

TREATMENT OF PROSTATE CANCER**CROSS-REFERENCE**

[0001] This application claims the benefit of U. S. Provisional Application Serial No. 63/517,672 filed August 4, 2023, U.S. Provisional Application Serial No. 63/603,336 filed November 28, 2023, U.S. Provisional Application Serial No. 63/617,895 filed January 5, 2024, and U.S. Provisional Application Serial No. 63/670,485 filed July 12, 2024; which are hereby incorporated by reference in their entirety.

BACKGROUND OF THE INVENTION

[0002] Prostate cancer is the most common cancer among men in the United States, with about one in nine men diagnosed in their lifetime. Despite high incidence rates, mortality rates continue to remain low due to screening methods that allow for early intervention and new effective treatments. However, even with low mortality rates, prostate cancer remains one of the leading causes of death among men.

[0003] The range of new therapeutic agents has significantly improved the treatment landscape of both castration-sensitive (localized and metastatic) and castration-resistant prostate cancer. Despite prolonged clinical responses, improved survival and quality of life, systemic treatments eventually fail in nearly all patients as indicated by rising prostate-specific antigen (PSA) levels and/or radiographic disease progression. After progression on these systemic therapies, chemotherapy is the remaining option providing modest improvements in overall survival at the expense of significant morbidity.

[0004] A consequence of the use of androgen receptor signaling inhibitors (ARSIs), such as CYP17 inhibitor abiraterone, and androgen receptor inhibitors apalutamide, darolutamide, and enzalutamide, has been the expansion of tumor heterogeneity highlighted by the clinical emergence of diverse phenotypic states to bypass ARSIs, including cells with heightened plasticity and divergent differentiation. Increased plasticity is a highly reproducible feature of prostate cancer tumors following ARSI therapy. Thus, there is a need to develop new therapeutic regimens to overcome or circumvent the emergence of epigenetic plasticity throughout prostate cancer treatment.

[0005] The present disclosure relates to methods of treating prostate cancer in a subject, comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor.

SUMMARY OF THE INVENTION

[0006] Disclosed herein are methods of treating prostate cancer in a subject, comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor.

[0007] Also disclosed herein are methods of treating prostate cancer in a subject, wherein the prostate cancer in the subject has been determined to be resistant to one or more first androgen receptor signaling inhibitors (ARSI), comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor.

[0008] Further disclosed herein are methods of treating prostate cancer in a subject, wherein the prostate cancer in the subject has been determined to be resistant to a CYP17 inhibitor, comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor.

[0009] Also disclosed herein are methods of treating prostate cancer in a subject, wherein the prostate cancer in the subject has been determined to be resistant to abiraterone, comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor.

[0010] Further disclosed herein are methods of treating prostate cancer in a subject, wherein the subject has received prior administration of one or more CYP17 inhibitors, comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor.

[0011] Also disclosed herein are methods of treating prostate cancer in a subject, wherein the subject (i) has received prior administration of one or more CYP17 inhibitors, and (ii) is androgen receptor inhibitor naïve, comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor.

[0012] Further disclosed herein are any of the described methods of treating prostate cancer in a subject, wherein the prostate cancer in the subject is selected from metastatic prostate cancer, non-metastatic prostate cancer, metastatic castration-resistant prostate cancer, metastatic castration-sensitive prostate cancer, localized high risk prostate cancer, recurrent prostate cancer, non-metastatic castration-resistant prostate cancer, non-metastatic castration-sensitive prostate cancer, androgen receptor inhibitor-sensitive prostate cancer, androgen receptor inhibitor-resistant prostate cancer, androgen receptor-dependent prostate cancer, androgen receptor-independent prostate cancer, neuroendocrine prostate cancer (NEPC), metastatic neuroendocrine prostate cancer (NEPC), prostate cancer with small cell features, metastatic prostate cancer with small cell features, and aggressive-variant prostate cancer.

[0013] Also disclosed herein is a method of treating prostate cancer in a subject, comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor. In some embodiments, the subject has received prior administration of one or more CYP17 inhibitors. In some embodiments, the subject has received prior administration of abiraterone or abiraterone acetate. In some embodiments, the prostate cancer in the subject has been determined to be resistant to abiraterone. In some embodiments, the subject is CYP17 inhibitor-naïve prior to the administration to the subject of the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor. In some embodiments, the subject is androgen receptor inhibitor naïve prior to the administration to the subject of the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor. In some embodiments, the subject has been administered one or more prior androgen deprivation therapies prior to the administration to the subject of the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor. In some embodiments, the subject has been administered a gonadotropin-releasing hormone (GnRH) analog prior to the administration to the subject of the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor. In some embodiments, the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor are administered to the subject sequentially or

simultaneously. In some embodiments, the androgen receptor inhibitor is selected from apalutamide, darolutamide, and enzalutamide. In some embodiments, the embryonic ectoderm development (EED) inhibitor is selected from EED226, A-395, APG-5918, BR-001, BR-002, EEDi-5285, EEDi-1056, FTX-6058, HJM-353, and MAK683. In some embodiments, the prostate cancer in the subject is selected from metastatic prostate cancer, non-metastatic prostate cancer, metastatic castration-resistant prostate cancer, metastatic castration-sensitive prostate cancer, localized high risk prostate cancer, recurrent prostate cancer, non-metastatic castration-resistant prostate cancer, non-metastatic castration-sensitive prostate cancer, androgen receptor inhibitor-sensitive prostate cancer, androgen receptor inhibitor-resistant prostate cancer, androgen receptor-dependent prostate cancer, androgen receptor-independent prostate cancer, neuroendocrine prostate cancer (NEPC), metastatic neuroendocrine prostate cancer (NEPC), prostate cancer with small cell features, metastatic prostate cancer with small cell features, and aggressive-variant prostate cancer.

[0014] In some embodiments, the prostate cancer in the subject is metastatic prostate cancer.

[0015] In some embodiments, the prostate cancer in the subject is metastatic castration-resistant prostate cancer.

INCORPORATION BY REFERENCE

[0016] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE FIGURES

[0017] FIG. 1 depicts the antitumor effect of Compound 4 in combination with darolutamide in a non-castrated prostate cancer model setting using the 22Rv1 cell line xenograft model in intact male BALB/c nude mice as described in Example 4.

[0018] FIG. 2 depicts the antitumor effect of Compound 4 and darolutamide in darolutamide acquired resistant 22Rv1 xenografts in intact male BALB/c nude mice as described in Example 5.

[0019] FIG. 3 depicts the antitumor effect of Compound 4, PF-06821497, and darolutamide in subcutaneous C4-2 castrated prostate cancer xenograft model in male NPG mice as described in Example 6.

[0020] FIG. 4 depicts the antitumor effect of Compound 4, PF-06821497, and darolutamide in subcutaneous C4-2 intact prostate cancer xenograft model in male NPG mice as described in Example 7.

[0021] FIG. 5 depicts the concentration of Compound 4 versus time on cycle 2, day 1 (C2D1) in the plasma of subjects administered Compound at doses of 100 mg once per day (QD), 200 mg QD, 400 mg QD, 600 mg QD, and 900 mg QD in the form of a pharmaceutical composition as described in Example 9.

[0022] FIG. 6 depicts the percent change from baseline of H3K27me3/H3 in monocytes (mean $\pm$ SEM) at cycle 1, day 15 in subjects that were orally administered Compound 4 at doses of 200 mg QD, 400 mg QD,

600 mg QD, 700 mg QD, 800 mg QD, or 900 mg QD in the form of a pharmaceutical composition as described in Example 9.

[0023] FIG. 7 depicts the change from baseline of the ratio of H3K27me3 to histone 3.1 (H3.1) in cell-free nucleosomes from subjects at cycle 1, day 15 (C1D15) that were orally administered Compound 4 at doses of 100 mg once per day (QD), 200 mg QD, 400 mg QD, 600 mg QD, 700 mg QD, or 900 mg QD in the form of a pharmaceutical composition as described in Example 9.

[0024] FIG. 8 depicts the change from baseline of the ratio of H3K27me3 to histone 3.1 (H3.1) in cell-free nucleosomes from subjects at cycle 2, day 1 (C2D1) that were orally administered Compound 4 at doses of 100 mg once per day (QD), 200 mg QD, 400 mg QD, 600 mg QD, 700 mg QD, or 900 mg QD in the form of a pharmaceutical composition as described in Example 9.

[0025] FIG. 9 depicts the assessment of treatment groups based on progression free survival (PFS) in a xenograft subcutaneous C4-2 castrated prostate cancer model in male NPG mice treated with Compound 4, PF-06821497, or darolutamide as described in Example 6.

[0026] FIG. 10 depicts the assessment of treatment groups based on progression free survival (PFS) in a xenograft subcutaneous C4-2 intact prostate cancer model in male NPG mice treated with Compound 4, PF-06821497, or darolutamide as described in Example 7.

DETAILED DESCRIPTION OF THE INVENTION

[0027] As used in the specification and appended claims, unless specified to the contrary, the following terms have the meaning indicated below.

[0028] As used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “an agent” includes a plurality of such agents, and reference to “the cell” includes reference to one or more cells (or to a plurality of cells) and equivalents thereof known to those skilled in the art, and so forth. When ranges are used herein for physical properties, such as molecular weight, or chemical properties, such as chemical formulae, all combinations and sub-combinations of ranges and specific embodiments therein are intended to be included. The term “about” when referring to a number or a numerical range means that the number or numerical range referred to is an approximation within experimental variability (or within statistical experimental error), and thus the number or numerical range, in some instances, will vary between 1% and 15% of the stated number or numerical range. The term “comprising” (and related terms such as “comprise” or “comprises” or “having” or “including”) is not intended to exclude that in other certain embodiments, for example, an embodiment of any composition of matter, composition, method, or process, or the like, described herein, “consist of” or “consist essentially of” the described features.

[0029] “Administering” when used in conjunction with a therapeutic, including androgen receptor inhibitors and embryonic ectoderm development (EED) inhibitors, means to administer a therapeutic systemically or locally, as directly into or onto a target tissue, or to administer a therapeutic to a subject whereby the therapeutic positively impacts the tissue to which it is targeted. Thus, as used herein, the term

“administering”, when used in conjunction with a composition described herein, can include, but is not limited to, providing a composition into or onto the target tissue; providing a composition systemically to a subject by, e.g., oral administration whereby the therapeutic reaches the target tissue or cells.

“Administering” a composition may be accomplished by injection, topical administration, and oral administration or by other methods alone or in combination with other known techniques.

[0030] The terms “androgen receptor signaling inhibitor,” “ARSI,” “androgen receptor pathway inhibitors”, and “ARPI,” as used herein, mean an agent that when administered to a subject having prostate cancer affect androgen signaling pathways in one or more cells comprising the prostate cancer. Androgen receptor signaling inhibitors include, but are not limited to, agents that inhibit androgen biosynthesis or modulate the function of the androgen receptor, including by inhibiting the function of the androgen receptor, by for example, binding to the receptor and interfering with the binding of androgen(s) to the androgen receptors. Examples of agents that inhibit androgen biosynthesis are known to those of ordinary skill in the art and include, but are not limited to, agents that inhibit enzymes responsible for androgen biosynthesis, such as 17 α -hydroxylase/C17,20-lyase (CYP17). Examples of CYP17 inhibitors are known to those of ordinary skill in the art and include abiraterone and abiraterone prodrugs, including abiraterone acetate.

[0031] The term “androgen receptor inhibitor,” as used herein, means an agent that bind to and modulate the function of androgen receptors and include, but are not limited to, agents that inhibit the binding of androgen(s) to androgen receptors, and agents that inhibit nuclear translocation of androgen receptors and their interaction with DNA. Examples of androgen receptor inhibitors are known to those having skill in the art and include, but are not limited to, apalutamide, darolutamide, and enzalutamide.

[0032] The term “animal” as used herein includes, but is not limited to, humans and non-human vertebrates such as wild, domestic and farm animals. As used herein, the terms “subject,” “subject” and “individual” are intended to include living organisms in which certain conditions as described herein can occur. Examples include humans, monkeys, cows, sheep, goats, dogs, cats, mice, rats, and transgenic species thereof. In a preferred embodiment, the subject is a primate. In certain embodiments, the primate or subject is a human. In certain instances, the human is an adult. In certain instances, the human is child. In further instances, the human is under the age of 12 years. In certain instances, the human is elderly. In other instances, the human is 60 years of age or older. Other examples of subjects include experimental animals such as mice, rats, dogs, cats, goats, sheep, pigs, and cows. The experimental animal can be an animal model for a disorder, e.g., a transgenic mouse with hypertensive pathology.

[0033] The term “castration-sensitive prostate cancer,” as used herein, would be understood by one of ordinary skill in the art as describing the condition also termed hormone-sensitive prostate cancer.

[0034] The terms “determine,” “determined,” and “determining,” and the like, as used herein mean that it has been established that a pre-condition in a subject exists, or a condition precedent with respect to a subject has been satisfied, prior to the administration to the subject of androgen receptor inhibitors and embryonic ectoderm development (EED) inhibitors.

[0035] The term “embryonic ectoderm development (EED) inhibitor,” as used herein, means an agent that inhibits the function of the embryonic ectoderm development (EED) protein, including by binding to the protein.

[0036] The term “pharmaceutically acceptable”, as used herein, means a carrier, diluent or excipient that is compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

[0037] The term “pharmaceutical composition” means a composition comprising at least one active ingredient, whereby the composition is amenable to investigation for a specified, efficacious outcome in a mammal (for example, without limitation, a human). Those of ordinary skill in the art will understand and appreciate the techniques appropriate for determining whether an active ingredient has a desired efficacious outcome based upon the needs of the artisan.

[0038] The terms “progressing,” “relapsed,” “refractory,” “resistant,” and the like as used herein refer to prostate cancer in a subject that appears to grow again after a period of remission, and/or being no longer responsive to the treatment that is currently being administered to the subject or that had been administered to the subject (e.g., prior treatment of a prostate cancer in a subject with a CYP17 inhibitor such as abiraterone). A determination of whether a cancer, or one or more cells comprising a cancer, in a subject is progressing, has relapsed, or has become refractory or resistant to a specific treatment modality, such as a CYP17 inhibitor such as abiraterone, can be made by methods known to those of ordinary skill in the art. For example, responsiveness, or non-responsiveness, as the case may be, of a cancer in a subject, or one or more cells comprising the cancer in a subject, can be assessed by measuring a subject’s clinical symptoms, conducting a biopsy of one or more relevant tissue types, reduction in the size and/or number of tumor lesions, duration of response, or progression-free survival. Further, prostate cancer in a subject can be determined to be resistant to a prior line of therapy by reference to the criteria set forth in the Prostate Cancer Clinical Trials Working Group 3 (PCWG3) criteria (see, for example, Scher et al. J. Clinical Oncology, 2016, vol. 34, number 12, pp. 1402-1418 and included appendices), including, but not limited to, (a) rising levels of prostate-specific antigen (PSA) defined as a minimum of 2 rising values obtained a minimum of one week apart with the latest result being at least 2.0 ng/mL (or 1.0 ng/mL if PSA rise is the only indication of progression), (b) confirmation of 2 new bone lesions while being administered the last systemic therapy, (c) soft tissue progression per RECIST 1.1, and (d) radiographic progression.

[0039] As used herein, the term “therapeutic” means an agent utilized to treat, combat, ameliorate, prevent, or improve an unwanted condition or disease of a subject.

[0040] A “therapeutically effective amount” or “effective amount” as used herein refers to the amount of active compound or pharmaceutical agent that elicits a biological or medicinal response in a tissue, system, animal, individual or human that is being sought by a researcher, veterinarian, medical doctor or other clinician, which includes one or more of the following: (1) preventing the disease; for example, preventing a disease, condition or disorder in an individual that may be predisposed to the disease, condition or disorder but does not yet experience or display the pathology or symptomatology of the disease, (2) inhibiting the disease; for example, inhibiting a disease, condition or disorder in an individual that is experiencing or

displaying the pathology or symptomatology of the disease, condition or disorder (i.e., arresting further development of the pathology and/or symptomatology), and (3) ameliorating the disease; for example, ameliorating a disease, condition or disorder in an individual that is experiencing or displaying the pathology or symptomatology of the disease, condition or disorder (i.e., reversing the pathology and/or symptomatology).

[0041] The terms “treat,” “treated,” “treatment,” or “treating” as used herein refers to both therapeutic treatment in some embodiments and prophylactic or preventative measures in other embodiments, wherein the object is to prevent or slow (lessen) an undesired physiological condition, disorder, or disease, or to obtain beneficial or desired clinical results. For the purposes described herein, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms; diminishment of the extent of the condition, disorder or disease; stabilization (i.e., not worsening) of the state of the condition, disorder or disease; delay in onset or slowing of the progression of the condition, disorder or disease; amelioration of the condition, disorder or disease state; and remission (whether partial or total), whether detectable or undetectable, or enhancement or improvement of the condition, disorder or disease. Treatment includes eliciting a clinically significant response without excessive levels of side effects. Treatment also includes prolonging survival as compared to expected survival if not receiving treatment. A prophylactic benefit of treatment includes prevention of a condition, retarding the progress of a condition, stabilization of a condition, or decreasing the likelihood of occurrence of a condition.

[0042] For simplicity, chemical moieties are defined and referred to throughout primarily as univalent chemical moieties (e.g., alkyl, aryl, etc.). Nevertheless, such terms may also be used to convey corresponding multivalent moieties under the appropriate structural circumstances clear to those skilled in the art. For example, while an “alkyl” moiety generally refers to a monovalent radical (e.g. $\text{CH}_3\text{-CH}_2\text{-}$), in certain circumstances a bivalent linking moiety can be “alkyl,” in which case those skilled in the art will understand the alkyl to be a divalent radical (e.g., $\text{-CH}_2\text{-CH}_2\text{-}$), which is equivalent to the term “alkylene.” (Similarly, in circumstances in which a divalent moiety is required and is stated as being “aryl,” those skilled in the art will understand that the term “aryl” refers to the corresponding divalent moiety, arylene.) All atoms are understood to have their normal number of valences for bond formation (i.e., 4 for carbon, 3 for N, 2 for O, and 2, 4, or 6 for S, depending on the oxidation state of the S).

[0043] The term “amino” as used herein refers to -NH_2 .

[0044] The term “acetyl” as used herein refers to -C(O)CH_3 .

[0045] The term “acyl” as used herein refers to an alkylcarbonyl or arylcarbonyl substituent wherein the alkyl and aryl portions are as defined herein.

[0046] The term “alkyl” as used herein refers to straight and branched chain aliphatic groups having from 1 to 12 carbon atoms. As such, “alkyl” encompasses C_1 , C_2 , C_3 , C_4 , C_5 , C_6 , C_7 , C_8 , C_9 , C_{10} , C_{11} and C_{12} groups. Examples of alkyl groups include, without limitation, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, and hexyl.

[0047] The term “alkenyl” as used herein means an unsaturated straight or branched chain aliphatic group with one or more carbon-carbon double bonds, having from 2 to 12 carbon atoms. As such, “alkenyl” encompasses C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁ and C₁₂ groups. Examples of alkenyl groups include, without limitation, ethenyl, propenyl, butenyl, pentenyl, and hexenyl.

[0048] The term “alkynyl” as used herein means an unsaturated straight or branched chain aliphatic group with one or more carbon-carbon triple bonds, having from 2 to 12 carbon atoms. As such, “alkynyl” encompasses C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁ and C₁₂ groups. Examples of alkynyl groups include, without limitation, ethynyl, propynyl, butynyl, pentynyl, and hexynyl.

[0049] The terms “alkylene,” “alkenylene,” and “alkynylene” as used herein mean an alkyl, alkenyl, or alkynyl group, as defined hereinabove, that is positioned between and serves to connect two other chemical groups. Examples of alkylene groups include, without limitation, methylene, ethylene, propylene, and butylene. Exemplary alkenylene groups include, without limitation, ethenylene, propenylene, and butenylene. Exemplary alkynylene groups include, without limitation, ethynylene, propynylene, and butynylene.

[0050] The term “alkoxy” as used herein refers to -OC₁-C₆ alkyl.

[0051] The term “cycloalkyl” as used herein as employed herein is a saturated and partially unsaturated cyclic hydrocarbon group having 3 to 12 carbons. As such, “cycloalkyl” includes C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁ and C₁₂ cyclic hydrocarbon groups. Examples of cycloalkyl groups include, without limitation, cyclopropyl, cyclobutyl, cyclopentyl, cyclopentenyl, cyclohexyl, cyclohexenyl, cycloheptyl, and cyclooctyl.

[0052] The term “heteroalkyl” as used herein refers to an alkyl group, as defined hereinabove, wherein one or more carbon atoms in the chain are independently replaced by O, S, or NR^x, wherein R^x is hydrogen or C₁-C₃ alkyl. Examples of heteroalkyl groups include methoxymethyl, methoxyethyl and methoxypropyl.

[0053] The term “aryl” as used herein means a C₆-C₁₄ aromatic moiety comprising one to three aromatic rings. As such, “aryl” includes C₆, C₁₀, C₁₃, and C₁₄ cyclic hydrocarbon groups. An exemplary aryl group is a C₆-C₁₀ aryl group. Particular aryl groups include, without limitation, phenyl, naphthyl, anthracenyl, and fluorenyl.

[0054] The terms “aralkyl” and “arylalkyl” as used herein mean an aryl group covalently linked to an alkylene group wherein the moiety is linked to another group via the alkyl moiety. An exemplary aralkyl group is -(C₁-C₆)alkyl(C₆-C₁₀)aryl, including, without limitation, benzyl, phenethyl, and naphthylmethyl.

[0055] The terms “heterocyclyl” and “heterocyclic” as used herein mean a mono- or bicyclic (fused or spiro) ring structure having from 3 to 12 atoms, (3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 atoms), for example 4 to 8 atoms, wherein one or more ring atoms are independently -C(O)-, N, NR⁵, O, or S, and the remainder of the ring atoms are quaternary or carbonyl carbons. Examples of heterocyclic groups include, without limitation, epoxy, oxiranyl, oxetanyl, azetidiny, aziridiny, tetrahydrofuranyl, tetrahydropyranyl, tetrahydrothiophenyl, pyrrolidiny, piperidiny, piperaziny, imidazolidiny, thiazolidiny, thiatanyl, dithianyl, trithianyl, azathianyl, oxathianyl, dioxolanyl, oxazolidiny, oxazolidinonyl, decahydroquinolinyl, piperidiny, 4-piperidonyl, thiomorpholinyl, dimethyl-morpholinyl, and morpholinyl. Specifically excluded from the scope of this term are compounds having adjacent ring O and/or S atoms.

[0056] As used herein, term “L-heterocyclyl” as used herein means a heterocyclyl group covalently linked to another group via an alkylene linker L, where L is C₁-C₄ alkylene.

[0057] The term “heteroaryl” as used herein means a group having 5 to 14 ring atoms, preferably 5, 6, 10, 13 or 14 ring atoms comprising an aromatic heterocyclic ring (e.g., having 6, 10, or 14 π electrons shared in a cyclic array), and having, in addition to carbon atoms, from one to three heteroatoms that are each independently N, O, or S. “Heteroaryl” also includes fused multicyclic (e.g., bicyclic) ring systems in which one or more of the fused rings is non-aromatic, provided that at least one ring is aromatic and at least one ring contains an N, O, or S ring atom.

[0058] Examples of heteroaryl groups include acridinyl, azocinyl, benzimidazolyl, benzofuranyl, benzo[d]oxazol-2(3H)-one, 2H-benzo[b][1,4]oxazin-3(4H)-one, benzothiofuranyl, benzothiophenyl, benzoxazolyl, benzthiazolyl, benztriazolyl, benztetrazolyl, benzisoxazolyl, benzisothiazolyl, benzimidazoliny, carbazolyl, 4aH-carbazolyl, carbolinyl, chromanyl, chromenyl, cinnolinyl, furanyl, furazanyl, imidazoliny, imidazolyl, 1H-indazolyl, indolenyl, indoliny, indoliziny, indolyl, 3H-indolyl, isobenzofuranyl, isochromanyl, isoindazolyl, isoindoliny, isoindolyl, isoquinolinyl, isothiazolyl, isoxazolyl, naphthyridinyl, octahydroisoquinolinyl, oxadiazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, oxazolidinyl, oxazolyl, oxazolidinyl, pyrimidinyl, phenanthridinyl, phenanthrolinyl, phenaziny, phenothiazinyl, phenoxathiinyl, phenoxazinyl, phthalazinyl, piperonyl, pteridinyl, purinyl, pyranyl, pyrazinyl, pyrazolidinyl, pyrazolinyl, pyrazolyl, pyridazinyl, pyridooxazole, pyridoimidazole, pyridothiazole, pyridinyl, pyridyl, pyrimidinyl, pyrrolinyl, 2H-pyrrolyl, pyrrolyl, quinazolinyl, quinolinyl, 4H-quinoliziny, quinoxaliny, quinuclidinyl, tetrahydroisoquinolinyl, tetrahydroquinolinyl, tetrazolyl, 6H-1,2,5-thiadiazinyl, 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, 1,2,5-thiadiazolyl, 1,3,4-thiadiazolyl, thianthrenyl, thiazolyl, thienyl, thienothiazolyl, thienooxazolyl, thienoimidazolyl, thiophenyl, triazinyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1,2,5-triazolyl, 1,3,4-triazolyl, and xanthenyl.

[0059] The terms “L-heteroaryl”, “heteroaralkyl” and “heteroarylalkyl” as used herein mean a group comprising a heteroaryl group covalently linked to another group via an alkylene linker. Examples of heteroaralkyl groups comprise a C₁-C₆ alkyl group and a heteroaryl group having 5, 6, 9, or 10 ring atoms. Examples of heteroaralkyl groups include pyridylmethyl, pyridylethyl, pyrrolylmethyl, pyrrolylethyl, imidazolylmethyl, imidazolylethyl, thiazolylmethyl, thiazolylethyl, benzimidazolylmethyl, benzimidazolylethyl, quinazolinylmethyl, quinolinylmethyl, quinolinylethyl, benzofuranylmethyl, indolinylethyl, isoquinolinylmethyl, isoinodylmethyl, cinnolinylmethyl, and benzothiophenylethyl. Specifically excluded from the scope of this term are compounds having adjacent ring O and/or S atoms.

[0060] The terms “arylene,” “heteroarylene,” and “heterocyclylene” as used herein mean an bivalent aryl, heteroaryl, or heterocyclyl group, respectively, as defined hereinabove, that is positioned between and serves to connect two other chemical groups.

[0061] As employed herein, when a moiety (e.g., cycloalkyl, aryl, heteroaryl, heterocyclyl, urea, etc.) is described as “optionally substituted” without expressly stating the substituents it is meant that the group

optionally has from one to four, preferably from one to three, more preferably one or two, non-hydrogen substituents.

[0062] The terms “halogen” and “halo” as used herein mean chlorine, bromine, fluorine, or iodine.

[0063] The term “haloalkyl” as used herein means an alkyl chain in which one or more hydrogens have been replaced by a halogen. Exemplary haloalkyls are trifluoromethyl, difluoromethyl, fluorochloromethyl, chloromethyl, and fluoromethyl.

[0064] The term “hydroxyalkyl” as used herein means an alkyl chain, as defined herein, wherein at least one hydrogen of the alkyl chain has been replaced by hydroxyl.

[0065] Disclosed herein are methods of treating prostate cancer in a subject, comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor. In some embodiments, the androgen receptor inhibitors is selected from apalutamide, darolutamide, and enzalutamide. In some embodiments, the androgen receptor inhibitor is apalutamide. In some embodiments, the androgen receptor inhibitor is darolutamide. In some embodiments, the androgen receptor inhibitor is enzalutamide.

