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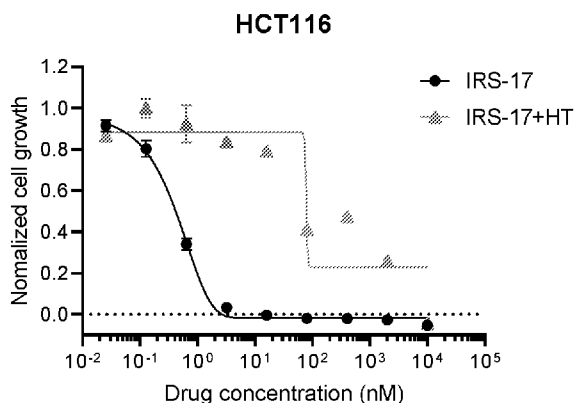


FIG. 1A

(57) Abstract: In one aspect, compounds and associated pharmaceutical compositions are described herein for the treatment of cancer. In some embodiments, for example, a pharmaceutical composition comprises a compound of Formula (I) in an amount sufficient to exhibit anti cancer activity.



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COMPOUNDS HAVING ANTICANCER ACTIVITY

RELATED APPLICATION DATA

The present application claims priority pursuant to Article 8 of the Patent Cooperation
5 Treaty to United States Provisional Patent Application Serial Number 63/047,612 filed July 2,
2020 which is incorporated herein by reference in its entirety.

STATEMENT OF GOVERNMENT RIGHTS

This invention was made with government support under Grant No. DP1AI124669
10 awarded by the National Institutes of Health (NIH). The government has certain rights in the
invention.

FIELD

The present invention relates to anticancer compounds and modes of action associated
15 with the compounds.

BACKGROUND

Cancer is responsible for a significant number of annual deaths in the United States and
worldwide. Many strategies for the treatment of cancer currently exist, such as radiation therapy,
20 chemotherapy, and surgery including the complete removal of cancerous tissue as well as
cytoreduction and palliation. In a number of cases, cancer treatment strategies comprise adjuvant
therapies such as surgery and chemotherapy. In cytoreductive procedures, for example, any
abnormal tissue remaining after the surgery can be treated with chemotherapy.

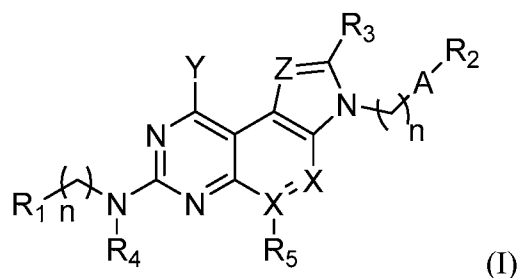
The inherently destructive nature of cancer therapies often results in harmful side-effects
25 such as damage to healthy, non-cancerous tissues. The cytotoxicity of various chemotherapeutic
agents, for example, can result in anemia, alopecia (hair loss), nausea and vomiting, damage to
nerves leading to burning, numbness, tingling or shooting pain. Chemotherapy can additionally
precipitate immunosuppression and myelosuppression thereby increasing a patient's chances for
infection and other disease.

30 Alternative strategies have also been developed, such as hypothermic technologies
employing nanoparticle compositions. Nanoparticle therapies have several disadvantages,
including the inability to treat deep tumor tissue and the negative immuno-response of

nanoparticles collecting in various areas of the lymphatic system. Accordingly, new cancer treatments are needed which can be effective at nanomolar concentrations.

SUMMARY

In one aspect, compounds and associated pharmaceutical compositions are described herein for the treatment of cancer. In some embodiments, for example, a pharmaceutical composition comprises a compound of Formula (I) and/or salts thereof:



wherein R₁, R₃, R₄ and R₅ are independently selected from the group consisting of hydrogen, alkyl, alkenyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, imine, cyanoimine, alkylene-aryl, alkylene-heteroaryl, amide, sulfonamide, acid, halo, and urea, wherein the alkyl, alkenyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, alkylene-aryl, alkylene-heteroaryl, amide and sulfonamide are optionally substituted with one or more substituents selected from the group consisting of (C₁–C₁₀)-alkyl, (C₁–C₁₀)-alkenyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, alkoxy, amide, sulfonamide, urea, halo, cyano, hydroxy, C(O)OR₆, and C(O)R₇, wherein R₆ is selected from the group consisting of hydrogen, alkyl and alkenyl and R₇ is selected from the group consisting of hydrogen, alkyl, alkenyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl and NR₈R₉, wherein R₈ and R₉ are independently selected from the group consisting of hydrogen, alkyl, alkenyl, aryl and heteroaryl; and

wherein R₂ is selected from the group consisting of alkyl, cycloalkyl, heterocycloalkyl, alkynyl, alkenyl, alkynylene-alkyl, alkynylene-cycloalkyl, alkynylene-heterocycloalkyl, alkynylene-aryl, alkynylene-heteroaryl, alkynylene-amine, alkynylene-protected amine, alkynylene-alkylsilane, fluoroalkyl, fluoro, bromo, B(OH)₂, nitro, cyano, and alkoxy; and wherein A is selected from the group consisting of aryl and heteroaryl; and

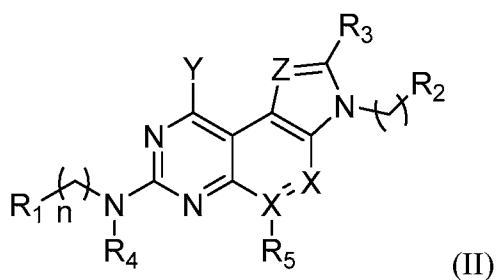
wherein X and Z are independently selected from the group consisting of C, N, O, S, SO₂, and NR₁₀R₁₁, wherein R₁₀ and R₁₁ are independently selected from the group consisting of hydrogen, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, alkenyl, aryl, heteroaryl, amide, sulfonamide, urea and C(O)R₁₂ wherein R₁₂ is selected from the group consisting of hydrogen, alkyl, alkenyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl and heteroaryl and wherein R₁₀ and R₁₁ may optionally form a ring structure; and

wherein Y is selected from the group consisting of OH, alkoxy, and NR₁₃R₁₄, wherein R₁₃ and R₁₄ are independently selected from the group consisting of hydrogen, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, alkenyl, aryl, heteroaryl, amide, sulfonamide, urea, alkylene-aryl, alkylene-heteroaryl, and C(O)R₁₅ wherein R₁₅ is selected from the group consisting of hydrogen, alkyl, alkenyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl and heteroaryl and wherein R₁₃ and R₁₄ may optionally form a ring structure, wherein the aryl, heteroaryl, alkylene-aryl and alkylene heteroaryl are optionally substituted with one or more substituents selected from the group consisting of alkyl, alkenyl, alkynyl, halo, and alkynylene-alkylsilane; and

n is an integer from 0 to 5,

wherein the compound of Formula (I) is present in the pharmaceutical composition in an amount sufficient to exhibit anticancer properties.

In some embodiments, a pharmaceutical composition comprises a compound of Formula (II) and/or salts thereof:



wherein R₁, R₃, R₄ and R₅ are independently selected from the group consisting of hydrogen, alkyl, alkenyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, alkylene-aryl, alkylene-heteroaryl, amide, sulfonamide, acid, halo, and urea, wherein the alkyl, alkenyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, alkylene-aryl, alkylene-heteroaryl, amide and sulfonamide are optionally substituted with one or more substituents selected from the group consisting of (C₁-C₁₀)-alkyl, (C₁-C₁₀)-alkenyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl,

alkoxy, amide, sulfonamide, urea, halo, hydroxy, C(O)OR₆, and C(O)R₇, wherein R₆ is selected from the group consisting of hydrogen, alkyl and alkenyl and R₇ is selected from the group consisting of hydrogen, alkyl, alkenyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl and NR₈R₉, wherein R₈ and R₉ are independently selected from the group consisting of

5 hydrogen, alkyl, alkenyl, aryl and heteroaryl; and

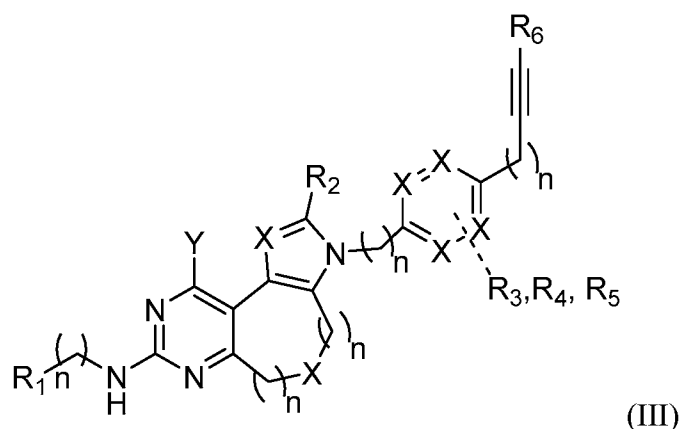
wherein R₂ is selected from the group consisting of arylene-alkynyl, heteroarylene-alkynyl, arylene-alkenyl, heteroarylene-alkenyl, alkynylene-alkyl, alkynylene-cycloalkyl, alkynylene-heterocycloalkyl, alkynylene-aryl, alkynylene-heteroaryl, alkenylene-aryl, alkenylene-heteroaryl, alkynylene-amine, alkynylene-protected amine, and alkynylene-alkylsilane; and

10 wherein X and Z are independently selected from the group consisting of C, N, O, S, SO₂, and NR₁₀R₁₁, wherein R₁₀ and R₁₁ are independently selected from the group consisting of hydrogen, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, alkenyl, aryl, heteroaryl, amide, sulfonamide, urea and C(O)R₁₂ wherein R₁₂ is selected from the group consisting of hydrogen, alkyl, alkenyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl and heteroaryl and wherein R₁₀ and R₁₁ may optionally form a ring structure; and

wherein Y is selected from the group consisting of OH and NR₁₂R₁₃, wherein R₁₃ and R₁₄ are independently selected from the group consisting of hydrogen, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, alkenyl, aryl, heteroaryl, amide, sulfonamide, urea and C(O)R₁₅ wherein R₁₅ is selected from the group consisting of hydrogen, alkyl, alkenyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl and heteroaryl and wherein R₁₃ and R₁₄ may optionally form a ring structure; and n is an integer from 0 to 5,

wherein the compound of Formula (II) is present in the pharmaceutical composition in an amount sufficient to exhibit anticancer properties.

25 In another aspect, a pharmaceutical composition comprises a compound of Formula (III) and/or salts thereof:



wherein $R_1 - R_6$ are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, amide, sulfonamide, halo, urea, and $-C(O)OR_7$, wherein R_7 is selected from the group consisting of hydrogen and alkyl, and wherein each X is

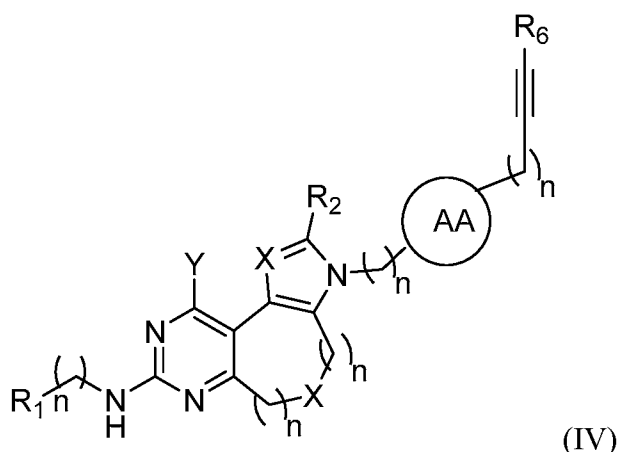
5 independently selected from the group consisting of C, N, O, S, SO_2 , and NR_8R_9 , wherein R_8 and R_9 are independently selected from the group consisting of hydrogen, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, alkenyl, aryl, heteroaryl, amide, sulfonamide, urea and $C(O)R_{10}$ wherein R_{10} is selected from the group consisting of hydrogen, alkyl, alkenyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl and heteroaryl and wherein R_8 and R_9 may optionally form a

10 ring structure; and wherein Y is selected from the group consisting of OH and $NR_{11}R_{12}$, wherein R_{11} and R_{12} are independently selected from the group consisting of hydrogen, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, alkenyl, aryl, heteroaryl, amide, sulfonamide, urea and $C(O)R_{13}$ wherein R_{13} is selected from the group consisting of hydrogen, alkyl, alkenyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl and heteroaryl and wherein R_{11} and R_{12} may optionally form a

15 ring structure; and n is an integer from 0 to 5,

wherein the compound of Formula (III) is present in the pharmaceutical composition in an amount sufficient to exhibit anticancer properties.

In another aspect, a pharmaceutical composition comprises a compound of Formula (IV) and/or salts thereof:

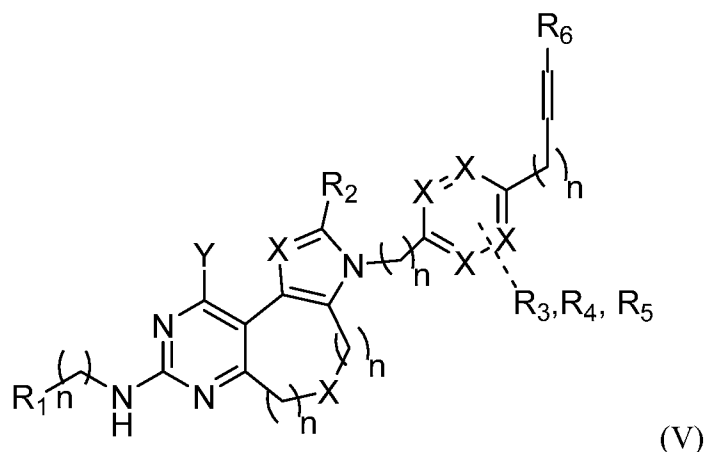


(IV)

wherein $R_1 - R_6$ are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, amide, sulfonamide, halo, urea, and $-C(O)OR_7$, wherein R_7 is selected from the group consisting of hydrogen and alkyl, and wherein each X is independently selected from the group consisting of C, N, O, S, SO_2 , and NR_8R_9 , wherein R_8 and R_9 are independently selected from the group consisting of hydrogen, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, alkenyl, aryl, heteroaryl, amide, sulfonamide, urea and $C(O)R_{10}$ wherein R_{10} is selected from the group consisting of hydrogen, alkyl, alkenyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl and heteroaryl and wherein R_8 and R_9 may optionally form a ring structure; and wherein Y is selected from the group consisting of OH and $NR_{11}R_{12}$, wherein R_{11} and R_{12} are independently selected from the group consisting of hydrogen, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, alkenyl, aryl, heteroaryl, amide, sulfonamide, urea and $C(O)R_{13}$ wherein R_{13} is selected from the group consisting of hydrogen, alkyl, alkenyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl and heteroaryl and wherein R_{11} and R_{12} may optionally form a ring structure; and wherein AA is selected from the group consisting of arylene, heteroarylene, cycloalkylene, and heterocycloalkylene, and n is an integer from 0 to 5,

wherein the compound of Formula (IV) is present in the pharmaceutical composition in an amount sufficient to exhibit anticancer properties.

