CORTISTATIN ANALOGUES AND SYNTHESES AND USES THEREOF

Related Applications

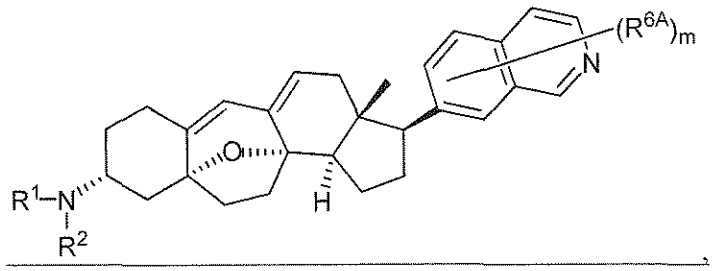
[0001] The present application claims priority under 35 U.S.C. § 119(e) to U.S. provisional patent applications, U.S.S.N. 61/920,674 filed December 24, 2013, U.S.S.N. 61/935,240 filed February 3, 2014, and U.S.S.N. 61/993,329 filed May 15, 2014, the entire contents of each of which is incorporated herein by reference.

Background

[0002] The cortistatins are a group of anti-angiogenic steroidal alkaloids first isolated in 2006 from the marine sponge *Corticium simplex*. See, *e.g.*, Aoki, *et al.*, *JACS* (2006) 128: 3148–9. From the date of isolation to the present, these natural products have been the subject of much study, especially in the development of total syntheses and of new unnatural biologically active analogs. See, *e.g.*, Aoki *et al.*, *Bioorganic & Medicinal Chemistry* (2007) 15: 6758–62. Mousseau *et al.*, *Cell Host & Microbe* (2012) 12: 97–108; Chen *et al.*, *Organic & Biomolecular Chemistry* (2010) 8: 2900; Hardin *et al.*, *European Journal of Organic Chemistry* (2010) 19: 3553. Thus, there is an active interest in the development of new cortistatin analogs and methods of their preparation.

Summary ~~of the Invention~~

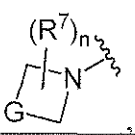
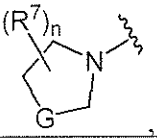
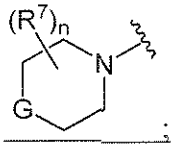
[0002a] In one aspect of the present disclosure, there is provided a compound of Formula:



or a pharmaceutically acceptable salt or quaternary amine salt thereof;

wherein:

m is 0, 1, 2, 3, or 4;

R^1 and R^2 are joined to form a ring of formula: , , or ;

n is 0, 1, or 2;

each instance of R^{6A} is independently halogen, $-NO_2$, -CN, $-OR^{6C}$, SR^{6C}, $N(R^{6C})_2$, $-C(=O)R^{6C}$, $-C(=O)OR^{6C}$, $-C(=O)N(R^{6C})_2$, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

wherein each instance of R^{6C} is independently hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, an oxygen protecting group when attached to oxygen, a sulfur protecting group when attached to sulfur, or a nitrogen protecting group when attached to nitrogen, optionally when attached to N the two R^{6C} groups may be joined to form an optionally substituted heterocyclyl or optionally substituted heteroaryl ring,

G is -NH-, $-NR^7$-, $-CH_2$-, $-CH(R^7)$-, or $-C(R^7)_2$-;

each instance of R^7 is independently amino, hydroxyl, halogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, substituted amino, substituted hydroxyl, thiol, substituted thiol, carbonyl, sulfonyl, sulfinyl, or a nitrogen protecting group when attached to a nitrogen atom; and

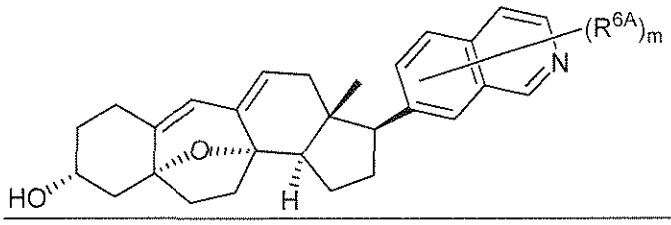
optionally wherein two R^7 groups are joined to form an optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl ring, or an oxo (=O) group.