[0066] Also disclosed herein are methods of treating prostate cancer in a subject, wherein the prostate cancer in the subject has been determined to be resistant to one or more first androgen receptor signaling inhibitors (ARSI), comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor. In some embodiments, the one or more first androgen receptor signaling inhibitors (ARSI) is selected from one or more first CYP17 inhibitors and one or more first androgen receptor inhibitors. In some embodiments, the one or more first androgen receptor signaling inhibitors (ARSI) is selected from one or more first CYP17 inhibitors. In some embodiments, the one or more first CYP17 inhibitor is abiraterone acetate. In some embodiments, the one or more first androgen receptor signaling inhibitors (ARSI) is selected from one or more androgen receptor inhibitors. In some embodiments, the one or more first androgen receptor inhibitors is selected from apalutamide, darolutamide, and enzalutamide. In some embodiments, the one or more first androgen receptor inhibitor is apalutamide. In some embodiments, the one or more first androgen receptor inhibitor is darolutamide. In some embodiments, the one or more first androgen receptor inhibitor is enzalutamide.

[0067] Also disclosed herein are methods of treating prostate cancer in a subject, wherein the prostate cancer in the subject has been determined to be resistant to a CYP17 inhibitor, comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor. In some embodiments, the CYP17 inhibitor is abiraterone acetate. In some embodiments, the androgen receptor inhibitor is selected from apalutamide, darolutamide, and enzalutamide. In some embodiments, the androgen receptor inhibitor is apalutamide. In some embodiments, the androgen receptor inhibitor is darolutamide. In some embodiments, the androgen receptor inhibitor is enzalutamide.

[0068] Also disclosed herein are methods of treating prostate cancer in a subject, wherein the prostate cancer in the subject has been determined to be resistant to abiraterone, comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor. In

some embodiments, the androgen receptor inhibitors is selected from apalutamide, darolutamide, and enzalutamide. In some embodiments, the androgen receptor inhibitor is apalutamide. In some embodiments, the androgen receptor inhibitor is darolutamide. In some embodiments, the androgen receptor inhibitor is enzalutamide.

[0069] Also disclosed herein are methods of treating prostate cancer in a subject, wherein the subject has received prior administration of one or more CYP17 inhibitors, comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor. In some embodiments, the CYP17 inhibitor is abiraterone acetate. In some embodiments, the androgen receptor inhibitors is selected from apalutamide, darolutamide, and enzalutamide. In some embodiments, the androgen receptor inhibitor is apalutamide. In some embodiments, the androgen receptor inhibitor is darolutamide. In some embodiments, the androgen receptor inhibitor is enzalutamide.

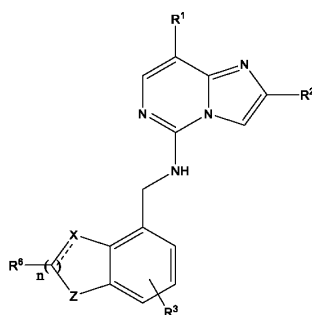
[0070] Further disclosed herein are methods of treating prostate cancer in a subject, wherein the subject (i) has received prior administration of one or more CYP17 inhibitors, and (ii) is androgen receptor inhibitor naïve, comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor. In some embodiments, the CYP17 inhibitor is abiraterone acetate. In some embodiments, the androgen receptor inhibitors is selected from apalutamide, darolutamide, and enzalutamide. In some embodiments, the androgen receptor inhibitor is apalutamide. In some embodiments, the androgen receptor inhibitor is darolutamide. In some embodiments, the androgen receptor inhibitor is enzalutamide.

[0071] In another embodiment are any of the methods of treating prostate cancer disclosed herein, wherein the embryonic ectoderm development (EED) inhibitor is a small molecule having a molecular weight of less than or equal to 1000 Daltons. In some embodiments, the embryonic ectoderm development (EED) inhibitor is a small molecule having a molecular weight of less than or equal to 900 Daltons, or less than or equal to 800 Daltons, or less than or equal to 750 Daltons, or less than or equal to 700 Daltons, or less than or equal to 650 Daltons, or less than or equal to 600 Daltons, or less than or equal to 575 Daltons, or less than or equal to 525 Daltons, or less than or equal to 500 Daltons, or less than or equal to 475 Daltons, or less than or equal to 450 Daltons, or less than or equal to 425 Daltons, or less than or equal to 400 Daltons, or less than or equal to 375 Daltons, or less than or equal to 350 Daltons, or less than or equal to 325 Daltons, or less than or equal to 300 Daltons, or less than or equal to 275 Daltons, or less than or equal to 250 Daltons, or less than or equal to 200 Daltons.

[0072] In another embodiment are any of the methods of treating prostate cancer disclosed herein, wherein the embryonic ectoderm development (EED) inhibitor is selected from EED226, A-395, APG-5918, BR-001, BR-002, EEDi-5285, EEDi-1056, FTX-6058, HJM-353, and MAK683. In some embodiments, the ectoderm development (EED) inhibitor is EED226. In some embodiments, the ectoderm development (EED) inhibitor is A-395. In some embodiments, the ectoderm development (EED) inhibitor is APG-5918. In some embodiments, the ectoderm development (EED) inhibitor is BR-001. In some embodiments, the ectoderm development (EED) inhibitor is BR-002. In some embodiments, the ectoderm development (EED) inhibitor

is EEDi-5285. In some embodiments, the ectoderm development (EED) inhibitor is EEDi-1056. In some embodiments, the ectoderm development (EED) inhibitor is FTX-6058. In some embodiments, the ectoderm development (EED) inhibitor is HJM-353. In some embodiments, the ectoderm development (EED) inhibitor is MAK683.

[0073] In another embodiment are any of the methods of treating prostate cancer disclosed herein, wherein the embryonic ectoderm development (EED) inhibitor is a compound of Formula (I)



Formula (I)

or a pharmaceutically acceptable salt thereof:

wherein:

===== represents a single or a double bond;

Z is O or S;

X is O, CR⁵, CR⁵OH, or C(R⁵)₂, wherein:

when X is O, ===== is a single bond;

when X is C(R⁵)₂, ===== is a single bond;

when X is CR⁵OH, ===== is a single bond; or

when X is CR⁵, ===== is a double bond;

R¹ is aryl, heteroaryl, L-cycloalkyl, -N(R⁵)heterocyclyl, or L-heterocyclyl, wherein the aryl, the heteroaryl or the cyclyl portion of the L-cycloalkyl, -N(R⁵)heterocyclyl, or L-heterocyclyl is optionally substituted with one or more R⁴;

R² is cyano, -COOR⁵, -C(O)N(R⁵)₂, or -C(O)N(R⁵)₂ wherein each R⁵ taken together with the nitrogen atom to which they are attached form a 5 - 8 membered heterocyclic ring optionally substituted with one or more R⁴;

each R³ is independently C₁-C₃ alkyl or halogen;

each R⁴ is independently oxo, cyano, halogen, -PO₃(C₁-C₃ alkyl)₂, hydroxyl, alkoxy, hydroxyalkyl, heteroalkyl, aralkyl, haloalkyl, -COOR⁵, -Y²-haloalkyl, -Y¹-C₁-C₆ alkyl, -Y²-C₁-C₆ alkyl, -L-cycloalkyl, -L-heteroaryl, -L-heterocyclyl, -Y¹-heterocyclyl, -Y²-heterocyclyl, -L-N(R⁵)₂, -O-L-N(R⁵)₂, -C(CF₃)N(R⁵)₂, -Y¹-N(R⁵)₂, or -Y²-N(R⁵)₂, wherein the ring portion of the aralkyl, -L-cycloalkyl, -L-heteroaryl, -L-heterocyclyl or -Y¹-heterocyclyl is optionally substituted with one or more R⁷;

L is a bond or C₁-C₄ alkylene;

Y^1 is a bond, $-C(O)-$, or $-NHC(O)-$;

Y^2 is a bond, $-S-$, $-SO-$, $-SO_2-$, or $-NR^5SO_2-$,

each R^5 is hydrogen or C_1-C_3 alkyl;

R^6 is hydrogen, C_1-C_3 alkyl, halogen, haloalkyl, hydroxyalkyl, or heteroalkyl;

each R^7 is oxo, cyano, hydroxyl, alkoxy, halogen, haloalkyl, hydroxyalkyl, heteroalkyl, cycloalkyl, -

$L-N(R^5)_2$, C_1-C_6 alkyl, or $-Y^1$ -heterocyclyl; and

n is 1 or 2.

[0074] In some embodiments, the embryonic ectoderm development (EED) inhibitor is a compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein Z is O. In some embodiments, the embryonic ectoderm development (EED) inhibitor is a compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein Z is S.

[0075] In some embodiments, the embryonic ectoderm development (EED) inhibitor is a compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein n is 1.

[0076] In some embodiments, the embryonic ectoderm development (EED) inhibitor is a compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein R^2 is cyano. In some embodiments, the embryonic ectoderm development (EED) inhibitor is a compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein R^2 is $-COOR^5$. In some embodiments, the embryonic ectoderm development (EED) inhibitor is a compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein R^2 is $-C(O)N(R^5)_2$.

[0077] In some embodiments, the embryonic ectoderm development (EED) inhibitor is a compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein R^3 is halogen. In some embodiments, the embryonic ectoderm development (EED) inhibitor is a compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein R^3 is fluorine.

[0078] In some embodiments, the embryonic ectoderm development (EED) inhibitor is a compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein X is $C(R^5)_2$ and $-----$ is a single bond.

[0079] In some embodiments, the embryonic ectoderm development (EED) inhibitor is a compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein X is CR^5 and $=====$ is a double bond.

[0080] In some embodiments, the embryonic ectoderm development (EED) inhibitor is a compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein X is O and $-----$ is a single bond.

[0081] In some embodiments, the embryonic ectoderm development (EED) inhibitor is a compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein R^1 is aryl optionally substituted with one or more R^4 . In some embodiments, R^1 is phenyl optionally substituted with one or more R^4 . In some embodiments, R^1 is phenyl is substituted with one, two or three R^4 . In some embodiments, the one, two or three R^4 are each independently halogen, $-PO_3(C_1-C_3 \text{ alkyl})_2$, hydroxyl, hydroxyalkyl, aralkyl, haloalkyl, $-COOR^5$, $-Y^1-C_1-C_6$ alkyl, $Y^2-C_1-C_6$ alkyl, $-L-N(R^5)_2$, $-O-L-N(R^5)_2$, $-C(CF_3)N(R^5)_2$, $-Y^1-N(R^5)_2$, $-Y^2-N(R^5)_2$, Y^2 -haloalkyl, $-L$ -heteroaryl, $-L$ -heterocyclyl, or $-Y^1$ -heterocyclyl, wherein the heterocyclyl portion of the $-L$ -heterocyclyl or $-Y^1$ -heterocyclyl is optionally substituted with one or more R^7 . In some embodiments, R^4 is -

Y^1 - C_1 - C_6 alkyl and Y^1 is a bond and the C_1 - C_6 alkyl is methyl, ethyl, isopropyl, butyl, or pentyl. In some embodiments, R^4 is $-Y^2$ - C_1 - C_6 alkyl and Y^2 is a $-SO_2$ - and the C_1 - C_6 alkyl is methyl. In some embodiments, R^4 is $-Y^2$ -haloalkyl and Y^2 is $-S$ - or $-SO_2$ - and the haloalkyl is trifluoromethyl. In some embodiments, R^4 is $-L-N(R^5)_2$ and L is a bond and each R^5 is hydrogen, each R^5 is methyl, or one R^5 is methyl and one R^5 is hydrogen. In some embodiments, R^4 is $-L-N(R^5)_2$ and L is methylene or ethylene and each R^5 is hydrogen, each R^5 is methyl or one R^5 is methyl and one R^5 is hydrogen. In some embodiments, R^4 is $-Y^1-N(R^5)_2$, Y^1 is $-C(O)-$ and each R^5 independently is hydrogen, each R^5 is independently methyl or one R^5 is methyl and one R^5 is hydrogen. In some embodiments, R^4 is $-Y^2-N(R^5)_2$, Y^2 is $-SO_2$ - and each R^5 independently is hydrogen, each R^5 is methyl, or one R^5 is methyl and one R^5 is independently hydrogen. In some embodiments, R^4 is $-Y^1$ -heterocyclyl and Y^1 is $-C(O)-$ and the heterocyclyl portion of the L -heterocyclyl is piperazinyl or 4-methyl-piperazinyl. In some embodiments, R^4 is $-L$ -heterocyclyl and L is a bond and the heterocyclyl portion of the L -heterocyclyl is azetidiny, oxetanyl, pyrrolidinyl, tetrahydrofuranyl, piperidinyl, piperazinyl, or $3\lambda^2$ -azabicyclo[3.1.0]hexanyl, each optionally substituted with one or more R^7 selected from oxo, C_1 - C_3 alkyl, alkoxy, hydroxyl and halogen. In some embodiments, R^4 is $-L$ -heterocyclyl, wherein L is a methylene and the heterocyclyl portion of the L -heterocyclyl is azetidiny, oxetanyl, pyrrolidinyl piperidinyl, each optionally substituted with one or more R^7 selected from C_1 - C_3 alkyl, alkoxy, hydroxyl and halogen. In some embodiments, R^4 is $-Y^1$ -heterocyclyl and Y^1 is $-C(O)-$ and the heterocyclyl portion of the Y^1 -heterocyclyl is morpholinyl optionally substituted with one or more C_1 - C_3 alkyl. In some embodiments, R^4 is $-L$ -heteroaryl optionally substituted with one or more R^7 . In some embodiments, R^4 is tetrazolyl. In some embodiments, R^4 is $-PO_3(C_1-C_3 \text{ alkyl})_2$. In some embodiments, R^4 is $-COOR^5$. In some embodiments, R^4 is hydroxyalkyl. In some embodiments, R^4 is $-O-L-N(R^5)_2$. In some embodiments, R^4 is aralkyl.

[0082] In some embodiments, the embryonic ectoderm development (EED) inhibitor is a compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein R^1 is heteroaryl optionally substituted with one or more R^4 . In some embodiments, R^1 is pyrazolyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, triazinyl, pyridyl, pyridinyl-2-one, pyrazinyl, pyridazinyl, pyrimidinyl, isoxazolyl, isoindolinyl, naphthyridinyl, 1,2,3,4-tetrahydroisoquinolinyl, or 5,6-dihydro-4H-pyrrolo[1,2-b]pyrazolyl, each optionally substituted with one or more R^4 . In some embodiments, R^1 is substituted with one or more R^4 , wherein each R^4 is independently cyano, halogen, $-Y^1$ - C_1 - C_6 alkyl, $-Y^2$ - C_1 - C_6 alkyl, alkoxy, hydroxyalkyl, heteroalkyl, haloalkyl, $-L$ -cycloalkyl, $-L-N(R^5)_2$, $-Y^1-N(R^5)_2$, $-L$ -heteroaryl, $-L$ -heterocyclyl, or $-Y^1$ -heterocyclyl, wherein the heteroaryl of the $-L$ -heteroaryl or the heterocyclyl portion of the L -heterocyclyl, or Y^1 -heterocyclyl is optionally substituted with one or more R^7 .

[0083] In some embodiments, the embryonic ectoderm development (EED) inhibitor is a compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein R^1 is pyrazolyl optionally substituted with one R^4 independently selected from hydroxyalkyl, heteroalkyl, haloalkyl, $-Y^1$ - C_1 - C_6 alkyl, $-L-N(R^5)_2$, L -heterocyclyl or L -heteroaryl, wherein the heteroaryl of the L -heteroaryl or the heterocyclyl portion of the L -heterocyclyl is optionally substituted with one or more R^7 . In some embodiments, R^4 is $-L$ -heteroaryl and L is methylene wherein the heteroaryl is pyridyl optional substituted with one or more R^7 . In some embodiments,

R^4 is -L-heterocyclyl optionally substituted with one or more R^7 where L is a bond and the heterocyclyl portion of the L-heterocyclyl is oxetanyl, tetrahydrofuranyl, tetrahydropyranyl, piperazinyl, or 4-methylpiperazinyl. In some embodiments, R^4 is -L-heterocyclyl optionally substituted with one or more R^7 where L is methylene and the heterocyclyl portion of the L-heterocyclyl is azetidiny, oxetanyl, pyrrolidinyl, pyrrolidinone, tetrahydrofuranyl, tetrahydropyranyl, morpholinyl, piperazinyl, or 4-methylpiperazinyl. In some embodiments, R^4 is -L-N(R^5)₂ where L is methylene and each R^5 is independently hydrogen, each R^5 is independently C₁-C₃ alkyl or one R^5 is C₁-C₃ alkyl and one R^5 is hydrogen. In some embodiments, R^4 is -Y¹-C₁-C₆ alkyl where Y¹ is a bond and the C₁-C₆ alkyl is methyl, ethyl, or isopropyl.

[0084] In some embodiments, the embryonic ectoderm development (EED) inhibitor is a compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein R^1 is pyrazolyl optionally substituted with two R^4 groups each independently selected from hydroxyalkyl, heteroalkyl, haloalkyl, and -Y¹-C₁-C₆ alkyl.

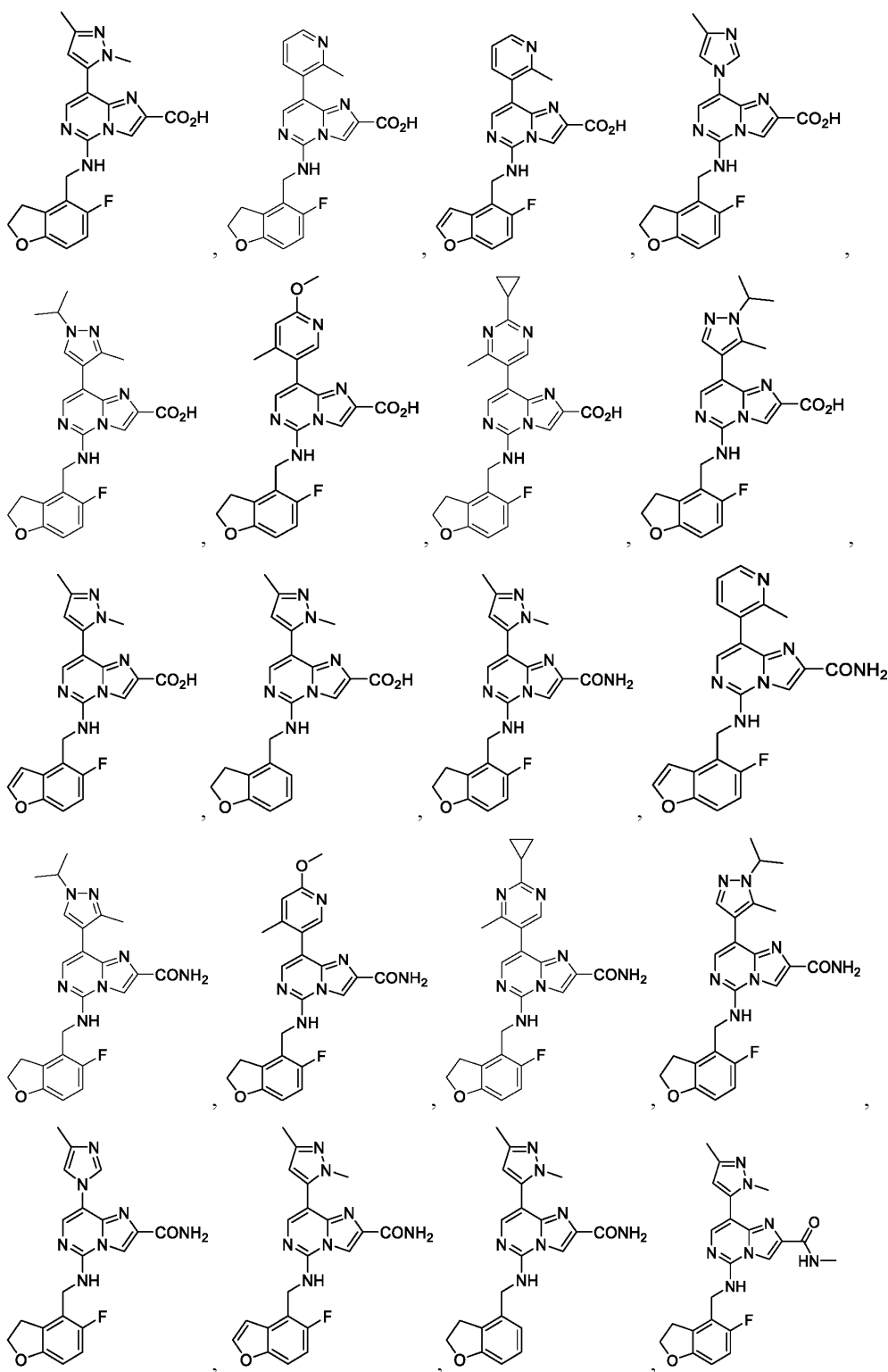
[0085] In some embodiments, the embryonic ectoderm development (EED) inhibitor is a compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein R^1 is pyridyl optionally substituted with one R^4 independently selected from cyano, halogen, alkoxy, hydroxyalkyl, heteroalkyl, haloalkyl, -Y¹-C₁-C₆ alkyl, -L-N(R^5)₂, -Y¹-N(R^5)₂, -L-cycloalkyl, or -L-heterocyclyl optionally substituted with one or more R^7 .

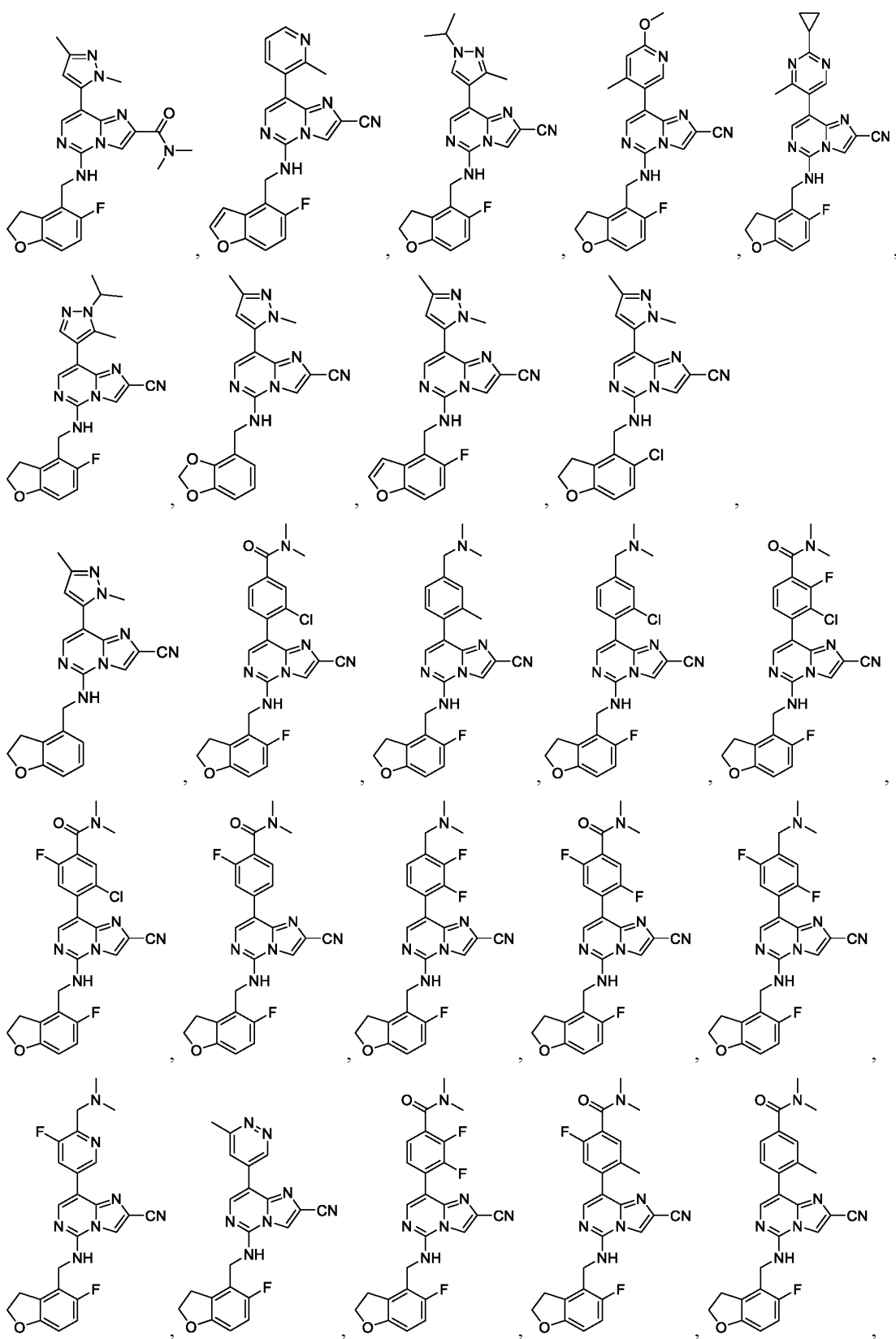
[0086] In some embodiments, the embryonic ectoderm development (EED) inhibitor is a compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein R^1 is -L-cycloalkyl optionally substituted with one or more R^4 .

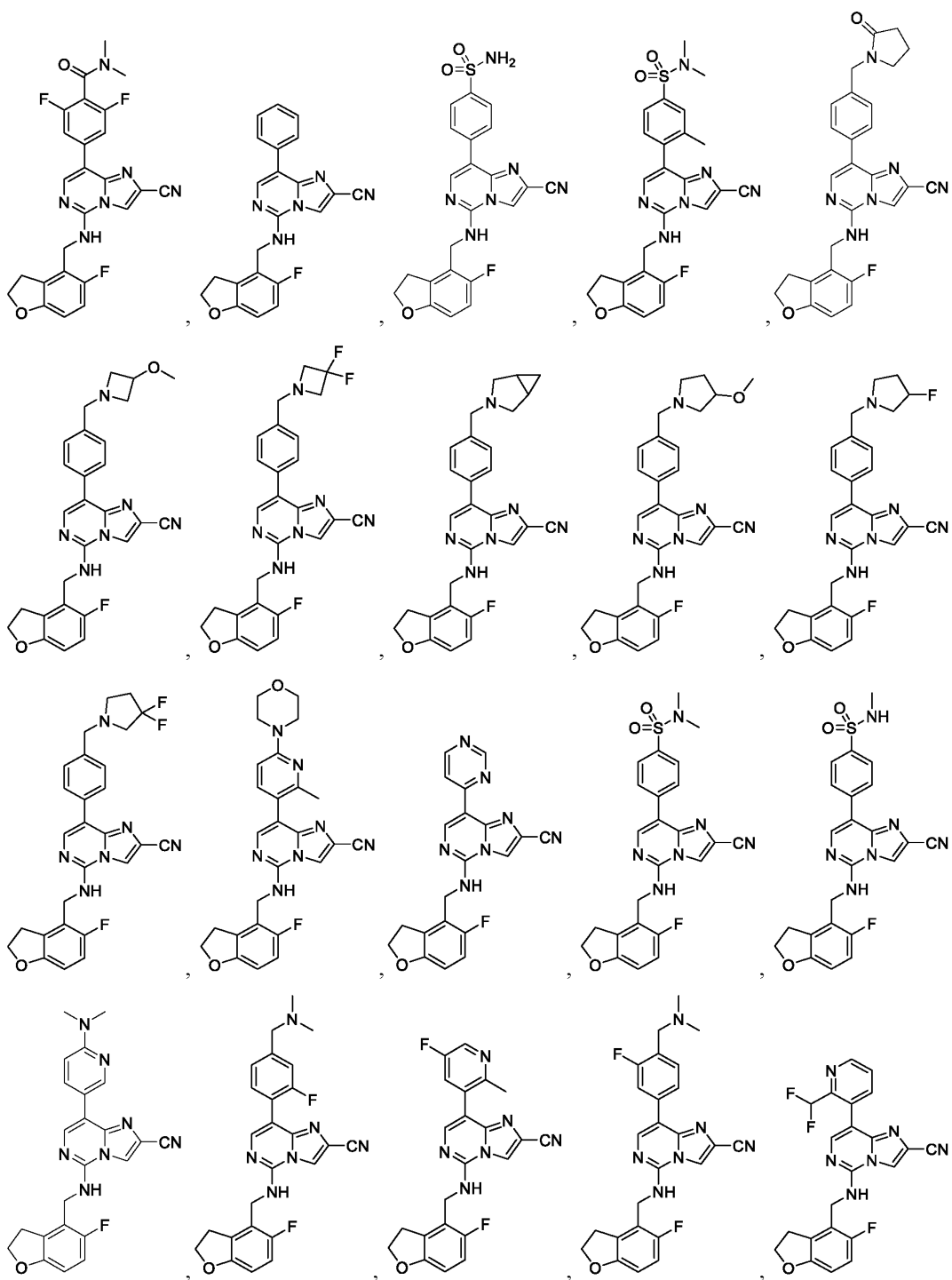
[0087] In some embodiments, the embryonic ectoderm development (EED) inhibitor is a compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein R^1 is -L-heterocyclyl optionally substituted with one or more R^4 .