In another aspect, a pharmaceutical composition comprises a compound of Formula (V) and/or salts thereof:

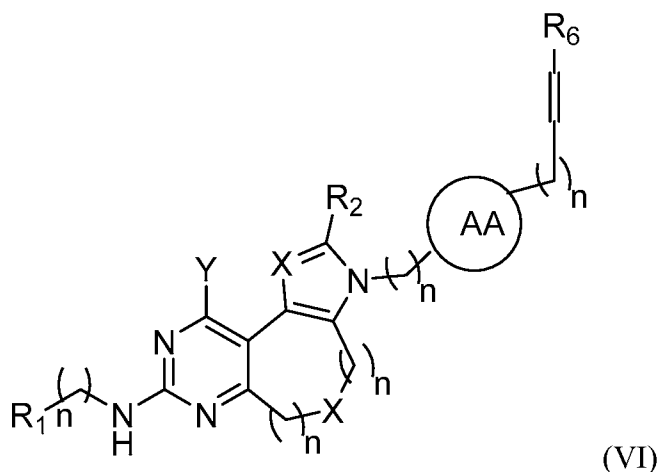


(V)

wherein $R_1 - R_6$ are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, amide, sulfonamide, halo, urea, and $-C(O)OR_7$, wherein R_7 is selected from the group consisting of hydrogen and alkyl, and wherein each X is
 5 independently selected from the group consisting of C, N, O, S, SO_2 , and NR_8R_9 , wherein R_8 and R_9 are independently selected from the group consisting of hydrogen, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, alkenyl, aryl, heteroaryl, amide, sulfonamide, urea and $C(O)R_{10}$ wherein R_{10} is selected from the group consisting of hydrogen, alkyl, alkenyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl and heteroaryl and wherein R_8 and R_9 may optionally form a
 10 ring structure; and wherein Y is selected from the group consisting of OH and $NR_{11}R_{12}$, wherein R_{11} and R_{12} are independently selected from the group consisting of hydrogen, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, alkenyl, aryl, heteroaryl, amide, sulfonamide, urea and $C(O)R_{13}$ wherein R_{13} is selected from the group consisting of hydrogen, alkyl, alkenyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl and heteroaryl and wherein R_{11} and R_{12} may optionally form a
 15 ring structure; and n is an integer from 0 to 5,

wherein the compound of Formula (V) is present in the pharmaceutical composition in an amount sufficient to exhibit anticancer properties.

In another aspect, a pharmaceutical composition comprises a compound of Formula (VI) and/or salts thereof:



wherein R₁ – R₆ are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, amide, sulfonamide, halo, urea, and –C(O)OR₇, wherein R₇ is selected from the group consisting of hydrogen and alkyl, and wherein each X is independently selected from the group consisting of C, N, O, S, SO₂, and NR₈R₉, wherein R₈ and R₉ are independently selected from the group consisting of hydrogen, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, alkenyl, aryl, heteroaryl, amide, sulfonamide, urea and C(O)R₁₀ wherein R₁₀ is selected from the group consisting of hydrogen, alkyl, alkenyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl and heteroaryl and wherein R₈ and R₉ may optionally form a ring structure; and wherein Y is selected from the group consisting of OH and NR₁₁R₁₂, wherein R₁₁ and R₁₂ are independently selected from the group consisting of hydrogen, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, alkenyl, aryl, heteroaryl, amide, sulfonamide, urea and C(O)R₁₃ wherein R₁₃ is selected from the group consisting of hydrogen, alkyl, alkenyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl and heteroaryl and wherein R₁₁ and R₁₂ may optionally form a ring structure; and wherein AA is selected from the group consisting of arylene, heteroarylene, cycloalkylene, and heterocycloalkylene, and n is an integer from 0 to 5,

wherein the compound of Formula (VI) is present in the pharmaceutical composition in an amount sufficient to exhibit anticancer properties.

In some embodiments, one or more compounds falling under one or more of Formulas (I)-(VI) exhibit anticancer properties at nanomolar (nM) concentrations. In some embodiments, for example, the one or more compounds exhibit anticancer properties at a concentration of 0.01 nM to greater than 1 μ M.

In another aspect, methods of treating cancerous tissue are described herein. In some embodiments, a method comprises administering to a patient having cancerous tissue one or more compounds of Formula(s) I-VI in therapeutically effective amount. In some embodiments, a therapeutically effective amount stops cancerous cell growth and/or tumor growth. A

5 therapeutically effective amount may also reduce tumor size, in some embodiments.

These and other embodiments are further described in the following detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A and 1B illustrate inhibition of cancer cell growth by a compound of Formula I
10 described herein according to some embodiments.

FIG. 2 illustrates folate competition from a compound of Formula I according to some embodiments.

FIGS. 3A and 3B illustrate change in tumor volume over time and tumor doubling time, respectively, in response to treatment with a compound of Formula I, according to some
15 embodiments.

FIG. 3C illustrates levels of intermediates in purine synthesis glycineamide ribonucleotide (GAR) and 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) in tumors treated with a compound of Formula 1 according to some embodiments.

FIGS. 4A and 4B illustrate change in tumor volume over time and tumor doubling time, respectively, in response to treatment with a compound of Formula I, according to some
20 embodiments.

FIG. 5A illustrate change in tumor volume in response to administration of a compound for Formula I according to some embodiments.

FIG. 5B illustrates pool size of circulating thymidine in response to administration of
25 Compound 1.

FIG. 5C illustrates thymidine ribonucleotide intermediate dUMP and purine intermediates GAR and AICAR levels in the tumors in response to administration of Compound 1.

DETAILED DESCRIPTION

Embodiments described herein can be understood more readily by reference to the following detailed description and examples and their previous and following descriptions. Elements, apparatus and methods described herein, however, are not limited to the specific
5 embodiments presented in the detailed description and examples. It should be recognized that these embodiments are merely illustrative of the principles of the present invention. Numerous modifications and adaptations will be readily apparent to those of skill in the art without departing from the spirit and scope of the invention.

10 Definitions

The term “alkyl” as used herein, alone or in combination, refers to a straight or branched saturated hydrocarbon group optionally substituted with one or more substituents. For example, an alkyl can be $C_1 - C_{30}$ or $C_1 - C_{18}$.

15 The term “alkenyl” as used herein, alone or in combination, refers to a straight or branched chain hydrocarbon group having at least one carbon-carbon double bond and optionally substituted with one or more substituents

The term “alkynyl” as used herein, alone or in combination, refers to a straight or branched chain hydrocarbon group having at least one carbon-carbon triple bond and optionally substituted with one or more substituents including, but not limited to, alkyl, cycloalkyl,
20 heterocycloalkyl, aryl, heteroaryl, amine, and/or alkylsilane.

The term “aryl” as used herein, alone or in combination, refers to an aromatic monocyclic or polycyclic ring system optionally substituted with one or more ring substituents.

The term “heteroaryl” as used herein, alone or in combination, refers to an aromatic monocyclic or polycyclic ring system in which one or more of the ring atoms is an element
25 other than carbon, such as nitrogen, oxygen and/or sulfur.

The term “cycloalkyl” as used herein, alone or in combination, refers to a non-aromatic, mono- or polycyclic ring system optionally substituted with one or more ring substituents.

The term “heterocycloalkyl” as used herein, alone or in combination, refers to a non-aromatic, mono- or polycyclic ring system in which one or more of the atoms in the ring system
30 is an element other than carbon, such as nitrogen, oxygen or sulfur, alone or in combination, and wherein the ring system is optionally substituted with one or more ring substituents.

The term “heteroalkyl” as used herein, alone or in combination, refers to an alkyl moiety as defined above, having one or more carbon atoms in the chain, for example one, two or three carbon atoms, replaced with one or more heteroatoms, which may be the same or different, where the point of attachment to the remainder of the molecule is through a carbon atom of the heteroalkyl radical.

The term “alkoxy” as used herein, alone or in combination, refers to the moiety RO-, where R is alkyl or alkenyl defined above.

The term “halo” as used herein, alone or in combination, refers to elements of Group VIIA of the Periodic Table (halogens). Depending on chemical environment, halo can be in a neutral or anionic state. Halo, for example, covers fluoro, chloro, bromo, and iodo.

I. Pharmaceutical Compositions for Treating Cancer

Various compounds are described herein. As discussed above and further illustrated in the examples below, the compounds can exhibit anticancer properties in some embodiments.

The compounds can fall under any one of Formulas I-VI described above. Compounds and/or salt(s) of Formulas I-VI can be individually administered in any amount consistent with precluding or inhibiting growth of cancerous tissue. In some embodiments, one or more of the compounds are administered in an amount or concentration of 0.001 nM to greater than 1 μ M. A compounds of any of Formulas I-VI can also be administered in an amount or concentration selected from Table I.

Table I – Amount of Compound of Formulas I-VI

0.01 nM – 500 nM
0.1 nM – 300 nM
1 nM to 150 nM
0.1 nM to 10 μ M
1 nM to 1 μ m

Additionally, compounds and/or salts of Formulas I-VI can be combined with one another and/or other chemotherapeutic agents or adjuvants in pharmaceutical compositions described herein. Compounds and/or salt(s) of Formulas I-VI. can also be combined with any physiologically suitable carrier or excipient.

The amount or concentration of compounds of Formulas I-VI employed in pharmaceutical compositions described herein can be dependent on the identity and/or nature of the cancer being treated. In some embodiments, compounds of Formulas I-VI are applied against cancer cell lines down-regulating reduced folate carrier (RFC). These cell lines, for example, can be resistant to pemetrexed and/or methotrexate. However, as shown herein, compounds of Formulas I-VI can exhibit higher potency to cell lines down-regulating RFC relative to pemetrexed and/or methotrexate. Compounds of Formulas I-VI, in some embodiments, can inhibit cancer cell growth by disrupting purine and/or thymidine biosynthesis. One or more compounds of Formulas I-VI, for example, may disrupt purine and/or thymidine biosynthesis in pancreatic cancer cells and/or colorectal cancer cells. In some embodiments, compounds of Formulas I-VI can exhibit an IC₅₀ for inhibiting cancer cell growth of 0.001 nM to 1 μM or 0.1 nM to 100 nM. The IC₅₀, in some embodiments, is greater than 1 μM.

II. Methods of Treating Cancer

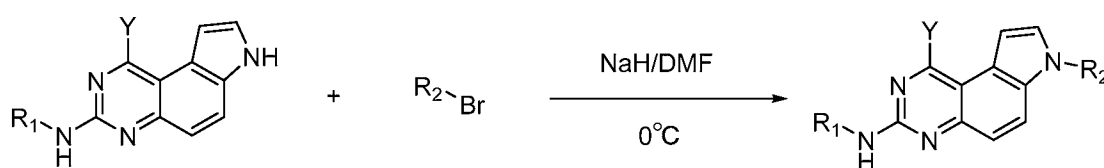
In another aspect, methods of treating cancerous tissue are described herein. In some embodiments, a method comprises administering to a patient having cancerous tissue one or more compounds of Formula(s) I-VI or salts thereof in therapeutically effective amount. In some embodiments, a therapeutically effective amount stops or inhibits cancerous cell growth and/or tumor growth. A therapeutically effective amount may also reduce tumor size, in some embodiments. In some embodiments, a compound of any of Formulas I-VI or salt thereof is administered in an amount selected from Table I. In some embodiments, a combination of two or more compounds of any of Formulas I-VI can be employed in treating cancerous cells and tissue. Moreover, compounds of Formulas I-VI can be combined with any adjuvants or other chemotherapeutic agents for the treatment of cancer.

These and other embodiments are further illustrated in the following non-limiting examples.

EXAMPLES – *Compounds Exhibiting Anticancer Activity*

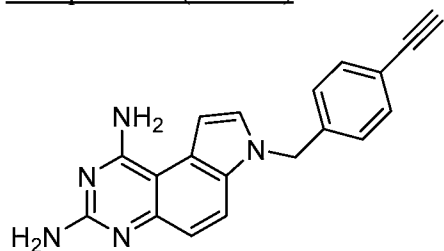
One or more of the following compounds according to at least one of Formulas I-VI, in some embodiments, exhibit anticancer activity. The compounds were prepared according to the following general reaction scheme. Common solvents were purified before use. All reagents

were reagent grade and purified where necessary. Reactions were monitored by thin-layer chromatography (TLC) using Whatman precoated silica gel plates. Flash column chromatography was performed over ultrapure silica gel (200–400 mesh) from Merck. ^1H NMR spectra were recorded on a Bruker AVANCE 300 (300 MHz), 400MHz or 500MHz spectrometer. Multiplicities for ^1H NMR are designated as s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, sext = sextet, dd = doublet of doublets, dt = doublet of triplets, m = multiplet, and br = broad. Electrospray impact (ESI) mass spectra were recorded on ISQEC mass spectrometer.

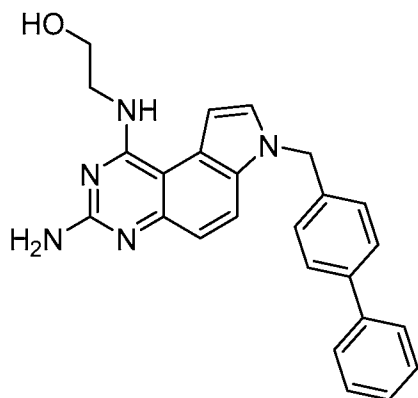


To a stirred solution of 7H-Pyrrolo[3,2-f]quinazoline-1,3-diamine (1.0 mmol) in dry DMF (20mL) was added NaH (1.2 mmol). The resulting reaction mixture was stirred at 0°C for 0.5 h. Then corresponding bromide (1.5mmol) was added. The reaction mixture was stirred at 0°C for 1 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel, eluting with 10:1 DCM : MeOH containing 1% Et₃N to give the desired compound as a solid. The following specific compounds were synthesized according to the foregoing procedure.

Compound 1 (IRS-17)



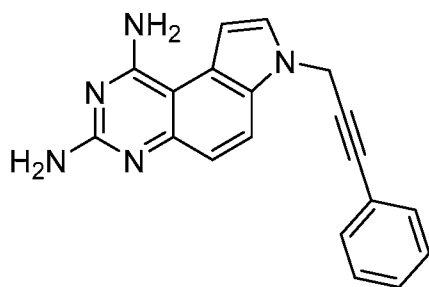
^1H NMR (500 MHz, DMSO-*d*₆) δ 7.71 (d, J = 9.0 Hz, 1H), 7.62 (d, J = 3.0 Hz, 1H), 7.41 (d, J = 8.0 Hz, 2H), 7.15 (d, J = 8.0 Hz, 2H), 7.12 (d, J = 3.0 Hz, 1H), 7.03 (d, J = 9.0 Hz, 1H), 6.78 (s, 2H), 5.89 (s, 2H), 5.53 (s, 2H), 4.14 (s, 1H). MS (ESI): $[\text{M}+\text{H}^+]$ 314.12.

Compound 2

- 5 ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.92 (d, $J = 9.0$ Hz, 1H), 7.85 (d, $J = 3.2$ Hz, 1H), 7.65 – 7.56 (m, 5H), 7.47 – 7.40 (m, 2H), 7.37 – 7.31 (m, 1H), 7.29 – 7.24 (m, 2H), 7.22 – 7.11 (m, 2H), 6.74 (s, 2H), 5.60 (s, 2H), 3.84 – 3.66 (m, 4H).

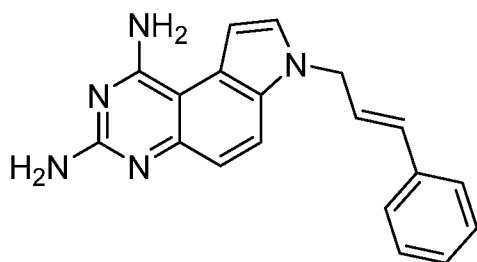
Compound 3

10



- ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 7.88 (d, $J = 9.0$ Hz, 1H), 7.58 (d, $J = 3.3$ Hz, 1H), 7.46 – 7.33 (m, 5H), 7.17 – 7.06 (m, 2H), 6.70 (s, 2H), 5.72 (s, 2H), 5.43 (s, 2H).
- 15 MS(ESI): $[\text{M}+\text{H}^+]$ 314.12.

