of  $R^2$  are optionally replaced by an O.

9. The compound of any of claims 1, 2, 5, or 6, wherein  $R^2$  is methyl.

10. The compound of any of claims 1-4, 7, or 8, wherein  $R^1$  is  $C_{1-10}$  alkyl,  $C_{2-10}$  alkenyl, or  $C_{2-8}$  alkynyl, optionally substituted with one or more  $R^4$ , or alternatively, X and  $R^1$  are combined to form  $-N_3$ .

11. The compound of any of claims 1-4 or 7-10, wherein X is  $-O-$  or  $-S-$ .

12. The compound of claim 11, wherein X is  $-O-$ .

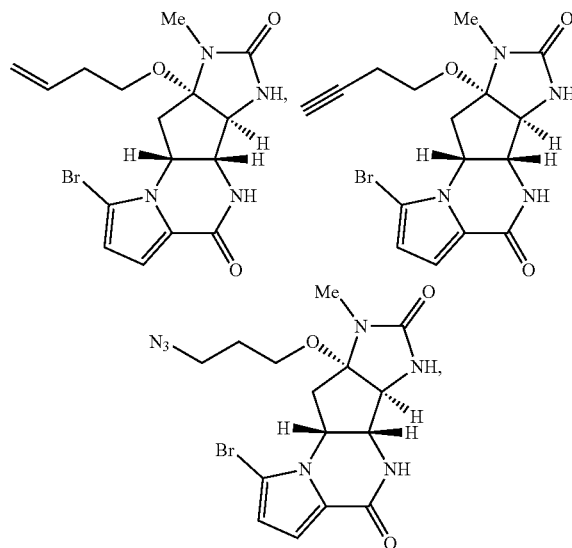
13. The compound of any of claims 1-12, wherein  $R^{3a}$  is Br or Cl,  $R^{3b}$  is H, and  $R^{3c}$  is H.

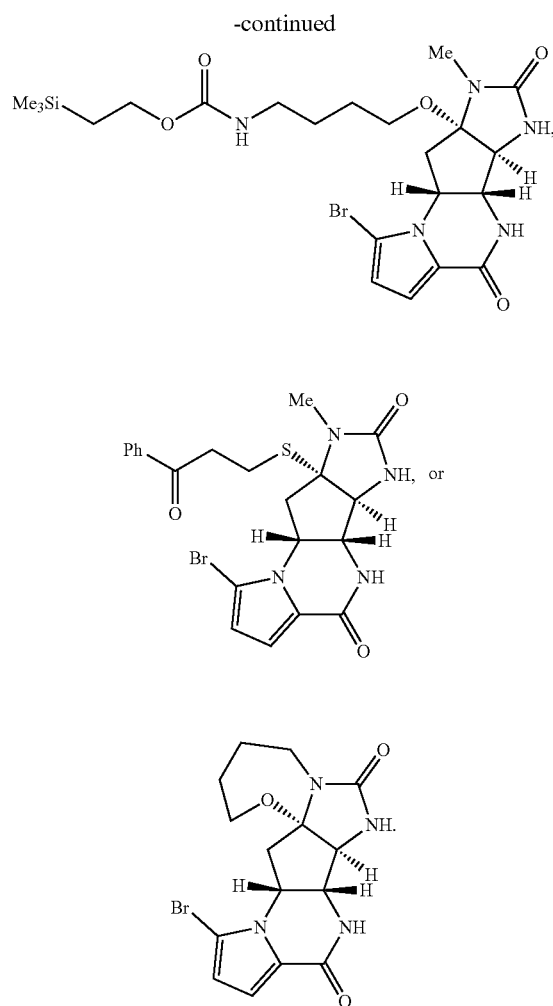
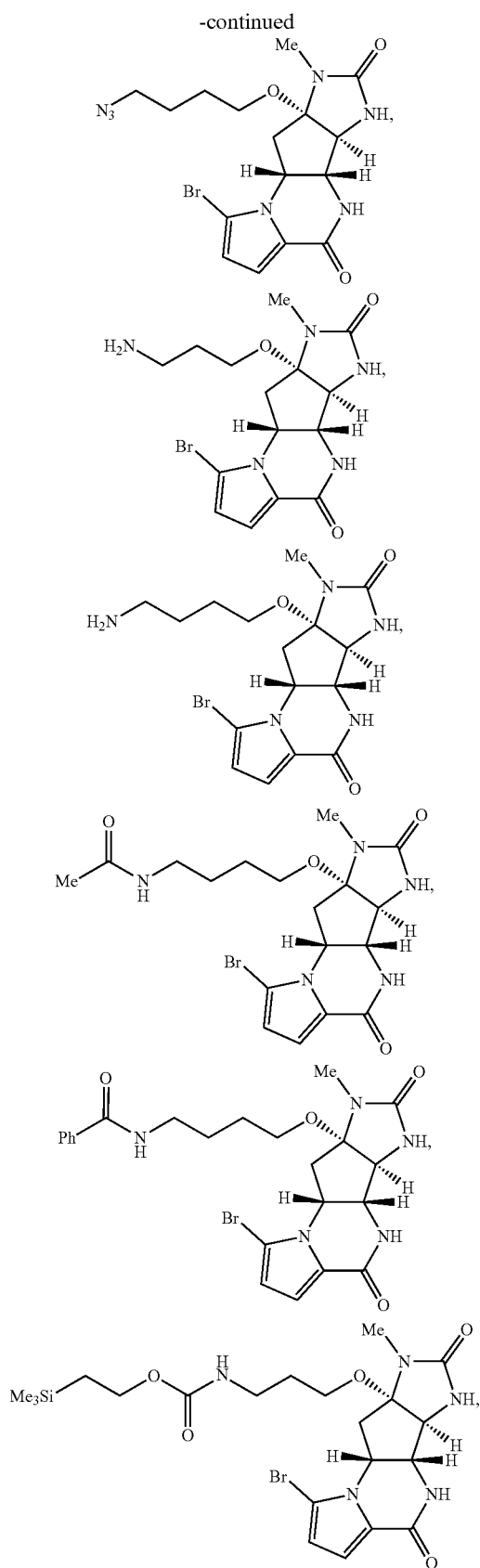
14. The compound of any of claims 1, 3, 5, or 7-12, wherein  $R^{3a}$  is Br,  $R^{3b}$  is H, and  $R^{3c}$  is H.