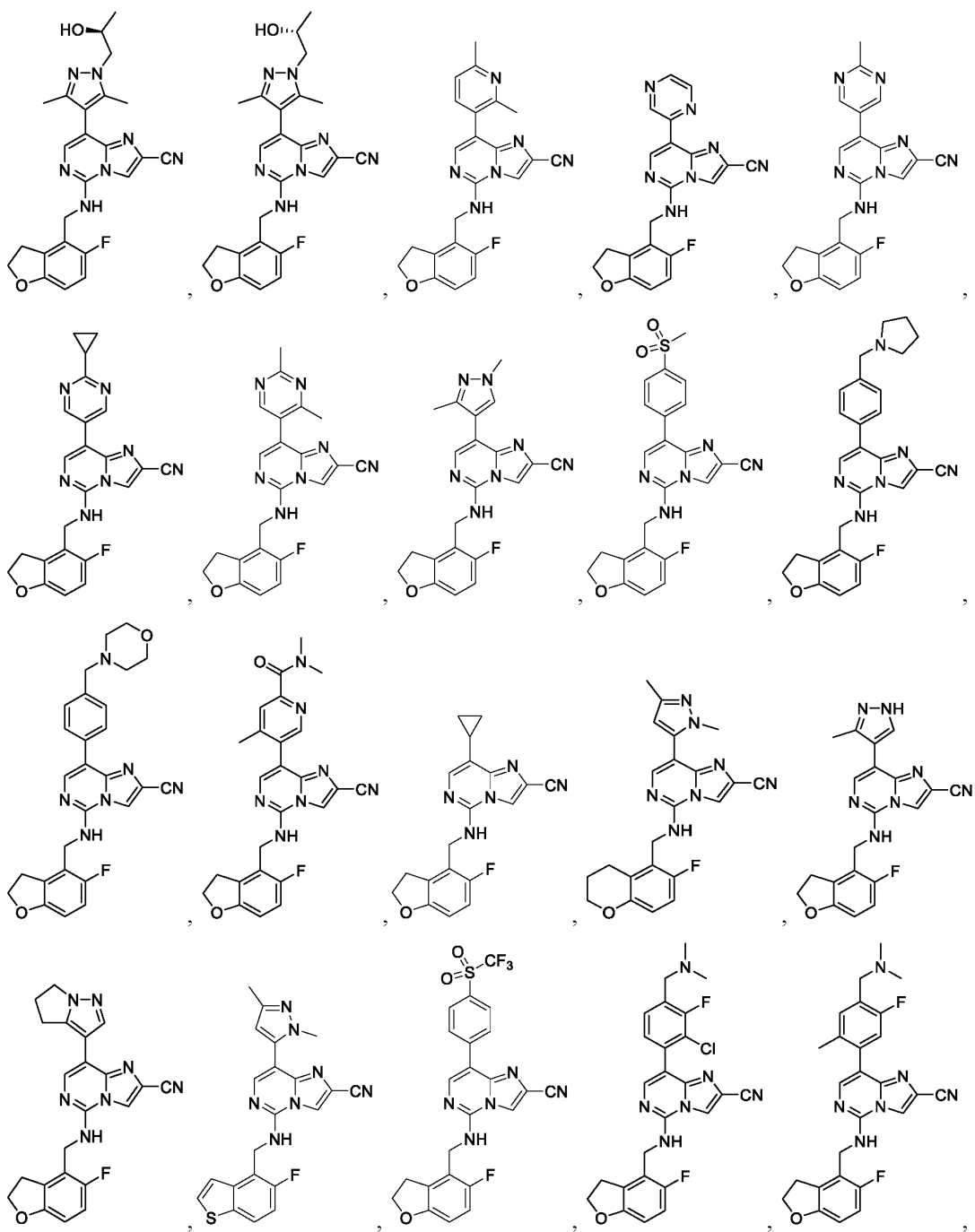
[0088] In some embodiments, the embryonic ectoderm development (EED) inhibitor is a compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein L is a bond and the heterocyclyl is piperidinyl or tetrahydropyranyl.

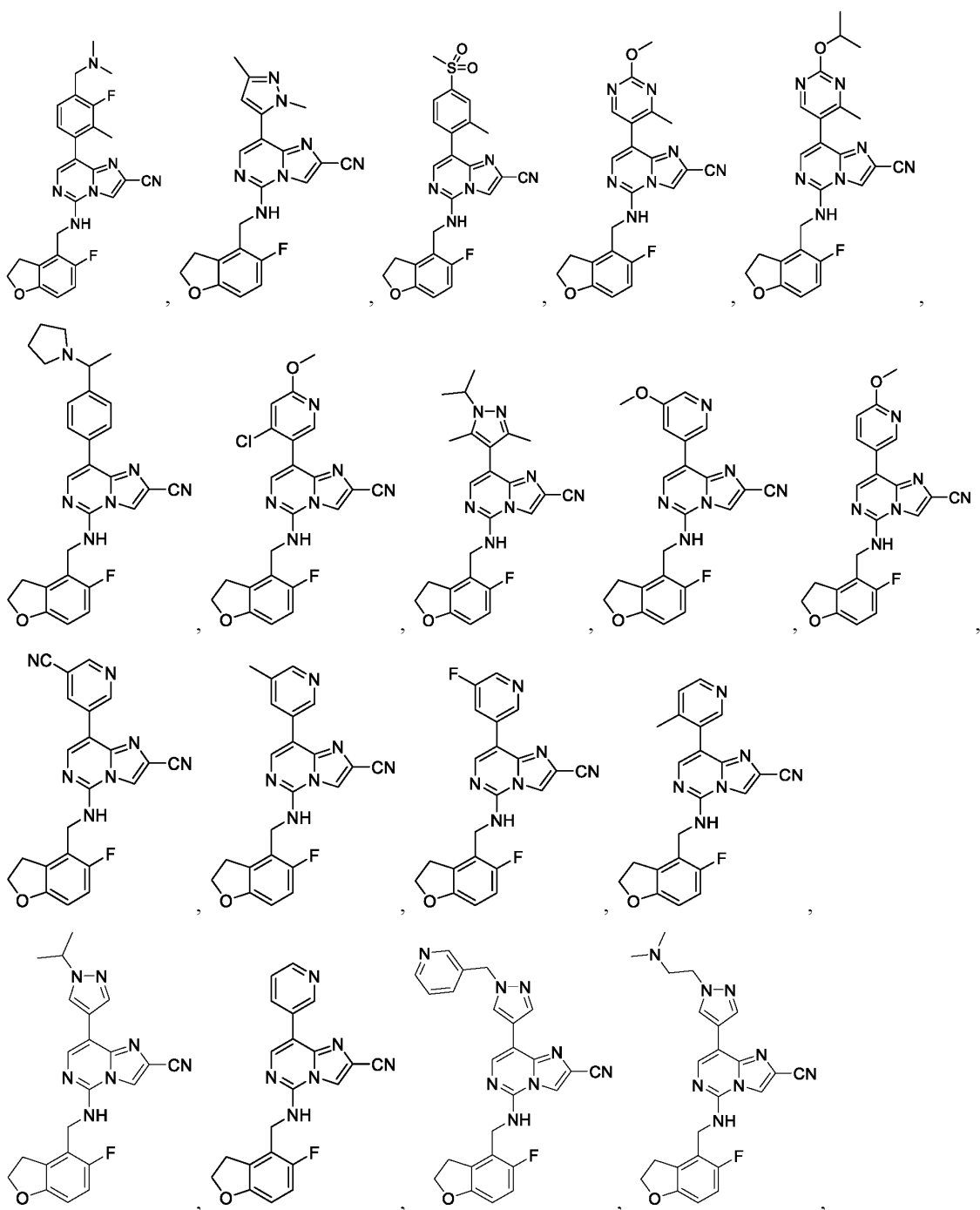
[0089] In some embodiments, the embryonic ectoderm development (EED) inhibitor is a compound selected from the group consisting of:

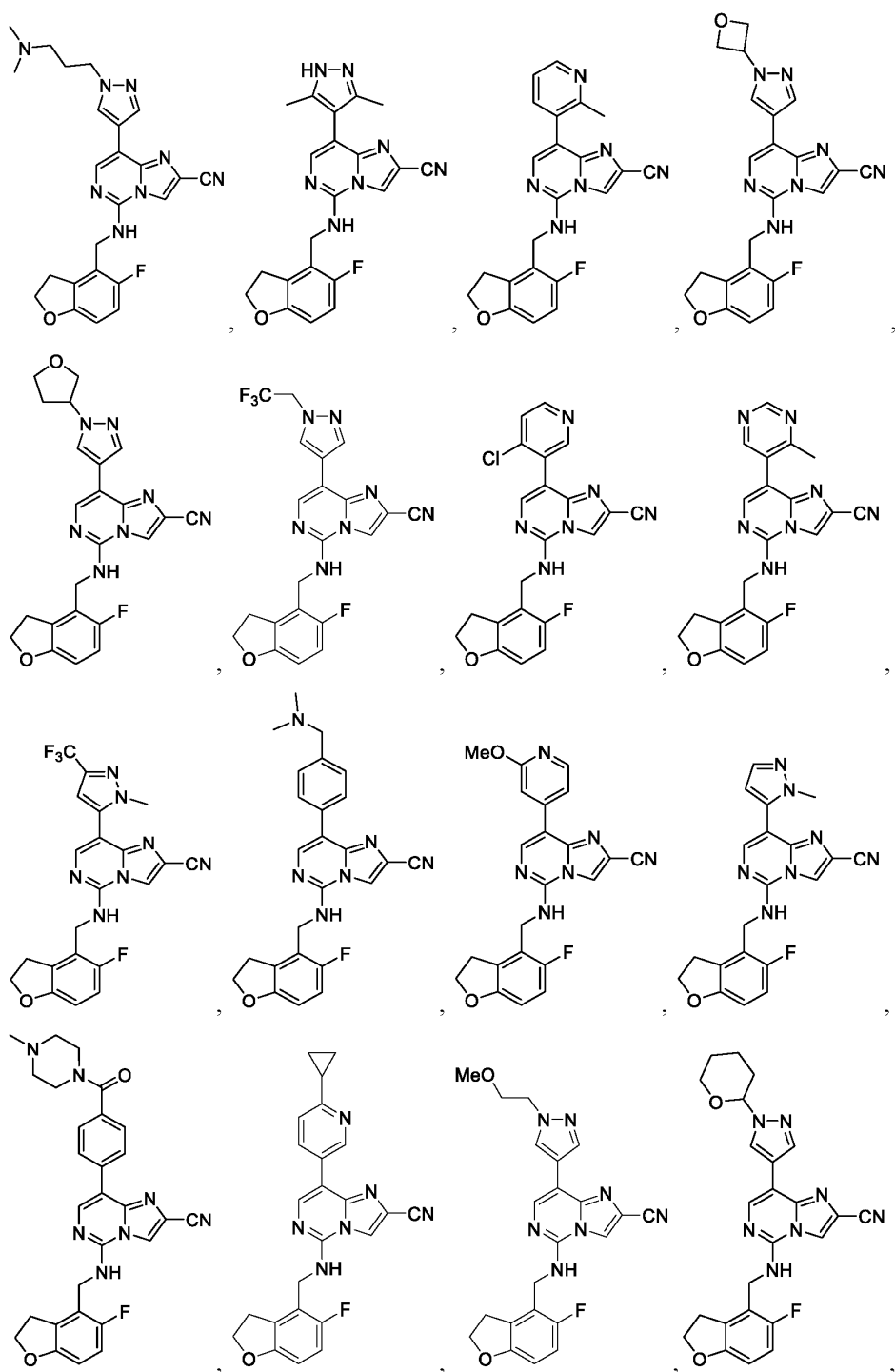


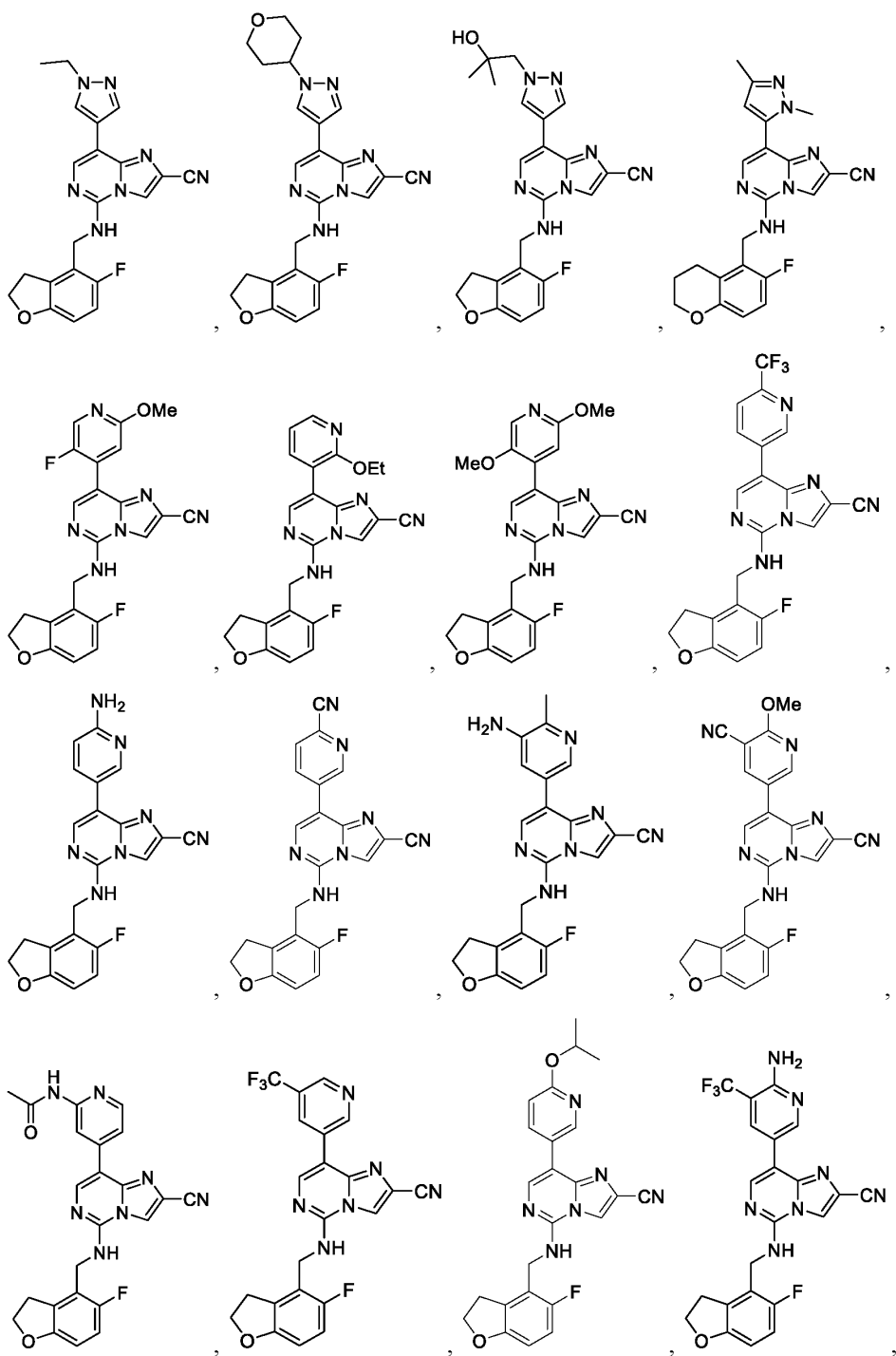


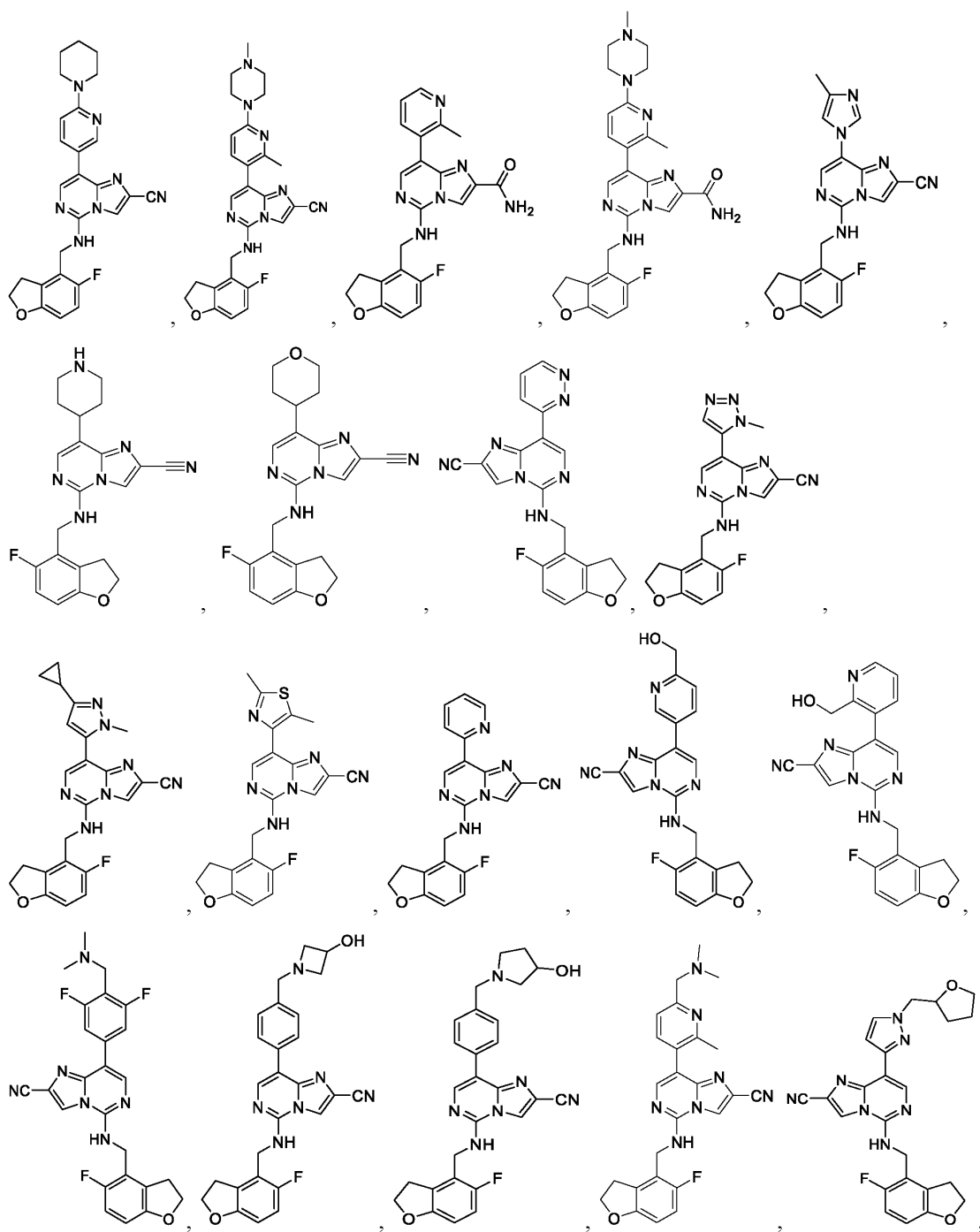


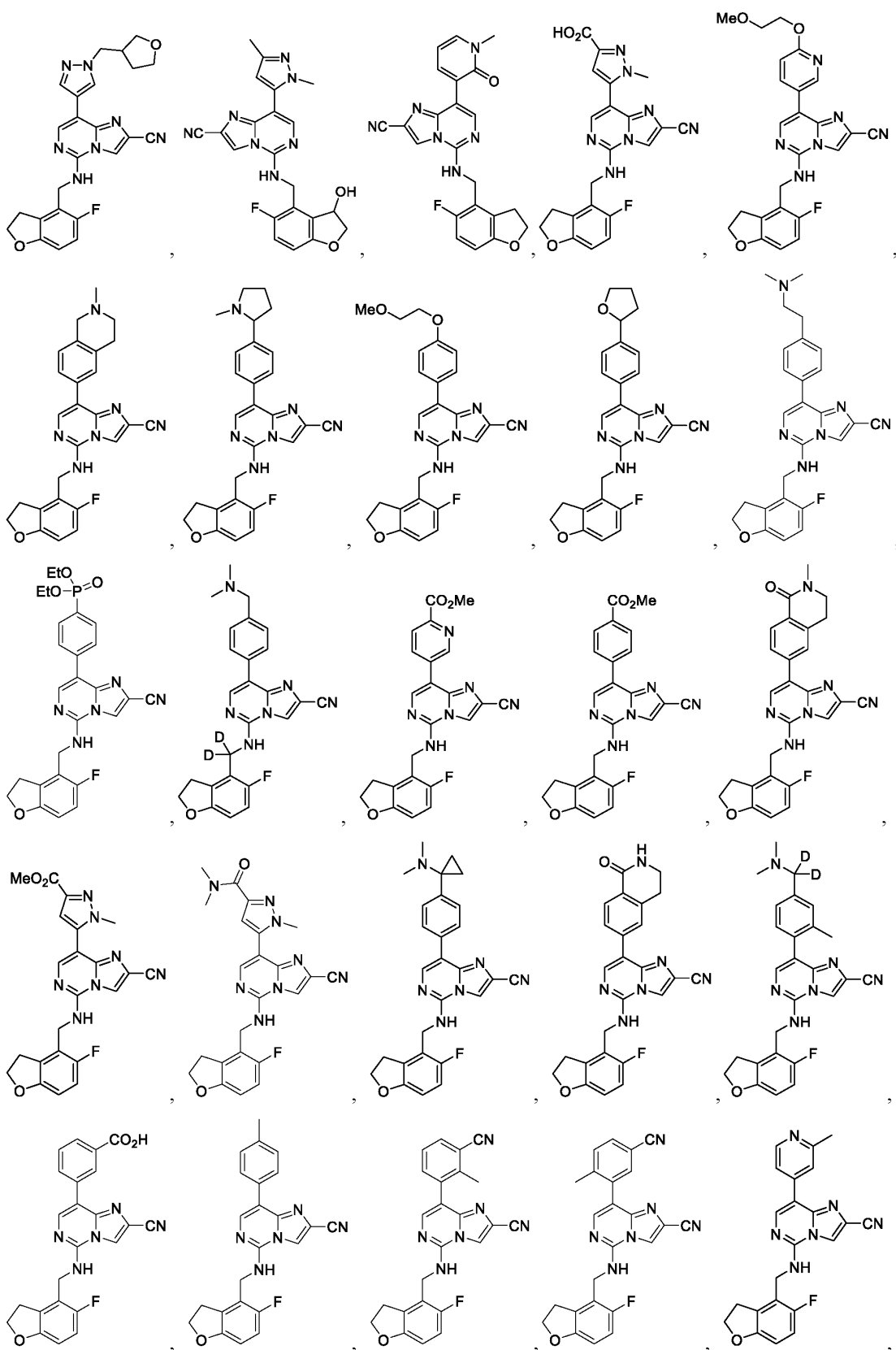


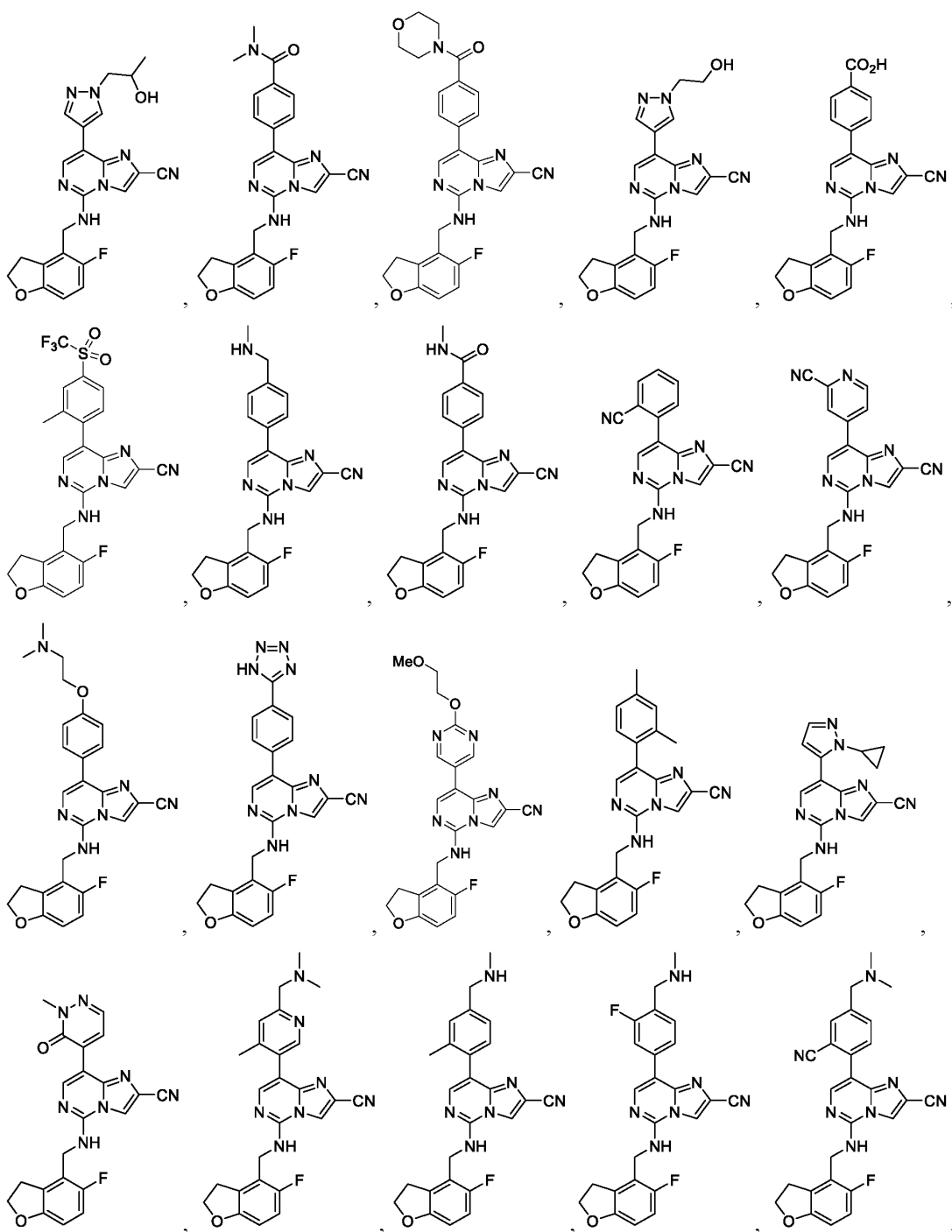


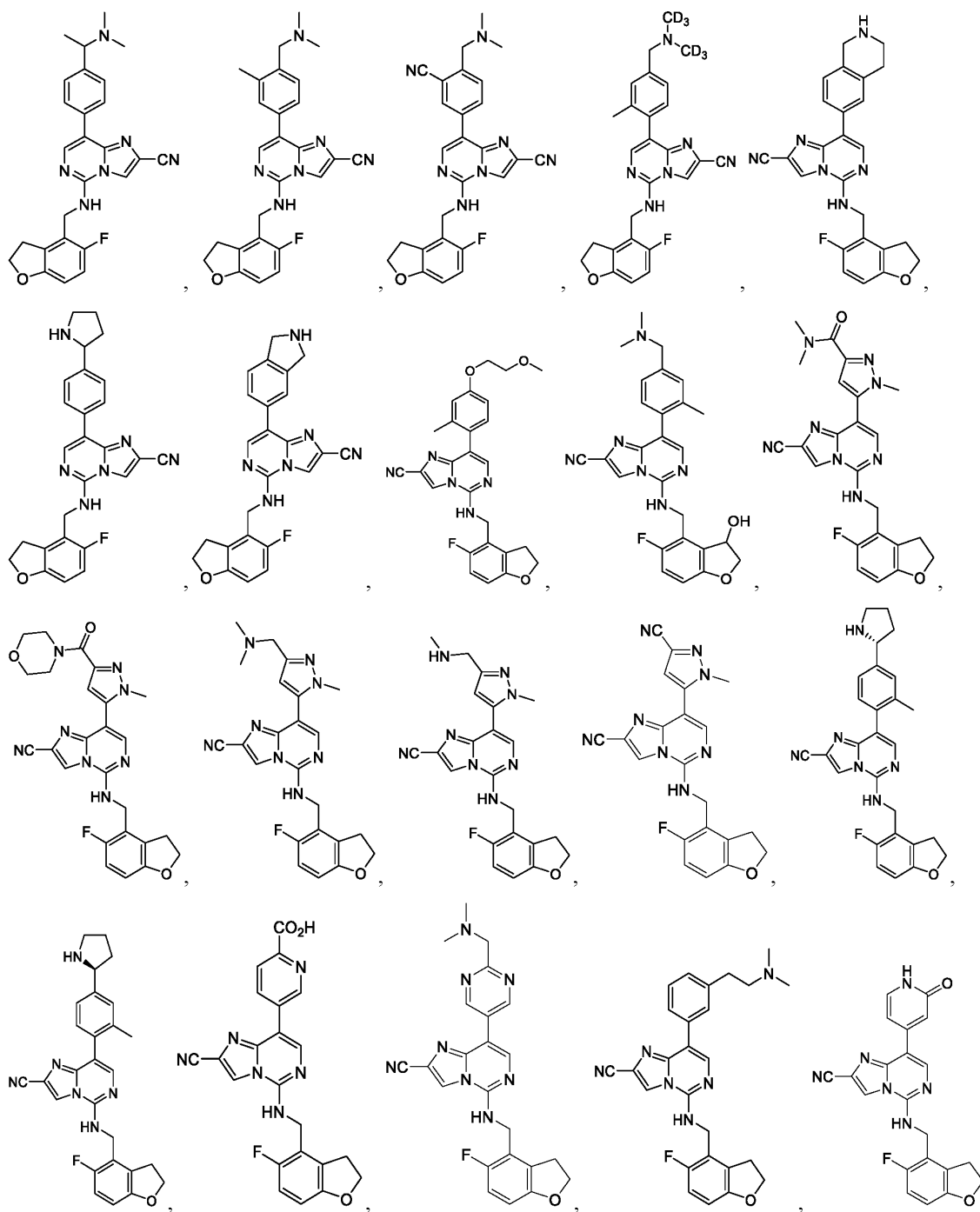






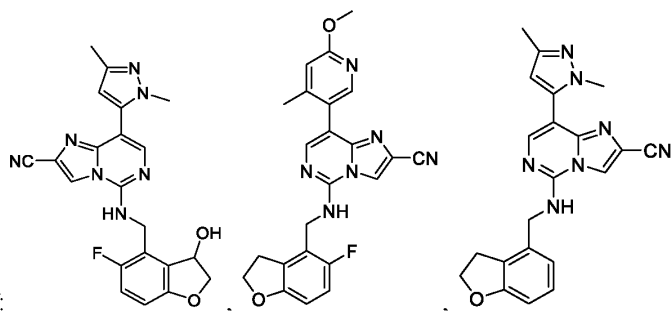




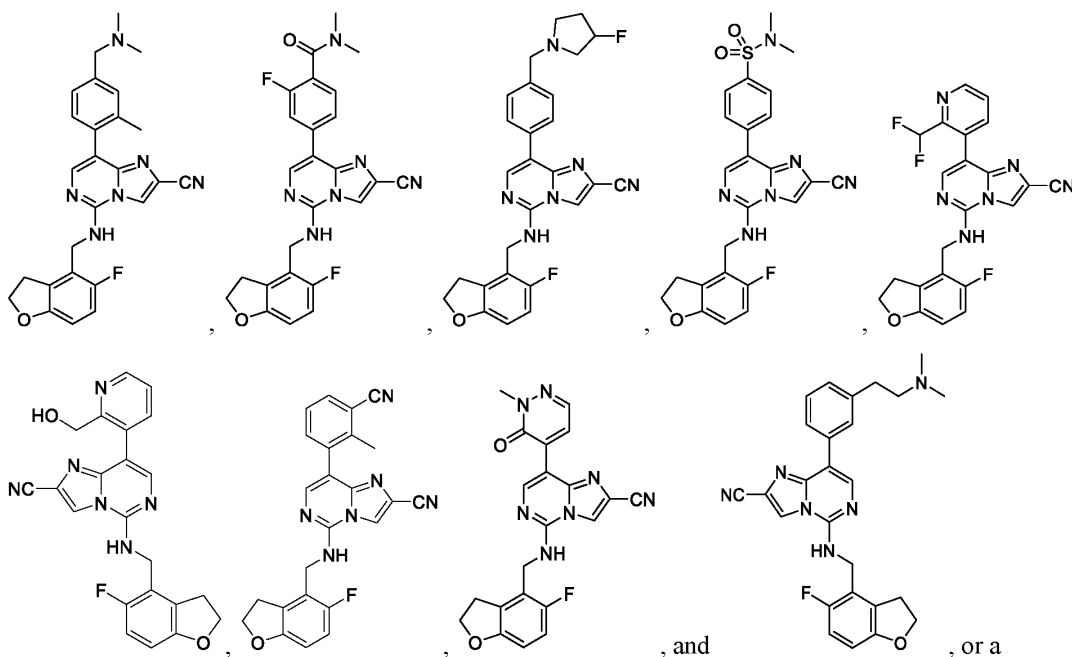




[0090] In some embodiments, the embryonic ectoderm development (EED) inhibitor is a compound selected

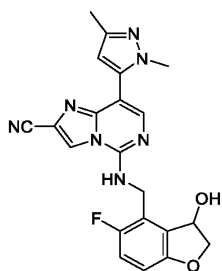


from the group consisting of:

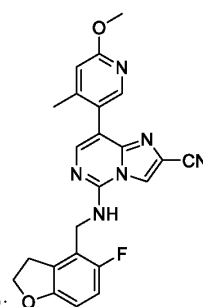


pharmaceutically acceptable salt thereof.

[0091] In some embodiments, the embryonic ectoderm development (EED) inhibitor is:

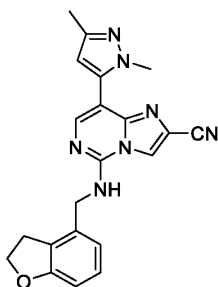


(Compound 1), or a pharmaceutically acceptable salt thereof.

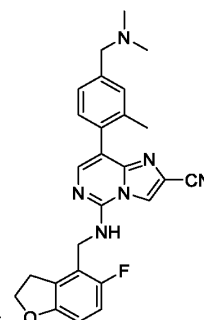


[0092] In some embodiments, the embryonic ectoderm development (EED) inhibitor is: (Compound 2), or a pharmaceutically acceptable salt thereof.

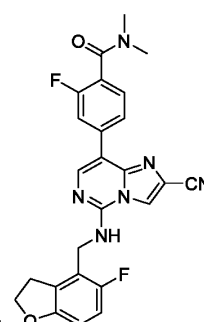
[0093] In some embodiments, the embryonic ectoderm development (EED) inhibitor is:



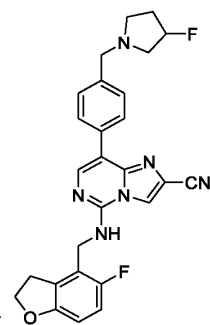
(Compound 3), or a pharmaceutically acceptable salt thereof.



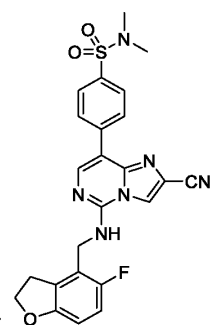
[0094] In some embodiments, the embryonic ectoderm development (EED) inhibitor is: (Compound 4), or a pharmaceutically acceptable salt thereof.



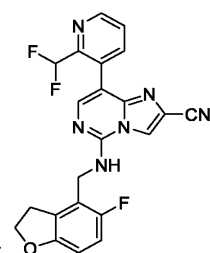
[0095] In some embodiments, the embryonic ectoderm development (EED) inhibitor is: (Compound 5), or a pharmaceutically acceptable salt thereof.



[0096] In some embodiments, the embryonic ectoderm development (EED) inhibitor is: (Compound 6), or a pharmaceutically acceptable salt thereof.

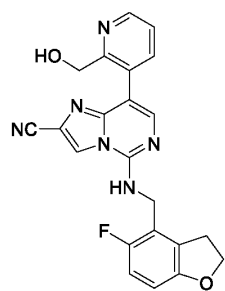


[0097] In some embodiments, the embryonic ectoderm development (EED) inhibitor is: (Compound 7), or a pharmaceutically acceptable salt thereof.

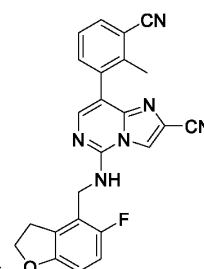


[0098] In some embodiments, the embryonic ectoderm development (EED) inhibitor is: (Compound 8), or a pharmaceutically acceptable salt thereof.

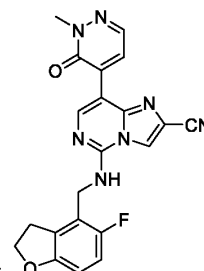
[0099] In some embodiments, the embryonic ectoderm development (EED) inhibitor is:



(Compound 9), or a pharmaceutically acceptable salt thereof.

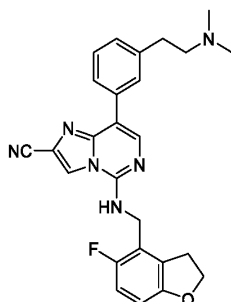


[00100] In some embodiments, the embryonic ectoderm development (EED) inhibitor is: (Compound 10), or a pharmaceutically acceptable salt thereof.



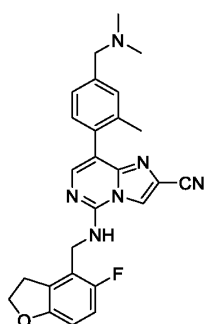
[00101] In some embodiments, the embryonic ectoderm development (EED) inhibitor is: (Compound 11), or a pharmaceutically acceptable salt thereof.

[00102] In some embodiments, the embryonic ectoderm development (EED) inhibitor is:



(Compound 12), or a pharmaceutically acceptable salt thereof.

[00103] Also provided herein are methods of treating prostate cancer in a subject disclosed herein, comprising administering to the subject a therapeutically effective amount of Compound 4:



(Compound 4), or a pharmaceutically acceptable salt thereof, wherein Compound 4 is in

crystalline form. Also provided herein are such methods wherein the crystalline form of Compound 4 is an anhydrous form. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a peak in an x-ray powder diffraction (XRPD) pattern at $8.1^\circ \pm 0.2^\circ$ 2-theta. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a further peak in an x-ray powder

diffraction (XRPD) pattern at $9.6^{\circ} \pm 0.2^{\circ}$ 2-theta. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits further peaks in an x-ray powder diffraction (XRPD) pattern at $5.7^{\circ} \pm 0.2^{\circ}$ 2-theta, $19.7^{\circ} \pm 0.2^{\circ}$ 2-theta, and $22.0^{\circ} \pm 0.2^{\circ}$ 2-theta. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits further peaks in an x-ray powder diffraction (XRPD) pattern at $9.8^{\circ} \pm 0.2^{\circ}$ 2-theta, $15.2^{\circ} \pm 0.2^{\circ}$ 2-theta, and $17.7^{\circ} \pm 0.2^{\circ}$ 2-theta. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a peak in a differential scanning calorimetry pattern of about 172°C . Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a peak in a differential scanning calorimetry pattern of from about 205°C to about 210°C . Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a peak in a differential scanning calorimetry pattern of from about 206°C to about 210°C , or from about 207°C to about 210°C , or from about 208°C to about 210°C , or from about 209°C to about 210°C . Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a loss in mass in a thermal gravimetric analysis of less than about 1% upon heating the sample from about 25°C to a temperature prior to melting. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a loss in mass in a thermal gravimetric analysis of less than about 1% upon heating the sample from about 25°C to about 380°C .

[00104] Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a peak in an x-ray powder diffraction (XRPD) pattern at $7.7^{\circ} \pm 0.2^{\circ}$ 2-theta. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits further peaks in an x-ray powder diffraction (XRPD) pattern at $13.7^{\circ} \pm 0.2^{\circ}$ 2-theta and $19.2^{\circ} \pm 0.2^{\circ}$ 2-theta. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits further peaks in an x-ray powder diffraction (XRPD) pattern at $5.5^{\circ} \pm 0.2^{\circ}$ 2-theta, $8.6^{\circ} \pm 0.2^{\circ}$ 2-theta, $15.9^{\circ} \pm 0.2^{\circ}$ 2-theta, $19.9^{\circ} \pm 0.2^{\circ}$ 2-theta, and $24.1^{\circ} \pm 0.2^{\circ}$ 2-theta. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits further peaks in an x-ray powder diffraction (XRPD) pattern at $10.6^{\circ} \pm 0.2^{\circ}$ 2-theta, $11.0^{\circ} \pm 0.2^{\circ}$ 2-theta, $15.4^{\circ} \pm 0.2^{\circ}$ 2-theta, $21.0^{\circ} \pm 0.2^{\circ}$ 2-theta, and $26.3^{\circ} \pm 0.2^{\circ}$ 2-theta. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a peak in a differential scanning calorimetry pattern of from about 203°C to about 210°C . Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a peak in a differential scanning calorimetry pattern of from about 203°C to about 208°C , or from about 203°C to about 206°C , or from about 203°C to about 205°C . Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a loss in mass in a thermal gravimetric analysis of less than about 2% upon heating the sample from about 25°C to about 380°C . Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a loss in mass in a thermal gravimetric analysis of less than about 2% upon heating the sample from about 25°C to about 210°C .

[00105] Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a peak in an x-ray powder diffraction (XRPD) pattern at $7.7^{\circ} \pm 0.2^{\circ}$ 2-theta. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits further a peak in an x-ray powder diffraction (XRPD) pattern at $15.4^{\circ} \pm 0.2^{\circ}$ 2-theta. Also provided herein are such methods wherein the crystalline form of

Compound 4 exhibits further a peak in an x-ray powder diffraction (XRPD) pattern at $19.2^{\circ} \pm 0.2^{\circ}$ 2-theta. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a further peak in an x-ray powder diffraction (XRPD) pattern at $13.7^{\circ} \pm 0.2^{\circ}$ 2-theta. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits further peaks in an x-ray powder diffraction (XRPD) pattern at $5.5^{\circ} \pm 0.2^{\circ}$ 2-theta, $8.6^{\circ} \pm 0.2^{\circ}$ 2-theta, $15.9^{\circ} \pm 0.2^{\circ}$ 2-theta, $19.9^{\circ} \pm 0.2^{\circ}$ 2-theta, and $24.1^{\circ} \pm 0.2^{\circ}$ 2-theta. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits further peaks in an x-ray powder diffraction (XRPD) pattern at $10.6^{\circ} \pm 0.2^{\circ}$ 2-theta, $11.0^{\circ} \pm 0.2^{\circ}$ 2-theta, $21.0^{\circ} \pm 0.2^{\circ}$ 2-theta, and $26.3^{\circ} \pm 0.2^{\circ}$ 2-theta. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a peak in a differential scanning calorimetry pattern of from about 203 °C to about 210 °C. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a peak in a differential scanning calorimetry pattern of from about 206 °C to about 210 °C. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a peak in a differential scanning calorimetry pattern of from about 203 °C to about 208 °C, or from about 203 °C to about 206 °C, or from about 203 °C to about 205 °C. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a loss in mass in a thermal gravimetric analysis of less than about 2% upon heating the sample from about 25 °C to about 380 °C. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a loss in mass in a thermal gravimetric analysis of less than about 2% upon heating the sample from about 25 °C to about 210 °C.

[00106] Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits peaks in an x-ray powder diffraction (XRPD) pattern at $7.7^{\circ} \pm 0.2^{\circ}$ 2-theta and $15.4^{\circ} \pm 0.2^{\circ}$ 2-theta. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a peak in an x-ray powder diffraction (XRPD) pattern at $19.2^{\circ} \pm 0.2^{\circ}$ 2-theta. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a further peak in an x-ray powder diffraction (XRPD) pattern at $13.7^{\circ} \pm 0.2^{\circ}$ 2-theta. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits further peaks in an x-ray powder diffraction (XRPD) pattern at $5.5^{\circ} \pm 0.2^{\circ}$ 2-theta, $8.6^{\circ} \pm 0.2^{\circ}$ 2-theta, $15.9^{\circ} \pm 0.2^{\circ}$ 2-theta, $19.9^{\circ} \pm 0.2^{\circ}$ 2-theta, and $24.1^{\circ} \pm 0.2^{\circ}$ 2-theta. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits further peaks in an x-ray powder diffraction (XRPD) pattern at $10.6^{\circ} \pm 0.2^{\circ}$ 2-theta, $11.0^{\circ} \pm 0.2^{\circ}$ 2-theta, $21.0^{\circ} \pm 0.2^{\circ}$ 2-theta, and $26.3^{\circ} \pm 0.2^{\circ}$ 2-theta. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a peak in a differential scanning calorimetry pattern of from about 203 °C to about 210 °C. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a peak in a differential scanning calorimetry pattern of from about 206 °C to about 210 °C. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a peak in a differential scanning calorimetry pattern of from about 203 °C to about 208 °C, or from about 203 °C to about 206 °C, or from about 203 °C to about 205 °C. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a loss in mass in a thermal gravimetric analysis of less than about 2% upon heating the sample from about 25 °C to about 380 °C. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a loss in mass in a

thermal gravimetric analysis of less than about 2% upon heating the sample from about 25 °C to about 210 °C.

[00107] Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits peaks in an x-ray powder diffraction (XRPD) pattern at $7.7^{\circ} \pm 0.2^{\circ}$ 2-theta and $19.2^{\circ} \pm 0.2^{\circ}$ 2-theta. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a peak in an x-ray powder diffraction (XRPD) pattern at $15.4^{\circ} \pm 0.2^{\circ}$ 2-theta. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a further peak in an x-ray powder diffraction (XRPD) pattern at $13.7^{\circ} \pm 0.2^{\circ}$ 2-theta. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits further peaks in an x-ray powder diffraction (XRPD) pattern at $5.5^{\circ} \pm 0.2^{\circ}$ 2-theta, $8.6^{\circ} \pm 0.2^{\circ}$ 2-theta, $15.9^{\circ} \pm 0.2^{\circ}$ 2-theta, $19.9^{\circ} \pm 0.2^{\circ}$ 2-theta, and $24.1^{\circ} \pm 0.2^{\circ}$ 2-theta. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits further peaks in an x-ray powder diffraction (XRPD) pattern at $10.6^{\circ} \pm 0.2^{\circ}$ 2-theta, $11.0^{\circ} \pm 0.2^{\circ}$ 2-theta, $21.0^{\circ} \pm 0.2^{\circ}$ 2-theta, and $26.3^{\circ} \pm 0.2^{\circ}$ 2-theta. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a peak in a differential scanning calorimetry pattern of from about 203 °C to about 210 °C. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a peak in a differential scanning calorimetry pattern of from about 206 °C to about 210 °C. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a peak in a differential scanning calorimetry pattern of from about 203 °C to about 208 °C, or from about 203 °C to about 206 °C, or from about 203 °C to about 205 °C. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a loss in mass in a thermal gravimetric analysis of less than about 2% upon heating the sample from about 25 °C to about 380 °C. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits a loss in mass in a thermal gravimetric analysis of less than about 2% upon heating the sample from about 25 °C to about 210 °C.

[00108] Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits less than about 10% degradation when stored at 25 °C and 60% relative humidity for at least 7 days. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits less than about 1%, or less than about 2%, or less than about 3%, or less than about 4%, or less than about 5%, or less than about 6%, or less than about 7%, or less than about 8%, or less than about 9% degradation when the crystalline form is stored at 25 °C and 60% relative humidity for at least 7 days.

[00109] Also provided herein are such methods wherein the crystalline form of Compound 4 (a) exhibits a peak in an x-ray powder diffraction (XRPD) pattern at $8.1^{\circ} \pm 0.2^{\circ}$ 2-theta, and (b) exhibits less than about 10% degradation when the crystalline form is stored at 25 °C and 60% relative humidity for at least 7 days. Also provided herein are such methods wherein the crystalline form of Compound 4 (a) exhibits peaks in an x-ray powder diffraction (XRPD) pattern at $9.6^{\circ} \pm 0.2^{\circ}$ 2-theta, $5.7^{\circ} \pm 0.2^{\circ}$ 2-theta, $19.7^{\circ} \pm 0.2^{\circ}$ 2-theta, and $22.0^{\circ} \pm 0.2^{\circ}$ 2-theta, and (b) less than about 10% degradation when the crystalline form is stored at 25 °C and 60% relative humidity for at least 7 days. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits less than about 1%, or less than about 2%, or less than about 3%, or less than

about 4%, or less than about 5%, or less than about 6%, or less than about 7%, or less than about 8%, or less than about 9% degradation when the crystalline form is stored at 25 °C and 60% relative humidity for at least 7 days.

[00110] Also provided herein are such methods wherein the crystalline form of Compound 4 (a) exhibits a peak in an x-ray powder diffraction (XRPD) pattern at $7.7^\circ \pm 0.2^\circ$ 2-theta, and (b) exhibits less than about 10% degradation when the crystalline form is stored at 25 °C and 60% relative humidity for at least 7 days. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits (a) peaks in an x-ray powder diffraction (XRPD) pattern at $7.7^\circ \pm 0.2^\circ$ 2-theta, $13.7^\circ \pm 0.2^\circ$ 2-theta, and $19.2^\circ \pm 0.2^\circ$ 2-theta, and (b) less than about 10% degradation when the crystalline form is stored at 25 °C and 60% relative humidity for at least 7 days. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits less than about 1%, or less than about 2%, or less than about 3%, or less than about 4%, or less than about 5%, or less than about 6%, or less than about 7%, or less than about 8%, or less than about 9% degradation when the crystalline form is stored at 25 °C and 60% relative humidity for at least 7 days.

[00111] Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits less than about 10% degradation when the crystalline forms are stored at 40 °C and 75% relative humidity for at least 7 days. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits less than about 1%, or less than about 2%, or less than about 3%, or less than about 4%, or less than about 5%, or less than about 6%, or less than about 7%, or less than about 8%, or less than about 9% degradation when the crystalline form is stored at 40 °C and 75% relative humidity for at least 7 days.

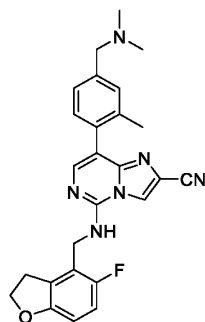
[00112] Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits (a) a peak in an x-ray powder diffraction (XRPD) pattern at $8.1^\circ \pm 0.2^\circ$ 2-theta, and (b) less than about 10% degradation when the crystalline form is stored at 40 °C and 75% relative humidity for at least 7 days. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits (a) peaks in an x-ray powder diffraction (XRPD) pattern at $9.6^\circ \pm 0.2^\circ$ 2-theta, $5.7^\circ \pm 0.2^\circ$ 2-theta, $19.7^\circ \pm 0.2^\circ$ 2-theta, and $22.0^\circ \pm 0.2^\circ$ 2-theta, and (b) less than about 10% degradation when the crystalline form is stored at 40 °C and 75% relative humidity for at least 7 days. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits less than about 1%, or less than about 2%, or less than about 3%, or less than about 4%, or less than about 5%, or less than about 6%, or less than about 7%, or less than about 8%, or less than about 9% degradation when the crystalline form is stored at 40 °C and 75% relative humidity for at least 7 days.

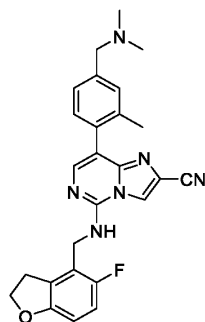
[00113] Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits (a) a peak in an x-ray powder diffraction (XRPD) pattern at $7.7^\circ \pm 0.2^\circ$ 2-theta, and (b) less than about 10% degradation when the crystalline form is stored at 40 °C and 75% relative humidity for at least 7 days. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits (a) peaks in an x-ray powder diffraction (XRPD) pattern at $7.7^\circ \pm 0.2^\circ$ 2-theta, $13.7^\circ \pm 0.2^\circ$ 2-theta, and $19.2^\circ \pm 0.2^\circ$ 2-theta, and (b) less than about 10% degradation when the crystalline form is stored at 40 °C and 75% relative humidity

for at least 7 days. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits less than about 1%, or less than about 2%, or less than about 3%, or less than about 4%, or less than about 5%, or less than about 6%, or less than about 7%, or less than about 8%, or less than about 9% degradation when the crystalline form is stored at 40 °C and 75% relative humidity for at least 7 days.

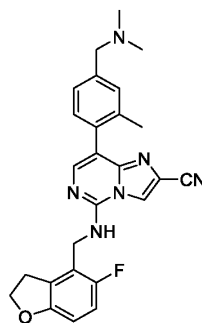
[00114] Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits less than about 10% degradation when the crystalline form is stored at 60 °C for at least one week. Also provided herein are such methods wherein the crystalline form of Compound 4 exhibits less than about 1%, or less than about 2%, or less than about 3%, or less than about 4%, or less than about 5%, or less than about 6%, or less than about 7%, or less than about 8%, or less than about 9% degradation when the crystalline form is stored at 60 °C for at least one week.

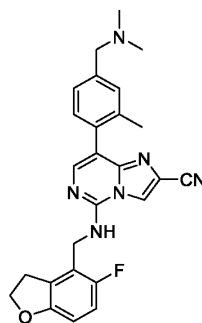
[00115] Also disclosed herein are methods of treating prostate cancer in a subject, comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED)



inhibitor of formula , or a pharmaceutically acceptable salt thereof. In some embodiments, the androgen receptor inhibitor is selected from apalutamide, darolutamide, and enzalutamide. In some embodiments, the androgen receptor inhibitor is apalutamide. In some embodiments, the androgen receptor inhibitor is darolutamide. In some embodiments, the androgen receptor inhibitor is enzalutamide.