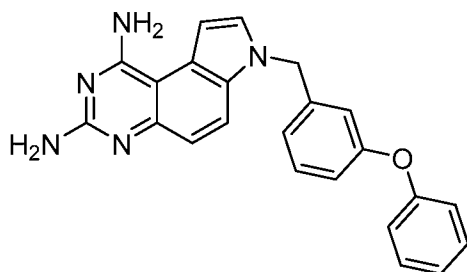
Compound 4



¹H NMR (300 MHz, DMSO-*d*₆) δ 7.80 (d, *J* = 9.0 Hz, 1H), 7.52 (d, *J* = 3.0 Hz, 1H), 7.43 – 7.37 (m, 2H), 7.34 – 7.26 (m, 2H), 7.26 – 7.22 (m, 1H), 7.08 (d, *J* = 3.0 Hz, 1H), 7.05 (d, *J* = 9.0 Hz, 1H), 6.67 (s, 2H), 6.52 – 6.46 (m, 2H), 5.67 (s, 2H), 5.09 – 5.01 (m, 2H).

MS(ESI): [M+H⁺] 316.15.

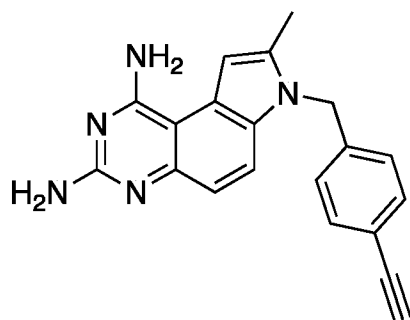
Compound 5



¹H NMR (300 MHz, DMSO-*d*₆) δ 7.71 (d, *J* = 9.0 Hz, 1H), 7.60 (d, *J* = 3.0 Hz, 1H), 7.39 – 7.25 (m, 3H), 7.13 (d, *J* = 7.2 Hz, 1H), 7.08 (d, *J* = 3.0 Hz, 1H), 7.02 (d, *J* = 9.0 Hz, 1H), 6.97 – 6.89 (m, 3H), 6.87 – 6.80 (m, 2H), 6.68 (s, 2H), 5.69 (s, 2H), 5.50 (s, 2H).

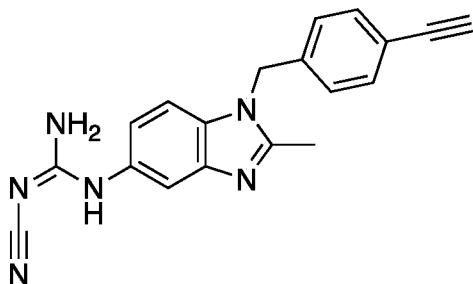
MS(ESI): [M+H⁺] 382.12.

Compound 6



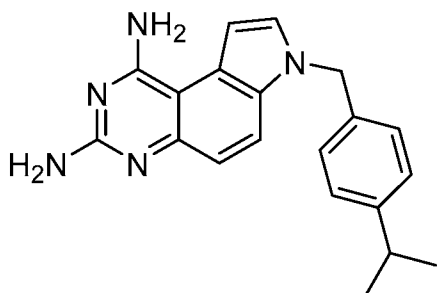
PPC-002-0036-1

Compound 7



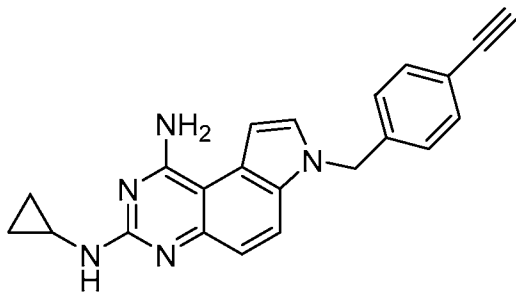
PPC-002-0053-1

5

Compound 8

^1H NMR (500 MHz, DMSO- d_6) δ 7.73 (d, $J = 9.0$ Hz, 1H), 7.59 (d, $J = 3.0$ Hz, 1H), 7.15 (d, $J = 8.0$ Hz, 2H), 7.11 (d, $J = 8.0$ Hz, 2H), 7.06 (d, $J = 3.0$ Hz, 1H), 7.01 (d, $J = 8.9$ Hz, 1H), 6.65 (s, 2H), 5.65 (s, 2H), 5.43 (s, 2H), 2.80 (p, $J = 7.0$ Hz, 1H), 1.12 (d, $J = 6.9$ Hz, 6H).

10

MS(ESI):[M+H⁺]332.42.Compound 9

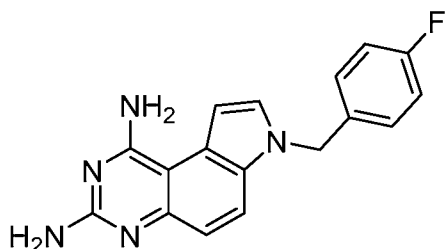
15

^1H NMR (300 MHz, DMSO- d_6) δ 7.70 (d, $J = 9.0$ Hz, 1H), 7.62 (d, $J = 3.0$ Hz, 1H), 7.41 (d, $J = 8.2$ Hz, 2H), 7.14 (d, $J = 8.2$ Hz, 2H), 7.11 (d, $J = 3.0$ Hz, 1H), 7.09 (d, $J = 9.0$ Hz, 1H), 6.68 (s,

2H), 6.47 – 6.30 (m, 1H), 5.53 (s, 2H), 4.16 (s, 1H), 2.84 – 2.70 (m, 1H), 0.68 – 0.57 (m, 2H), 0.48 – 0.39 (m, 2H).

MS(ESI): [M+H⁺] 354.15.

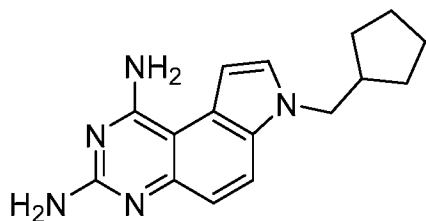
5 Compound 10



¹H NMR (500 MHz, DMSO-*d*₆) δ 8.08 (d, *J* = 9.0 Hz, 1H), 7.91 (d, *J* = 3.0 Hz, 1H), 7.60 (s, 2H), 7.37 (d, *J* = 3.0 Hz, 1H), 7.31 – 7.24 (m, 2H), 7.21 (d, *J* = 9.0 Hz, 1H), 7.18 – 7.11 (m, 2H), 5.56 (s, 2H).

MS(ESI): [M+H⁺] 308.15.

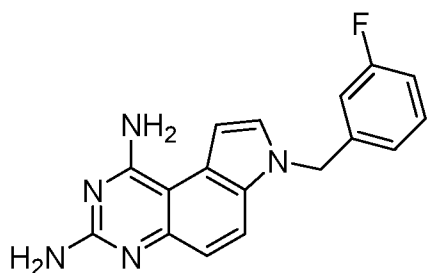
Compound 11



¹H NMR (500 MHz, DMSO-*d*₆) δ 7.78 (d, *J* = 9.0 Hz, 1H), 7.47 (d, *J* = 3.0 Hz, 1H), 7.05 (d, *J* = 9.0 Hz, 1H), 7.00 (d, *J* = 3.0 Hz, 1H), 6.68 (s, 2H), 5.69 (s, 2H), 4.13 (d, *J* = 7.5 Hz, 2H), 2.42 – 2.29 (m, 1H), 1.66 – 1.58 (m, 2H), 1.58 – 1.52 (m, 2H), 1.51 – 1.44 (m, 2H), 1.28 – 1.20 (m, 2H).

MS(ESI): [M+H⁺] 282.24.

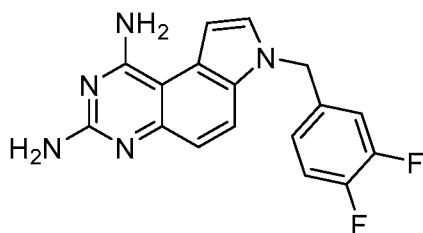
Compound 12



^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.92 – 7.82 (m, 1H), 7.80 – 7.72 (m, 1H), 7.39 – 7.31 (m, 1H), 7.25 – 7.18 (m, 1H), 7.14 – 7.05 (m, 2H), 7.04 – 6.96 (m, 2H), 5.55 (s, 2H).

5 MS(ESI): $[\text{M}+\text{H}^+]$ 308.06.

Compound 13

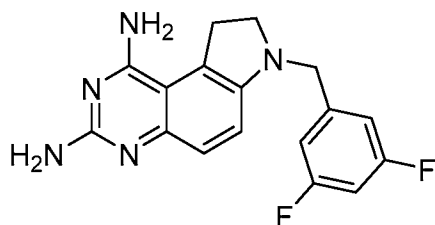


10 ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.75 (d, $J = 9.0$ Hz, 1H), 7.63 (d, $J = 3.0$ Hz, 1H), 7.41 – 7.34 (m, 1H), 7.34 – 7.26 (m, 1H), 7.11 (d, $J = 3.0$ Hz, 1H), 7.03 (d, $J = 9.0$ Hz, 1H), 7.02 – 6.99 (m, 1H), 6.72 (s, 2H), 5.73 (s, 2H), 5.49 (s, 2H).

MS(ESI): $[\text{M}+\text{H}^+]$ 326.10.

Compound 14

15

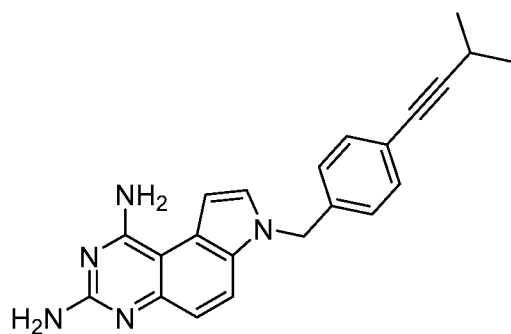


^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.76 (d, $J = 9.0$ Hz, 1H), 7.66 (d, $J = 3.2$ Hz, 1H), 7.18 – 7.09 (m, 2H), 7.05 (d, $J = 9.0$ Hz, 1H), 6.90 – 6.84 (m, 2H), 6.78 (s, 2H), 5.81 (s, 2H), 5.54 (s, 2H).

MS(ESI): $[\text{M}+\text{H}^+]$ 326.10.

20

Compound 15

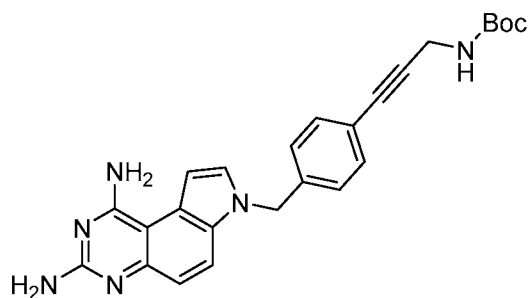


PPC-002-0076-1

^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.69 (d, $J = 9.0$ Hz, 1H), 7.60 (d, $J = 3.2$ Hz, 1H), 7.27 (d, $J = 8.0$ Hz, 2H), 7.09 (d, $J = 8.0$ Hz, 3H), 7.01 (d, $J = 9.0$ Hz, 1H), 6.77 (s, 2H), 5.78 (s, 2H), 5.48 (s, 2H), 2.81 – 2.65 (m, 1H), 1.15 (d, $J = 6.8$ Hz, 6H).

MS (ESI): $[\text{M}+\text{H}^+]$ 356.20

Compound 16

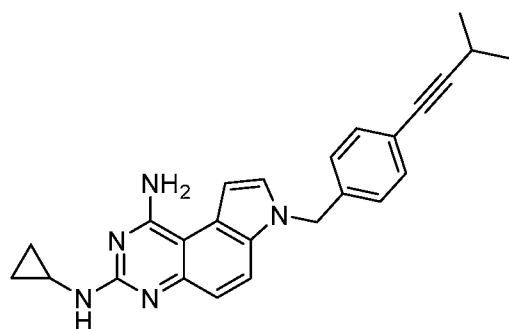


PPC-002-0077-1

^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 7.70 (d, $J = 9.0$ Hz, 1H), 7.61 (d, $J = 3.1$ Hz, 1H), 7.34 – 7.29 (m, 2H), 7.29 (s, 1H), 7.18 – 7.05 (m, 3H), 7.01 (d, $J = 9.0$ Hz, 1H), 6.79 (s, 2H), 5.79 (s, 2H), 5.49 (s, 2H), 3.91 (d, $J = 6.0$ Hz, 2H), 1.35 (s, 9H).

MS (ESI): $[\text{M}+\text{H}^+]$ 443.53

Compound 17

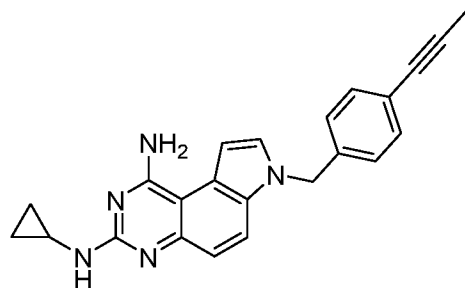


PPC-002-0078-1

^1H NMR (400 MHz, DMSO- d_6) δ 7.68 (m, 1H), 7.64 – 7.59 (m, 1H), 7.34 – 7.26 (m, 2H), 7.15 – 7.06 (m, 4H), 6.67 (s, 2H), 6.37 (s, 1H), 5.51 (s, 2H), 2.83 – 2.74 (m, 1H), 1.18 (d, J = 6.9 Hz, 6H), 0.63 (m, 2H), 0.51 – 0.37 (m, 2H).

MS (ESI): $[\text{M}+\text{H}^+]$ 396.50

Compound 18

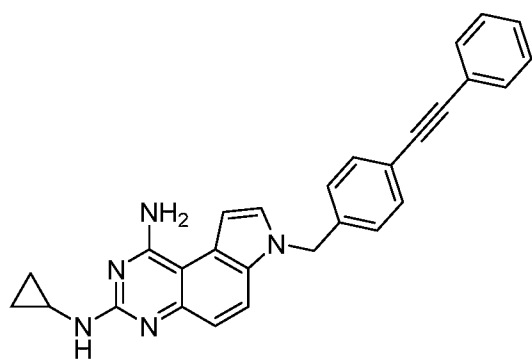


PPC-002-0079-1

^1H NMR (400 MHz, DMSO- d_6) δ 7.73 (d, J = 9.2 Hz, 1H), 7.69 – 7.60 (m, 1H), 7.32 (d, J = 6.4 Hz, 2H), 7.15 – 7.07 (m, 4H), 5.51 (s, 2H), 2.88 – 2.72 (m, 1H), 0.86 (t, J = 6.4 Hz, 2H), 0.64 (dd, J = 6.4, 2.4 Hz, 2H).

MS (ESI): $[\text{M}+\text{H}^+]$ 368.45

Compound 19

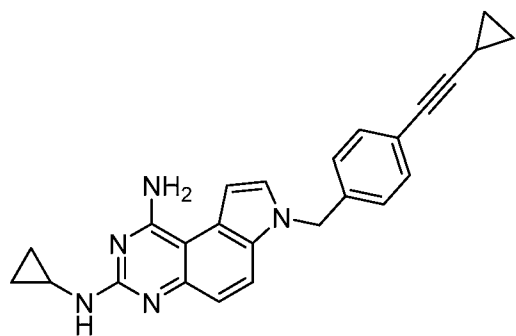


PPC-002-0080-1

^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.74 (d, $J = 8.8$ Hz, 1H), 7.66 (d, $J = 3.2$ Hz, 1H), 7.55 – 7.49 (m, 4H), 7.42 (d, $J = 3.2$ Hz, 3H), 7.20 (d, $J = 8.0$ Hz, 2H), 7.15 – 7.11 (m, 2H), 6.75 (s, 2H), 6.46 (s, 1H), 5.57 (s, 2H), 2.79 (m, 1H), 0.64 (m, 2H), 0.52 – 0.40 (m, 2H).