[0002b] In another aspect of the present disclosure, there is provided a pharmaceutical composition comprising a compound as defined herein, or a pharmaceutically acceptable salt thereof, in combination with a pharmaceutically acceptable excipient.

[0002c] In yet another aspect of the present disclosure, there is provided use of a compound as defined herein, or a pharmaceutically acceptable salt, in the manufacture of a

medicament for the treatment of a medical condition by inhibiting CDK8 and/or CDK19 kinase activity in a subject.

[0002d] In a further aspect of the present disclosure, there is provided a compound of formula:



(D-2-h-ii),

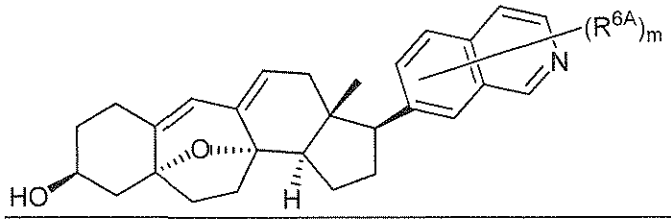
or a pharmaceutically acceptable salt thereof;

wherein:

m is 0, 1, 2, 3, or 4;

each instance of R^{6A} is independently halogen, $-NO_2$, -CN, $-OR^{6C}$, SR^{6C}, $N(R^{6C})_2$, $-C(=O)R^{6C}$, $-C(=O)OR^{6C}$, $-C(=O)N(R^{6C})_2$, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl; and wherein each instance of R^{6C} is independently hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, an oxygen protecting group when attached to oxygen, a sulfur protecting group when attached to sulfur, or a nitrogen protecting group when attached to nitrogen, optionally when attached to N the two R^{6C} groups may be joined to form an optionally substituted heterocyclyl or optionally substituted heteroaryl ring.

[0002e] In another aspect of the present disclosure, there is provided a compound of formula:



(D-1-h-ii)

or a pharmaceutically acceptable salt thereof;

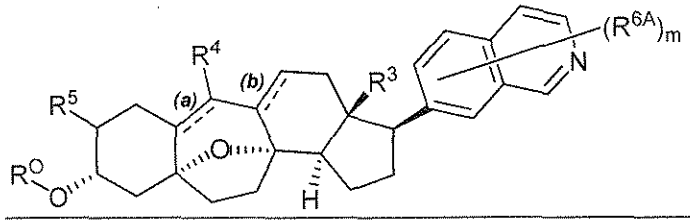
wherein:

m is 0, 1, 2, 3, or 4;

each instance of R^{6A} is independently halogen, $-NO_2$, -CN, $-OR^{6C}$, SR^{6C}, $N(R^{6C})_2$, $-C(=O)R^{6C}$, $-C(=O)OR^{6C}$, $-C(=O)N(R^{6C})_2$, optionally substituted alkyl, optionally substituted

alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl; and wherein each instance of R^{6C} is independently hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, an oxygen protecting group when attached to oxygen, a sulfur protecting group when attached to sulfur, or a nitrogen protecting group when attached to nitrogen, optionally when attached to N the two R^{6C} groups may be joined to form an optionally substituted heterocyclyl or optionally substituted heteroaryl ring.