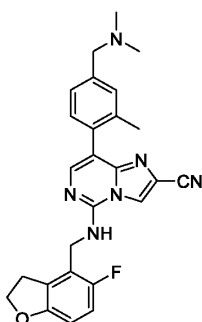
[00116] Also disclosed herein are methods of treating prostate cancer in a subject, wherein the prostate cancer in the subject has been determined to be resistant to one or more first androgen receptor signaling inhibitors (ARSI), comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an

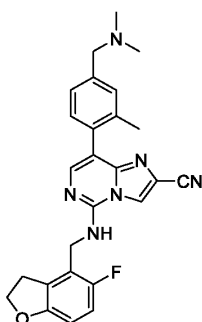


embryonic ectoderm development (EED) inhibitor of formula , or a pharmaceutically acceptable salt thereof. In some embodiments, the one or more first androgen receptor signaling inhibitors (ARSI) is selected from CYP17 inhibitors and androgen receptor inhibitors. In some embodiments, the one or more first androgen receptor signaling inhibitors (ARSI) is selected from CYP17 inhibitors. In some embodiments, the CYP17 inhibitor is abiraterone acetate. In some embodiments, the one or more first

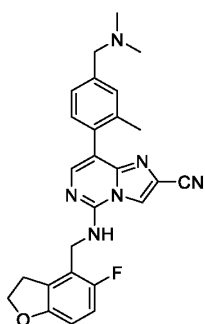
androgen receptor signaling inhibitors (ARSI) is selected from one or more androgen receptor inhibitors. In some embodiments, the one or more first androgen receptor inhibitors is selected from apalutamide, darolutamide, and enzalutamide. In some embodiments, the one or more first androgen receptor inhibitor is apalutamide. In some embodiments, the one or more first androgen receptor inhibitor is darolutamide. In some embodiments, the one or more first androgen receptor inhibitor is enzalutamide.

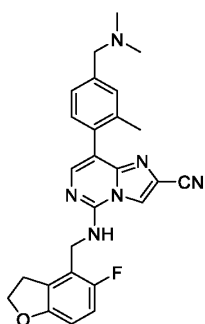
[00117] Also disclosed herein are methods of treating prostate cancer in a subject, wherein the prostate cancer in the subject has been determined to be resistant to a CYP17 inhibitor, comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor



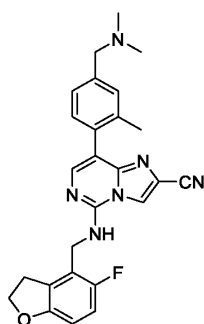
of formula , or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP17 inhibitor is abiraterone acetate. In some embodiments, the one androgen receptor inhibitor is selected from apalutamide, darolutamide, and enzalutamide. In some embodiments, the androgen receptor inhibitor is apalutamide. In some embodiments, the androgen receptor inhibitor is darolutamide. In some embodiments, the androgen receptor inhibitor is enzalutamide.

[00118] Also disclosed herein are methods of treating prostate cancer in a subject, wherein the prostate cancer in the subject has been determined to be resistant to abiraterone, comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor of



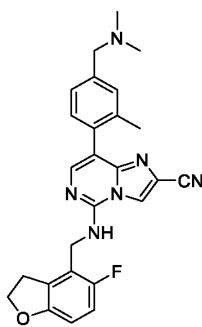
formula , or a pharmaceutically acceptable salt thereof. In some embodiments, the androgen receptor inhibitors is selected from apalutamide, darolutamide, and enzalutamide. In some embodiments, the androgen receptor inhibitor is apalutamide. In some embodiments, the androgen receptor inhibitor is darolutamide. In some embodiments, the androgen receptor inhibitor is enzalutamide.

[00119] Also disclosed herein are methods of treating prostate cancer in a subject, wherein the subject has received prior administration of one or more CYP17 inhibitors, comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor of formula



, or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP17 inhibitor is abiraterone acetate. In some embodiments, the androgen receptor inhibitor is selected from apalutamide, darolutamide, and enzalutamide. In some embodiments, the androgen receptor inhibitor is apalutamide. In some embodiments, the androgen receptor inhibitor is darolutamide. In some embodiments, the androgen receptor inhibitor is enzalutamide.

[00120] Also disclosed herein are methods of treating prostate cancer in a subject, wherein the subject (i) has received prior administration of one or more CYP17 inhibitors, and (ii) is androgen receptor inhibitor naïve, comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm



development (EED) inhibitor of formula , or a pharmaceutically acceptable salt thereof.

In some embodiments, the CYP17 inhibitor is abiraterone acetate. In some embodiments, the androgen receptor inhibitor is selected from apalutamide, darolutamide, and enzalutamide. In some embodiments, the androgen receptor inhibitor is apalutamide. In some embodiments, the androgen receptor inhibitor is darolutamide. In some embodiments, the androgen receptor inhibitor is enzalutamide.

[00121] Also disclosed herein are any of the methods of treating prostate cancer disclosed herein, wherein the subject has received one or more prior chemotherapeutic treatments prior the administration of the androgen receptor inhibitor, and the embryonic ectoderm development (EED) inhibitor. In an embodiment, the subject has received up to one prior chemotherapeutic treatments prior the administration of the androgen receptor inhibitor, and the embryonic ectoderm development (EED) inhibitor.

[00122] Also disclosed herein are any of the methods of treating prostate cancer disclosed herein, wherein the subject is CYP17 inhibitor-naïve prior to the administration to the subject of the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor.

[00123] Also disclosed herein are any of the methods of treating prostate cancer disclosed herein, wherein the subject is androgen receptor inhibitor naïve prior to the administration to the subject of the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor.

[00124] Also disclosed herein are any of the methods of treating prostate cancer disclosed herein, wherein the subject has not been administered a CYP17 inhibitor or an androgen receptor inhibitor prior to the administration to the subject of the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor. In some embodiments, the subject has not been administered abiraterone or an androgen receptor inhibitor selected from apalutamide, darolutamide, or enzalutamide prior to the administration to the subject of the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor. In some embodiments, the subject has not been administered abiraterone or apalutamide prior to the administration to the subject of the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor. In some embodiments, the subject has not been administered abiraterone or darolutamide prior to the administration to the subject of the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor. In some embodiments, the subject has not been administered abiraterone or enzalutamide prior to the administration to the subject of the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor. In some embodiments, the ectoderm development (EED) inhibitor is Compound 4.

[00125] Also disclosed herein are any of the methods of treating prostate cancer disclosed herein, wherein the subject has not been administered abiraterone or an androgen receptor inhibitor selected from apalutamide, darolutamide, or enzalutamide prior to the administration to the subject of apalutamide and Compound 4. In some embodiments, the subject has not been administered abiraterone or an androgen receptor inhibitor selected from apalutamide, darolutamide, or enzalutamide prior to the administration to the subject of darolutamide and Compound 4. In some embodiments, the subject has not been administered abiraterone or an androgen receptor inhibitor selected from apalutamide, darolutamide, or enzalutamide prior to the administration to the subject of enzalutamide and Compound 4.

[00126] Also disclosed herein are any of the methods of treating prostate cancer disclosed herein, wherein the subject has not been administered abiraterone or apalutamide prior to the administration to the subject of apalutamide and Compound 4. In some embodiments, the subject has not been administered abiraterone or darolutamide prior to the administration to the subject of apalutamide and Compound 4. In some embodiments, the subject has not been administered abiraterone or enzalutamide prior to the administration to the subject of apalutamide and Compound 4.

[00127] Also disclosed herein are any of the methods of treating prostate cancer disclosed herein, wherein the subject has not been administered abiraterone or apalutamide prior to the administration to the subject of darolutamide and Compound 4. In some embodiments, the subject has not been administered abiraterone or darolutamide prior to the administration to the subject of darolutamide and Compound 4. In some embodiments, the subject has not been administered abiraterone or enzalutamide prior to the administration to the subject of darolutamide and Compound 4.

[00128] Also disclosed herein are any of the methods of treating prostate cancer disclosed herein, wherein the subject has not been administered abiraterone or apalutamide prior to the administration to the subject of enzalutamide and Compound 4. In some embodiments, the subject has not been administered abiraterone or

darolutamide prior to the administration to the subject of enzalutamide and Compound 4. In some embodiments, the subject has not been administered abiraterone or enzalutamide prior to the administration to the subject of enzalutamide and Compound 4.

[00129] Also disclosed herein are any of the methods of treating prostate cancer disclosed herein, wherein the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor are administered to the subject sequentially or simultaneously. In an embodiment, the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor are administered to the subject sequentially. In an embodiment, the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor are administered to the subject simultaneously.

[00130] Also disclosed herein are any of the methods of treating prostate cancer disclosed herein, wherein the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor are administered to the subject on the same day.

[00131] Also disclosed herein are any of the methods of treating prostate cancer disclosed herein, wherein the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor are administered to the subject within a 24-hour period. In another embodiment, the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor are administered to the subject within a 12-hour period, or a 10-hour period, or an 8-hour period, or a 6-hour period, or a 4-hour period, or a 2-hour period, or within an hour of each of other.

[00132] Also disclosed herein are any of the methods of treating prostate cancer disclosed herein, wherein the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor are administered to the subject once per day or twice per day. In an embodiment, the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor are administered to the subject once per day. In an embodiment, the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor are administered to the subject twice per day. In an embodiment, the androgen receptor inhibitor is administered to the subject twice per day and the embryonic ectoderm development (EED) inhibitor is administered to the subject once per day. In an embodiment, the androgen receptor inhibitor is administered to the subject once per day and the embryonic ectoderm development (EED) inhibitor is administered to the subject twice per day.

[00133] Also disclosed herein are any of the methods of treating prostate cancer disclosed herein, wherein the androgen receptor inhibitor is administered to the subject with food or without food. In an embodiment, the androgen receptor inhibitor is administered to the subject with food. In an embodiment, the androgen receptor inhibitor is administered to the subject without food.

[00134] Also disclosed herein are any of the methods of treating prostate cancer disclosed herein, wherein the subject has been administered one or more prior androgen deprivation therapies prior to the administration to the subject of the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor.

[00135] Also disclosed herein are any of the methods of treating prostate cancer disclosed herein, wherein the subject has been administered a gonadotropin-releasing hormone (GnRH) analog prior to the administration to the subject of the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor.

[00136] Also disclosed herein are any of the methods of treating prostate cancer disclosed herein, wherein the subject receives a gonadotropin-releasing hormone (GnRH) analog concurrently with the administration to the subject of the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor. In an embodiment, the GnRH analog is selected from leuprolide, goserelin, histrelin, triptorelin, degarelix, and relugolix. In an embodiment, the GnRH analog is leuprolide. In an embodiment, the GnRH analog is goserelin. In an embodiment, the GnRH analog is histrelin. In an embodiment, the GnRH analog is triptorelin. In an embodiment, the GnRH analog is degarelix. In an embodiment, the GnRH analog is relugolix.

[00137] Also disclosed herein are any of the methods of treating prostate cancer disclosed herein, wherein the subject had a bilateral orchiectomy prior to the administration to the subject of the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor.

[00138] Also disclosed herein are any of the methods of treating prostate cancer disclosed herein, wherein the subject is not administered a compound that is a substrate of CYP3A4, CYP2C19, CYP2C8, CYP2C9, UGT, P-gp, BCRP, or OATP1B1 during the time period in which the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor are administered to the subject.

[00139] Also disclosed herein are any of the methods of treating prostate cancer disclosed herein, wherein the subject is not administered a compound that is a substrate of CYP3A4, CYP2C9, UGT, P-gp, BCRP, or OATP1B1 during the time period in which the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor are administered to the subject.

[00140] Also disclosed herein are any of the methods of treating prostate cancer disclosed herein, wherein the subject is not administered a compound that is (a) a CYP2C8 inhibitor, (b) a CYP3A4 inducer, or (c) a substrate of CYP3A4, CYP2C9, or CYP2C19 during the time period in which the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor are administered to the subject.

[00141] Also disclosed herein are any of the methods of treating prostate cancer disclosed herein, wherein the subject is not administered a compound that is (a) a CYP3A4 inducer, (b) a PG-p inhibitor, (c) a CYP3A4 inhibitor, (d) a BCRP substrate, (e) an OATP1B1 substrate, or (f) a OATP1B3 substrate during the time period in which the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor are administered to the subject.

[00142] Also disclosed herein are any of the methods of treating prostate cancer disclosed herein, wherein the prostate cancer in the subject is selected from metastatic prostate cancer, non-metastatic prostate cancer, metastatic castration-resistant prostate cancer, metastatic castration-sensitive prostate cancer, localized high risk prostate cancer, recurrent prostate cancer, non-metastatic castration-resistant prostate cancer, non-metastatic castration-sensitive prostate cancer, androgen receptor inhibitor-sensitive prostate cancer, androgen receptor inhibitor-resistant prostate cancer, androgen receptor-dependent prostate cancer, androgen

receptor-independent prostate cancer, neuroendocrine prostate cancer (NEPC), metastatic neuroendocrine prostate cancer (NEPC), prostate cancer with small cell features, metastatic prostate cancer with small cell features, and aggressive-variant prostate cancer. In some embodiments, the prostate cancer in the subject is metastatic prostate cancer. In some embodiments, the prostate cancer in the subject is non-metastatic prostate cancer. In some embodiments, the prostate cancer in the subject is metastatic castration-resistant prostate cancer. In some embodiments, the prostate cancer in the subject is metastatic castration-sensitive prostate cancer. In some embodiments, the prostate cancer in the subject is localized high risk prostate cancer. In some embodiments, the prostate cancer in the subject is recurrent prostate cancer. In some embodiments, the prostate cancer in the subject is non-metastatic castration-resistant prostate cancer. In some embodiments, the prostate cancer in the subject is non-metastatic castration-sensitive prostate cancer. In some embodiments, the prostate cancer in the subject is androgen receptor inhibitor-sensitive prostate cancer. In some embodiments, the prostate cancer in the subject is androgen receptor inhibitor-resistant prostate cancer. In some embodiments, the prostate cancer in the subject is androgen receptor-dependent prostate cancer. In some embodiments, the prostate cancer in the subject is androgen receptor-independent prostate cancer. In some embodiments, the prostate cancer in the subject is neuroendocrine prostate cancer (NEPC). In some embodiments, the prostate cancer in the subject is metastatic neuroendocrine prostate cancer (NEPC). In some embodiments, the prostate cancer in the subject is prostate cancer with small cell features. In some embodiments, the prostate cancer in the subject is metastatic prostate cancer with small cell features. In some embodiments, the prostate cancer in the subject is aggressive-variant prostate cancer.

[00143] In other embodiments of the methods of treating prostate cancer in a subject disclosed herein, a subject having prostate cancer that may benefit from the administration to the subject of an androgen receptor inhibitor, and an embryonic ectoderm development (EED) inhibitor may include one or more of the following criteria: (a) the subject has undergone bilateral orchiectomy or be willing to continue GnRH analogue or antagonist to maintain castrate levels of testosterone; (b) the subject has progressed after at least one line of ARSI (abiraterone, enzalutamide, apalutamide, darolutamide) and must not have received more than 2 chemotherapy regimens in the mCRPC setting; (c) a subject exhibiting evidence of progressive disease by PCWG3 criteria, including rising PSA, defined as a minimum of 2 rising values obtained a minimum of one week apart with the latest result being at least 2.0 ng/mL (or 1.0 ng/mL if PSA rise is the only indication of progression), or confirmation of 2 new bone lesions on last systemic therapy, or soft tissue progression per RECIST 1.1; (d) subject having measurable and/or evaluable disease by RECIST 1.1; (e) an ECOG performance status of 0 or 1; and (f) adequate organ function.

[00144] In other embodiments are provided the methods of treating prostate cancer in a subject described herein, wherein the subject is administered a therapeutically effective amount of (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor, and is further administered one or more additional therapeutic agents. In further embodiments are provided such methods, wherein the one or more additional therapeutic agents is selected from an androgen receptor degrader, a chemotherapeutic agent, a mitotic inhibitors, an antimetabolites, a platinum-based agents, histone deacetylase (HDAC) inhibitors,

CD30-directed antibody-drug conjugates, farnesyl transferase inhibitors, SYK inhibitors, JAK inhibitors, PI3K pathway inhibitors, immunomodulatory agents, AKT inhibitors, radiopharmaceuticals, PARP inhibitors, or combinations thereof. In further embodiments are provided such methods, wherein the one or more additional therapeutic agents are selected from chemotherapeutic agents.

[00145] In other embodiments are provided such methods wherein the androgen receptor degrader is a PROteolytic-TArgeting-Chimera (PROTAC®) androgen receptor (AR) degrader. In further embodiments, the androgen receptor degrader is bavdegalutamide (ARV-110).

[00146] In further embodiments are provided such methods, wherein the chemotherapeutic agents are selected from actinomycin, azacytidine, azathioprine, bendamustine, bleomycin, bortezomib, chlorambucil, cyclophosphamide, daunorubicin, doxifluridine, doxorubicin, epirubicin, epothilone, etoposide, idarubicin, irinotecan, lurbinectedin, mechlorethamine, mitoxantrone, teniposide, topotecan, valrubicin, vemurafenib, vinblastine, vincristine, and vindesine. In some embodiments, the chemotherapeutic agent is actinomycin. In some embodiments, the chemotherapeutic agent is azacytidine. In some embodiments, the chemotherapeutic agent is azathioprine. In some embodiments, the chemotherapeutic agent is bleomycin. In some embodiments, the chemotherapeutic agent is bortezomib. In some embodiments, the chemotherapeutic agent is chlorambucil. In some embodiments, the chemotherapeutic agent is cyclophosphamide. In some embodiments, the chemotherapeutic agent is daunorubicin. In some embodiments, the chemotherapeutic agent is doxifluridine. In some embodiments, the chemotherapeutic agent is doxorubicin. In some embodiments, the chemotherapeutic agent is epirubicin. In some embodiments, the chemotherapeutic agent is epothilone. In some embodiments, the chemotherapeutic agent is etoposide. In some embodiments, the chemotherapeutic agent is idarubicin. In some embodiments, the chemotherapeutic agent is irinotecan. In some embodiments, the chemotherapeutic agent is lurbinectedin. In some embodiments, the chemotherapeutic agent is mechlorethamine. In some embodiments, the chemotherapeutic agent is mitoxantrone. In some embodiments, the chemotherapeutic agent is teniposide. In some embodiments, the chemotherapeutic agent is topotecan. In some embodiments, the chemotherapeutic agent is valrubicin. In some embodiments, the chemotherapeutic agent is vemurafenib. In some embodiments, the chemotherapeutic agent is vinblastine. In some embodiments, the chemotherapeutic agent is vincristine. In some embodiments, the chemotherapeutic agent is vindesine.

[00147] In further embodiments are provided such methods, wherein the one or more additional therapeutic agents are selected from mitotic inhibitors. In further embodiments are provided such methods, wherein the mitotic inhibitors are selected from paclitaxel, docetaxel, cabazitaxel, tesetaxel, and nab-paclitaxel. In some embodiments, the mitotic inhibitor is paclitaxel. In some embodiments, the mitotic inhibitor is docetaxel. In some embodiments, the mitotic inhibitor is cabazitaxel. In some embodiments, the mitotic inhibitor is tesetaxel. In some embodiments, the mitotic inhibitor is nab-paclitaxel.

[00148] In yet other embodiments, the one or more additional therapeutic agents are selected from antimetabolites. In some embodiments, the one or more antimetabolites are selected from azacytidine, 6-mercaptopurine, capecitabine, hydroxyurea, cladribine, pralatrexate, thioguanine, decitabine, clofarabine,

nelarabine, fludarabine, 5-fluorouracil, gemcitabine, cytarabine, pemetrexed, and methotrexate, cytarabine (Ara-C), floxuridine, fludarabine, pentostatin, and trifluridine/tipiracil combination. In some embodiments, the one or more antimetabolites are selected from 6-mercaptopurine, capecitabine, hydroxyurea, cladribine, pralatrexate, thioguanine, decitabine, clofarabine, nelarabine, fludarabine, 5-fluorouracil, gemcitabine, cytarabine, pemetrexed, and methotrexate.

[00149] In some embodiments, the antimetabolite is azacytidine. In some embodiments, the antimetabolite is 6-mercaptopurine. In some embodiments, the antimetabolite is capecitabine. In some embodiments, the antimetabolite is hydroxyurea. In some embodiments, the antimetabolite is cladribine. In some embodiments, the antimetabolite is pralatrexate. In some embodiments, the antimetabolite is thioguanine. In some embodiments, the antimetabolite is decitabine. In some embodiments, the antimetabolite is clofarabine. In some embodiments, the antimetabolite is nelarabine. In some embodiments, the antimetabolite is fludarabine. In some embodiments, the antimetabolite is 5-fluorouracil. In some embodiments, the antimetabolite is gemcitabine. In some embodiments, the antimetabolite is cytarabine. In some embodiments, the antimetabolite is pemetrexed. In some embodiments, the antimetabolite is methotrexate. In some embodiments, the antimetabolite is cytarabine (Ara-C). In some embodiments, the antimetabolite is floxuridine. In some embodiments, the antimetabolite is fludarabine. In some embodiments, the antimetabolite is pentostatin. In some embodiments, the antimetabolite is a trifluridine/tipiracil combination.

[00150] In some embodiments, the one or more additional therapeutic agents are selected from platinum-based agents. In some embodiments, the platinum-based agents are selected from cisplatin, carboplatin, oxaliplatin, nedaplatin, lobaplatin, triplatin tetranitrate, pheanthriplatin, picoplatin, and satraplatin. In further embodiments, the platinum-based agent is cisplatin. In further embodiments, the platinum-based agent is carboplatin. In further embodiments, the platinum-based agent is oxaliplatin. In further embodiments, the platinum-based agent is nedaplatin. In further embodiments, the platinum-based agent is lobaplatin. In further embodiments, the platinum-based agent is triplatin tetranitrate. In further embodiments, the platinum-based agent is pheanthriplatin. In further embodiments, the platinum-based agent is picoplatin. In further embodiments, the platinum-based agent is satraplatin.

[00151] In further embodiments are provided such methods, wherein the one or more additional therapeutic agents are selected from histone deacetylase (HDAC) inhibitors. In further embodiments are provided such methods, wherein the histone deacetylase (HDAC) inhibitors are selected from vorinostat, romidepsin, belinostat, tucidinostat, panobinostat, mocetinostat, givinostat, resmiostat, abexinostat, ricolinostat, entinostat, tinostamustin, fimepinostat, CXD-101, quisinostat, and chidamide. In further embodiments, the histone deacetylase (HDAC) inhibitor is vorinostat. In further embodiments, the histone deacetylase (HDAC) inhibitor is romidepsin. In further embodiments, the histone deacetylase (HDAC) inhibitor is belinostat. In further embodiments, the histone deacetylase (HDAC) inhibitor is tucidinostat. In further embodiments, the histone deacetylase (HDAC) inhibitor is Panobinostat. In further embodiments, the histone deacetylase (HDAC) inhibitor is mocetinostat. In further embodiments, the histone deacetylase (HDAC) inhibitor is givinostat. In further embodiments, the histone deacetylase (HDAC) inhibitor is resmiostat. In further

embodiments, the histone deacetylase (HDAC) inhibitor is abexinostat. In further embodiments, the histone deacetylase (HDAC) inhibitor is ricolinostat. In further embodiments, the histone deacetylase (HDAC) inhibitor is entinostat. In further embodiments, the histone deacetylase (HDAC) inhibitor is tinostamustin. In further embodiments, the histone deacetylase (HDAC) inhibitor is fimepinostat. In further embodiments, the histone deacetylase (HDAC) inhibitor is CXD-101. In further embodiments, the histone deacetylase (HDAC) inhibitor is quisinostat. In further embodiments, the histone deacetylase (HDAC) inhibitor is chidamide.

[00152] In further embodiments are provided such methods, wherein the one or more additional therapeutic agents are selected from CD30-directed antibody-drug conjugates. In further embodiments are provided such methods, wherein the CD30-directed antibody-drug conjugates are selected from brentuximab vedotin and SGN-CD30C. In further embodiments, the CD30-directed antibody-drug conjugate is brentuximab vedotin. In further embodiments, the CD30-directed antibody-drug conjugate is SGN-CD30C.

[00153] In further embodiments are provided such methods, wherein the one or more additional therapeutic agents are selected from farnesyl transferase inhibitors. In further embodiments are provided such methods, wherein the farnesyl transferase inhibitors are selected from antroquinonol, BMS-214662, L778123, L744832, FTI-276, FTI-277, manumycin A, LB-42708, moverastin, PD169541, ABT-100, FTI-2153, tipifarnib and lonafamib. In further embodiments, the farnesyl transferase inhibitor is antroquinonol. In further embodiments, the farnesyl transferase inhibitor is BMS-214662. In further embodiments, the farnesyl transferase inhibitor is L778123. In further embodiments, the farnesyl transferase inhibitor is L744832. In further embodiments, the farnesyl transferase inhibitor is FTI-276. In further embodiments, the farnesyl transferase inhibitor is FTI-277. In further embodiments, the farnesyl transferase inhibitor is manumycin A. In further embodiments, the farnesyl transferase inhibitor is LB-42708. In further embodiments, the farnesyl transferase inhibitor is moverastin. In further embodiments, the farnesyl transferase inhibitor is PD169541. In further embodiments, the farnesyl transferase inhibitor is ABT-100. In further embodiments, the farnesyl transferase inhibitor is FTI-2153. In further embodiments, the farnesyl transferase inhibitor is tipifarnib. In further embodiments, the farnesyl transferase inhibitor is lonafamib.

[00154] In further embodiments are provided such methods, wherein the one or more additional therapeutic agents are selected from SYK inhibitors. In further embodiments are provided such methods, wherein the SYK inhibitors are selected from fostamatinib (R788), entospletinib (GS-9973), cerdulatinib (PRT062070), and TAK-659. In further embodiments, the SYK inhibitor is fostamatinib (R788). In further embodiments, the SYK inhibitor is entospletinib (GS-9973). In further embodiments, the SYK inhibitor is cerdulatinib (PRT062070). In further embodiments, the SYK inhibitor is TAK-659.

[00155] In further embodiments are provided such methods, wherein the one or more additional therapeutic agents are selected from JAK inhibitors. In further embodiments are provided such methods, wherein the JAK inhibitors are selected from tofacitinib, baricitinib, ruxolitinib, upadacitinib, fedratinib, abrocitinib, and ruxolitinib. In further embodiments, the JAK inhibitor is tofacitinib. In further embodiments, the JAK inhibitor is baricitinib. In further embodiments, the JAK inhibitor is ruxolitinib. In further embodiments, the

JAK inhibitor is upadacitinib. In further embodiments, the JAK inhibitor is fedratinib. In further embodiments, the JAK inhibitor is abrocitinib. In further embodiments, the JAK inhibitor is ruxolitinib.

[00156] In further embodiments are provided such methods, wherein the one or more additional therapeutic agents are selected from PI3K pathway inhibitors. In further embodiments are provided such methods, wherein the PI3K pathway inhibitors are selected from taselisib (GDC-0032), GDC-0077, perifosine, idelalisib, buparlisib (BKM120), duvelisib, (IPI-145), copanlisib (BAY 80-6946), PX-866, dactolisib, CUDC-907, voxtalisib (SAR245409, XL765), ME-401, IPI-549, SF1126, RP6530, INK1117, pictilisib (GDC-0941), XL147 (SAR245408), palomid 529, GSK1059615, ZSTK474, and PWT33597. In further embodiments, the PI3K pathway inhibitor is taselisib (GDC-0032). In further embodiments, the PI3K pathway inhibitor is GDC-0077. In further embodiments, the PI3K pathway inhibitor is perifosine. In further embodiments, the PI3K pathway inhibitor is idelalisib. In further embodiments, the PI3K pathway inhibitor is buparlisib (BKM120). In further embodiments, the PI3K pathway inhibitor is duvelisib. In further embodiments, the PI3K pathway inhibitor is (IPI-145). In further embodiments, the PI3K pathway inhibitor is copanlisib (BAY 80-6946). In further embodiments, the PI3K pathway inhibitor is PX-866. In further embodiments, the PI3K pathway inhibitor is dactolisib. In further embodiments, the PI3K pathway inhibitor is CUDC-907. In further embodiments, the PI3K pathway inhibitor is voxtalisib (SAR245409, XL765). In further embodiments, the PI3K pathway inhibitor is ME-401. In further embodiments, the PI3K pathway inhibitor is IPI-549. In further embodiments, the PI3K pathway inhibitor is SF1126. In further embodiments, the PI3K pathway inhibitor is RP6530. In further embodiments, the PI3K pathway inhibitor is INK1117. In further embodiments, the PI3K pathway inhibitor is pictilisib (GDC-0941). In further embodiments, the PI3K pathway inhibitor is XL147 (SAR245408). In further embodiments, the PI3K pathway inhibitor is palomid 529. In further embodiments, the PI3K pathway inhibitor is GSK1059615. In further embodiments, the PI3K pathway inhibitor is ZSTK474. In further embodiments, the PI3K pathway inhibitor is PWT33597.