MS (ESI): $[\text{M}+\text{H}^+]$ 430.49

Compound 20

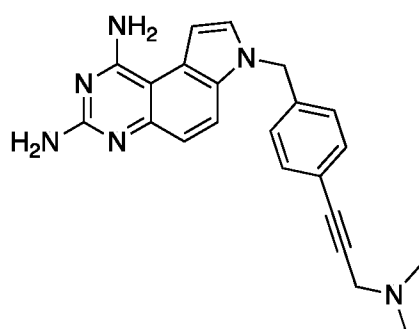


PPC-002-0081-1

^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.71 (d, $J = 9.2$ Hz, 1H), 7.61 (d, $J = 3.2$ Hz, 1H), 7.33 – 7.26 (m, 2H), 7.16 – 7.06 (m, 4H), 6.69 (s, 2H), 6.40 (s, 1H), 5.50 (s, 2H), 2.79 (m, 1H), 1.51 (m, 1H), 0.91 – 0.81 (m, 4H), 0.73 – 0.59 (m, 4H)

MS (ESI): $[\text{M}+\text{H}^+]$ 394.19

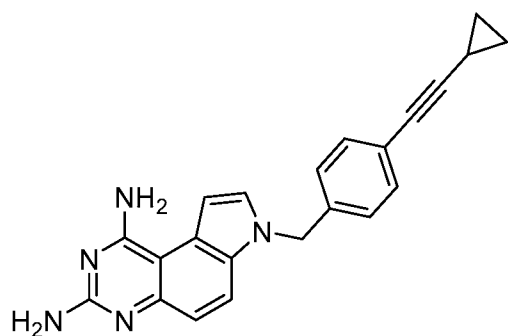
Compound 21



PPC-002-0082-1

Compound 22

5

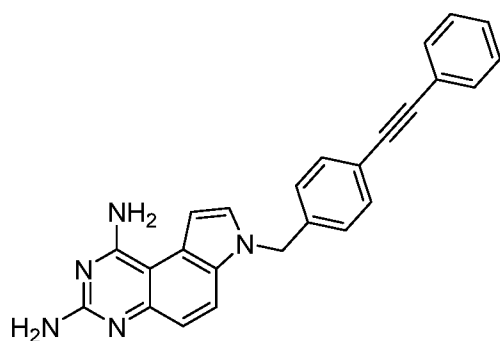


PPC-002-0083-1

^1H NMR (400 MHz, DMSO- d_6) δ 7.70 (d, J = 8.8 Hz, 1H), 7.61 (d, J = 3.2 Hz, 1H), 7.29 (d, J = 8.0 Hz, 2H), 7.17 – 7.08 (m, 3H), 7.02 (d, J = 8.8 Hz, 1H), 6.73 (s, 2H), 5.76 (s, 2H), 5.50 (s, 2H), 1.50 (m, 1H), 0.91 – 0.79 (m, 2H), 0.72 – 0.64 (m, 2H).

MS (ESI): $[\text{M}+\text{H}^+]$ 354.46Compound 23

15

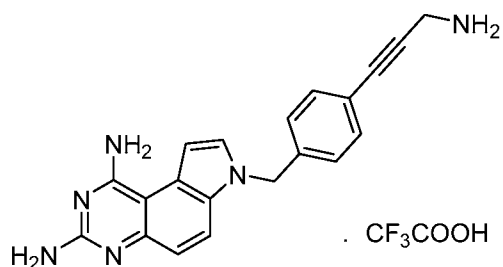


PPC-002-0084-1

^1H NMR (400 MHz, DMSO- d_6) δ 7.80 (d, $J = 8.8\text{ Hz}$, 1H), 7.71 (d, $J = 3.2\text{ Hz}$, 1H), 7.57 – 7.46 (m, 4H), 7.42 (q, $J = 2.8\text{ Hz}$, 3H), 7.25 – 7.16 (m, 3H), 7.08 (d, $J = 8.8\text{ Hz}$, 1H), 6.13 (s, 2H), 5.58 (s, 2H).

MS (ESI): $[\text{M}+\text{H}^+]$ 390.41

Compound 24

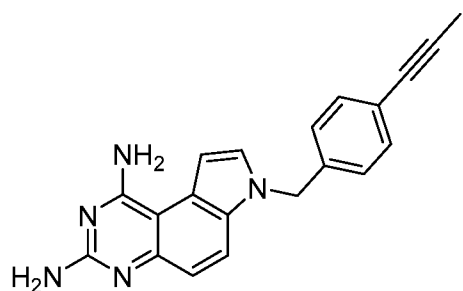


PPC-002-0085-1

^1H NMR (400 MHz, DMSO- d_6) δ 8.06 (d, $J = 9.0\text{ Hz}$, 1H), 7.93 (d, $J = 3.2\text{ Hz}$, 1H), 7.67 (s, 2H), 7.47 – 7.37 (m, 3H), 7.22 (dd, $J = 9.0, 2.4\text{ Hz}$, 3H), 5.64 (s, 2H), 3.98 (s, 2H).

MS (ESI): $[\text{M}+\text{H}^+]$ 343.38

Compound 25

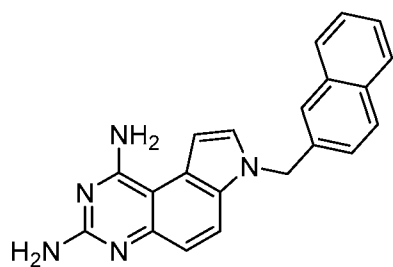


PPC-002-0087-1

^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.75 (d, $J = 9.0$ Hz, 1H), 7.65 (d, $J = 3.2$ Hz, 1H), 7.37 – 7.26 (m, 2H), 7.17 – 7.10 (m, 3H), 7.04 (d, $J = 9.0$ Hz, 1H), 6.88 (s, 2H), 5.93 (s, 2H), 5.51 (s, 2H),
 5 2.00 (s, 3H).

MS (ESI): $[\text{M}+\text{H}^+]$ 328.37

Compound 26

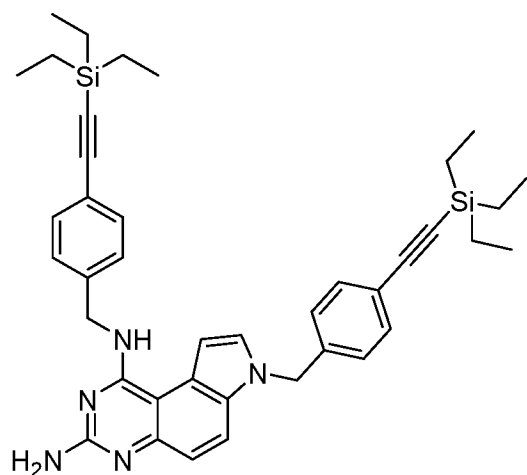


PPC-002-0089-1

^1H NMR (300 MHz, $\text{DMSO-}d_6$) δ 7.90 – 7.79 (m, 4H), 7.77 (d, $J = 3.2$ Hz, 1H), 7.72 (s, 1H), 7.52 – 7.43 (m, 2H), 7.33 (dd, $J = 8.5, 1.8$ Hz, 1H), 7.19 (d, $J = 3.2$ Hz, 1H), 7.06 (d, $J = 9.0$ Hz, 1H), 6.22 (s, 2H), 5.68 (s, 2H).

MS (ESI): $[\text{M}+\text{H}^+]$ 340.35

Compound 27

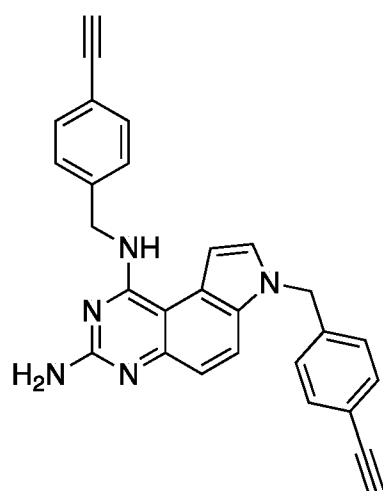


PPC-002-0090-1

^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 7.70 (s, 1H), 7.65 (d, $J = 3.5$ Hz, 1H), 7.41 – 7.34 (m, 6H), 7.31 (s, 1H), 7.23 (d, $J = 3.0$ Hz, 1H), 7.14 (d, $J = 8.0$ Hz, 2H), 5.86 (s, 2H), 5.51 (s, 2H), 4.83 (d, $J = 6.0$ Hz, 2H), 0.95 (m, 18H), 0.59 (m, 12H).

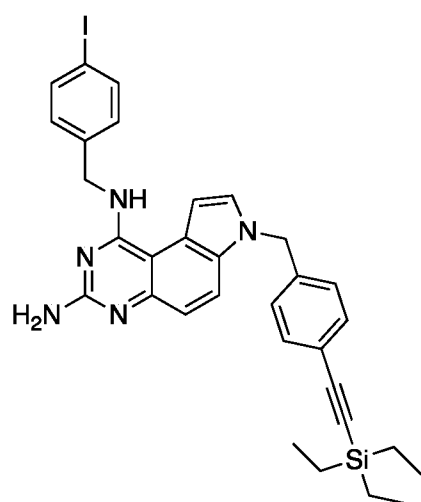
MS (ESI): $[\text{M}+\text{H}^+]$ 657.03

Compound 28

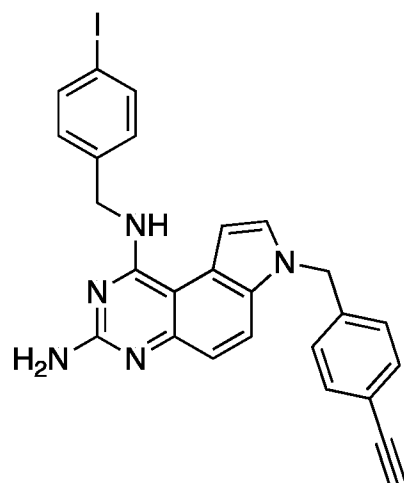


10 PPC-002-0091-1

Compound 29

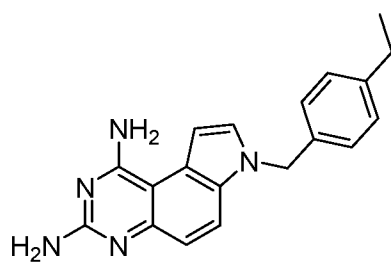


PPC-002-0092-1

Compound 30

5

PPC-002-0093-1

Compound 31

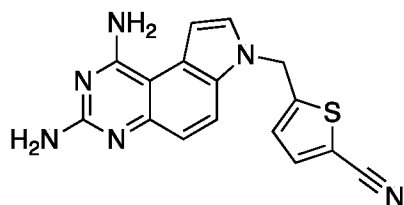
10

PPC-002-0094-1

^1H NMR (400 MHz, DMSO- d_6) δ 7.79 (d, J = 9.0 Hz, 1H), 7.65 (d, J = 3.2 Hz, 1H), 7.13 (t, J = 3.2 Hz, 5H), 7.05 (d, J = 9.0 Hz, 1H), 6.92 (s, 2H), 5.98 (s, 2H), 5.46 (s, 2H), 2.55 (m, 2H), 1.12 (t, J = 7.6 Hz, 3H).

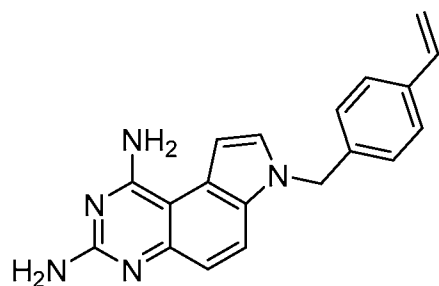
5 MS (ESI): $[\text{M}+\text{H}^+]$ 318.36

Compound 32



10 PPC-002-0095-1

Compound 33



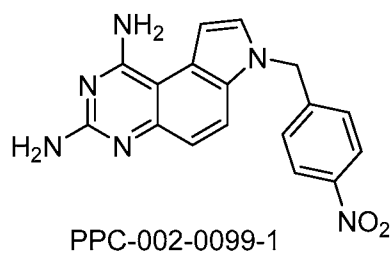
PPC-002-0096-1

15

^1H NMR (500 MHz, DMSO- d_6) δ 7.71 (d, J = 9.0 Hz, 1H), 7.60 (d, J = 3.0 Hz, 1H), 7.41 – 7.34 (m, 2H), 7.13 (d, J = 8.2 Hz, 2H), 7.07 (d, J = 3.0 Hz, 1H), 7.00 (d, J = 9.0 Hz, 1H), 6.74 (s, 2H), 6.65 (dd, J = 17.6, 11.0 Hz, 1H), 5.81 – 5.68 (m, 3H), 5.46 (s, 2H), 5.19 (dd, J = 10.9, 1.0 Hz, 1H).

20 MS (ESI): $[\text{M}+\text{H}^+]$ 316.40

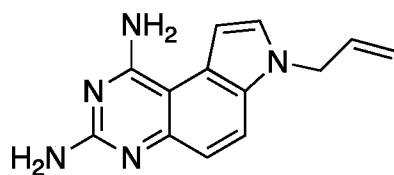
Compound 34



^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.22 – 8.17 (m, 2H), 7.96 (d, $J = 9.0$ Hz, 1H), 7.91 (d, $J = 3.2$ Hz, 1H), 7.43 – 7.35 (m, 3H), 7.19 (d, $J = 9.0$ Hz, 1H), 7.12 (s, 2H), 5.76 (s, 2H).

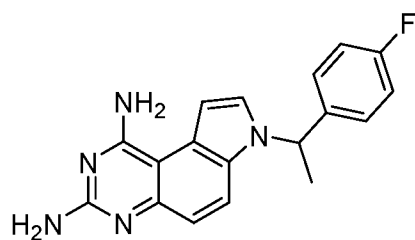
5 MS (ESI): $[\text{M}+\text{H}^+]$ 335.08

Compound 35



10

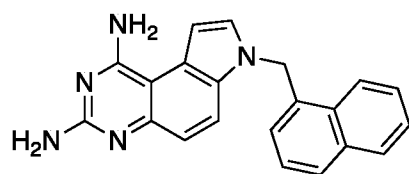
Compound 36



15 ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.80 (d, $J = 3.2$ Hz, 1H), 7.74 (d, $J = 8.8$ Hz, 1H), 7.33 – 7.23 (m, 2H), 7.19 – 7.07 (m, 3H), 7.01 (d, $J = 8.8$ Hz, 1H), 6.80 (s, 2H), 5.95 (q, $J = 6.8$ Hz, 1H), 5.81 (s, 2H), 1.90 (d, $J = 6.8$ Hz, 3H).

MS (ESI): $[\text{M}+\text{H}^+]$ 322.32

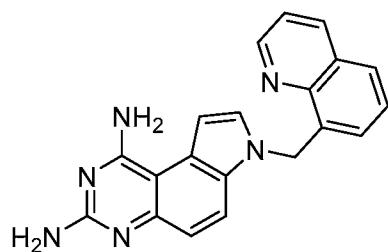
20 Compound 37



PPC-002-0114

Compound 38

5

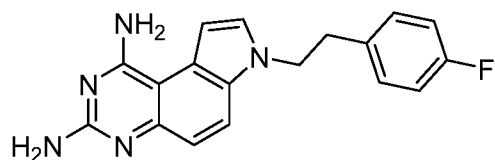


PPC-002-0115-1

^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 9.07 (dd, $J = 4.0, 2.0$ Hz, 1H), 8.43 (dd, $J = 8.4, 2.0$ Hz, 1H), 7.92 (d, $J = 8.0$ Hz, 1H), 7.80 (d, $J = 8.8$ Hz, 1H), 7.73 (d, $J = 3.2$ Hz, 1H), 7.65 (dd, $J = 8.4, 4.4$ Hz, 1H), 7.47 (t, $J = 8.0$ Hz, 1H), 7.16 (d, $J = 3.2$ Hz, 1H), 7.03 (dd, $J = 8.4, 4.4$ Hz, 2H), 6.92 (s, 2H), 6.15 (s, 2H), 5.95 (s, 2H).