15. The compound of any of claims 1-12, wherein  $R^{3a}$  is Br or Cl,  $R^{3b}$  is Br or Cl, and  $R^{3c}$  is H.

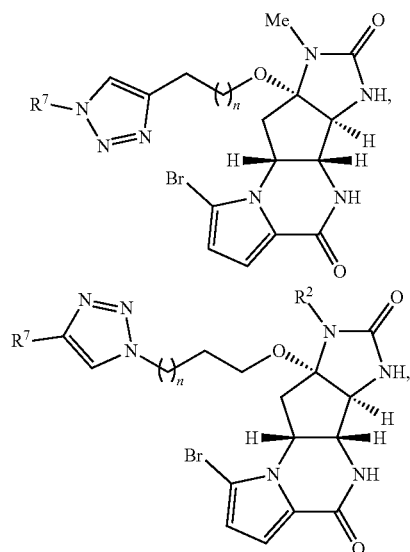
16. The compound of any of claims 1-12, wherein  $R^{3a}$  is Br,  $R^{3b}$  is Br, and  $R^{3c}$  is H.

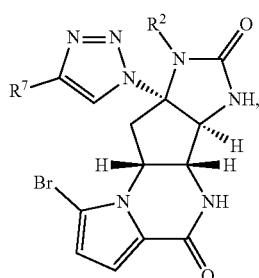
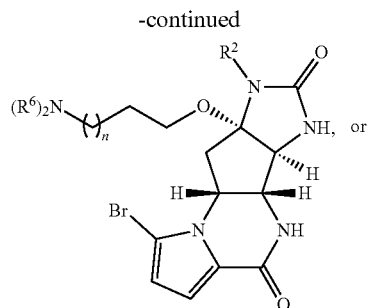
17. The compound of claim 1, selected from:





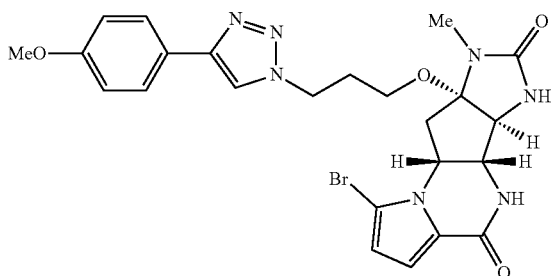
18. The compound of claim 1, selected from:



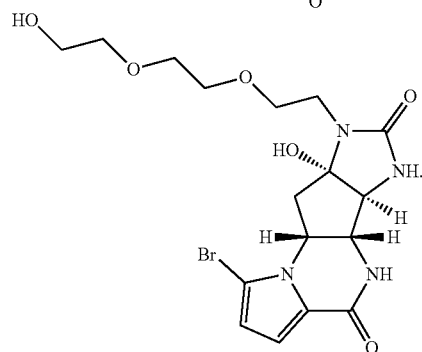
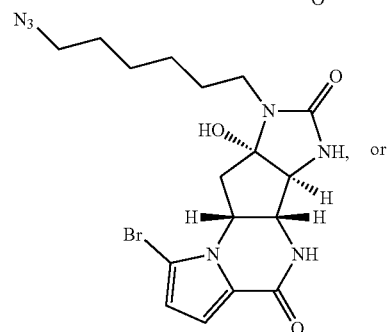
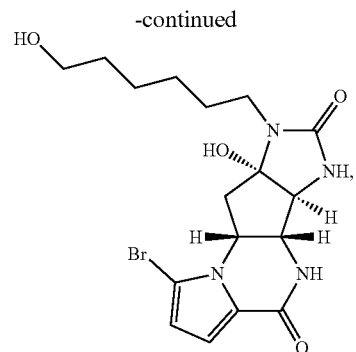
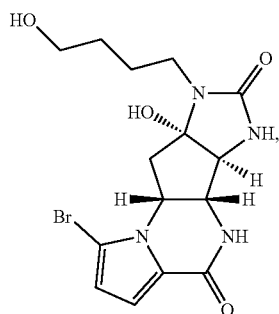


wherein R<sup>2</sup> is H or Me.

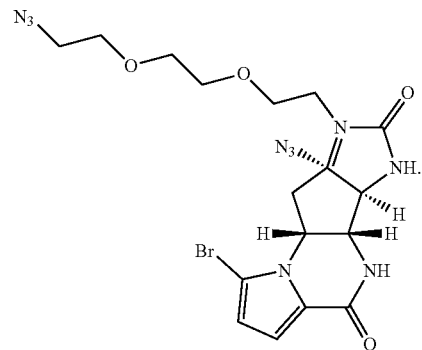
19. The compound of claim 18, wherein the compound is:



20. The compound of claim 1, selected from:



21. The compound of claim 1, wherein the compound is:



22. A pharmaceutical composition comprising a compound of any of claims 1-21 and a pharmaceutically acceptable excipient.

23. A method for the treatment, prevention, or delay of cancer, comprising administering a therapeutically effect



amount of a compound of any of claims 1-22, a pharmaceutically acceptable salt thereof, or a composition thereof to a subject in need thereof.

24. The method of any of claim 23, wherein the cancer is breast cancer, lung cancer, colorectal cancer, stomach cancer, ovarian cancer, papillary thyroid carcinoma, melanoma, prostate cancer, esophageal cancer, liver cancer, bladder cancer, renal cancer, head and neck cancers, salivary gland cancer, endometrial cancer, cervical cancer, pancreatic cancer, sarcoma, glioblastoma and glioma, or pleural mesothelioma.

25. The method of any of claim 24, wherein the cancer is breast cancer.

26. The method of claim 25, wherein the breast cancer is selected from the group consisting of ductal carcinoma in situ (DCIS), invasive ductal carcinoma (IDC), tubular carcinoma, medullary carcinoma, mucinous carcinoma, papillary carcinoma, cribriform carcinoma, invasive lobular carcinoma (ILC), inflammatory breast cancer, lobular carcinoma in situ (LCIS), luminal A, luminal B, triple-negative/basal-like, and HER2-enriched, normal-like breast cancer.

27. The method of any of claims 23-26, wherein the cancer is recurrent cancer at the primary site, metastatic cancer, or recurrent metastatic cancer.

28. The method of claim 25 or 26, wherein the breast cancer is metastatic breast cancer or recurrent metastatic breast cancer.

29. The method of any of claims 23-28, wherein the cancer is prevented or delayed.

30. The method of any of claims 23-29, wherein the administering is before surgery and/or radiotherapy and/or systemic medical therapy.

31. The method of any of claims 23-29, wherein the administering is after surgery and/or radiotherapy and/or systemic medical therapy.