[00157] In further embodiments are provided such methods, wherein the one or more additional therapeutic agents are selected from immunomodulatory agents. In further embodiments are provided such methods, wherein the immunomodulatory agents are selected from lenalidomide, PD-1 inhibitors, PD-L1 inhibitors, CTLA-4 agents, T-cell immunoglobulin and ITIM domain (TIGIT) agents, TIM-3 inhibitors, and LAG-3 inhibitors.

[00158] In other embodiments are provided the methods disclosed herein, wherein the one or more additional therapeutic agents are selected from PD-1 inhibitors. In some embodiments, the PD-1 inhibitors are selected from pembrolizumab, nivolumab, cemiplimab, JTX-4014, spartalizumab (PDR001), camrelizumab (SHR1210), sintilimab (IBI308), tislelizumab (BGB-A317), toripalimab (JS 001), dostarlimab (TSR-042, WBP-285), INCMGA00012 (MGA012), AMP-224, and AMP-514 (MEDI0680).

[00159] In some embodiments, the PD-1 inhibitor is pembrolizumab. In some embodiments, the PD-1 inhibitor is nivolumab. In some embodiments, the PD-1 inhibitor is cemiplimab. In some embodiments, the PD-1 inhibitor is spartalizumab (PDR001). In some embodiments, the PD-1 inhibitor is camrelizumab (SHR1210). In some embodiments, the PD-1 inhibitor is sintilimab (IBI308). In some embodiments, the PD-

1 inhibitor is tislelizumab (BGB-A317). In some embodiments, the PD-1 inhibitor is toripalimab (JS 001). In some embodiments, the PD-1 inhibitor is dostarlimab (TSR-042, WBP-285). In some embodiments, the PD-1 inhibitor is INCMGA00012 (MGA012). In some embodiments, the PD-1 inhibitor is AMP-224. In some embodiments, the PD-1 inhibitor is AMP-514 (MEDI0680).

[00160] In other embodiments are provided the methods disclosed herein, wherein the one or more additional therapeutic agents are selected from PD-L1 inhibitors. In some embodiments, the PD-L1 inhibitors are selected from atezolizumab, avelumab, durvalumab, MPDL3280A (RG7446), MDX-1105 (BMS-936559), BMS-935559, MSB0010718C, and MEDI4736.

[00161] In some embodiments, the PD-L1 inhibitors are selected from atezolizumab, avelumab, durvalumab, KN035, CK-301, AUNP12, CA-170, and BMS-986189.

[00162] In some embodiments, the PD-L1 inhibitor is atezolizumab. In some embodiments, the PD-L1 inhibitor is avelumab. In some embodiments, the PD-L1 inhibitor is durvalumab. In some embodiments, the PD-L1 inhibitor is MPDL3280A (RG7446). In some embodiments, the PD-L1 inhibitor is MDX-1105 (BMS-936559). In some embodiments, the PD-L1 inhibitor is BMS-935559. In some embodiments, the PD-L1 inhibitor is MSB0010718C. In some embodiments, the PD-L1 inhibitor is MEDI4736.

[00163] In further embodiments are provided such methods, wherein the CTLA-4 inhibitors are selected from ipilimumab and tremelimumab. In further embodiments, the CTLA-4 inhibitor is ipilimumab. In further embodiments, the CTLA-4 inhibitor is tremelimumab.

[00164] In further embodiments are provided such methods, wherein the T-cell immunoglobulin and ITIM domain (TIGIT) agents are selected from BMS-986207, BGB-A1217, tiragolumab, AB154, ASP8374, MK-7684, CD112RCOM701, and LY3435151. In further embodiments, the TIGIT agent is BMS-986207. In further embodiments, the TIGIT agent is BGB-A1217. In further embodiments, the TIGIT agent is tiragolumab. In further embodiments, the TIGIT agent is AB154. In further embodiments, the TIGIT agent is ASP8374. In further embodiments, the TIGIT agent is MK-7684. In further embodiments, the TIGIT agent is CD112RCOM701. In further embodiments, the TIGIT agent is LY3435151.

[00165] In further embodiments are provided such methods, wherein the TIM-3 inhibitors are selected from Sym023, INCAGN02390, LY331367, Sym021, MBG453, BGB-A425, TSR-022, RO7121661, and LU3415244. In further embodiments, the TIM-3 inhibitor is Sym023. In further embodiments, the TIM-3 inhibitor is INCAGN02390. In further embodiments, the TIM-3 inhibitor is LY331367. In further embodiments, the TIM-3 inhibitor is Sym021. In further embodiments, the TIM-3 inhibitor is MBG453. In further embodiments, the TIM-3 inhibitor is BGB-A425. In further embodiments, the TIM-3 inhibitor is TSR-022. In further embodiments, the TIM-3 inhibitor is RO7121661. In further embodiments, the TIM-3 inhibitor is LU3415244.

[00166] In further embodiments are provided such methods, wherein the LAG-3 inhibitors are selected from relatlimab, tebotelimab, chlorogenic acid, RO-7247669, favezelimab, INCAGN-2385, IBI-110, eftilagimod alpha, Sym-022, LBL-007, ABL-501, HLX 26, IBI-323, ieramylimab, FS 118, EMB-02, and finalimab. In further embodiments, the LAG-3 inhibitor is relatlimab. In further embodiments, the LAG-3 inhibitor is

tebotelimab. In further embodiments, the LAG-3 inhibitor is chlorogenic acid. In further embodiments, the LAG-3 inhibitor is RO-7247669. In further embodiments, the LAG-3 inhibitor is favezelimab. In further embodiments, the LAG-3 inhibitor is INCAGN-2385. In further embodiments, the LAG-3 inhibitor is IBI-110,=. In further embodiments, the LAG-3 inhibitor is eftilagimod alpha. In further embodiments, the LAG-3 inhibitor is Sym-022. In further embodiments, the LAG-3 inhibitor is LBL-007. In further embodiments, the LAG-3 inhibitor is ABL-501. In further embodiments, the LAG-3 inhibitor is HLX 26,=. In further embodiments, the LAG-3 inhibitor is IBI-323. In further embodiments, the LAG-3 inhibitor is ieramilimab. In further embodiments, the LAG-3 inhibitor is FS 118. In further embodiments, the LAG-3 inhibitor is EMB-02. In further embodiments, the LAG-3 inhibitor is finalimab.

[00167] In further embodiments are provided such methods, wherein the AKT inhibitor is ipatasertib (GDC-0068), capivasertib (AZD5363), MK2206, afuresertib (GSK2110183), uprosertib (GSK2141795), perifosine (KRX-0401), PHT-427 (CS-0223), or Akti-1/2. In some embodiments, the AKT inhibitor is ipatasertib (GDC-0068). In some embodiments, the PI3K inhibitor is taselisib (GDC-0032), GDC-0077, perifosine, idelalisib, buparlisib (BKM120), duvelisib, (IPI-145), copanlisib (BAY 80-6946), PX-866, dactolisib, CUDC-907, voxalisib (SAR245409, XL765), ME-401, IPI-549, SF1126, RP6530, INK1117, pictilisib (GDC-0941), XL147 (SAR245408), palomid 529, GSK1059615, ZSTK474, or PWT33597.

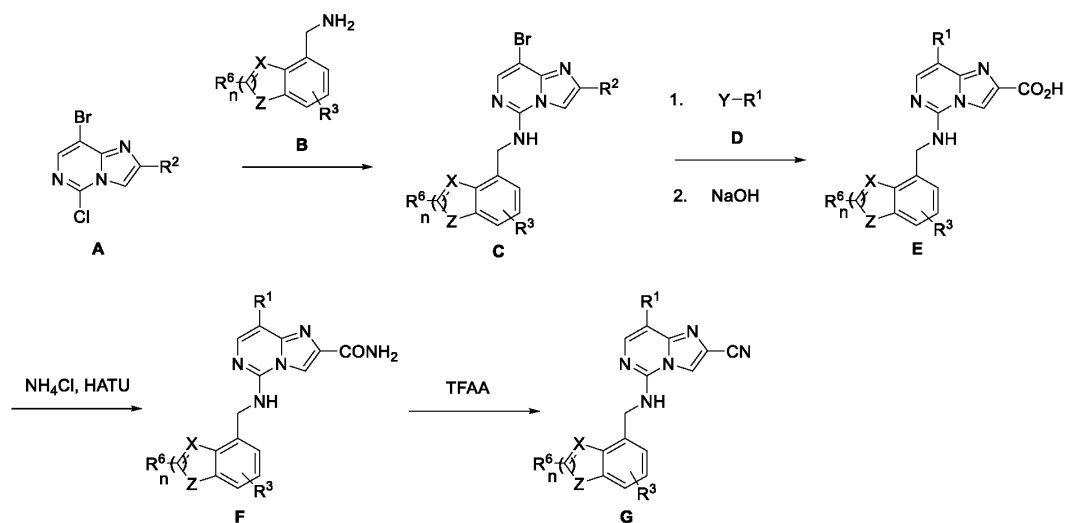
[00168] In further embodiments are provided such methods, wherein the radiopharmaceuticals are selected from radio-ligand therapy agents. In some embodiments, the radiopharmaceutical is lutetium Lu 177 vipivotide tetraxetan.

[00169] In further embodiments are provided such methods, wherein the PARP inhibitors are selected from olaparib, rucaparib, niraparib, veliparib, fuzolaparib, CEP 9722, E7016, talazoparib, veliparib, pamiparib, AZD5305, AZD5135, AZD9574, IMP1734, DM5167, KU-0059436 (AZD2281), NMS-293, SNV-001, compounds disclosed in WO 2022/225934, compounds disclosed in WO 2023/056039, compounds disclosed in WO 2022/247816, and compounds disclosed in CN 115677688 A.

[00170] The compounds of Formula (I), or pharmaceutically acceptable salts thereof, may be prepared using commercially available reagents and intermediates in the synthetic methods and reaction schemes described herein, those described in United States Patent No. 11,091,495, or may be prepared using other reagents and conventional methods well known to those skilled in the art. The contents of United States Patent No. 11,091,495 are hereby incorporated by reference for that purpose.

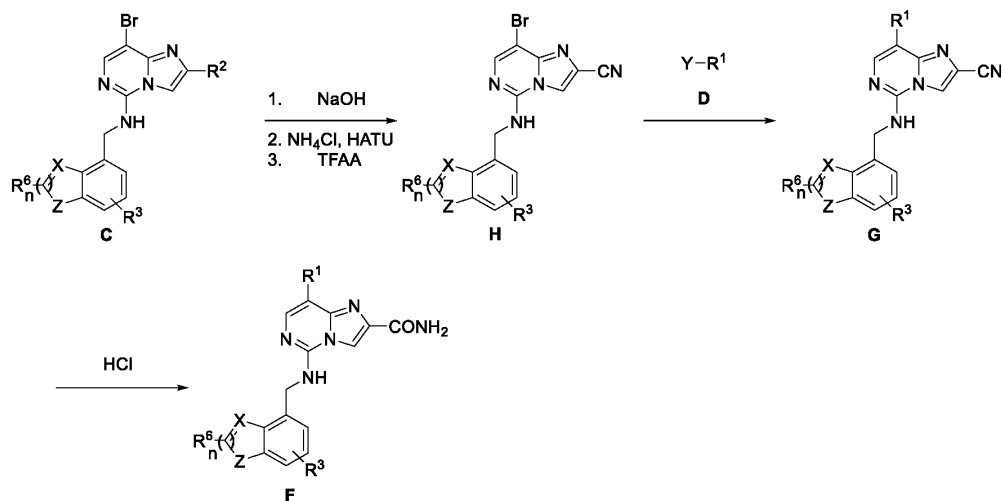
[00171] For instance, intermediates for compounds and compounds of Formula (I) of the present invention may be prepared according to General Reaction Schemes I or II:

General Reaction Scheme I



[00172] In General Reaction Scheme I, R²-ester substituted imidazo[1,2-c]pyrimidine A is coupled to R³ optionally substituted intermediate amine B by nucleophilic substitution to yield Intermediate C. A boronic acid derivative (Y)-R¹ D is coupled via a Suzuki reaction with halogen substituted Intermediate C in the presence of a suitable base, e.g., sodium carbonate, and the R² ester is converted to the acid by saponification with NaOH to generate intermediate acid E. The acid is converted to the corresponding amide, which is dehydrated to form title compound nitrile G.

General Reaction Scheme II



[00173] In General Reaction Scheme II, halogenated Intermediate C containing a suitable R² reactant, e.g., an ester, in the presence of a suitable base is converted to acid intermediate by saponification, then treated with NH₄Cl in the presence of HATU to form the amide which is subsequently dehydrated to form nitrile Intermediate H. R¹ is coupled to Intermediate H via a Suzuki reaction using boronic acid derivative (Y) in

the presence of base. The nitrile group of R1-containing Intermediate G is hydrolyzed in the presence of acid and water to afford title compound amide F.

[00174] In some embodiments are provided methods of treatment of prostate cancer in a subject, comprising administering to the subject a pharmaceutically acceptable salts of the compounds of Formula (I). The desired salt may be prepared by any suitable method available in the art, for example, treatment of the free base with an inorganic acid, such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, or with an organic acid, such as acetic acid, maleic acid, succinic acid, mandelic acid, fumaric acid, malonic acid, pyruvic acid, oxalic acid, glycolic acid, salicylic acid, a pyranosidyl acid, such as glucuronic acid or galacturonic acid, an alpha-hydroxy acid, such as citric acid or tartaric acid, an amino acid, such as aspartic acid or glutamic acid, an aromatic acid, such as benzoic acid or cinnamic acid, a sulfonic acid, such as p-toluenesulfonic acid or ethanesulfonic acid, or the like. It is specifically contemplated herein that references to the compounds of Formula (I), also refer in the alternative to pharmaceutically acceptable salts of compounds of Formula (I).

[00175] If the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is a solid, it is understood by those skilled in the art that the compounds or salts thereof may exist in different crystal or polymorphic forms, all of which are intended to be within the scope of the present invention and specified formulas.

[00176] Also provided herein are uses of isotopically-labeled compounds of Formula (I), or a pharmaceutically acceptable thereof, wherein one or more atoms is replaced by an atom having the same atomic number, but an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes suitable for inclusion in the compounds of the invention include isotopes of hydrogen, such as ^2H and ^3H , carbon, such as ^{11}C , ^{13}C and ^{14}C , chlorine, such as ^{36}Cl , fluorine, such as ^{18}F , iodine, such as ^{123}I and ^{125}I , nitrogen, such as ^{13}N and ^{15}N , oxygen, such as ^{15}O , ^{17}O and ^{18}O , phosphorus, such as ^{32}P , and sulfur, such as ^{35}S . Certain isotopically-labeled compounds of the invention, for example, those incorporating a radioactive isotope, are useful in drug and/or substrate tissue distribution studies. The radioactive isotopes tritium (^3H) and carbon-14 (^{14}C) are particularly useful for this purpose in view of their ease of incorporation and ready means of detection. Substitution with heavier isotopes such as deuterium, ^2H , may afford certain therapeutic advantages resulting from greater metabolic stability, for example, increased in vivo half-life or reduced dosage requirements, and hence may be preferred in some circumstances. Substitution with positron emitting isotopes, such as ^{11}C , ^{18}F , ^{15}O and ^{13}N , can be useful in Positron Emission Topography (PET) studies for examining substrate receptor occupancy. Isotopically-labeled compounds of Formula (I), or a pharmaceutically acceptable salt thereof, can generally be prepared by conventional techniques known to those skilled in the art or by processes analogous to those described herein, using an appropriate isotopically-labeled reagent in place of the non-labeled reagent otherwise employed.

[00177] In one aspect, the compositions described herein comprising a compound of Formula (I), or a pharmaceutically acceptable salt thereof, are used for the treatment of prostate cancer in subjects. Such

compositions may be prepared in pharmaceutically acceptable dosage forms for administration to subjects. Pharmaceutically acceptable dosage forms include, for example, liquids, suspensions, powders for reconstitution, tablets, pills, sachets, or capsules of hard or soft gelatin (*See, e.g., Remington: The Science and Practice of Pharmacy* (Gennaro, 21st Ed. Mack Pub. Co., Easton, PA (2005)). The compounds of Formula (I), or a pharmaceutically acceptable salt thereof, may be formulated into pharmaceutical compositions as described below in any pharmaceutical form recognizable to the skilled artisan as being suitable. Pharmaceutical compositions of the invention comprise a therapeutically effective amount of at least one compound of Formula (I), or a pharmaceutically acceptable salt thereof, and an inert, pharmaceutically acceptable carrier or diluent.

[00178] The pharmaceutical carriers employed may be either solid or liquid. Exemplary solid carriers are lactose, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid, and the like. Exemplary liquid carriers are syrup, peanut oil, olive oil, water, and the like. Similarly, the compositions may include time-delay or time-release material known in the art, such as glyceryl monostearate or glyceryl distearate alone or with a wax, ethylcellulose, hydroxypropylmethylcellulose, methylmethacrylate or the like. Further additives or excipients may be added to achieve the desired formulation properties. For example, a bioavailability enhancer, such as Labrasol, Gelucire or the like, or formulator, such as CMC (carboxymethylcellulose), PG (propyleneglycol), or PEG (polyethyleneglycol), may be added. Gelucire, a semi-solid vehicle that protects active ingredients from light, moisture, and oxidation, may be added, e.g., when preparing a capsule formulation.

[00179] If a solid carrier is used, the preparation can be tableted, placed in a hard gelatin capsule in powder or pellet form, or formed into a troche or lozenge. The amount of solid carrier may vary, but generally will be from about 25 mg to about 1 g. If a liquid carrier is used, the preparation may be in the form of syrup, emulsion, soft gelatin capsule, sterile injectable solution or suspension in an ampoule or vial or non-aqueous liquid suspension. If a semi-solid carrier is used, the preparation may be in the form of hard and soft gelatin capsule formulations. The inventive compositions are prepared in unit-dosage form appropriate for the mode of administration, e.g. parenteral or oral administration.

[00180] To obtain a stable water-soluble dose form, a compound of Formula (I), or a pharmaceutically acceptable salt thereof, may be dissolved in an aqueous solution of an organic or inorganic acid, such as a 0.3 M solution of succinic acid or citric acid. If a soluble salt form is not available, the compound, or a pharmaceutically acceptable salt thereof, may be dissolved in a suitable co-solvent or combinations of co-solvents. Examples of suitable co-solvents include alcohol, propylene glycol, polyethylene glycol 300, polysorbate 80, glycerin and the like in concentrations ranging from 0 to 60% of the total volume. In an exemplary embodiment, a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is dissolved in DMSO and diluted with water. The composition may also be in the form of a solution of a salt form of the active ingredient in an appropriate aqueous vehicle such as water or isotonic saline or dextrose solution.

[00181] Proper formulation is dependent upon the route of administration selected. For injection, a compound of Formula (I), or a pharmaceutically acceptable salt thereof, may be formulated into aqueous solutions, preferably in physiologically compatible buffers such as Hanks solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

[00182] For oral administration, the compounds can be formulated by combining the active compounds with pharmaceutically acceptable carriers known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for oral ingestion by a subject to be treated. Pharmaceutical preparations for oral use can be obtained using a solid excipient in admixture with the active ingredient (agent), optionally grinding the resulting mixture, and processing the mixture of granules after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients include: fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; and cellulose preparations, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as crosslinked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

[00183] Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, polyvinyl pyrrolidone, Carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active agents.

[00184] Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with fillers such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active agents may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration. For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

[00185] For administration intranasally or by inhalation, a compound of Formula (I), or a pharmaceutically acceptable salt thereof, may be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of gelatin for use in an inhaler or insufflator and the like may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[00186] The compounds may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit-dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

[00187] Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active agents may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

[00188] Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g. sterile pyrogen-free water, before use.

[00189] In addition to the formulations described above, a compound of Formula (I), or a pharmaceutically acceptable salt thereof, may also be formulated as a depot preparation. Such long-acting formulations may be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example, as an emulsion in an acceptable oil) or ion-exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt. A pharmaceutical carrier for hydrophobic compounds is a co-solvent system comprising benzyl alcohol, a non-polar surfactant, a water-miscible organic polymer, and an aqueous phase. The co-solvent system may be a VPD co-solvent system. VPD is a solution of 3% w/v benzyl alcohol, 8% w/v of the non-polar surfactant polysorbate 80, and 65% w/v polyethylene glycol 300, made up to volume in absolute ethanol. The VPD co-solvent system (VPD: 5W) contains VPD diluted 1:1 with a 5% dextrose in water solution. This co-solvent system dissolves hydrophobic compounds well, and itself produces low toxicity upon systemic administration. The proportions of a co-solvent system may be suitably varied without destroying its solubility and toxicity characteristics. Furthermore, the identity of the co-solvent components may be varied: for example, other low-toxicity non-polar surfactants may be used instead of polysorbate 80; the fraction size of polyethylene glycol may be varied; other biocompatible polymers may replace polyethylene glycol, e.g. polyvinyl pyrrolidone; and other sugars or polysaccharides may be substituted for dextrose.

[00190] Alternatively, other delivery systems for hydrophobic pharmaceutical compounds may be employed. Liposomes and emulsions are known examples of delivery vehicles or carriers for hydrophobic drugs. Certain organic solvents such as dimethylsulfoxide (DMSO) also may be employed, although usually at the cost of greater toxicity due to the toxic nature of DMSO. Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing

the therapeutic agent. Various sustained-release materials have been established and are known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein stabilization may be employed.

[00191] The pharmaceutical compositions also may comprise suitable solid- or gel-phase carriers or excipients. These carriers and excipients may provide marked improvement in the bioavailability of poorly soluble drugs. Examples of such carriers or excipients include calcium carbonate, calcium phosphate, sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

[00192] Further, the pharmaceutical composition may be incorporated into a skin patch for delivery of the drug directly onto the skin.

[00193] Additionally, the pharmaceutically acceptable formulations of the compounds of Formula (I), or a pharmaceutically acceptable salt thereof, that may be used to practice the methods disclosed herein may contain a compound of Formula (I), or a pharmaceutically acceptable salt thereof, in an amount of from about 0.5 w/w % to about 95 w/w %, or from about 1 w/w % to about 95 w/w %, or from about 1 w/w % to about 75 w/w %, or from about 5 w/w % to about 75 w/w %, or from about 10 w/w % to about 75 w/w %, or from about 10 w/w % to about 50 w/w %.

[00194] It will be appreciated that the actual dosages of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, to be administered to a subject in need thereof, will vary according to the particular agent being used, the particular composition formulated, the mode of administration, and the particular site, host, and disease being treated. Those skilled in the art using conventional dosage-determination tests in view of the experimental data for a given compound may ascertain optimal dosages for a given set of conditions. For oral administration, an exemplary daily dose generally employed will be from about 0.001 to about 1000 mg/kg of body weight, with courses of treatment repeated at appropriate intervals. In some embodiments are provided the methods disclosed herein, wherein the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject in an amount between about 0.01 mg/kg per day to about 300 mg/kg per day. In other embodiments are provided the methods disclosed herein, wherein the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject in an amount between about 0.1 mg/kg per day to about 100 mg/kg per day. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in an amount between about 10 mg to 500 mg per day. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in an amount between about 100 mg to about 400 mg per day. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in an amount between about 150 mg to about 350 mg per day. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in an amount between about 150 mg to about 300 mg per day. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in an amount between about 160 mg to about 300 mg per day. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt

thereof, is administered in an amount of about 160 mg per day. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in an amount of about 200 mg per day. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in an amount of about 240 mg per day. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in an amount of about 280 mg per day. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in an amount of about 320 mg per day.

[00195] In some embodiments are provided the methods disclosed herein, wherein the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject in an amount between about 100 mg and about 1000 mg once per day, between about 100 mg and about 900 mg once per day, between about 100 mg and about 850 mg once per day, between about 100 mg and about 800 mg once per day, between about 100 mg and about 750 mg once per day, between about 100 mg and about 700 mg once per day, between about 100 mg and about 650 mg once per day, between about 100 mg and about 600 mg once per day, between about 100 mg and about 550 mg once per day, or between about 100 mg and about 500 mg once per day.

[00196] In some embodiments are provided the methods disclosed herein, wherein the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject in an amount of about 100 mg once per day, about 150 mg once per day, about 200 mg once per day, about 300 mg once per day, about 225 mg once per day, about 275 mg once per day, about 300 mg once per day, about 325 mg once per day, about 350 mg once per day, about 375 mg once per day, about 400 mg once per day, about 425 mg once per day, about 450 mg once per day, about 475 mg once per day, about 500 mg once per day, about 525 mg once per day, about 550 mg once per day, about 575 mg once per day, about 600 mg once per day, about 625 mg once per day, about 650 mg once per day, about 675 mg once per day, about 700 mg once per day, about 725 mg once per day, about 750 mg once per day, about 775 mg once per day, about 800 mg once per day, about 825 mg once per day, about 850 mg once per day, about 875 mg once per day, about 900 mg once per day, about 925 mg once per day, about 950 mg once per day, about 975 mg once per day, or about 1000 mg once per day.

[00197] In some embodiments are provided the methods disclosed herein, wherein the Compound 4 is administered to the subject in an amount between about 100 mg and about 1000 mg once per day, between about 100 mg and about 900 mg once per day, between about 100 mg and about 850 mg once per day, between about 100 mg and about 800 mg once per day, between about 100 mg and about 750 mg once per day, between about 100 mg and about 700 mg once per day, between about 100 mg and about 650 mg once per day, between about 100 mg and about 600 mg once per day, between about 100 mg and about 550 mg once per day, or between about 100 mg and about 500 mg once per day.

[00198] In some embodiments are provided the methods disclosed herein, wherein the Compound 4 is administered to the subject in an amount of about 100 mg once per day, about 150 mg once per day, about 200 mg once per day, about 300 mg once per day, about 225 mg once per day, about 275 mg once per day,

about 300 mg once per day, about 325 mg once per day, about 350 mg once per day, about 375 mg once per day, about 400 mg once per day, about 425 mg once per day, about 450 mg once per day, about 475 mg once per day, about 500 mg once per day, about 525 mg once per day, about 550 mg once per day, about 575 mg once per day, about 600 mg once per day, about 625 mg once per day, about 650 mg once per day, about 675 mg once per day, about 700 mg once per day, about 725 mg once per day, about 750 mg once per day, about 775 mg once per day, about 800 mg once per day, about 825 mg once per day, about 850 mg once per day, about 875 mg once per day, about 900 mg once per day, about 925 mg once per day, about 950 mg once per day, about 975 mg once per day, or about 1000 mg once per day.

[00199] In some embodiments are provided the methods disclosed herein, wherein Compound 4 is administered to the subject in an amount of about 100 mg once per day. In some embodiments are provided the methods disclosed herein, wherein Compound 4 is administered to the subject in an amount of about 200 mg once per day. In some embodiments are provided the methods disclosed herein, wherein Compound 4 is administered to the subject in an amount of about 400 mg once per day. In some embodiments are provided the methods disclosed herein, wherein Compound 4 is administered to the subject in an amount of about 500 mg once per day. In some embodiments are provided the methods disclosed herein, wherein Compound 4 is administered to the subject in an amount of about 600 mg once per day. In some embodiments are provided the methods disclosed herein, wherein Compound 4 is administered to the subject in an amount of about 700 mg once per day. In some embodiments are provided the methods disclosed herein, wherein Compound 4 is administered to the subject in an amount of about 800 mg once per day. In some embodiments are provided the methods disclosed herein, wherein Compound 4 is administered to the subject in an amount of about 900 mg once per day.