MS (ESI): $[\text{M}+\text{H}^+]$ 341.33Compound 39

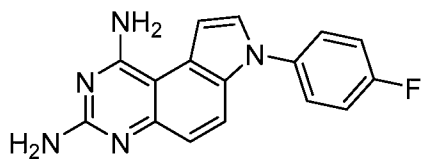
15



PPC-002-0116-1

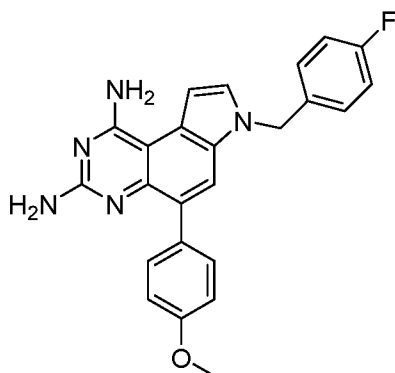
^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.78 (d, $J = 8.8$ Hz, 1H), 7.37 (d, $J = 3.2$ Hz, 1H), 7.25 – 7.14 (m, 2H), 7.12 – 6.94 (m, 4H), 6.73 (s, 2H), 5.79 (s, 2H), 4.47 (t, $J = 7.2$ Hz, 2H), 3.08 (t, $J = 7.2$ Hz, 2H).

MS (ESI): $[\text{M}+\text{H}^+]$ 341.33

Compound 40

PPC-002-0117-1

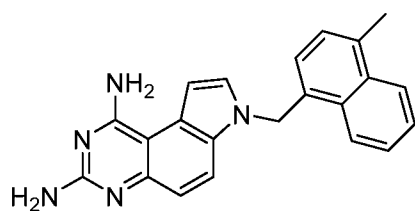
- 5 ^1H NMR (300 MHz, Methanol- d_4) δ 7.78 (dd, $J = 9.1, 0.9$ Hz, 1H), 7.66 (d, $J = 3.3$ Hz, 1H), 7.62 – 7.55 (m, 2H), 7.41 – 7.30 (m, 2H), 7.25 – 7.18 (m, 2H).

MS (ESI): $[\text{M}+\text{H}^+]$ 294.30Compound 41

PPC-002-0123-1

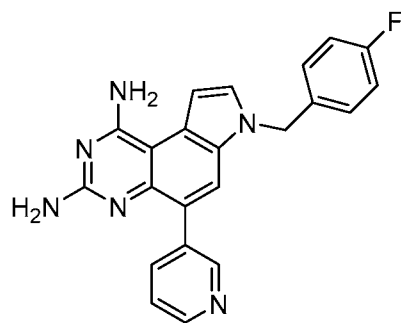
- 10 ^1H NMR (400 MHz, DMSO- d_6) δ 7.74 (s, 1H), 7.65 (d, $J = 3.2$ Hz, 1H), 7.55 – 7.46 (m, 2H), 7.30 – 7.20 (m, 2H), 7.20 – 7.09 (m, 3H), 7.01 – 6.94 (m, 2H), 6.83 (s, 2H), 5.71 (s, 2H), 5.54 (s, 2H), 3.80 (s, 3H).
- 15 MS (ESI): $[\text{M}+\text{H}^+]$ 414.48

Compound 42



PPC-002-0125-1

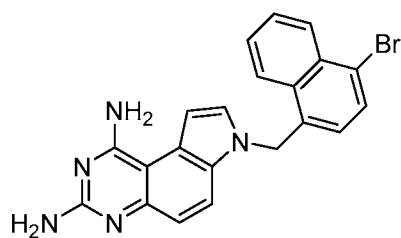
^1H NMR (400 MHz, DMSO- d_6) δ 8.22 – 8.13 (m, 1H), 8.12 – 8.03 (m, 1H), 7.72 (d, J = 9.2 Hz, 1H), 7.66 – 7.57 (m, 2H), 7.48 (d, J = 3.2 Hz, 1H), 7.24 (d, J = 7.2 Hz, 1H), 7.11 (d, J = 3.2 Hz, 1H), 7.02 (d, J = 9.2 Hz, 1H), 6.70 (s, 2H), 6.68 (s, 1H), 5.97 (s, 2H), 5.70 (s, 2H), 2.62 (s, 3H).
 MS (ESI): $[\text{M}+\text{H}^+]$ 354.44

Compound 43

PPC-002-0126-1

^1H NMR (400 MHz, Methanol- d_4) δ 8.70 (dd, J = 2.2, 0.8 Hz, 1H), 8.57 (dd, J = 4.8, 1.6 Hz, 1H), 8.04 (dt, J = 8.0, 2.0 Hz, 1H), 7.83 (d, J = 0.8 Hz, 1H), 7.66 (d, J = 3.2 Hz, 1H), 7.56 (m, 1H), 7.29 – 7.18 (m, 2H), 7.13 (dd, J = 3.2, 0.8 Hz, 1H), 7.10 – 7.00 (m, 2H), 5.56 (s, 2H).
 MS (ESI): $[\text{M}+\text{H}^+]$ 385.41

Compound 44

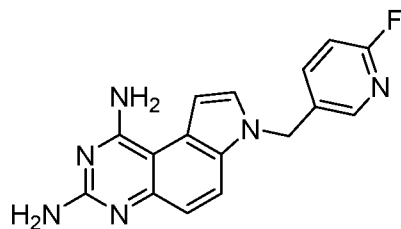


PPC-002-0127-1

^1H NMR (400 MHz, DMSO- d_6) δ 8.25 (m, 2H), 7.79 – 7.70 (m, 4H), 7.55 (d, J = 3.1 Hz, 1H), 7.17 (d, J = 3.1 Hz, 1H), 7.03 (d, J = 8.9 Hz, 1H), 6.76 (s, 2H), 6.54 (d, J = 7.7 Hz, 1H), 6.04 (s, 2H), 5.76 (s, 2H).

MS (ESI): $[\text{M}+\text{H}^+]$ 418.31

Compound 45

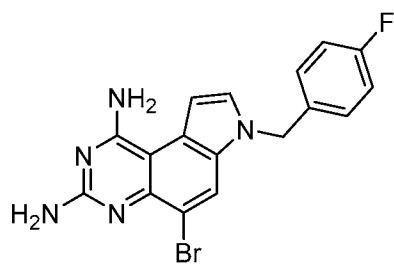


PPC-002-0129-1

^1H NMR (400 MHz, DMSO- d_6) δ 8.24 (d, J = 2.4 Hz, 1H), 7.99 (d, J = 8.9 Hz, 1H), 7.82 (t, J = 5.5 Hz, 2H), 7.47 (s, 2H), 7.25 (d, J = 3.2 Hz, 1H), 7.15 (dd, J = 8.9, 2.4 Hz, 2H), 6.58 (s, 2H), 5.59 (s, 2H).

MS (ESI): $[\text{M}+\text{H}^+]$ 309.36

Compound 46



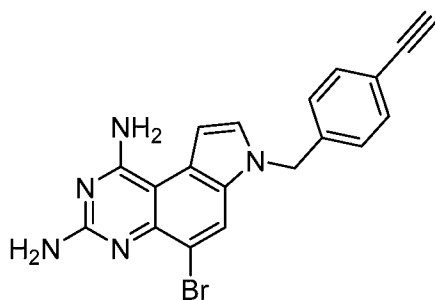
PPC-002-0130-1

^1H NMR (400 MHz, DMSO- d_6) δ 8.21 (s, 1H), 7.66 (d, J = 3.2 Hz, 1H), 7.29 – 7.20 (m, 2H), 7.20 – 7.09 (m, 3H), 6.85 (s, 2H), 5.94 (s, 2H), 5.51 (s, 2H).

MS (ESI): $[\text{M}+\text{H}^+]$ 386.21

5

Compound 47



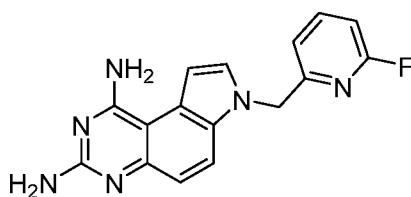
PPC-002-0131-1

^1H NMR (400 MHz, Chloroform- d +MeOD) δ 7.82 (d, J = 1.2 Hz, 1H), 7.30 (d, J = 8.0 Hz, 2H), 7.25 – 7.23 (m, 1H), 6.90 (d, J = 8.0 Hz, 2H), 6.72 (d, J = 3.2 Hz, 1H), 5.29 (s, 2H), 3.03 (s, 1H).

10

MS (ESI): $[\text{M}+\text{H}^+]$ 393.25

Compound 48



PPC-002-0132-1

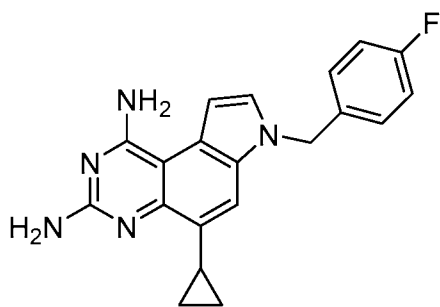
^1H NMR (400 MHz, DMSO- d_6) δ 8.00 – 7.86 (m, 2H), 7.77 (d, J = 3.0 Hz, 1H), 7.65 (s, 1H), 7.29 (d, J = 3.0 Hz, 1H), 7.15 (d, J = 8.8 Hz, 1H), 7.09 (dd, J = 8.0, 2.4 Hz, 1H), 6.95 (dd, J = 8.0, 2.4 Hz, 1H), 6.76 (s, 2H), 5.63 (s, 2H).

15

MS (ESI): $[\text{M}+\text{H}^+]$ 309.33

20

Compound 49

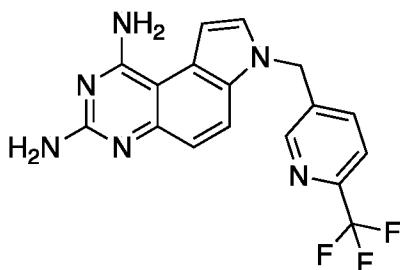


PPC-002-0133-1

^1H NMR (400 MHz, Methanol- d_4) δ 7.59 (d, $J = 4.4$ Hz, 2H), 7.27 – 7.15 (m, 2H), 7.13 – 6.98 (m, 3H), 5.53 (s, 2H), 2.20 (m, 1H), 1.15 – 1.03 (m, 2H), 0.76 – 0.65 (m, 2H).

5 MS (ESI): $[\text{M}+\text{H}^+]$ 348.45

Compound 50

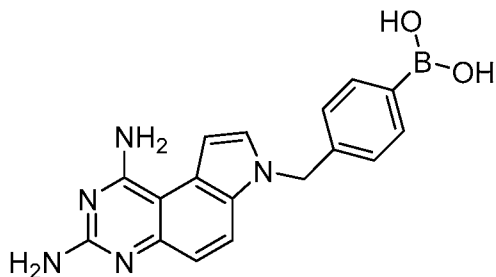


10 PPC-002-0134-1

^1H NMR (400 MHz, Chloroform- d +*MeOD*) δ 8.35 (d, $J = 2.0$ Hz, 1H), 7.45 (m 2H), 7.35 (dd, $J = 8.4, 2.0$ Hz, 1H), 7.28 (s, 1H), 7.03 (d, $J = 8.4$ Hz, 1H), 6.77 (d, $J = 3.2$ Hz, 1H), 5.42 (s, 2H).

15 MS (ESI): $[\text{M}+\text{H}^+]$ 359.30

Compound 51

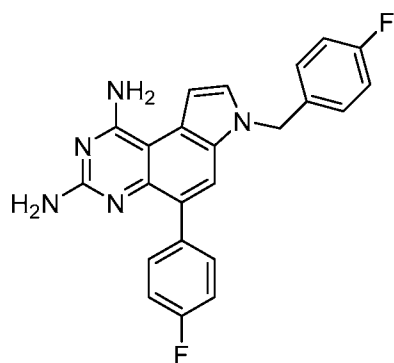


PPC-002-0135-1

^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.03 (m, 3H), 7.92 (d, $J = 3.2$ Hz, 1H), 7.71 (d, $J = 7.6$ Hz, 2H), 7.45 (s, 2H), 7.38 (d, $J = 3.2$ Hz, 1H), 7.21 (d, $J = 8.8$ Hz, 1H), 7.15 (d, $J = 7.6$ Hz, 2H), 5.59 (s, 2H).

5 MS (ESI): $[\text{M}+\text{H}^+]$ 334.17

Compound 52

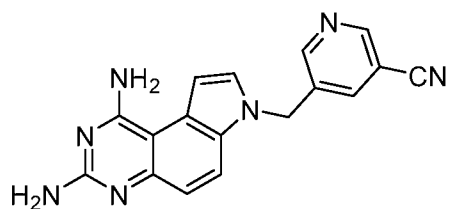


PPC-002-0136-1

^1H NMR (400 MHz, $\text{Methanol}-d_4$) δ 7.79 (d, $J = 0.8$ Hz, 1H), 7.69 (d, $J = 3.2$ Hz, 1H), 7.56 – 7.44 (m, 2H), 7.30 – 7.19 (m, 4H), 7.16 (dd, $J = 3.2, 0.8$ Hz, 1H), 7.10 – 7.01 (m, 2H), 5.55 (s, 2H).

15 MS (ESI): $[\text{M}+\text{H}^+]$ 402.40

Compound 53

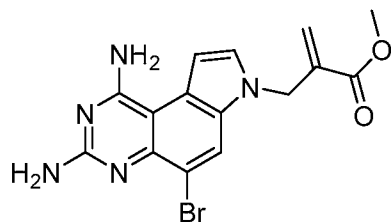


PPC-002-0139-1

^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.94 (d, $J = 2.0$ Hz, 1H), 8.76 (d, $J = 2.0$ Hz, 1H), 8.18 (d, $J = 2.0$ Hz, 1H), 7.94 (d, $J = 9.0$ Hz, 1H), 7.78 (d, $J = 3.2$ Hz, 1H), 7.23 (d, $J = 3.2$ Hz, 1H), 7.11 (d, $J = 9.0$ Hz, 1H), 6.28 (s, 2H), 5.64 (s, 2H).

MS (ESI): $[M+H]^+$ 316.31

Compound 54

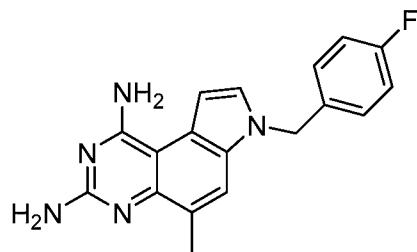


5 PPC-002-0140-1

^1H NMR (400 MHz, Methanol- d_4) δ 8.11 (d, $J = 1.2$ Hz, 1H), 7.45 (d, $J = 3.2$ Hz, 1H), 6.96 (dd, $J = 3.2, 0.8$ Hz, 1H), 6.27 (d, $J = 1.0$ Hz, 1H), 5.31 (d, $J = 1.0$ Hz, 1H), 5.14 (t, $J = 1.6$ Hz, 2H), 3.79 (s, 3H).

10 MS (ESI): $[M+H]^+$ 376.22

Compound 55

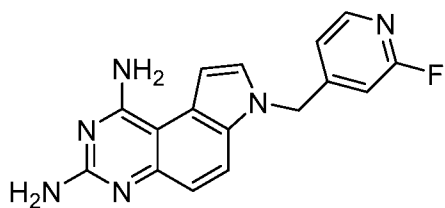


15 PPC-002-0141-1

^1H NMR (400 MHz, Methanol- d_4) δ 7.60 (d, $J = 3.2$ Hz, 1H), 7.41 (t, $J = 3.2$ Hz, 1H), 7.19 – 7.14 (m, 2H), 7.04 (dd, $J = 8.8, 3.2$ Hz, 2H), 6.95 (t, $J = 3.2$ Hz, 1H), 5.44 (d, $J = 3.2$ Hz, 2H), 2.52 (d, $J = 3.2$ Hz, 3H).