[0002d] In yet another aspect of the present disclosure, there is provided a compound of formula:



(E-2-h-i),

or a pharmaceutically acceptable salt thereof;

wherein:

m is 0, 1, 2, 3, or 4;

R^4 is hydrogen, halogen, optionally substituted alkyl, or $-Si(R^A)_3$;

R^5 is hydrogen, halogen, or optionally substituted alkyl;

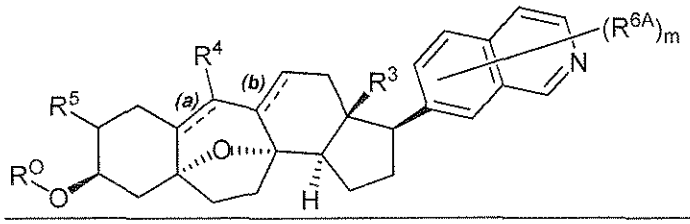
each instance of ----, designated as (*a*) and (*b*) represents a single or double bond;

R^O is optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, $-C(=O)R^A$, $-C(=O)OR^A$, $-C(=O)N(R^A)_2$, or an oxygen protecting group.

each instance of R^A is independently hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, carbonyl, silyl, an oxygen protecting group when attached to oxygen, a sulfur protecting group when attached to sulfur, or a nitrogen protecting group when attached to nitrogen; optionally when attached to N the two R^A groups may be joined to form an optionally substituted heterocyclyl or optionally substituted heteroaryl ring;

each instance of R^{6A} is independently halogen, $-NO_2$, -CN, $-OR^{6C}$, SR^{6C}, $N(R^{6C})_2$, $-C(=O)R^{6C}$, $-C(=O)OR^{6C}$, $-C(=O)N(R^{6C})_2$, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl; and wherein each instance of R^{6C} is independently hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, an oxygen protecting group when attached to oxygen, a sulfur protecting group when attached to sulfur, or a nitrogen protecting group when attached to nitrogen, optionally when attached to N the two R^{6C} groups may be joined to form an optionally substituted heterocyclyl or optionally substituted heteroaryl ring.

[0002e] In a further aspect of the present disclosure, there is provided a compound of formula:



(E-1-h-i),

or a pharmaceutically acceptable salt thereof;

wherein:

m is 0, 1, 2, 3, or 4;

R^4 is hydrogen, halogen, optionally substituted alkyl, or $-Si(R^A)_3$;

R^5 is hydrogen, halogen, or optionally substituted alkyl;

each instance of ----, designated as (*a*) and (*b*) represents a single or double bond;

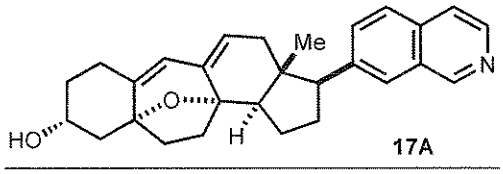
R^O is optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, $-C(=O)R^A$, $-C(=O)OR^A$, $-C(=O)N(R^A)_2$, or an oxygen protecting group.

each instance of R^A is independently hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, carbonyl, silyl, an oxygen protecting group when attached to oxygen, a sulfur protecting group when attached to sulfur, or a nitrogen protecting group when attached

to nitrogen; optionally when attached to N the two R^A groups may be joined to form an optionally substituted heterocyclyl or optionally substituted heteroaryl ring;

each instance of R^{6A} is independently halogen, $-NO_2$, -CN, $-OR^{6C}$, SR^{6C}, $N(R^{6C})_2$, $-C(=O)R^{6C}$, $-C(=O)OR^{6C}$, $-C(=O)N(R^{6C})_2$, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl; and wherein each instance of R^{6C} is independently hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, an oxygen protecting group when attached to oxygen, a sulfur protecting group when attached to sulfur, or a nitrogen protecting group when attached to nitrogen, optionally when attached to N the two R^{6C} groups may be joined to form an optionally substituted heterocyclyl or optionally substituted heteroaryl ring.

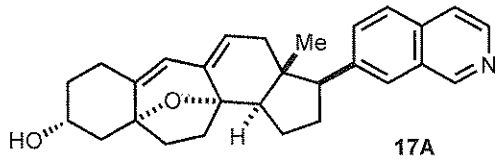
[0002f] In another aspect of the present disclosure, there is provided a compound of Formula:



17A

or a pharmaceutically acceptable salt thereof.