32. The method of any of claims 23-29, wherein the administering is concurrent with systemic medical therapy.

33. The method of any of claims 30-32, wherein systemic medical therapy includes chemotherapies, hormonal therapies, targeted biologic therapies, and/or immunotherapies.

34. The method of any of claims 23-33, wherein treatment results in inhibition of induced transcription of osteopontin (OPN) in fibroblasts, inflammatory cells, and immune cells of the tumor microenvironment.

35. The method of claim 34, wherein the fibroblasts are mammary fibroblasts.

36. The method of any of claims 23-35, wherein the cancer is characterized by a tumor microenvironment exhibiting down regulation of fibroblast Tiam1 and upregulation of fibroblast OPN.

37. The method of any of claims 23-35, wherein the cancer is characterized by a tumor microenvironment exhibiting upregulation of fibroblast OPN.

38. The method of any of claims 34-37, wherein the inhibition occurs at or below the cytotoxic range determined for the cancer cells being treated.

39. The method of any of claims 34-37, wherein the transcription is of splice variants of OPN.

40. The method of claim 39, wherein the splice variants are osteopontin-a, osteopontin-b, or osteopontin-c.

41. The method of any of claim 38, wherein the inhibition results in interference with cancer cell adhesion, cancer cell invasion, and cancer stem cell populations.

42. The method of claim 23, further comprising coadministration to the subject one of the following:

an antitumor agent selected from the group consisting of paclitaxel, doxorubicin, daunorubicin, cyclophosphamide, methotrexate, 5-fluorouracil, thiotepa, busulfan, improsulfan, piposulfan, benzodopa, carboquone, meturedopa, uredopa, altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide, trimethylolmelamine, bullatacin, bullatacinone, camptothecin, bryostatin, callystatin, cryptophycin 1, cryptophycin 8, dolastatin, duocarmycin, eleutherobin, pancratistatin, sarcodictyin, spongistatin, chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard, carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine, calicheamicin, dynemicin, clodronate, esperamicin, aclacinomycin, actinomycin, authramycin, azaserine, bleomycin, cactinomycin, carabycin, carminomycin, carzinophilin, chromomycin, dactinomycin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycin C, mycophenolic acid, nogalamycin, olivomycin, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin, denopterin, pteropterin, trimetrexate, fludarabine, 6-mercaptopurine, thiamiprine, thioguanine, ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, androgens such as calusterone, dromostanolone propionate, epitostanol, mepitiostane, testolactone, aminoglutethimide, mitotane, trilostane, frolic acid, aceglatone, aldophosphamide glycoside, aminolevulinic acid, eniluracil, amsacrine, bestrabucil, bisantrene, edatraxate, defofamine, demecolcine, diaziquone, elfornithine, elliptinium acetate, epothilone, etoglucid, lentinan, lonidamine, maytansine, ansamitocine, mitoguzone, mitoxantrone, mopidanmol, nitraerine, pentostatin, phenamet, pirarubicin, losoxantrone, podophyllinic acid, 2-ethylhydrazide, procarbazine, razoxane, rhizoxin, schizophyllan, spirogermanium, tenuazonic acid, triaziquone, roridine A, anguidine, urethane, vindesine, dacarbazine, mannomustine, mitobronitol, mitolactol, pipobroman, gacytosine, docetaxel, chlorambucil, gemcitabine, 6-thioguanine, mercaptopurine, cisplatin, oxaliplatin, carboplatin, vinblastine, etoposide, ifosfamide, mitoxantrone, vincristine, vinorelbine, novantrone, teniposide, edatrexate, daunomycin, aminopterin, xeloda, ibandronate, irinotecan, topoisomerase inhibitor, difluoromethylornithine (DMFO), retinoic acid, capecitabine, and pharmacologically acceptable salts or derivatives thereof;

an anti-metabolite agent selected from the group consisting of, 5-fluorouracil, 5-fluorouracil prodrugs (e.g., capecitabine), 5-fluorodeoxyuridine monophosphate, cytarabine, 5-azacytidine, gemcitabine, mercaptopurine, thioguanine, azathioprine, adenosine, pentostatin, erythrohydroxynonyladenine, cladribine and pharmacologically acceptable salts or derivatives thereof,

an anti-estrogen agent selected from the group consisting of selective estrogen receptor modulators, pure receptor

antagonists, aromatase inhibitors, and anti-gonadotropins and pharmacologically acceptable salts or derivatives thereof; wherein

the selective estrogen receptor modulator (SERM) is selected from the group consisting of anordrin, bazedoxifene, broparestrol, clomifene, cyclofenil, lasofoxifene, ormeloxifene, ospemifene, raloxifene, tamoxifen citrate, toremifene citrate, and pharmacologically acceptable salts or derivatives thereof;