20 MS (ESI): $[M+H]^+$ 322.34

Compound 56

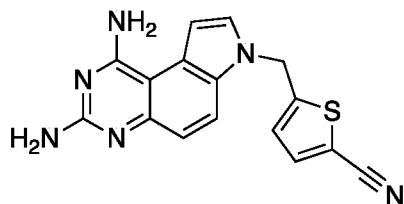


PPC-002-0143-1

^1H NMR (400 MHz, DMSO- d_6) δ 8.16 (d, J = 5.2 Hz, 1H), 7.71 (d, J = 9.0 Hz, 1H), 7.66 (d, J = 3.2 Hz, 1H), 7.18 (d, J = 3.2 Hz, 1H), 7.05 (d, J = 9.0 Hz, 1H), 7.00 – 6.98 (m, 1H), 6.84 (d, J = 1.5 Hz, 1H), 6.79 (s, 2H), 5.80 (s, 2H), 5.64 (s, 2H).

5 MS (ESI): $[\text{M}+\text{H}^+]$ 309.36

Compound 57



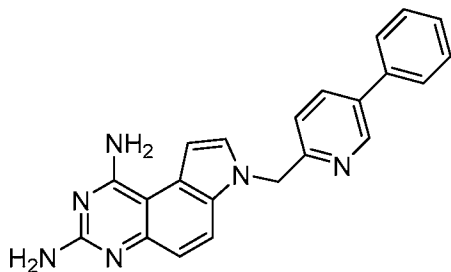
10 PPC-002-0144-1

^1H NMR (400 MHz, DMSO- d_6) δ 7.91 – 7.79 (m, 3H), 7.63 (d, J = 3.2 Hz, 1H), 7.11 (d, J = 3.2 Hz, 1H), 7.05 (d, J = 8.8 Hz, 1H), 6.74 (s, 2H), 5.77 (s, 2H), 5.51 (s, 2H).

MS (ESI): $[\text{M}+\text{H}^+]$ 321.38

15

Compound 58

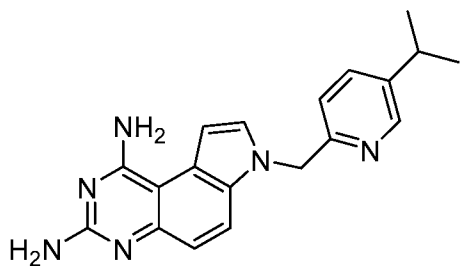


PPC-002-0145-1

^1H NMR (400 MHz, DMSO- d_6) δ 8.85 (d, J = 2.4 Hz, 1H), 8.01 (dd, J = 8.4, 2.4 Hz, 1H), 7.78 (d, J = 9.2 Hz, 1H), 7.74 – 7.63 (m, 3H), 7.48 (t, J = 7.2 Hz, 2H), 7.41 (t, J = 7.2 Hz, 1H), 7.15 (d, J = 3.2 Hz, 1H), 7.05 (d, J = 8.4 Hz, 2H), 6.82 (s, 2H), 5.83 (s, 2H), 5.65 (s, 2H).

20

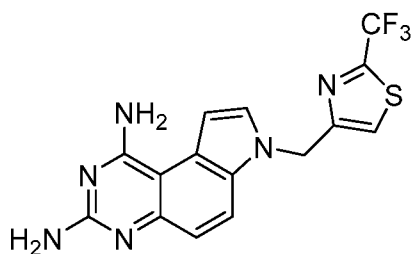
MS (ESI): $[\text{M}+\text{H}^+]$ 367.41

Compound 59

PPC-002-0146-1

- 5 ^1H NMR (400 MHz, DMSO- d_6) δ 8.42 (d, $J = 2.4$ Hz, 1H), 7.77 (d, $J = 8.8$ Hz, 1H), 7.68 – 7.54 (m, 2H), 7.13 (d, $J = 3.2$ Hz, 1H), 7.04 (d, $J = 8.8$ Hz, 1H), 6.92 (d, $J = 8.0$ Hz, 1H), 6.87 (s, 2H), 5.91 (s, 2H), 5.55 (s, 2H), 2.88 (m, 1H), 1.17 (d, $J = 6.8$ Hz, 6H).

MS (ESI): $[\text{M}+\text{H}^+]$ 333.40

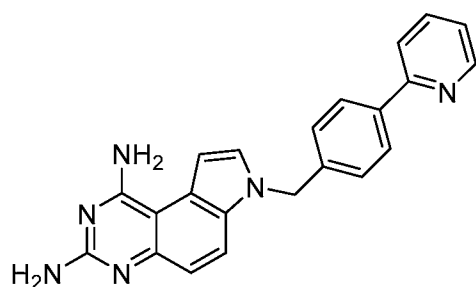
10 Compound 60

PPC-002-0147-1

^1H NMR (400 MHz, DMSO- d_6) δ 7.92 – 7.82 (m, 2H), 7.62 (d, $J = 3.2$ Hz, 1H), 7.13 (d, $J = 3.2$ Hz, 1H), 7.07 (d, $J = 9.2$ Hz, 1H), 6.83 (s, 2H), 5.87 (s, 2H), 5.71 (s, 2H).

- 15 MS (ESI): $[\text{M}+\text{H}^+]$ 365.33

Compound 61

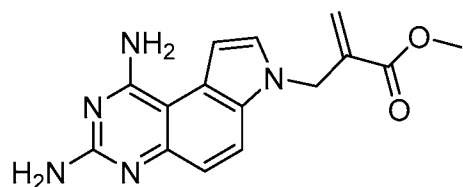


PPC-002-0148-1

^1H NMR (400 MHz, DMSO- d_6) δ 8.63 (dt, $J = 4.8, 1.6$ Hz, 1H), 8.06 – 7.98 (m, 2H), 7.94 – 7.81 (m, 2H), 7.78 (d, $J = 9.0$ Hz, 1H), 7.69 (d, $J = 3.2$ Hz, 1H), 7.35 – 7.25 (m, 3H), 7.15 (d, $J = 3.2$ Hz, 1H), 7.05 (d, $J = 9.0$ Hz, 1H), 6.85 (s, 2H), 5.93 – 5.80 (m, 2H), 5.58 (s, 2H).

5 MS (ESI): $[\text{M}+\text{H}^+]$ 367.41

Compound 62

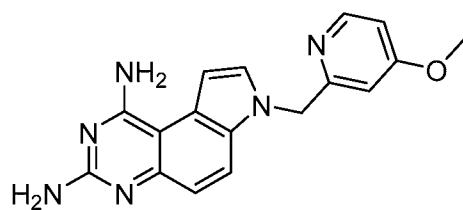


PPC-002-0149-1

10 ^1H NMR (400 MHz, DMSO- d_6) δ 7.71 (d, $J = 8.8$ Hz, 1H), 7.45 (d, $J = 3.2$ Hz, 1H), 7.14 – 6.99 (m, 2H), 6.66 (s, 2H), 6.15 (s, 1H), 5.67 (s, 2H), 5.24 (s, 1H), 5.14 (s, 2H), 3.73 (s, 3H).

MS (ESI): $[\text{M}+\text{H}^+]$ 298.11

Compound 63



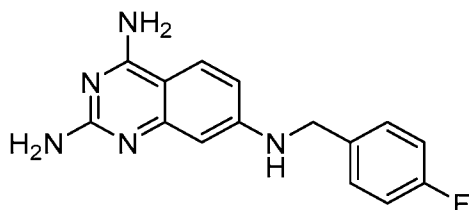
PPC-002-00150-1

15

^1H NMR (400 MHz, DMSO- d_6) δ 8.47 – 8.25 (m, 1H), 7.91 – 7.55 (m, 2H), 7.01 (m, 5H), 6.59 (s, 1H), 6.04 (s, 2H), 5.52 (s, 2H), 3.73 (s, 3H).

MS (ESI): $[\text{M}+\text{H}^+]$ 321.20.

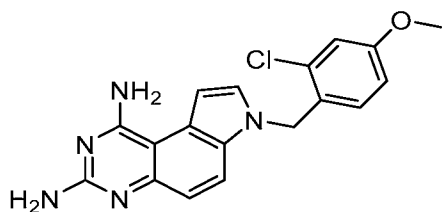
20

Compound 64

PPC-002-0152-1

¹H NMR (400 MHz, DMSO-*d*₆) δ 7.83 (s, 2H), 7.51 – 7.38 (m, 2H), 7.19 – 7.11 (m, 4H), 7.08 (s, 1H), 6.55 (s, 2H), 6.29 (t, *J* = 6.4 Hz, 1H), 4.29 (d, *J* = 6.0 Hz, 2H).

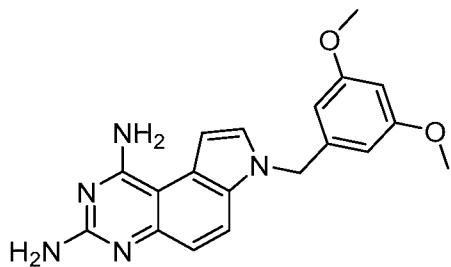
MS (ESI): [M+H⁺] 284.30

Compound 65

PPC-002-0153-1

¹H NMR (400 MHz, DMSO-*d*₆) δ 7.70 (d, *J* = 9.2 Hz, 1H), 7.51 (d, *J* = 3.2 Hz, 1H), 7.11 (dd, *J* = 8.0, 2.8 Hz, 2H), 7.05 (d, *J* = 9.2 Hz, 1H), 6.84 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.76 (s, 2H), 6.73 (d, *J* = 8.4 Hz, 1H), 5.78 (s, 2H), 5.50 (s, 2H), 3.74 (s, 3H).

MS (ESI): [M+H⁺] 354.79

Compound 66

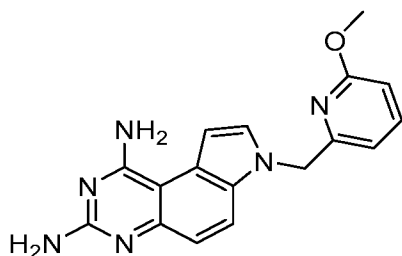
PPC-002-0154-1

^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.74 (dd, $J = 9.0, 0.8$ Hz, 1H), 7.62 (d, $J = 3.2$ Hz, 1H), 7.13 – 7.08 (m, 1H), 7.04 (d, $J = 9.0$ Hz, 1H), 6.75 (s, 2H), 6.38 (t, $J = 2.4$ Hz, 1H), 6.34 (d, $J = 2.4$ Hz, 2H), 5.76 (s, 2H), 5.41 (s, 2H), 3.67 (s, 6H).

MS (ESI): $[\text{M}+\text{H}^+]$ 350.34

5

Compound 67



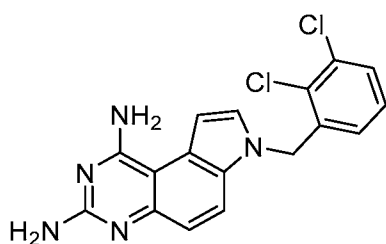
PPC-002-0156-1

^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.79 (dd, $J = 9.0, 0.8$ Hz, 1H), 7.69 – 7.50 (m, 2H), 7.18 – 7.09 (m, 1H), 7.05 (d, $J = 8.8$ Hz, 1H), 6.78 (s, 2H), 6.69 (dd, $J = 8.8, 0.8$ Hz, 1H), 6.51 (dd, $J = 7.2, 0.8$ Hz, 1H), 5.80 (s, 2H), 5.49 (s, 2H), 3.82 (s, 3H).

10

MS (ESI): $[\text{M}+\text{H}^+]$ 321.37

Compound 68



PPC-002-0157-1

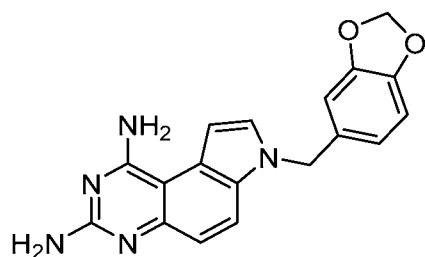
^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.67 (d, $J = 9.0$ Hz, 1H), 7.62 – 7.51 (m, 2H), 7.33 – 7.13 (m, 2H), 7.04 (d, $J = 9.0$ Hz, 1H), 6.76 (s, 2H), 6.39 (dd, $J = 7.6, 1.2$ Hz, 1H), 5.76 (s, 2H), 5.65 (s, 2H).

15

MS (ESI): $[\text{M}+\text{H}^+]$ 359.21

20

Compound 69

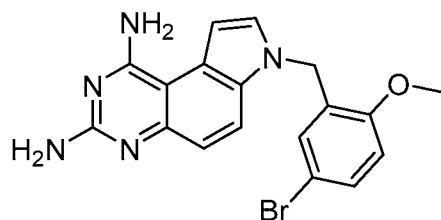


PPC-002-0158-1

¹H NMR (400 MHz, DMSO-*d*₆) δ 7.78 (d, *J* = 8.8 Hz, 1H), 7.62 (d, *J* = 3.0 Hz, 1H), 7.08 (d, *J* = 3.0 Hz, 1H), 7.03 (d, *J* = 8.8 Hz, 1H), 6.88 – 6.79 (m, 2H), 6.76 – 6.73 (m, 1H), 5.96 (s, 2H), 5.76 (s, 2H), 5.38 (s, 2H).

MS (ESI): [M+H⁺] 334.36

Compound 70

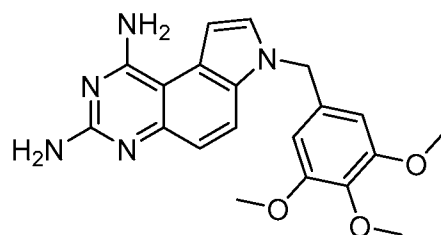


PPC-002-00161-1

¹H NMR (400 MHz, DMSO-*d*₆) δ 7.71 (d, *J* = 8.8 Hz, 1H), 7.55 (d, *J* = 3.2 Hz, 1H), 7.43 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.10 (d, *J* = 3.2 Hz, 1H), 7.07 – 7.01 (m, 2H), 6.79 (d, *J* = 2.4 Hz, 1H), 6.73 (s, 2H), 5.75 (s, 2H), 5.42 (s, 2H), 3.87 (s, 3H).

MS (ESI): [M+H⁺] 398.24.

Compound 71



PPC-002-00162-1

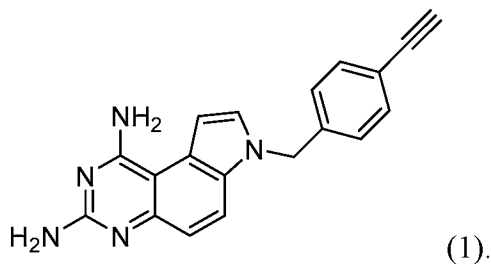
^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.83 (d, $J = 9.2$ Hz, 1H), 7.64 (d, $J = 3.2$ Hz, 1H), 7.09 (d, $J = 3.2$ Hz, 1H), 7.04 (d, $J = 9.2$ Hz, 1H), 6.74 (s, 2H), 6.61 (s, 2H), 5.76 (s, 2H), 5.38 (s, 2H), 3.68 (s, 6H), 3.59 (s, 3H).

MS (ESI): $[\text{M}+\text{H}^+]$ 398.24

5

Anticancer Properties of Compound 1 (IRS-17)

The anticancer properties of Compound 1 were investigated as follows:



10

A. *Compound 1 – Purine and Thymidine Inhibition*

Cells of (a) HCT116 and (b) Panc1 cell lines were each seeded in 96-well plates at the density of 3000/well in 80 μL of DMEM media supplemented with 10 vol% dialyzed FBS. The next day, 10 μL of media with or without hypoxanthine (1 mM) and thymidine (160 μM) was added to each well. 10 μL of Compound 1 diluted in media as 10x stock of desired concentration was also added. Relative cell number is measured by resazurin right before and 4 days after the addition of Compound 1. Namely, resazurin dissolved in phosphate-buffered saline (PBS) was added to each well to a final concentration of 10 $\mu\text{g/mL}$ followed by 1-hour incubation at 37 $^{\circ}\text{C}$. Fluorescence (excitation 550 nm, emission 590 nm) is then measured by a microplate reader.