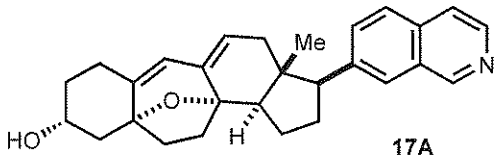
[0002g] In yet another aspect of the present disclosure, there is provided a pharmaceutical composition comprising compound:



17A

or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

[0002h] In a further aspect of the present disclosure, there is provided use of a compound:

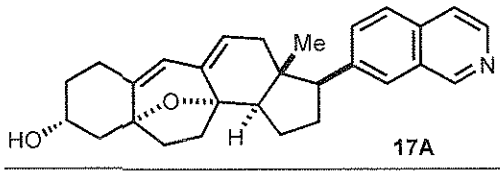


17A

or a pharmaceutically acceptable salt optionally in a pharmaceutical composition thereof for the manufacture of a medicament for the treatment of a medical condition by inhibiting CDK8 and/or CDK19 kinase activity in a subject.

[0002i] In another aspect of the present disclosure, there is provided a compound as defined herein, or a pharmaceutically acceptable salt, optionally in a pharmaceutical composition, for use in therapy.

[0002j] In yet another aspect of the present disclosure, there is provided a compound:



17A

or a pharmaceutically acceptable salt optionally in a pharmaceutical composition thereof for use in therapy.

[0003] Provided herein are new cortistatin analogs of Formula (**A**), (**B**), and (**C**), and pharmaceutically acceptable salts, quaternary amine salts, and N-oxides thereof, synthesized, in part, by reductive amination of a ketone of Formula (**B**) to provide the aminated product of Formula (**A**), as depicted in *Scheme 1*, optionally *via* an imine intermediate of Formula (**C**).

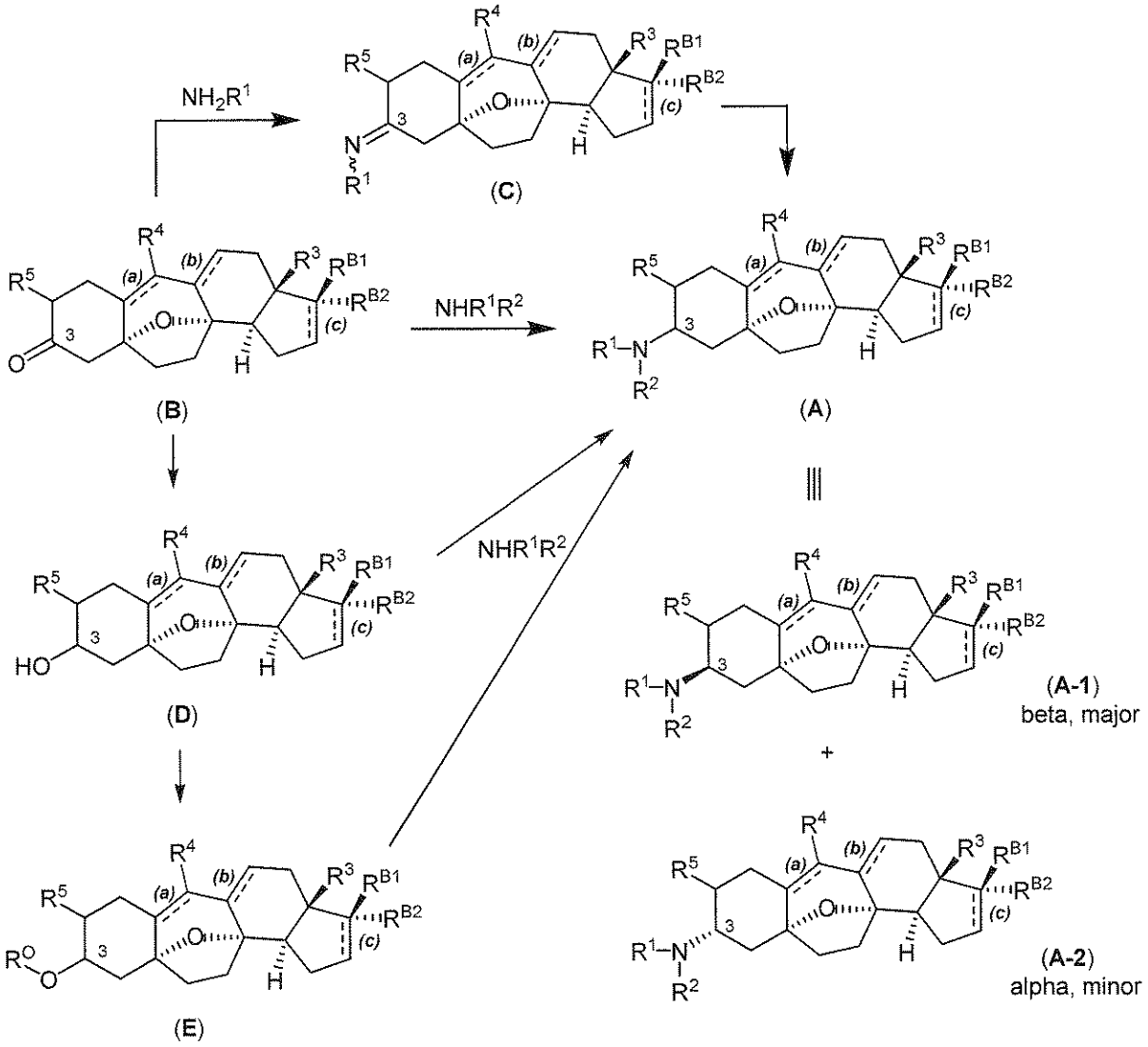
[0004] Further provided are new cortistatin analogs prepared by reduction of the ketone of Formula (**B**) to provide a C3-hydroxyl compound of Formula (**D**). Still yet further provided is a compound of Formula (**E**), prepared by substitution of the compound of Formula (**D**). Such compounds may also be converted to a compound of Formula (**A**) upon treatment with an amine under suitable conditions.