[00200] In some embodiments are provided the methods disclosed herein, wherein Compound 4 is administered to the subject in an amount that provides a concentration of Compound 4 in the plasma of the subject of equal to or greater than 100 nM for at least 4 hours following administration. In some embodiments are provided the methods disclosed herein, wherein Compound 4 is administered to the subject in an amount that provides a concentration of Compound 4 in the plasma of the subject of equal to or greater than 100 nM for at least 8 hours following administration. In some embodiments are provided the methods disclosed herein, wherein Compound 4 is administered to the subject in an amount that provides a concentration of Compound 4 in the plasma of the subject of equal to or greater than 100 nM for at least 12 hours following administration. In some embodiments are provided the methods disclosed herein, wherein Compound 4 is administered to the subject in an amount that provides a concentration of Compound 4 in the plasma of the subject of equal to or greater than 100 nM for at least 16 hours following administration. In some embodiments are provided the methods disclosed herein, wherein Compound 4 is administered to the subject in an amount that provides a concentration of Compound 4 in the plasma of the subject of equal to or greater than 100 nM for at least 20 hours following administration. In some embodiments are provided the methods disclosed herein, wherein Compound 4 is administered to the subject in an amount that provides a

[00202] In some embodiments are provided the methods disclosed herein, wherein Compound 4 is administered to the subject in an amount that provides a concentration of Compound 4 in the plasma of the subject of equal to or greater than 250 nM for at least 4 hours following administration. In some embodiments are provided the methods disclosed herein, wherein Compound 4 is administered to the subject in an amount that provides a concentration of Compound 4 in the plasma of the subject of equal to or greater than 250 nM for at least 8 hours following administration. In some embodiments are provided the methods disclosed herein, wherein Compound 4 is administered to the subject in an amount that provides a concentration of Compound 4 in the plasma of the subject of equal to or greater than 250 nM for at least 12 hours following administration. In some embodiments are provided the methods disclosed herein, wherein Compound 4 is administered to the subject in an amount that provides a concentration of Compound 4 in the plasma of the subject of equal to or greater than 250 nM for at least 16 hours following administration. In some embodiments are provided the methods disclosed herein, wherein Compound 4 is administered to the subject in an amount that provides a concentration of Compound 4 in the plasma of the subject of equal to or greater than 250 nM for at least 20 hours following administration. In some embodiments are provided the methods disclosed herein, wherein Compound 4 is administered to the subject in an amount that provides a concentration of Compound 4 in the plasma of the subject of equal to or greater than 250 nM for at least 24 hours following administration.

[00216] Furthermore, the pharmaceutically acceptable formulations of the compounds of Formula (I), or a pharmaceutically acceptable salt thereof, that may be used to practice the methods disclosed herein may contain a compound of Formula (I), or a pharmaceutically acceptable salt thereof, in an amount of about 10 mg to about 2000 mg, or from about 10 mg to about 1500 mg, or from about 10 mg to about 1000 mg, or from about 10 mg to about 750 mg, or from about 10 mg to about 500 mg, or from about 25 mg to about 500 mg, or from about 50 mg to about 500 mg, or from about 100 mg to about 500 mg.

[00217] In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to a subject in need thereof once a day. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to a subject in need thereof twice a day. the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to a subject in need thereof three times a day.

[00218] In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to a subject in need thereof in 28-day cycles. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to a subject in need thereof in multiple 28-day cycles. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to a subject in need thereof for at least one 28-day cycle. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to a subject in need thereof on each day of each 28-day cycle.

[00219] In some instances, the methods described herein comprise administering the compositions and formulations comprising the compounds of Formula (I), or a pharmaceutically acceptable salt thereof, in combination with one or more additional therapeutic agents, to the subject or subject in need thereof in multiple cycles repeated on a regular schedule with periods of rest in between each cycle. For example, in some instances, treatment given for one week followed by three weeks of rest is one treatment cycle. The length of a treatment cycle depends on the treatment being given. In some embodiments, the length of a treatment cycle ranges from two to six weeks. In some embodiments, the length of a treatment cycle ranges from three to six weeks. In some embodiments, the length of a treatment cycle ranges from three to four weeks. In some embodiments, the length of a treatment cycle is three weeks (or 21 days). In some embodiments, the length of a treatment cycle is four weeks (28 days). In some embodiments, the length of a treatment cycle is 56 days. In some embodiments, a treatment cycle lasts one, two, three, or four weeks. In some embodiments, a treatment cycle lasts three weeks. In some embodiments, a treatment cycle lasts four weeks. The number of treatment doses scheduled within each cycle also varies depending on the drugs being given.

[00220] Dosages of compositions described herein can be determined by any suitable method. Maximum tolerated doses (MTD) and maximum response doses (MRD) for the compounds of Formula (I), or a pharmaceutically acceptable salt thereof, and the additional therapeutic agents when administered to the subject, can be determined via established animal and human experimental protocols as well as in the examples described herein. For example, toxicity and therapeutic efficacy of the compound of Formula (I), or

a pharmaceutically acceptable salt thereof, and the additional therapeutic agent can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, including, but not limited to, for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between the toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio between LD₅₀ and ED₅₀. The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in a human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with minimal toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. Additional relative dosages, represented as a percent of maximal response or of maximum tolerated dose, are readily obtained via the protocols.

[00221] In some embodiments, the amount of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, and/or pharmaceutical formulations comprising them that corresponds to such an amount varies depending upon factors such as the particular salt or form, disease condition and its severity, the identity (*e.g.*, age, weight, sex) of the subject or host in need of treatment, but can nevertheless be determined according to the particular circumstances surrounding the case, including, *e.g.*, the specific agent being administered, the liquid formulation type, the condition being treated, and the subject or host being treated.

Methods of detecting biomarkers

[00222] The ARSI administered to the subject having prostate cancer in combination with the EED inhibitor may be used in an amount according to the information known to those of ordinary skill in the art regarding those agents. For example, abiraterone acetate, apalutamide, darolutamide, and enzalutamide are administered to a subject according to the methods disclosed herein in amounts, and under conditions, according to those approved by one more regulatory authorities (*e.g.*, the United States Food and Drug Administration) for the treatment of various types of prostate cancer using those agents. In one embodiment, abiraterone acetate may be administered to a subject having metastatic castration-resistant prostate according to the methods disclosed herein in an amount that is 1,000 mg orally once daily with prednisone 5 mg orally twice daily. In another embodiment, abiraterone acetate may be administered to a subject having metastatic castration-sensitive prostate cancer according to the methods disclosed herein in an amount that is 1,000 mg orally once daily with prednisone 5 mg orally once daily. In another embodiment, apalutamide may be administered to a subject having metastatic castration-sensitive prostate or non-metastatic castration-resistant prostate cancer according to the methods disclosed herein in an amount that is 240 mg administered orally once daily, with or without food. In another embodiment, darolutamide may be administered to a subject having non-metastatic castration-resistant prostate or metastatic hormone-sensitive prostate cancer (in combination with docetaxel) according to the methods disclosed herein in an amount that is 600 mg administered orally twice daily with food, and for subjects with hormone-sensitive prostate cancer being treated in combination with docetaxel, the first cycle of docetaxel is administered to the subject within 6 weeks after the start of treatment with darolutamide. In another embodiment, enzalutamide may be

administered to a subject having castration-resistant prostate cancer or metastatic castration-sensitive prostate cancer according to the methods disclosed herein in an amount that is 160 mg administered orally once daily.

[00223] In some embodiments are provided the methods disclosed herein wherein the measurement of one or more biomarkers is used to determine whether a subject would benefit from the administration of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, or whether the subject is responding to the administration of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Biomarkers that may be used according to the methods disclosed herein are those known to those having ordinary skill in the art, including, but not limited to, those disclosed in The Fifth edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms, Leukemia, July 2022, vol. 36, no. 7, pages 1720-1748.

[00224] Disclosed herein are methods comprising: (a) providing a biologic sample obtained from a subject having prostate cancer; (b) assaying to detect in the biologic sample obtained from the subject a presence or absence of a biomarker; (c) detecting the presence or absence of the biomarker in the biologic sample using the methods described herein; and (d) administering to the subject a therapeutically effective amount of a compound of an androgen receptor inhibitor, and an embryonic ectoderm development (EED) inhibitor, if the biomarker is present in the biological sample. In some embodiments, the presence of one or more biomarkers described herein indicate the prostate cancer in the subject is resistant to one or more prior therapies, such as the cancer being resistant to abiraterone or one or more androgen receptor inhibitors such as the prostate cancer being resistant to apalutamide, and/or darolutamide, and/or enzalutamide.

[00225] The presence, absence, or level, of such biomarkers may be measured, collectively or individually, in a biological sample obtained from a subject, such as a sample of a solid tumor, such as prostate cancer, or from a sample of a relevant biological fluid, such as a blood sample. In some instances, the one or more biomarkers are detected in plasma or serum that is derived from a blood sample obtained from the subject. In some instances, the methods of detection disclosed herein are useful for predicting a therapeutic response to a therapy described herein (*e.g.*, the administration to a subject of an androgen receptor inhibitor, and an embryonic ectoderm development (EED) inhibitor), monitor the treatment using the therapy of, and treating with the therapy, a proliferative disease or condition described herein in a subject.

[00226] In some embodiments, the expression of a biomarker in a biological sample from a subject is measured by use of immunohistochemistry (IHC) assays. Such immunohistochemistry (IHC) assays are commercially available, or may be developed and utilized according to methods known to those having ordinary skill in the art.

[00227] Immunohistochemistry techniques utilize an antibody to probe and visualize cellular antigens in situ, generally by chromogenic or fluorescent methods. In such techniques, antibodies or antisera, polyclonal antisera, or monoclonal antibodies specific for each marker are used to detect expression. The antibodies can be detected by direct labeling of the antibodies themselves, for example, with radioactive labels, fluorescent labels, hapten labels such as, biotin, or an enzyme such as horse radish peroxidase or alkaline phosphatase. Alternatively, unlabeled primary antibody is used in conjunction with a labeled secondary antibody,

comprising antisera, polyclonal antisera, or a monoclonal antibody specific for the primary antibody.

Immunohistochemistry protocols and kits are well known in the art and are commercially available.

[00228] Two general methods of IHC are generally available; direct and indirect assays. According to the first assay, binding of antibody to the target antigen is determined directly. This direct assay uses a labeled reagent, such as a fluorescent tag or an enzyme-labeled primary antibody, which can be visualized without further antibody interaction. In a typical indirect assay, unconjugated primary antibody binds to the antigen and then a labeled secondary antibody binds to the primary antibody. Where the secondary antibody is conjugated to an enzymatic label, a chromagenic or fluorogenic substrate is added to provide visualization of the antigen. Signal amplification occurs because several secondary antibodies may react with different epitopes on the primary antibody. The primary and/or secondary antibody used for immunohistochemistry typically will be labeled with a detectable moiety. Numerous labels are available which can be generally grouped into the following categories. First, are radioisotopes, such as ^{35}S , ^{14}C , ^{125}I , ^3H , and ^{131}I . The antibody can be labeled with the radioisotope using the techniques described in Current Protocols in Immunology, Volumes 1 and 2, Coligen et al., Ed. Wiley-Interscience, New York, N.Y., Pubs. (1991) for example and radioactivity can be measured using scintillation counting. Next, are colloidal gold particles. Third are fluorescent labels including, but are not limited to, rare earth chelates (europium chelates), Texas Red, rhodamine, fluorescein, dansyl, Lissamine, umbelliferone, phycocrytherin, phycocyanin, or commercially available fluorophores such SPECTRUM ORANGE® and SPECTRUM GREEN® and/or derivatives of any one or more of the above. The fluorescent labels can be conjugated to the antibody using the techniques disclosed in Current Protocols in Immunology, supra, for example. Fluorescence can be quantified using a fluorimeter. Fourth are various enzyme-substrate labels are available and U.S. Pat. No. 4,275,149 provides a review of some of these. The enzyme generally catalyzes a chemical alteration of the chromogenic substrate that can be measured using various techniques. For example, the enzyme may catalyze a color change in a substrate, which can be measured spectrophotometrically. Alternatively, the enzyme may alter the fluorescence or chemiluminescence of the substrate. Techniques for quantifying a change in fluorescence are described above. The chemiluminescent substrate becomes electronically excited by a chemical reaction and may then emit light which can be measured (using a chemiluminometer, for example) or donates energy to a fluorescent acceptor. Examples of enzymatic labels include luciferases (e.g., firefly luciferase and bacterial luciferase; U.S. Pat. No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, malate dehydrogenase, urease, peroxidase such as horseradish peroxidase (HRPO), alkaline phosphatase, β -galactosidase, glucoamylase, lysozyme, saccharide oxidases (e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase), heterocyclic oxidases (such as uricase and xanthine oxidase), lactoperoxidase, microperoxidase, and the like. Techniques for conjugating enzymes to antibodies are described in O'Sullivan et al. Methods for the Preparation of Enzyme-Antibody Conjugates for use in Enzyme Immunoassay, in Methods in Enzym. (ed J. Langone & H. Van Vunakis), Academic press, New York, 73:147-166 (1981). Examples of enzyme-substrate combinations include, for example (i) Horseradish peroxidase (HRPO) with hydrogen peroxide as a substrate, wherein the hydrogen peroxidase oxidizes a

dye precursor [e.g., orthophenylene diamine (OPD) or 3,3',5,5'-tetramethyl benzidine hydrochloride (TMB)]. 3,3-Diaminobenzidine (DAB) may also be used to visualize the HRP-labeled antibody; (ii) alkaline phosphatase (AP) with para-Nitrophenyl phosphate as chromogenic substrate; and (iii) β -D-galactosidase (β -D-Gal) with a chromogenic substrate (e.g., p-nitrophenyl- β -D-galactosidase) or fluorogenic substrate (e.g., 4-methylumbelliferyl- β -D-galactosidase). Numerous other enzyme-substrate combinations are available to those skilled in the art. These methods are generally described in U.S. Pat. Nos. 4,275,149 and 4,318,980. Sometimes, the label is indirectly conjugated with the antibody. The skilled artisan will be aware of various techniques for achieving this. For example, the antibody can be conjugated with biotin and any of the four broad categories of labels mentioned above can be conjugated with avidin, or vice versa. Biotin binds selectively to avidin and thus, the label can be conjugated with the antibody in this indirect manner. Alternatively, to achieve indirect conjugation of the label with the antibody, the antibody is conjugated with a small hapten and one of the different types of labels mentioned above is conjugated with an anti-hapten antibody. Thus, indirect conjugation of the label with the antibody can be achieved.

[00229] Biological samples obtained from subjects comprising tissue samples may be prepared according to protocols commonly used in the art. Typically, sections of paraffin-embedded cells or tissues are obtained by (1) preserving tissue in fixative, (2) dehydrating the fixed tissue, (3) infiltrating the tissue with fixative, (4) orienting the tissue such that the cut surface accurately represents the tissue, (5) embedding the tissue in paraffin (making a paraffin block), (6) cutting tissue paraffin block with a microtome in sections of 4-5 picometers, and (7) mounting sections onto slides. The slides may then be read by a pathologist or the like assessing for the presence or absence of a biomarker, or of abnormal or normal cells or a specific cell type and provides the loci of the cell types of interest. Thus, for example, a pathologist or the like would review the slides and identify normal cells and abnormal cells (such as abnormal or tumor cells). Any means of defining the loci of the cells of interest may be used (e.g., coordinates on an X-Y axis).

[00230] Aside from the sample preparation procedures discussed above, further treatment of the tissue section prior to, during or following IHC may be desired. For example, epitope retrieval methods, such as heating the tissue sample in citrate buffer may be carried out [see, e.g., Leong et al. Appl. Immunohistochem. 4(3):201 (1996)]. Following an optional blocking step, the tissue section is exposed to primary antibody for a sufficient period of time and under suitable conditions such that the primary antibody binds to the target protein antigen in the tissue sample. Appropriate conditions for achieving this can be determined by routine experimentation.

[00231] The extent of binding of antibody to the sample is determined by using any one of the detectable labels discussed above. For example, the label is an enzymatic label (e.g. HRPO) which catalyzes a chemical alteration of the chromogenic substrate such as 3,3'-diaminobenzidine chromogen. Preferably the enzymatic label is conjugated to antibody which binds specifically to the primary antibody (e.g. the primary antibody is rabbit polyclonal antibody and secondary antibody is goat anti-rabbit antibody). Specimens thus prepared may be mounted and coverslipped. Slide evaluation is then determined, e.g. using a microscope.

[00232] IHC may be combined with morphological staining, either prior to or thereafter. After deparaffinization, the sections mounted on slides may be stained with a morphological stain for evaluation. The morphological stain to be used provides for accurate morphological evaluation of a tissue section. The section may be stained with one or more dyes each of which distinctly stains different cellular components. In one embodiment, hematoxylin is used for staining cellular nucleic acid of the slides. Hematoxylin is widely available. An example of a suitable hematoxylin is Hematoxylin II (Ventana). When lighter blue nuclei are desired, a bluing reagent may be used following hematoxylin staining. One of skill in the art will appreciate that staining may be optimized for a given tissue by increasing or decreasing the length of time the slides remain in the dye.

[00233] Automated systems for slide preparation and IHC processing are available commercially. The Ventana® BenchMark XT system is an example of such an automated system.

[00234] After staining, the tissue section may be analyzed by standard techniques of microscopy. Generally, a pathologist or the like assesses the tissue for the presence of abnormal or normal cells or a specific cell type and provides the loci of the cell types of interest. Thus, for example, a pathologist or the like would review the slides and identify normal cells and abnormal cells (such as abnormal or tumor cells). Any means of defining the loci of the cells of interest may be used (e.g., coordinates on an X-Y axis).

[00235] In some embodiments, the presence, or an absence, and/or a level of expression of the biomarker is detected in the sample obtained from a subject by analyzing the genetic material in the sample. In some embodiments, the genetic material is obtained from blood, serum, plasma, sweat, hair, tears, urine, and other techniques known by one of skill in the art. In some embodiments the sample comprises circulating tumor RNA (ctRNA). In some embodiments the sample comprises peripheral blood mononuclear cells (PBMCs). In some embodiments the sample comprises circulating tumor cells (CTCs). In some cases, the genetic material is obtained from a tumor biopsy or liquid biopsy. In some embodiments, a tumor biopsy comprises a formalin-fixed paraffin embedded biopsy, a fresh frozen biopsy, a fresh biopsy, or a frozen biopsy. In some embodiments, a liquid biopsy comprises PBMCs, circulating tumor RNA, plasma cell-free RNA, or circulating tumor cells (CTCs). Tumor and liquid biopsies can undergo additional analytic processing for sample dissociation, cell sorting, and enrichment of cell populations of interest.

[00236] In some embodiments, methods of detecting a presence, absence, or level of a biomarker in a biologic sample obtained from the subject involve detecting a nucleic acid sequence. In some cases, the nucleic acid sequence comprises deoxyribonucleic acid (DNA), such as in the case of detecting complementary DNA (cDNA) of an mRNA transcript. In some instances, the nucleic acid sequence comprises a denatured DNA molecule or fragment thereof. In some instances, the nucleic acid sequence comprises DNA selected from: genomic DNA, viral DNA, mitochondrial DNA, plasmid DNA, amplified DNA, circular DNA, circulating DNA, cell-free DNA, or exosomal DNA. In some instances, the DNA is single-stranded DNA (ssDNA), double-stranded DNA, denaturing double-stranded DNA, synthetic DNA, and combinations thereof. The circular DNA may be cleaved or fragmented. In some instances, the nucleic acid sequence comprises ribonucleic acid (RNA). In some instances, the nucleic acid sequence comprises

fragmented RNA. In some instances, the nucleic acid sequence comprises partially degraded RNA. In some instances, the nucleic acid sequence comprises a microRNA or portion thereof. In some instances, the nucleic acid sequence comprises an RNA molecule or a fragmented RNA molecule (RNA fragments) selected from: a microRNA (miRNA), a pre-miRNA, a pri-miRNA, a mRNA, a pre-mRNA, a viral RNA, a viroid RNA, a virusoid RNA, circular RNA (circRNA), a ribosomal RNA (rRNA), a transfer RNA (tRNA), a pre-tRNA, a long non-coding RNA (lncRNA), a small nuclear RNA (snRNA), a circulating RNA, a cell-free RNA, an exosomal RNA, a vector-expressed RNA, an RNA transcript, a synthetic RNA, and combinations thereof.

[00237] Disclosed herein, in some embodiments, a biomarker is detected by subjecting a sample obtained from the subject to a nucleic acid-based detection assay. In some instances, the nucleic acid-based detection assay comprises quantitative polymerase chain reaction (qPCR), reverse transcription PCT (RT-qPCR), gel electrophoresis (including for e.g., Northern or Southern blot), immunohistochemistry (IHC), immunofluorescence (IF), in situ hybridization (ISH) such as fluorescent in situ hybridization (FISH), cytochemistry, microarray, or sequencing. In some embodiments, the sequencing technique comprises next generation sequencing. In some embodiments, the methods involve a hybridization assay such as fluorogenic qPCR (e.g., TaqMan™, SYBR green, SYBR green I, SYBR green II, SYBR gold, ethidium bromide, methylene blue, Pyronin Y, DAPI, acridine orange, Blue View or phycoerythrin), which involves a nucleic acid amplification reaction with a specific primer pair, and hybridization of the amplified nucleic acid probes comprising a detectable moiety or molecule that is specific to a target nucleic acid sequence. In some instances, a number of amplification cycles for detecting a target nucleic acid in a qPCR assay is about 5 to about 30 cycles. In some instances, the number of amplification cycles for detecting a target nucleic acid is at least about 5 cycles. In some instances, the number of amplification cycles for detecting a target nucleic acid is at most about 30 cycles. In some instances, the number of amplification cycles for detecting a target nucleic acid is about 5 to about 10, about 5 to about 15, about 5 to about 20, about 5 to about 25, about 5 to about 30, about 10 to about 15, about 10 to about 20, about 10 to about 25, about 10 to about 30, about 15 to about 20, about 15 to about 25, about 15 to about 30, about 20 to about 25, about 20 to about 30, or about 25 to about 30 cycles. For TaqMan™ methods, the probe may be a hydrolysable probe comprising a fluorophore and quencher that is hydrolyzed by DNA polymerase when hybridized to a target nucleic acid. In some cases, the presence of a target nucleic acid is determined when the number of amplification cycles to reach a threshold value is less than 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, or 20 cycles. In some instances, hybridization may occur at standard hybridization temperatures, e.g., between about 35 °C and about 65 °C in a standard PCR buffer.

[00238] An additional exemplary nucleic acid-based detection assay comprises the use of nucleic acid probes conjugated or otherwise immobilized on a bead, multi-well plate, or other substrate, wherein the nucleic acid probes are configured to hybridize with a target nucleic acid sequence. In some instances, the nucleic acid probe is specific to one or more of a polynucleotide sequence that encodes a relevant biomarker as disclosed herein. In some instances, the nucleic acid probe specific to a biomarker comprises a nucleic acid probe sequence sufficiently complementary to the polynucleotide sequence that encodes the relevant biomarker

protein. In some instances, the probe comprises a transcribed polynucleotide sequence (e.g., RNA, cDNA). In some embodiments, the nucleic acid probe can be, for example, a full-length cDNA, or a portion thereof, such as an oligonucleotide of at least about 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40, 45, or 50 nucleotides in length and sufficient to specifically hybridize under standard hybridization conditions to the target nucleic acid sequence. In some embodiments, the target nucleic acid sequence is immobilized on a solid surface and contacted with a probe, for example by running the isolated target nucleic acid sequence on an agarose gel and transferring the target nucleic acid sequence from the gel to a membrane, such as nitrocellulose. In some embodiments, the probe(s) are immobilized on a solid surface, for example, in an Affymetrix gene chip array, and the probe(s) are contacted with the target nucleic acid sequence.

[00239] In some embodiments, the term “probe” with regards to nucleic acids, refers to any nucleic acid molecule that is capable of selectively binding to a specifically intended target nucleic acid sequence. In some instances, probes are specifically designed to be labeled, for example, with a radioactive label, a fluorescent label, an enzyme, a chemiluminescent tag, a colorimetric tag, or other labels or tags that are known in the art. In some instances, the fluorescent label comprises a fluorophore. In some instances, the fluorophore is an aromatic or heteroaromatic compound. In some instances, the fluorophore is a pyrene, anthracene, naphthalene, acridine, stilbene, benzoxazole, indole, benzindole, oxazole, thiazole, benzothiazole, canine, carbocyanine, salicylate, anthranilate, xanthenes dye, coumarin. Exemplary xanthene dyes include, e.g., fluorescein and rhodamine dyes. Fluorescein and rhodamine dyes include, but are not limited to 6-carboxyfluorescein (FAM), 2'7'-dimethoxy-4'5'-dichloro-6-carboxyfluorescein (JOE), tetrachlorofluorescein (TET), 6-carboxyrhodamine (R6G), N,N,N', N'-tetramethyl-6-carboxyrhodamine (TAMRA), 6-carboxy-X-rhodamine (ROX). Suitable fluorescent probes also include the naphthylamine dyes that have an amino group in the alpha or beta position. For example, naphthylamino compounds include 1-dimethylaminonaphthyl-5-sulfonate, 1-anilino-8-naphthalene sulfonate, and 2-p-toluidinyl-6-naphthalene sulfonate, 5-(2'-aminoethyl)aminonaphthalene-1-sulfonic acid (EDANS). Exemplary coumarins include, e.g., 3-phenyl-7-isocyanatocoumarin; acridines, such as 9-isothiocyanatoacridine and acridine orange; N-(p-(2-benzoxazolyl)phenyl) maleimide; cyanines, such as, e.g., indodicarbocyanine 3 (Cy3), indodicarbocyanine 5 (Cy5), indodicarbocyanine 5.5 (Cy5.5), 3-(-carboxy-pentyl)-3'-ethyl-5,5'-dimethyloxacarboxyanine (CyA); 1H, 5H, 11H, 15H-Xantheno[2,3, 4-ij: 5,6, 7-i'j']diquinolizin-18-ium, 9-[2 (or 4)-[[[6-[2,5-dioxo-1-pyrrolidinyl)oxy]-6-oxohexyl]amino]sulfonyl]-4 (or 2)-sulfophenyl]-2,3, 6,7, 12,13, 16,17-octahydro-inner salt (TR or Texas Red); or BODIPY™ dyes. In some cases, the probe comprises FAM as the dye label.

[00240] In some embodiments, detecting the one or more biomarkers comprises sequencing genetic material obtained from a sample from the subject. Sequencing can be performed with any appropriate sequencing technology, including but not limited to single-molecule real-time (SMRT) sequencing, Polony sequencing, sequencing by ligation, reversible terminator sequencing, proton detection sequencing, ion semiconductor sequencing, nanopore sequencing, electronic sequencing, pyrosequencing, Maxam-Gilbert sequencing, chain termination (e.g., Sanger) sequencing, +S sequencing, or sequencing by synthesis. Sequencing methods also include next-generation sequencing, e.g., modern sequencing technologies such as Illumina sequencing (e.g.,

Solexa), Roche 454 sequencing, Ion torrent sequencing, and SOLiD sequencing. In some cases, next-generation sequencing involves high-throughput sequencing methods. Additional sequencing methods available to one of skill in the art may also be employed.