The results are provided in FIGS. 1A and 1B. As illustrated in FIGS. 1A and 1B, Compound 1 inhibited cell growth under supplement and non-supplement conditions. Inhibition of growth in both cancer cell lines was achieved via disruption of purine and thymine synthesis.

B. *Compound 1 – Folate Competition*

Compound 1 was tested relative to pemetrexed and methotrexate against two isogenic CHO cell lines with low and high RFC expression levels. IC_{50} values were determined in folic

25

acid free RPMI media supplemented with 10 vol.% dialyzed FBS and 25 nM 5-formyl THF. Table II provides the IC₅₀ values for Compound 1, Pemetrexed and Methotrexate.

Table II

	IC ₅₀ (nM)	
	Low RFC expression	High RFC expression
Compound 1	<0.002	0.06
Pemetrexed	800	150
Methotrexate	4000	480

5

As provided in Table II, Compound 1 exhibits higher potency in cells down-regulating RFC, which are resistant to Pemetrexed and Methotrexate. FIG. 2 also illustrates the higher potency of Compound 1 in low-RFC expression cells.

10 C. *Effect of Compound 1 on KPC Pancreatic Ductal Adenocarcinoma Allograft*

Tumor harvested from K-ras^{LSL.G12D/+}; Trp53^{R172H/+}; Pdx-1-Cre (KPC) mice was passaged at the flank of C57BL/6 subcutaneously. To initiate allografts, subcutaneous tumors were harvested and minced in DMEM media, mixed with equal volume of Matrigel basement membrane matrix (Corning 354234), and injected subcutaneously at the flank of male C57BL/6
 15 mice. Tumors were measured twice per week. Once the average tumor volume reaches 100 mm³, Compound 1 (5mg/kg body weight, dissolved in 10% 2-hydroxypropyl-β-cyclodextrin) and vehicle (10% 2-hydroxypropyl-β-cyclodextrin, 10mL/kg body weight) were injected intraperitoneally once daily, with continued tumor monitoring. At the end of experiment, mice were euthanized by cervical dislocation after being fasted for 6 hr. Tumors are collected into
 20 aluminum foil, clamped by a Wollenberg clamp pre-cooled in liquid N₂, and kept cold in liquid N₂.

To extract metabolites from tumor samples, tumors were first ground by a Cryomill (Retsch). About 10 mg of ground tissue is measured and extracted by extraction buffer (acetonitrile:methanol:H₂O=2:2:1, then supplemented with 0.5 vol% formic acid) at the ratio of

370 μ L buffer per 10 mg tissue. Samples were vortexed and adjusted to pH7 by 15% NH_4HCO_3 (30 μ L per 10 mg tissue). Samples were centrifuged (16000 g for 20 min) at 4 $^\circ\text{C}$, and supernatants are collected for measurement. Metabolite levels were measured by Q Exactive Plus Hybrid Quadrupole-Orbitrap coupled with hydrophobic interaction chromatography (HILIC) operating in the negative ionization mode.

FIG. 3A illustrates change in tumor volume over time, and FIG. 3B illustrates tumor doubling time. As provided in FIGS. 3A and 3B, Compound 1 substantially inhibited tumor growth over the study time period, and protracted tumor doubling time. FIG. 3C illustrates levels of intermediates in purine synthesis glycineamide ribonucleotide (GAR) and 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) in the tumors, indicating folate competition or interference.

D. *Effect of Compound 1 on HCT116 Colorectal Carcinoma Xenograft*

To initiate allografts, 2×10^6 MC38 cells were injected subcutaneously at the flank of female C57BL/6 mice. Once the average tumor volume reaches 50 mm^3 , Compound 1 (5mg/kg body weight, dissolved in 10% 2-hydroxypropyl- β -cyclodextrin) and vehicle (10% 2-hydroxypropyl- β -cyclodextrin, 10mL/kg body weight) are injected intraperitoneally once daily. After the final dose, mice were fasted for 12 hr. Then blood was collected by tail snip into a tube without anticoagulants. Serum was collected by taking the supernatant after centrifugation. Mice were then euthanized by cervical dislocation, and tumors were harvested and frozen in liquid N_2 . Tumor metabolites were extracted and measured as previously described in C. To extract serum metabolites, 2.5 μ L of serum was added into 80 μ L of methanol. Samples were centrifuged (16000 g for 10 min) at 4 $^\circ\text{C}$, and supernatant is collected.

FIGS. 4A illustrates change in tumor volume over time, and FIG. 4B illustrates tumor doubling time. As provided in FIGS. 4A and 4B, Compound 1 substantially inhibited tumor growth over the study time period, and protracted tumor doubling time.

E. *Effect of Compound 1 on MC38 Mouse Colon Adenocarcinoma Allograft*

To initiate allografts, 2×10^6 MC38 cells were injected subcutaneously at the flank of female C57BL/6 mice. Once the average tumor volume reaches 50 mm^3 , IRS-17 (5mg/kg body weight, dissolved in 10% 2-hydroxypropyl- β -cyclodextrin) and vehicle (10% 2-hydroxypropyl-

β -cyclodextrin, 10mL/kg body weight) are injected intraperitoneally once daily. After the final dose, mice were fasted for 12 hr. Then blood was collected by tail snip into a tube without anticoagulants. Serum was collected by taking the supernatant after centrifugation. Mice were then euthanized by cervical dislocation, and tumors are harvested and frozen in liquid N₂.

- 5 Tumor metabolites were extracted and measured as previously described in C. To extract serum metabolites, 2.5 μ L of serum was added into 80 μ L of methanol. Samples were centrifuged (16000 g for 10 min) at 4 °C, and supernatant is collected.

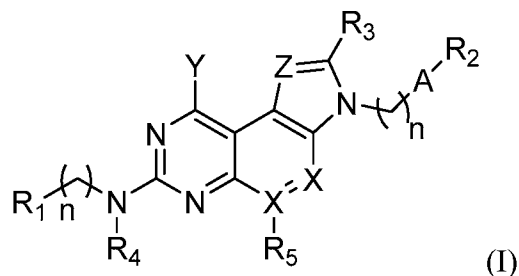
FIG. 5A illustrates some reduction in tumor volume over the treatment period. FIG. 5B illustrates pool size of circulating thymidine, and FIG. 5C illustrates thymidine ribonucleotide
10 intermediate dUMP and purine intermediates GAR and AICAR levels in the tumors in response to administration of Compound 1.

Various embodiments of the invention have been described in fulfillment of the various objects of the invention. It should be recognized that these embodiments are merely illustrative of the principles of the present invention. Numerous modifications and adaptations thereof will
15 be readily apparent to those skilled in the art without departing from the spirit and scope of the invention.

CLAIMS

1. A pharmaceutical composition comprising a compound of Formula (I) and/or salts thereof:

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wherein R₁, R₃, R₄ and R₅ are independently selected from the group consisting of hydrogen, alkyl, alkenyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, imine, cyanoimine, alkylene-aryl, alkylene-heteroaryl, amide, sulfonamide, acid, halo, and urea, wherein the alkyl, alkenyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, alkylene-aryl, alkylene-heteroaryl, amide and sulfonamide are optionally substituted with one or more substituents selected from the group consisting of (C₁–C₁₀)-alkyl, (C₁–C₁₀)-alkenyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, alkoxy, amide, sulfonamide, urea, halo, cyano, hydroxy, C(O)OR₆, and C(O)R₇, wherein R₆ is selected from the group consisting of hydrogen, alkyl and alkenyl and R₇ is selected from the group consisting of hydrogen, alkyl, alkenyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl and NR₈R₉, wherein R₈ and R₉ are independently selected from the group consisting of hydrogen, alkyl, alkenyl, aryl and heteroaryl; and

wherein R₂ is selected from the group consisting of alkyl, cycloalkyl, heterocycloalkyl, alkynyl, alkenyl, alkynylene-alkyl, alkynylene-cycloalkyl, alkynylene-heterocycloalkyl, alkynylene-aryl, alkynylene-heteroaryl, alkynylene-amine, alkynylene-protected amine, alkynylene-alkylsilane, fluoroalkyl, fluoro, bromo, B(OH)₂, nitro, cyano, and alkoxy; and

wherein A is selected from the group consisting of aryl and heteroaryl; and wherein X and Z are independently selected from the group consisting of C, N, O, S, SO₂, and NR₁₀R₁₁, wherein R₁₀ and R₁₁ are independently selected from the group consisting of hydrogen, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, alkenyl, aryl, heteroaryl, amide, sulfonamide, urea and C(O)R₁₂ wherein R₁₂ is selected from the group consisting of hydrogen,

alkyl, alkenyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl and heteroaryl and wherein R₁₀ and R₁₁ may optionally form a ring structure; and

wherein Y is selected from the group consisting of OH, alkoxy, and NR₁₃R₁₄, wherein R₁₃ and R₁₄ are independently selected from the group consisting of hydrogen, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, alkenyl, aryl, heteroaryl, amide, sulfonamide, urea, alkylene-aryl, alkylene-heteroaryl, and C(O)R₁₅ wherein R₁₅ is selected from the group consisting of hydrogen, alkyl, alkenyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl and heteroaryl and wherein R₁₃ and R₁₄ may optionally form a ring structure, wherein the aryl, heteroaryl, alkylene-aryl and alkylene heteroaryl are optionally substituted with one or more substituents selected from the group consisting of alkyl, alkenyl, alkynyl, halo, and alkynylene-alkylsilane; and

n is an integer from 0 to 5,

wherein the compound of Formula (I) is present in the pharmaceutical composition in an amount sufficient to exhibit anticancer properties.

2. The pharmaceutical composition of claim 1, wherein R₂ is selected from the group consisting of alkynyl, alkynylene-alkyl, alkynylene-cycloalkyl, alkynylene-heterocycloalkyl, alkynylene-aryl, alkynylene-heteroaryl, alkynylene-amine, alkynylene-protected amine, and alkynylene-alkylsilane.

3. The pharmaceutical composition of claim 1, wherein R₂ is selected from the group consisting of alkynyl, alkenyl, and alkynylene-alkyl.

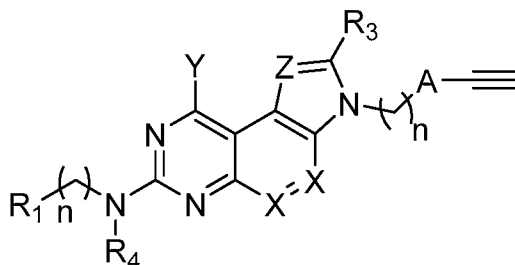
4. The pharmaceutical composition of claim 2, wherein R₁ and R₄ are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, and heterocycloalkyl.

5. The pharmaceutical composition of claim 2, wherein Y is NR₁₂R₁₃, wherein R₁₂ and R₁₃ are independently selected from the group consisting of hydrogen, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, alkenyl, aryl, and heteroaryl.

6. The pharmaceutical composition of claim 2, wherein X and Z are independently selected from C and N.

7. The pharmaceutical composition of claim 2, wherein R_3 is selected from the group consisting of hydrogen and alkyl.

5 8. The pharmaceutical composition of claim 2, wherein the compound of Formula (I) is:



9. The pharmaceutical composition of claim 8, wherein X and Z are selected from the group consisting of C and N, and wherein R_1 , R_3 , and R_4 are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, and heterocycloalkyl.

10

10. The pharmaceutical composition of claim 2, wherein the compound of Formula (I) and/or salt thereof is present in an amount of 0.01 nM to 1 μ M.

15 11. The pharmaceutical composition of claim 2, wherein the compound of Formula (I) and/or salt thereof is present in an amount of 0.1 nM to 500 nM.

12. The pharmaceutical composition of claim 2, wherein the compound of Formula (I) inhibits purine and/or thymine biosynthesis in cancer cells.

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13. The pharmaceutical composition of claim 12, wherein the cancer cells are pancreatic cancer cells.

14. The pharmaceutical composition of claim 2, wherein the compound of Formula (I) exhibits higher potency relative to pemetrexed or methotrexate for cancer cells down-regulating reduced folate carrier.

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15. The pharmaceutical composition of claim 2, wherein the compound of Formula (I) inhibits colorectal tumor growth.

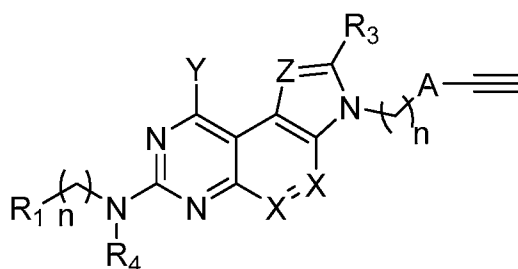
16. The pharmaceutical composition of claim 2 further comprising one more adjuvants or additional chemotherapeutic agents.

17. A method of treating cancer comprising:
administering to a patient having cancerous tissue the pharmaceutical composition of claim 1.