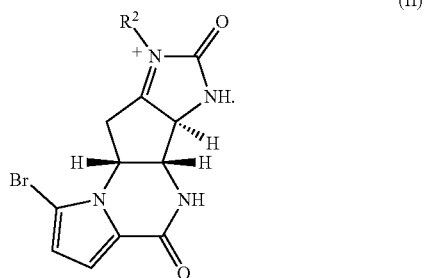
the pure receptor antagonist is selected from the group consisting of fulvestrant, brilanestrant, elacestrant, and pharmacologically acceptable salts or derivatives thereof;

the aromatase inhibitor is selected from the group consisting of anastrozole, letrozole, vorozole, exemestane, formestane and pharmacologically acceptable salts or derivatives thereof;

the anti-gonadotropin is selected from the group consisting of triptorelin, leuprolide acetate, and pharmacologically acceptable salts or derivatives thereof;

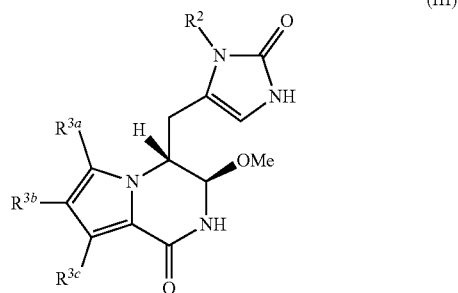
a tyrosine kinase inhibitor selected from the group consisting of trastuzumab, pertuzumab, imatinib, gefitinib, erlotinib, sunitinib, adavosertib, lapatinib and pharmacologically acceptable salts or derivatives thereof; and an immune checkpoint inhibitor selected from the group consisting of ipilimumab, pembrolizumab, nivolumab, avelumab, durvalunab, atezolizumab and pharmacologically acceptable salts or derivatives thereof.

**43.** A method of making a compound of claim 1, comprising addition of a nucleophile to an iminium intermediate of Formula (II):

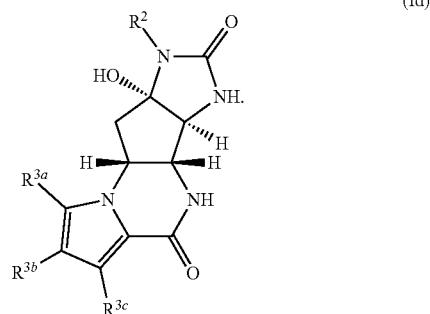


**44.** The method of claim 43, wherein the nucleophile is  $R^1-XH$  and X is  $-O-$ ,  $-S-$ , or  $-N(R^4)-$ .

**45.** A method of making a compound of claim 1, comprising acid-promoted cyclization of a compound of Formula (III) to afford a compound of Formula (Id):

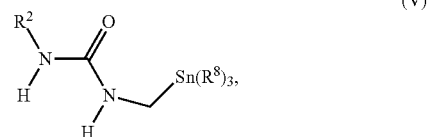
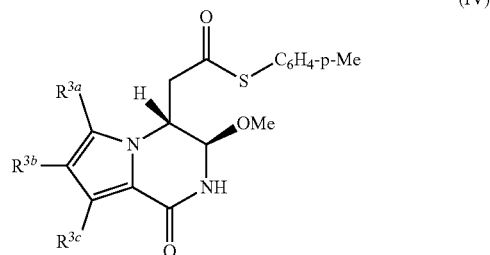


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**46.** The method of claim 47, wherein the acid is methanesulfonic acid, p-toluenesulfonic acid, sulfuric acid, hydrochloric acid, trifluoroacetic acid, trifluoromethanesulfonic acid, and nitric acid.

**47.** A method of making compound (III) of claim 45, comprising copper-mediated coupling between a compound of Formula (IV) and a compound of Formula (V):



wherein  $R^8$  is alkyl or cycloalkyl.

**48.** The method of claim 47, wherein the copper-mediated coupling is carried out with copper (I)-thiophene-2-carboxylate (CuTC) or copper(I) diphenylphosphinate (CuDPP).

**49.** The method of claim 47, further comprising treatment with an acid.

\* \* \* \* \*