[0005] It has been surprisingly found that the *beta* isomers of Formula (**A**), referred to as Formula (**A-1**), have been found to be equipotent, or more potent, than cortistatin A at inhibiting CDK8 kinase activity and the proliferation of AML cells, and it has also been found that the corresponding *alpha* isomers of Formula (**A**), referred to as Formula (**A-2**), are

also very potent. Furthermore, it has been discovered that compounds of Formula (**B**) have been found active against the growth of AML cell lines in culture and CDK8 kinase activity in cells.

[0006] Further provided are pharmaceutical compositions comprising a cortistatin analogs of Formula (**A**), (**B**), (**C**), (**D**), or (**E**) or a pharmaceutically acceptable salt, quaternary amine salt, or N-oxide thereof, and a pharmaceutically acceptable excipient. Further provided are methods of use and treatment.

Scheme 1.



[0007] Thus, as ~~as~~ further described herein, ~~in one aspect,~~ provided is a method of preparing a compound of Formula (**A**), or a pharmaceutically acceptable salt, quaternary amine salt, or N-oxide thereof; the method comprising contacting a compound of Formula (**B**), or a pharmaceutically acceptable salt, provided R^{B1} and R^{B2} are not joined to form an

oxo group; with an amine of formula HNR^1R^2, or salt thereof, under reductive amination conditions.

[0008] ~~In another aspect, provided is~~ The present disclosure also provides a method of preparing a compound of Formula (**D**), or a pharmaceutically acceptable salt thereof; the method comprising contacting a compound of Formula (**B**), or a pharmaceutically acceptable salt, with a reducing agent, to provide a compound of Formula (**D**).

[0009] ~~In another aspect, provided is~~ The present disclosure further provides a method of preparing a compound of Formula (**E**), or a pharmaceutically acceptable salt thereof; the method comprising contacting a compound of Formula (**D**), or a pharmaceutically acceptable salt thereof, with a compound of formula R^O-LG, wherein LG is a leaving group, to provide a compound of Formula (**E**).