18. The method of claim 17, wherein cells of the cancerous tissue down-regulate reduced folate carrier.

19. The method of claim 18, wherein IC₅₀ of the compound of Formula (I) is less than 0.1 nM.

20. The method of claims 18, wherein the compound of Formula (I) is:



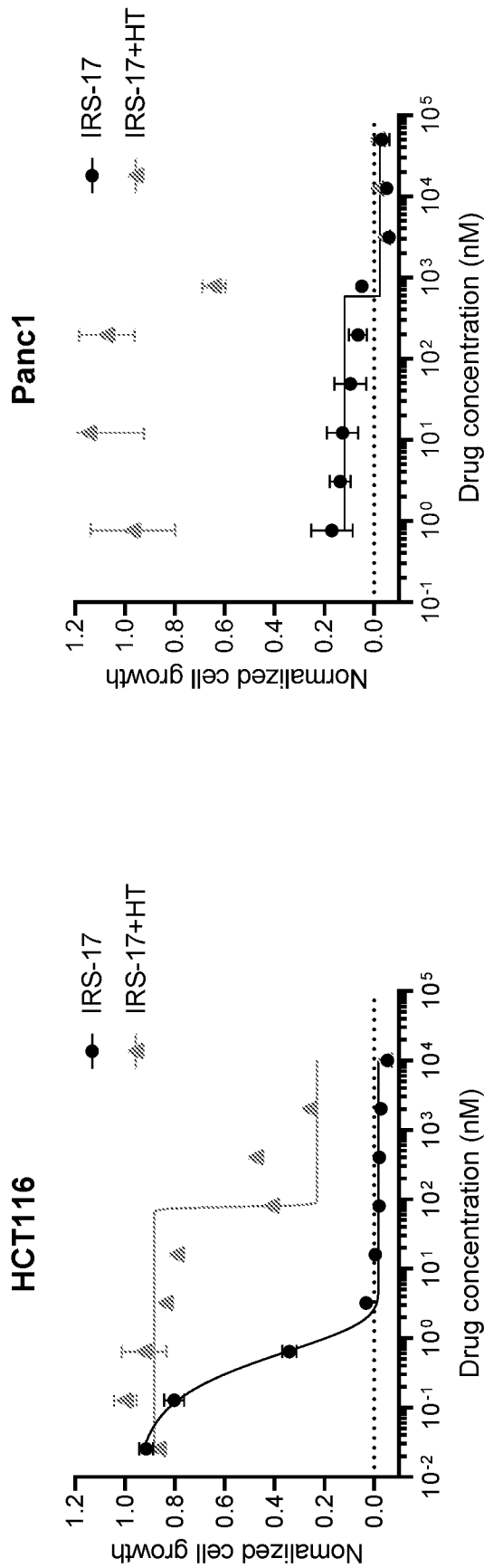


FIG. 1A

FIG. 1B

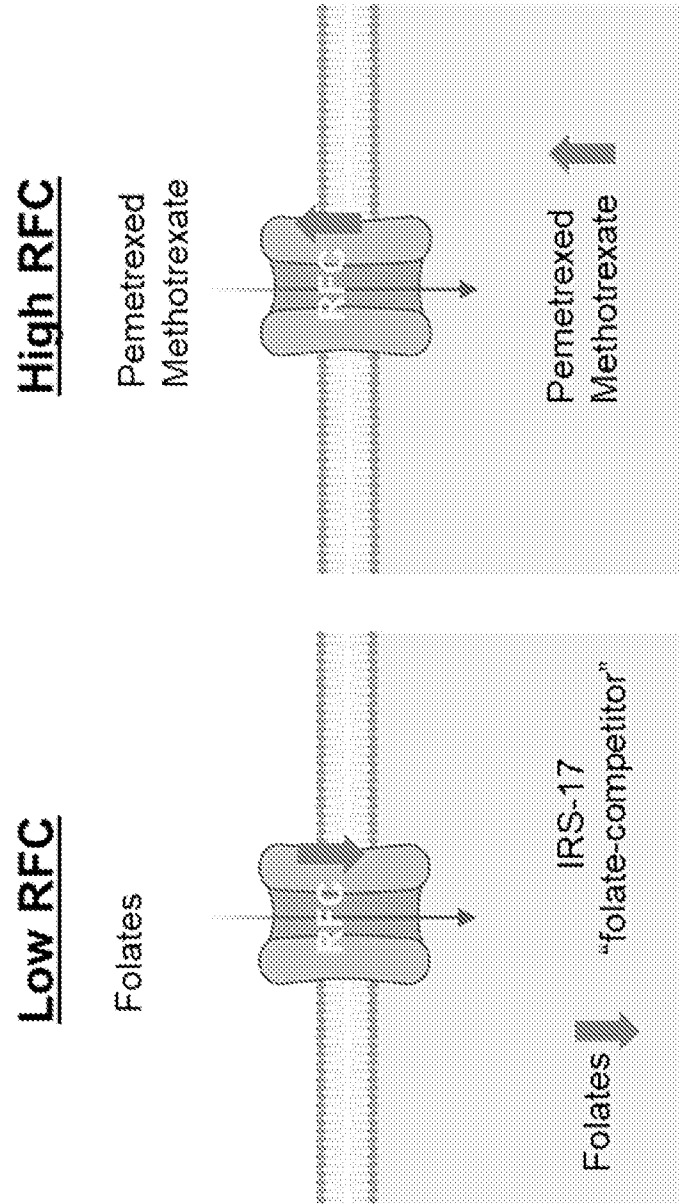


FIG. 2

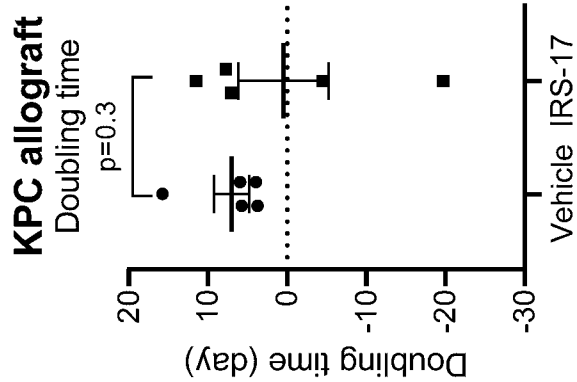


FIG. 3B

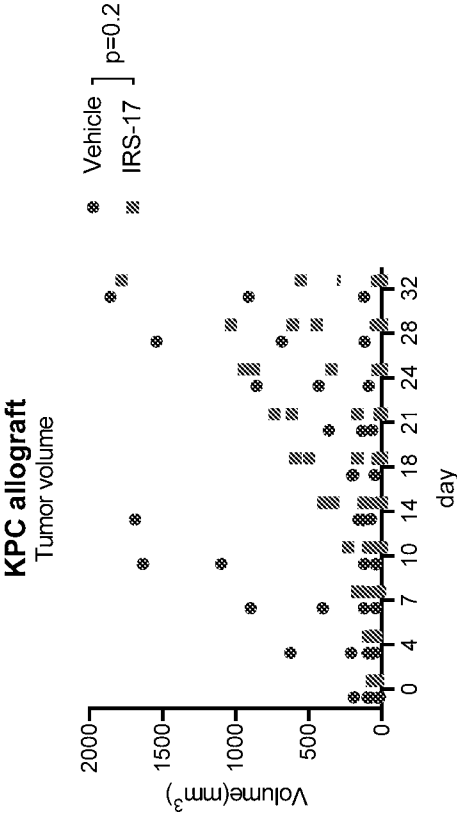


FIG. 3A

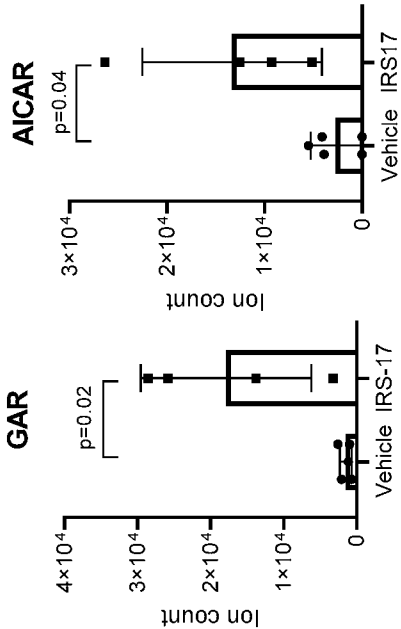


FIG. 3C

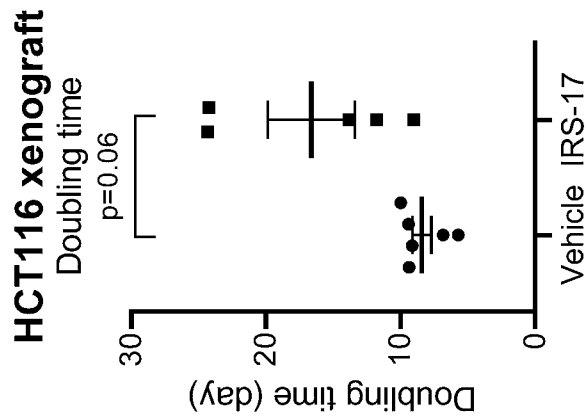


FIG. 4B

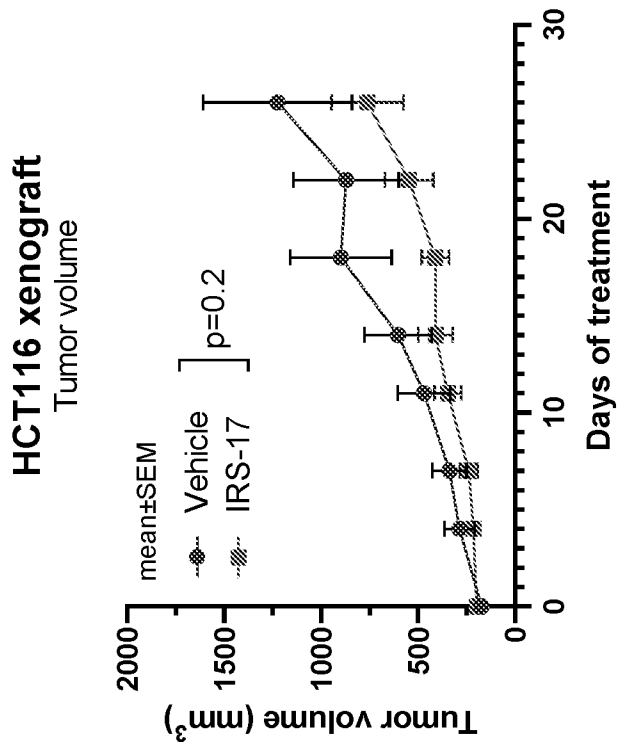


FIG. 4A

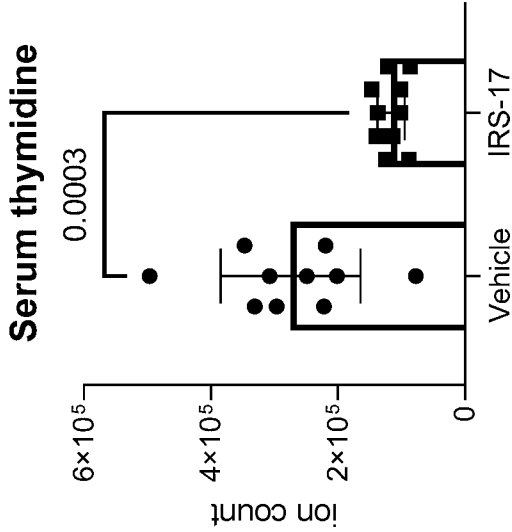


FIG. 5B

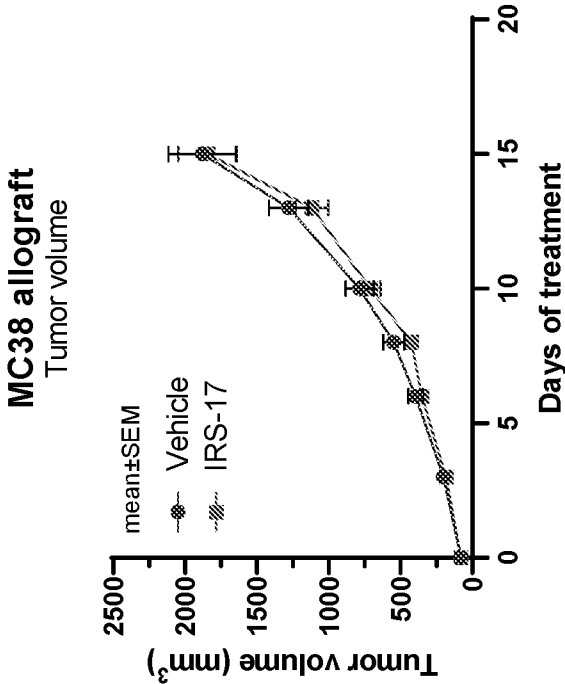


FIG. 5A

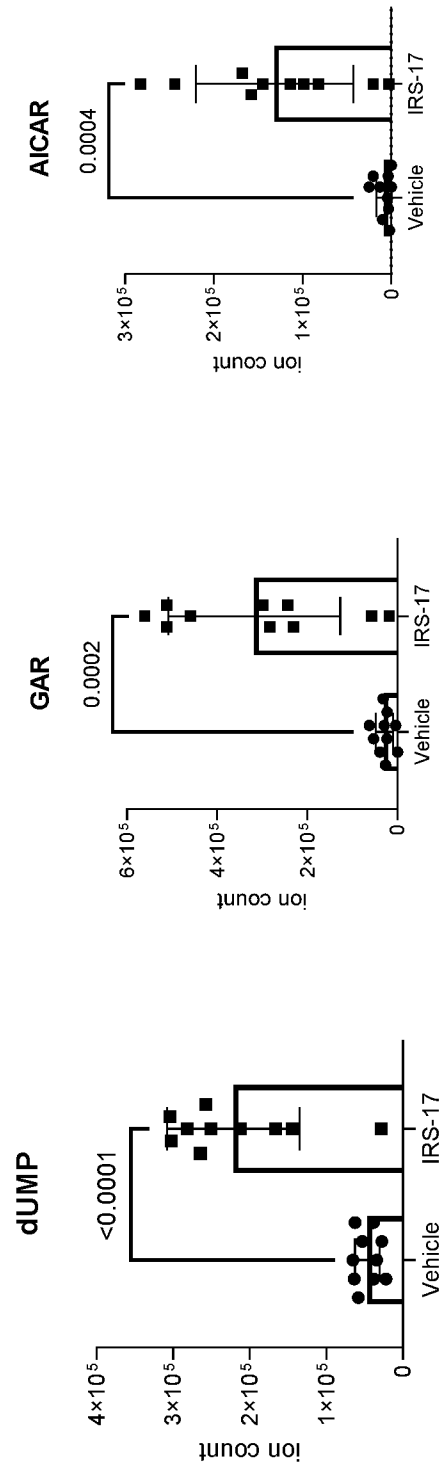


FIG. 5C

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/40171

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C07D 487/02; A61P 35/00 (2021.01)

CPC - C07D 487/02; A61P 35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2019/0201401 A1 (The Trustees of Princeton University) 04 July 2019 (04.07.2019) Figure 15; Figure 16	1
A	US 4,118,561 A (LEDIG) 03 October 1978 (03.10.1978) ENTIRE DOCUMENT	1
A	US 2014/0378486 A1 (XIAO, et.al.) 25 December 2014 (25.12.2014) ENTIRE DOCUMENT	1

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

09 NOVEMBER 2021

Date of mailing of the international search report

DEC 15 2021

Name and mailing address of the ISA/US

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Facsimile No. 571-273-8300

Authorized officer

Kari Rodriguez

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/40171

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
--Please see attached sheet--

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Attachment to Box.No.III:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I+: Claims 1-16 directed to a pharmaceutical composition comprising a compound of Formula (I), and/or salts thereof, wherein the compound of Formula (I) is present in the pharmaceutical composition in an amount sufficient to exhibit anticancer properties. The pharmaceutical composition will be searched to the extent that the composition encompasses the first species of claim 1, wherein R1, R3, R4 and R5 are each hydrogen; R2 is alkyl; A is aryl; X and Z are each C; Y is OH; and each n is 0. It is believed that claim 1 reads on this first named invention, and this claim will therefore be searched without fee, to the extent that it encompasses the composition described above. Applicant is invited to elect additional composition(s), wherein each additional composition elected will require one additional invention fee. Applicants must specify the claims that encompass any additionally elected composition. Applicants must further indicate, if applicable, the claims which encompass the first named invention of Group I+, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the '+' group(s) will result in only the first claimed invention of Group I+ to be searched. Additionally, an exemplary election wherein different actual variables are selected is suggested. An exemplary election for Group I+ would be a pharmaceutical composition comprising the compound specified in claim 8, wherein R1, R3 and R4 are each hydrogen; X and Z are each C; Y is NR12R13, where R12 and R13 are each hydrogen; A is aryl and each n is 1 (i.e., claims 1-16).

Group II: Claims 17-20, directed to a method of treating cancer comprising administering to a patient having cancerous tissue the pharmaceutical composition comprising a compound of Formula I and/or its salts.

The group of inventions listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I+ includes the technical feature of a unique pharmaceutical composition, which is not required by any other invention of Group I+.

Group II includes the technical feature of a method of treating cancer in a patient, not required by Group I+.

Common technical features:

The inventions of Group I+ share the technical feature of pharmaceutical composition comprising a compound of Formula I and/or salts thereof.

Groups I+ and II also share the technical feature of a pharmaceutical composition comprising a compound of Formula I and/or salts thereof.

This shared technical feature, however, does not provide a contribution over the prior art, as being anticipated by US 2019/0201401 A1 to The Trustees of Princeton University (hereinafter "Princeton"), which discloses a pharmaceutical composition comprising a compound of formula I (para [0006]) wherein R1 is cycloalkyl; R3 is C1-alkyl; R4 is H; R5 is H; R2 is alkyl; A is a heteroaryl; X and Z are each C; Y is NR13R14, wherein each of R13 and R14 is H; n as it relates to N(R4)-(CH2)n-R1 is 0 and n as it relates to -N-(CH2)n-A-R2 is 1 (Figure 15, Second row, Second Compound) and wherein the compound of formula I is present in the pharmaceutical composition in an amount sufficient to exhibit anticancer properties (para [0006];[0010]).

As said composition was known in the art at the time of the invention, this cannot be considered a special technical feature, that would otherwise unify the inventions of Groups I+-II.

The inventions of Group I+-II, thus lack unity under PCT Rule 13.