[0010] ~~In another aspect, provided is~~ The present disclosure also provides a method of preparing a compound of Formula (**A**), or a pharmaceutically acceptable salt, quaternary amine salt, or N-oxide thereof; the method comprising contacting a compound of Formula (**E**), wherein R^O is $C(=O)R^A$, or a pharmaceutically acceptable salt thereof, with a compound of formula NHR^1R^2, to provide a compound of Formula (**A**).

[0011] ~~In another aspect, provided are~~ The present disclosure further provides pharmaceutical compositions comprising a compound of Formula (**A**), (**B**), (**C**), (**D**), or (**E**), or a pharmaceutically acceptable salt, quaternary amine, or N-oxide thereof.

[0012] ~~In another aspect, provided is~~ The present disclosure also provides a method of treating a condition associated with angiogenesis comprising administering to a subject in need thereof a compound of Formula (**A**), (**B**), (**C**), (**D**), or (**E**), or a pharmaceutically acceptable salt, quaternary amine, or N-oxide thereof, or a pharmaceutical composition thereof. In certain embodiments, the condition is a diabetic condition, an inflammatory condition, macular degeneration, obesity, atherosclerosis, or a proliferative disorder.

[0013] ~~In yet another aspect, provided is~~ The present disclosure further provides a method of treating a condition associated with CDK8 and/or CDK19 kinase activity, comprising administering to a subject in need thereof a compound of Formula (**A**), (**B**), (**C**), (**D**), or (**E**), or a pharmaceutically acceptable salt, quaternary amine, or N-oxide thereof, or a pharmaceutical composition thereof. In certain embodiments, the condition is a proliferative disorder. In certain embodiments, the proliferative disorder is cancer. In certain embodiments,the cancer is a hematopoietic cancer. In certain embodiments, the hematopoietic cancer is lymphoma. In certain embodiments, the hematopoietic cancer is leukemia. In certain embodiments, the hematopoietic cancer is multiple myeloma. In certain embodiments, the leukemia is acute myelocytic leukemia (AML). In certain embodiments, the proliferative disorder is a myeloproliferative neoplasm.

In certain embodiments, the myeloproliferative neoplasm is primary myelofibrosis (PMF). In certain embodiments, the cancer is a solid tumor.

[0014] ~~In yet another aspect, provided is~~ The present disclosure also provides a method of inhibiting CDK8 and/or CDK19 kinase activity in a cell comprising contacting a compound of Formula (**A**), (**B**), (**C**), (**D**), or (**E**), or a pharmaceutically acceptable salt, quaternary amine, or N-oxide thereof, or a pharmaceutical composition thereof, with the cell.

[0015] ~~In yet another aspect, provided is~~ The present disclosure further provides a method of modulating the β-catenin pathway in a cell comprising contacting a compound of Formula (**A**), (**B**), (**C**), (**D**), or (**E**), or a pharmaceutically acceptable salt, quaternary amine, or N-oxide thereof, or a pharmaceutical composition thereof, with the cell.

[0016] ~~In yet another aspect, provided is~~ The present disclosure also provides a method of modulating STAT1 activity in a cell comprising contacting a compound of Formula (**A**), (**B**), (**C**), (**D**), or (**E**), or a pharmaceutically acceptable salt, quaternary amine, or N-oxide thereof, or a pharmaceutical composition thereof, with the cell.

[0017] ~~In yet another aspect, provided is~~ The present disclosure further provides a method of modulating the TGFβ/BMP pathway in a cell comprising contacting a compound of Formula (**A**), (**B**), (**C**), (**D**), or (**E**), or a pharmaceutically acceptable salt, quaternary amine, or N-oxide thereof, or a pharmaceutical composition thereof, with the cell.

[0018] ~~In yet another aspect, provided is~~ The present disclosure also provides a method of modulating HIF-1-A (HIF-1-alpha) activity in a cell comprising contacting a compound of Formula (**A**), (**B**), (**C**), (**D**), or (**E**), or a pharmaceutically acceptable salt, quaternary amine, or N-oxide thereof, or a pharmaceutical composition thereof, with the cell.

[0019] ~~In yet another aspect, provided is~~ The present disclosure futher provides a method of increasing BIM expression to induce apoptosis in a cell comprising contacting a compound of Formula (**A**), (**B**), (**C**), (**D**), or (**E**), or a pharmaceutically acceptable salt, quaternary amine, or N-oxide thereof, or a pharmaceutical composition thereof, with the cell.

[0020] In any of the above recited methods, the method is an *in vitro* method or an *in vivo* method.

[0021] Further provided are CDK8 or CDK19 Tpr105 point mutants, and methods of use thereof. For example, ~~in one aspect,~~ provided is a method of validating CDK8 and /or CDK19 kinase activity in a cell by contacting a CDK8 or CDK19 Trp105 point mutant and a cortistatin or cortistatin analog. ~~In another aspect, provided is~~ The present disclosure also provides a CDK8 Trp105 point mutant. In certain embodiments, the CDK8 Trp105 point mutant has an amino acid sequence that a

degree of homology to the amino acid sequence of SEQ ID NO: 1 of at least about 80%. Further provided is a protein that has a degree of homology to the amino acid sequence of SEQ ID NO: 1 of at least about 80%. ~~In yet another aspect, provided is~~ The present disclosure further provides a CDK19 Trp105 point mutant. In certain embodiments, the CDK19 Trp105 point mutant has an amino acid sequence that a degree of homology to the amino acid sequence of SEQ ID NO: 2 of at least about 80%. Further provided is a protein that has a degree of homology to the amino acid sequence of SEQ ID NO: 2 of at least about 80%.

[0022] The details of one or more embodiments of the ~~invention~~ present disclosure are set forth in the accompanying Figures. Other features, objects, and advantages of the ~~invention~~ present disclosure will be apparent from the Detailed Description, the Examples, and from the claims.

Brief Description of the Drawings

[0023] ***Figure 1*** depicts the structure of cortistatin A and other known cortistatins.

[0024] ***Figures 2A-2B*** depict an exemplary reductive amination of ketone 13 to provide compounds 14A and 14B, and their corresponding N-oxides (Figure 2A) and the molecular structure of compound 14B, wherein the dimethylamine is beta (axial) (Figure 2B).

[0025] ***Figures 3A-3M*** depict exemplary compounds of the present invention. Additional compounds are further described herein.

[0026] ***Figures 4A-4B*** depict the dose-dependent inhibition of proliferation of AML cell lines MV4;11 (4A) and MOLM-14 (4B) upon 7-day treatment with cortistatin A and the indicated analogs. Cells were passaged and fresh compounds were added on day 3 (mean+/-standard error, n=3).

[0027] ***Figures 5A-5B*** depict the dose-dependent inhibition of proliferation of AML cell line MV4;11 upon 3-day or 10-day treatment with the indicated cortistatin analogs. Cells were passaged and fresh compounds were added on days 3 and 7 (mean+/-standard error, n=3).

[0028] ***Figures 6A-6B*** depict the dose-dependent inhibition of proliferation of AML cell line MOLM-14 upon 3-day or 10-day treatment with the indicated cortistatin analogs. Cells were passaged and fresh compounds were added on days 3 and 7 (mean+/-standard error, n=3).

[0029] ***Figure 7*** demonstrates cortistatin A potently and selectively inhibits CDK8 module kinase activity in cells. CDK8 has been reported to inhibit interferon gamma-stimulated phosphorylation of STAT1 S727. See, *e.g.*, Bancerek *et al.*, *Immunity* (2013) 38:250–262. HepG2 cells were incubated with cortistatin A for 1 hour followed by the