[00241] In some instances, a number of nucleotides that are sequenced are at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 100, 150, 200, 300, 400, 500, 2000, 4000, 6000, 8000, 10000, 20000, 50000, 100000, or more than 100000 nucleotides. In some instances, the number of nucleotides sequenced is in a range of about 1 to about 100000 nucleotides, about 1 to about 10000 nucleotides, about 1 to about 1000 nucleotides, about 1 to about 500 nucleotides, about 1 to about 300 nucleotides, about 1 to about 200 nucleotides, about 1 to about 100 nucleotides, about 5 to about 100000 nucleotides, about 5 to about 10000 nucleotides, about 5 to about 1000 nucleotides, about 5 to about 500 nucleotides, about 5 to about 300 nucleotides, about 5 to about 200 nucleotides, about 5 to about 100 nucleotides, about 10 to about 100000 nucleotides, about 10 to about 10000 nucleotides, about 10 to about 1000 nucleotides, about 10 to about 500 nucleotides, about 10 to about 300 nucleotides, about 10 to about 200 nucleotides, about 10 to about 100 nucleotides, about 20 to about 100000 nucleotides, about 20 to about 10000 nucleotides, about 20 to about 1000 nucleotides, about 20 to about 500 nucleotides, about 20 to about 300 nucleotides, about 20 to about 200 nucleotides, about 20 to about 100 nucleotides, about 30 to about 100000 nucleotides, about 30 to about 10000 nucleotides, about 30 to about 1000 nucleotides, about 30 to about 500 nucleotides, about 30 to about 300 nucleotides, about 30 to about 200 nucleotides, about 30 to about 100 nucleotides, about 50 to about 100000 nucleotides, about 50 to about 10000 nucleotides, about 50 to about 1000 nucleotides, about 50 to about 500 nucleotides, about 50 to about 300 nucleotides, about 50 to about 200 nucleotides, or about 50 to about 100 nucleotides.

[00242] In some cases, a hybridization assay, such as those described herein, is used to detect the mRNA encoding the biomarker in the sample. Exemplary probe sequences that are hybridizable to a target nucleic acid sequence comprise at least 10, but no more than 100 contiguous nucleotides comprising the relevant sequence. In some cases, RNA sequencing (RNAseq) is used to detect the mRNA encoding the relevant biomarker protein.

[00243] Detection of the mRNA, in some cases, involves amplification of the subject's nucleic acid by the polymerase chain reaction (PCR). In some embodiments, the PCR assay involves use of a pair of primers capable of amplifying at least about 10 contiguous nucleobases within a nucleic acid sequence, thereby amplifying the one or more gene products in the biomarker. In fluorogenic quantitative PCR, quantitation is based on amount of fluorescence signals (TaqMan and SYBR green). In some embodiments, the nucleic acid probe is conjugated to a detectable molecule. The detectable molecule may be a fluorophore. The nucleic acid probe may also be conjugated to a quencher.

[00244] In some embodiments, the assay for detecting the presence or absence of mRNA encoding a relevant biomarker comprises reverse-transcribing the relevant mRNA molecule to produce a corresponding complementary DNA (cDNA) molecule. In some embodiments, the assay further comprises contacting the cDNA molecule with a nucleic acid probe comprising a nucleic acid sequence that is complementary to a nucleic acid sequence of the cDNA molecule. In some embodiments, the assay comprises detecting a double-

stranded hybridization product between the nucleic acid probe and the cDNA molecule. In some embodiments, the hybridization product is further amplified using a pair of primers. In some embodiments, the primers comprises a first primer with a nucleic acid sequence comprising at least 10 but not more than 50 contiguous nucleic acids within a relevant nucleic acid sequence that binds to a top strand of the double-stranded hybridization product; and a second primer with a nucleic acid sequence comprising at least 10 but not more than 50 contiguous nucleic acids within a nucleic acid sequence that is reverse complement to the relevant nucleic acid sequence that binds to a bottom strand of the double-stranded hybridization product.

[00245] Disclosed herein, in some embodiments, are methods comprising preparing a complementary DNA (cDNA) library. In some embodiments, the cDNA library is sequenced using suitable sequence methodologies disclosed herein. In some embodiments, the cDNA library is labeled, a plurality of nucleic acid probes is generated, and fixed to an immobile surface (such as a microarray). In some embodiments, the plurality of nucleic acid probes is capable of hybridizing to at least about 10 contiguous nucleotides of the two or more genes in a sample obtained from the subject. In some embodiments, detecting the presence of or absence of a biomarker includes detecting a high or a low level of expression of one or more genes as compared to a reference level.

[00246] Disclosed herein, in some embodiments, genetic material is extracted from a biologic sample obtained from a subject, e.g., a sample of blood, serum, or tissue. In certain embodiments where nucleic acids are extracted, the nucleic acids are extracted using any technique that does not interfere with subsequent analysis. In certain embodiments, this technique uses alcohol precipitation using ethanol, methanol, or isopropyl alcohol. In certain embodiments, this technique uses phenol, chloroform, or any combination thereof. In certain embodiments, this technique uses cesium chloride. In certain embodiments, this technique uses sodium, potassium or ammonium acetate or any other salt commonly used to precipitate DNA. In certain embodiments, this technique utilizes a column or resin based nucleic acid purification scheme such as those commonly sold commercially, one non-limiting example would be the GenElute Bacterial Genomic DNA Kit available from Sigma Aldrich. In certain embodiments, after extraction the nucleic acid is stored in water, Tris buffer, or Tris-EDTA buffer before subsequent analysis. In an exemplary embodiment, the nucleic acid material is extracted in water. In some cases, extraction does not comprise nucleic acid purification. In certain embodiments, RNA may be extracted from cells using RNA extraction techniques including, for example, using acid phenol/guanidine isothiocyanate extraction (RNAzol B; Biogenesis), RNeasy RNA preparation kits (Qiagen) or PAXgene (PreAnalytix, Switzerland).

[00247] In some aspects, circulating tumor RNA (ctRNA) is used to assess the expression levels of RNA molecules, shed by the tumor into the blood stream. In some embodiments, detection of ctRNA is useful, for example, for detecting and diagnosing a tumor. Because tumor DNA and RNA has acquired multiple genetic mutations, leading to tumor development, ctRNA are not an exact match to the individual's DNA and RNA, respectively. Finding DNA and RNA with genetic differences aids in tumor detection. Diagnosing the type of tumor using ctRNA can reduce the need for getting a sample of the tumor tissue (tumor biopsy), which can be challenging when a tumor is difficult to access, such as a tumor in the brain or lung.

[00248] In some embodiments, a decrease in the quantity of ctRNA suggests the solid tumor is shrinking and treatment with a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is effective. In some embodiments, a lack of ctRNA in the bloodstream indicates that the cancer has not returned after treatment with a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

[00249] Described herein are methods of assessing genetic alterations by ctRNA profiling. In some embodiments, the genomic profiling is performed after each treatment cycle with a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the gene alterations indicate that the cancer is becoming resistant to the treatment with a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the lack of gene alterations indicate that the cancer is not becoming resistant to the treatment with a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

[00250] Described herein are methods of assessing genetic alterations, including, but not limited to, mutations in certain genes and/or copy number alterations in certain genes, by circulating tumor DNA (ctDNA) and/or cell-free DNA (cfDNA) profiling. In some embodiments, the genomic profiling is performed after each treatment cycle with a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the gene alterations indicate that the cancer is becoming resistant to the treatment with a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the lack of gene alterations indicate that the cancer is not becoming resistant to the treatment with a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

[00251] In some embodiments, the expression of a biomarker is measured by immunofluorescence (IF) assays. In some embodiments, the expression of a biomarker is measured by in situ hybridization (ISH) assays. In some embodiments, the expression of a biomarker transcript levels are measured using assays such as quantitative polymerase chain reaction (qPCR), microarray, and RNA sequencing, or assays commercially available from companies such as Fluidigm and Nanostring.

[00252] Disclosed herein are methods of treating a subject having prostate cancer, comprising: (a) providing a biologic sample obtained from a subject having prostate cancer; (b) assaying to detect in the biologic sample obtained from the subject a presence or absence of a biomarker; (c) detecting the presence or absence of the biomarker in the biologic sample using the methods described herein; and (d) administering to the subject a therapeutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, if the biomarker is present in the biological sample. In some embodiments, expression of a biomarker is based on the expression level of the biomarker deviating from a reference expression level. In some embodiments, the expression level is high, relative to the reference expression level. In some embodiments, the expression level is low, relative to the reference expression level. In some embodiments, the reference expression level is derived from an individual, or a group of individuals, that do not have cancer. In some embodiments, the reference expression level is derived from an individual, or a group of individuals, that have cancer that does not therapeutically respond to the compound of Formula (I), or a

pharmaceutically acceptable salt thereof. In some embodiments, the expression level deviates from the reference expression level by at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100%.

[00253] In some embodiments, the determination of expression or the presence of a biomarker is defined based on the percentage of cells that stain weakly, moderately, or strongly for the relevant biomarker, with the threshold defining the minimal percentage of cells that are required to stain positive at the various intensity levels ($\geq a\%$ of tumor cells stain weakly, $\geq b\%$ of tumor cells stain moderately, $\geq c\%$ of tumor cells stain strongly, or a combination thereof). In some embodiments, the one or more of the cells comprising the cancer has been determined to express a biomarker when $\geq$ about 10%, $\geq$ about 15%, $\geq$ about 20%, $\geq$ about 25%, $\geq$ about 30%, $\geq$ about 35%, $\geq$ about 40%, $\geq$ about 45%, $\geq$ about 50%, $\geq$ about 55%, $\geq$ about 60%, $\geq$ about 65%, $\geq$ about 70%, $\geq$ about 75%, $\geq$ about 80%, $\geq$ about 85%, $\geq$ about 90%, or $\geq$ about 95% of the tumor cells stain weakly for the biomarker; when $\geq$ about 10%, $\geq$ about 15%, $\geq$ about 20%, $\geq$ about 25%, $\geq$ about 30%, $\geq$ about 35%, $\geq$ about 40%, $\geq$ about 45%, $\geq$ about 50%, $\geq$ about 55%, $\geq$ about 60%, $\geq$ about 65%, $\geq$ about 70%, $\geq$ about 75%, $\geq$ about 80%, $\geq$ about 85%, $\geq$ about 90%, or $\geq$ about 95% of the tumor cells stain moderately for the biomarker; when $\geq$ about 10%, $\geq$ about 15%, $\geq$ about 20%, $\geq$ about 25%, $\geq$ about 30%, $\geq$ about 35%, $\geq$ about 40%, $\geq$ about 45%, $\geq$ about 50%, $\geq$ about 55%, $\geq$ about 60%, $\geq$ about 65%, $\geq$ about 70%, $\geq$ about 75%, $\geq$ about 80%, $\geq$ about 85%, $\geq$ about 90%, or $\geq$ about 95% of the tumor cells stain strongly for the biomarker; or any combinations thereof.

Kits and articles of manufacture

[00254] Disclosed herein, in certain embodiments, are kits and articles of manufacture for use with one or more methods and compositions described herein. Such kits include a carrier, package, or container that is compartmentalized to receive one or more containers such as vials, tubes, and the like, each of the container(s) comprising one of the separate elements to be used in a method described herein. Suitable containers include, for example, bottles, vials, syringes, and test tubes. In one embodiment, the containers are formed from a variety of materials such as glass or plastic.

[00255] A kit typically includes labels listing contents and/or instructions for use, and package inserts with instructions for use. A set of instructions will also typically be included.

[00256] In one embodiment, a label is on or associated with the container. In one embodiment, a label is on a container when letters, numbers or other characters forming the label are attached, molded, or etched into the container itself, a label is associated with a container when it is present within a receptacle or carrier that also holds the container, e.g., as a package insert. In one embodiment, a label is used to indicate that the contents are to be used for a specific therapeutic application. The label also indicates directions for use of the contents, such as in the methods described herein.

[00257] In certain embodiments, the pharmaceutical compositions are presented in a pack or dispenser device which contains one or more unit dosage forms containing a compound provided herein. The pack, for example, contains metal or plastic foil, such as a blister pack. In one embodiment, the pack or dispenser device is accompanied by instructions for administration. In one embodiment, the pack or dispenser is also accompanied with a notice associated with the container in form prescribed by a governmental agency

regulating the manufacture, use, or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the drug for human or veterinary administration. Such notice, for example, is the labeling approved by the U.S. Food and Drug Administration for drugs, or the approved product insert. In one embodiment, compositions containing a compound provided herein formulated in a compatible pharmaceutical carrier are also prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

[00258] Disclosed herein is a kit comprising (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor, for use in treating prostate cancer in a subject in need thereof and a package insert comprising instructions for determining when the administration to the subject having prostate cancer measuring the expression of a biomarker described herein in one or more of the cells comprising the prostate cancer and (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor using if one or more of the cells comprising the prostate cancer has been determined to express the biomarker.

Embodiments

[00259] Embodiment 1: A method of treating prostate cancer in a subject, comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor.

[00260] Embodiment 2: A method of treating prostate cancer in a subject, wherein the prostate cancer in the subject has been determined to be resistant to one or more first androgen receptor signaling inhibitors (ARSI), comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor.

[00261] Embodiment 3: The method of embodiment 2, wherein the one or more first androgen receptor signaling inhibitors (ARSI) is selected from one or more first CYP17 inhibitors and one or more first androgen receptor inhibitors.

[00262] Embodiment 4: The method of embodiment 3, wherein the one or more first androgen receptor signaling inhibitors (ARSI) is selected from one or more first CYP17 inhibitors.

[00263] Embodiment 5: The method of embodiment 4, wherein the one or more first CYP17 inhibitor is abiraterone acetate.

[00264] Embodiment 6: The method of embodiment 2, wherein the one or more first androgen receptor signaling inhibitors (ARSI) is selected from one or more androgen receptor inhibitors.

[00265] Embodiment 7: The method of embodiment 6, wherein the one or more first androgen receptor inhibitors is selected from apalutamide, darolutamide, and enzalutamide.

[00266] Embodiment 8: A method of treating prostate cancer in a subject, wherein the prostate cancer in the subject has been determined to be resistant to a CYP17 inhibitor, comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor.

[00267] Embodiment 9: The method of embodiment 8, wherein the CYP17 inhibitor is abiraterone acetate.

[00268] Embodiment 10: A method of treating prostate cancer in a subject, wherein the prostate cancer in the subject has been determined to be resistant to abiraterone, comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor.

[00269] Embodiment 11: A method of treating prostate cancer in a subject, wherein the subject has received prior administration of one or more CYP17 inhibitors, comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor.

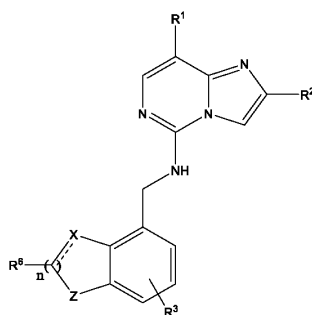
[00270] Embodiment 12: A method of treating prostate cancer in a subject, wherein the subject (i) has received prior administration of one or more CYP17 inhibitors, and (ii) is androgen receptor inhibitor naïve, comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor.

[00271] Embodiment 13: The method of any one of embodiments 1 to 12, wherein the androgen receptor inhibitor is selected from apalutamide, darolutamide, and enzalutamide.

[00272] Embodiment 14: The method of any one of embodiments 1 to 13, wherein the embryonic ectoderm development (EED) inhibitor is a small molecule having a molecular weight of less or equal to 1000 Daltons.

[00273] Embodiment 15: The method of any one of embodiments 1 to 14, wherein the embryonic ectoderm development (EED) inhibitor is selected from EED226, A-395, APG-5918, BR-001, BR-002, EEDi-5285, EEDi-1056, FTX-6058, HJM-353, and MAK683.

[00274] Embodiment 16: The method of any one of embodiments 1 to 13, wherein the embryonic ectoderm development (EED) inhibitor is a compound of Formula (I)



Formula (I)

or a pharmaceutically acceptable salt thereof:

wherein:

----- represents a single or a double bond;

Z is O or S;

X is O, CR⁵, CR⁵OH, or C(R⁵)₂, wherein:

when X is O, ----- is a single bond;

when X is C(R⁵)₂, ----- is a single bond;

when X is CR⁵OH, ----- is a single bond; or

when X is CR⁵, ----- is a double bond;

R¹ is aryl, heteroaryl, L-cycloalkyl, -N(R⁵)heterocyclyl, or L-heterocyclyl, wherein the aryl, the heteroaryl or the cyclyl portion of the L-cycloalkyl, -N(R⁵)heterocyclyl, or L-heterocyclyl is optionally substituted with one or more R⁴;

R² is cyano, -COOR⁵, -C(O)N(R⁵)₂, or -C(O)N(R⁵)₂ wherein each R⁵ taken together with the nitrogen atom to which they are attached form a 5 - 8 membered heterocyclic ring optionally substituted with one or more R⁴;

each R³ is independently C₁-C₃ alkyl or halogen;

each R⁴ is independently oxo, cyano, halogen, -PO₃(C₁-C₃ alkyl)₂, hydroxyl, alkoxy, hydroxyalkyl, heteroalkyl, aralkyl, haloalkyl, -COOR⁵, -Y²-haloalkyl, -Y¹-C₁-C₆ alkyl, -Y²-C₁-C₆ alkyl, -L-cycloalkyl, -L-heteroaryl, -L-heterocyclyl, -Y¹-heterocyclyl, -Y²-heterocyclyl, -L-N(R⁵)₂, -O-L-N(R⁵)₂, -C(CF₃)N(R⁵)₂, -Y¹-N(R⁵)₂, or -Y²-N(R⁵)₂, wherein the ring portion of the aralkyl, -L-cycloalkyl, -L-heteroaryl, -L-heterocyclyl or -Y¹-heterocyclyl is optionally substituted with one or more R⁷;

L is a bond or C₁-C₄ alkylene;

Y¹ is a bond, -C(O)-, or -NHC(O)-;

Y² is a bond, -S-, -SO-, -SO₂-, or -NR⁵SO₂-;

each R⁵ is hydrogen or C₁-C₃ alkyl;

R⁶ is hydrogen, C₁-C₃ alkyl, halogen, haloalkyl, hydroxyalkyl, or heteroalkyl;

each R⁷ is oxo, cyano, hydroxyl, alkoxy, halogen, haloalkyl, hydroxyalkyl, heteroalkyl, cycloalkyl, -L-N(R⁵)₂, C₁-C₆ alkyl, or -Y¹-heterocyclyl; and

n is 1 or 2.

[00275] Embodiment 17: The method of embodiment 16, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, Z is O.

[00276] Embodiment 18: The method of embodiment 17, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, Z is S.

[00277] Embodiment 19: The method of any one of embodiments 16 to 18, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, n is 1.

[00278] Embodiment 20: The method of any of embodiments 16 to 19, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R² is cyano.

[00279] Embodiment 21: The method of any of embodiments 16 to 19, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R² is -COOR⁵.

[00280] Embodiment 22: The method of any of embodiments 16 to 19, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R² is -C(O)N(R⁵)₂.

[00281] Embodiment 23: The method of any of embodiments 16 to 22, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R³ is halogen.

[00282] Embodiment 24: The method of embodiment 23, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R³ is fluorine.

[00283] Embodiment 25: The method of any of embodiments 16 to 24, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, X is $C(R^5)_2$ and ===== is a single bond.

[00284] Embodiment 26: The method of any of embodiments 16 to 24, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, X is CR^5 and ===== is a double bond.

[00285] Embodiment 27: The method of any of embodiments 16 to 24, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, X is O and ===== is a single bond.

[00286] Embodiment 28: The method of any of embodiments 16 to 27, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^1 is aryl optionally substituted with one or more R^4 .

[00287] Embodiment 29: The method of embodiment 28, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^1 is phenyl optionally substituted with one or more R^4 .

[00288] Embodiment 30: The method of embodiment 29, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^1 is phenyl is substituted with one, two or three R^4 .

[00289] Embodiment 31: The method of embodiment 30, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, the one, two or three R^4 are each independently halogen, $-PO_3(C_1-C_3 \text{ alkyl})_2$, hydroxyl, hydroxyalkyl, aralkyl, haloalkyl, $-COOR^5$, $-Y^1-C_1-C_6 \text{ alkyl}$, $Y^2-C_1-C_6 \text{ alkyl}$, $-L-N(R^5)_2$, $-O-L-N(R^5)_2$, $-C(CF_3)N(R^5)_2$, $-Y^1-N(R^5)_2$, $-Y^2-N(R^5)_2$, Y^2 -haloalkyl, $-L$ -heteroaryl, $-L$ -heterocyclyl, or $-Y^1$ -heterocyclyl, wherein the heterocyclyl portion of the $-L$ -heterocyclyl or $-Y^1$ -heterocyclyl is optionally substituted with one or more R^7 .

[00290] Embodiment 32: The method of embodiment 31, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^4 is $-Y^1-C_1-C_6 \text{ alkyl}$ and Y^1 is a bond and the $C_1-C_6 \text{ alkyl}$ is methyl, ethyl, isopropyl, butyl, or pentyl.

[00291] Embodiment 33: The method of embodiment 31, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^4 is $-Y^2-C_1-C_6 \text{ alkyl}$ and Y^2 is a $-SO_2-$ and the $C_1-C_6 \text{ alkyl}$ is methyl.

[00292] Embodiment 34: The method of embodiment 31, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^4 is $-Y^2$ -haloalkyl and Y^2 is $-S-$ or $-SO_2-$ and the haloalkyl is trifluoromethyl.

[00293] Embodiment 35: The method of embodiment 31, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^4 is $-L-N(R^5)_2$ and L is a bond and each R^5 is hydrogen, each R^5 is methyl, or one R^5 is methyl and one R^5 is hydrogen.

[00294] Embodiment 36: The method of embodiment 31, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^4 is $-L-N(R^5)_2$ and L is methylene or ethylene and each R^5 is hydrogen, each R^5 is methyl or one R^5 is methyl and one R^5 is hydrogen.

[00295] Embodiment 37: The method of embodiment 31, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^4 is $-Y^1-N(R^5)_2$, Y^1 is $-C(O)-$ and each R^5 independently is hydrogen, each R^5 is independently methyl or one R^5 is methyl and one R^5 is hydrogen.

[00296] Embodiment 38: The method of embodiment 31, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^4 is $-Y^2-N(R^5)_2$, Y^2 is $-SO_2-$ and each R^5 independently is hydrogen, each R^5 is methyl, or one R^5 is methyl and one R^5 is independently hydrogen.

[00297] Embodiment 39: The method of embodiment 31, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^4 is $-Y^1$ -heterocyclyl and Y^1 is $-C(O)-$ and the heterocyclyl portion of the L-heterocyclyl is piperazinyl or 4-methyl-piperazinyl.

[00298] Embodiment 40: The method of embodiment 31, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^4 is $-L$ -heterocyclyl and L is a bond and the heterocyclyl portion of the L-heterocyclyl is azetidiny, oxetanyl, pyrrolidinyl, tetrahydrofuranyl, piperidinyl, piperazinyl, or $3\lambda^2$ -azabicyclo[3.1.0]hexanyl, each optionally substituted with one or more R^7 selected from oxo, C_1 - C_3 alkyl, alkoxy, hydroxyl and halogen.

[00299] Embodiment 41: The method of embodiment 31, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^4 is $-L$ -heterocyclyl, wherein L is a methylene and the heterocyclyl portion of the L-heterocyclyl is azetidiny, oxetanyl, pyrrolidinyl piperidinyl, each optionally substituted with one or more R^7 selected from C_1 - C_3 alkyl, alkoxy, hydroxyl and halogen.

[00300] Embodiment 42: The method of embodiment 31, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^4 is $-Y^1$ -heterocyclyl and Y^1 is $-C(O)-$ and the heterocyclyl portion of the Y^1 -heterocyclyl is morpholinyl optionally substituted with one or more C_1 - C_3 alkyl.

[00301] Embodiment 43: The method of embodiment 31, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^4 is $-L$ -heteroaryl optionally substituted with one or more R^7 .

[00302] Embodiment 44: The method of embodiment 31, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^4 is tetrazolyl.

[00303] Embodiment 45: The method of embodiment 31, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^4 is $-PO_3(C_1-C_3 \text{ alkyl})_2$.

[00304] Embodiment 46: The method of embodiment 31, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^4 is $-COOR^5$.

[00305] Embodiment 47: The method of embodiment 31, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^4 is hydroxyalkyl.

[00306] Embodiment 48: The method of embodiment 31, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^4 is $-O-L-N(R^5)_2$.

[00307] Embodiment 49: The method of embodiment 31, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^4 is aralkyl.

[00308] Embodiment 50: The method of any of embodiments 15 to 26, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^1 is heteroaryl optionally substituted with one or more R^4 .

[00309] Embodiment 51: The method of embodiment 50, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^1 is pyrazolyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, triazinyl, pyridyl, pyridinyl-2-one, pyrazinyl, pyridazinyl, pyrimidinyl, isoxazolyl, isoindolinyl,

naphthyridinyl, 1,2,3,4-tetrahydroisoquinolinyl, or 5,6-dihydro-4H-pyrrolo[1,2-b]pyrazolyl, each optionally substituted with one or more R^4 .

[00310] Embodiment 52: The method of embodiment 50, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^1 is substituted with one or more R^4 ; wherein each R^4 is independently cyano, halogen, $-Y^1-C_1-C_6$ alkyl, $-Y^2-C_1-C_6$ alkyl, alkoxy, hydroxyalkyl, heteroalkyl, haloalkyl, $-L$ -cycloalkyl, $-L-N(R^5)_2$, $-Y^1-N(R^5)_2$, $-L$ -heteroaryl, $-L$ -heterocyclyl, or $-Y^1$ -heterocyclyl, wherein the heteroaryl of the $-L$ -heteroaryl or the heterocyclyl portion of the L -heterocyclyl, or Y^1 -heterocyclyl is optionally substituted with one or more R^7 .

[00311] Embodiment 53: The method of embodiment 50, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^1 is pyrazolyl optionally substituted with one R^4 independently selected from hydroxyalkyl, heteroalkyl, haloalkyl, $-Y^1-C_1-C_6$ alkyl, $-L-N(R^5)_2$, L -heterocyclyl or L -heteroaryl, wherein the heteroaryl of the L -heteroaryl or the heterocyclyl portion of the L -heterocyclyl is optionally substituted with one or more R^7 .

[00312] Embodiment 54: The method of embodiment 53, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^4 is $-L$ -heteroaryl and L is methylene wherein the heteroaryl is pyridyl optionally substituted with one or more R^7 .

[00313] Embodiment 55: The method of embodiment 53, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^4 is $-L$ -heterocyclyl optionally substituted with one or more R^7 where L is a bond and the heterocyclyl portion of the L -heterocyclyl is oxetanyl, tetrahydrofuranyl, tetrahydropyranyl, piperazinyl, or 4-methylpiperazinyl.

[00314] Embodiment 56: The method of embodiment 53, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^4 is $-L$ -heterocyclyl optionally substituted with one or more R^7 where L is methylene and the heterocyclyl portion of the L -heterocyclyl is azetidyl, oxetanyl, pyrrolidinyl, pyrrolidinone, tetrahydrofuranyl, tetrahydropyranyl, morpholinyl, piperazinyl, or 4-methylpiperazinyl.

[00315] Embodiment 57: The method of embodiment 53, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^4 is $-L-N(R^5)_2$ where L is methylene and each R^5 is independently hydrogen, each R^5 is independently C_1-C_3 alkyl or one R^5 is C_1-C_3 alkyl and one R^5 is hydrogen.

[00316] Embodiment 58: The method of embodiment 53, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^4 is $-Y^1-C_1-C_6$ alkyl where Y^1 is a bond and the C_1-C_6 alkyl is methyl, ethyl, or isopropyl.

[00317] Embodiment 59: The method of embodiment 50, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^1 is pyrazolyl optionally substituted with two R^4 groups each independently selected from hydroxyalkyl, heteroalkyl, haloalkyl, and $-Y^1-C_1-C_6$ alkyl.

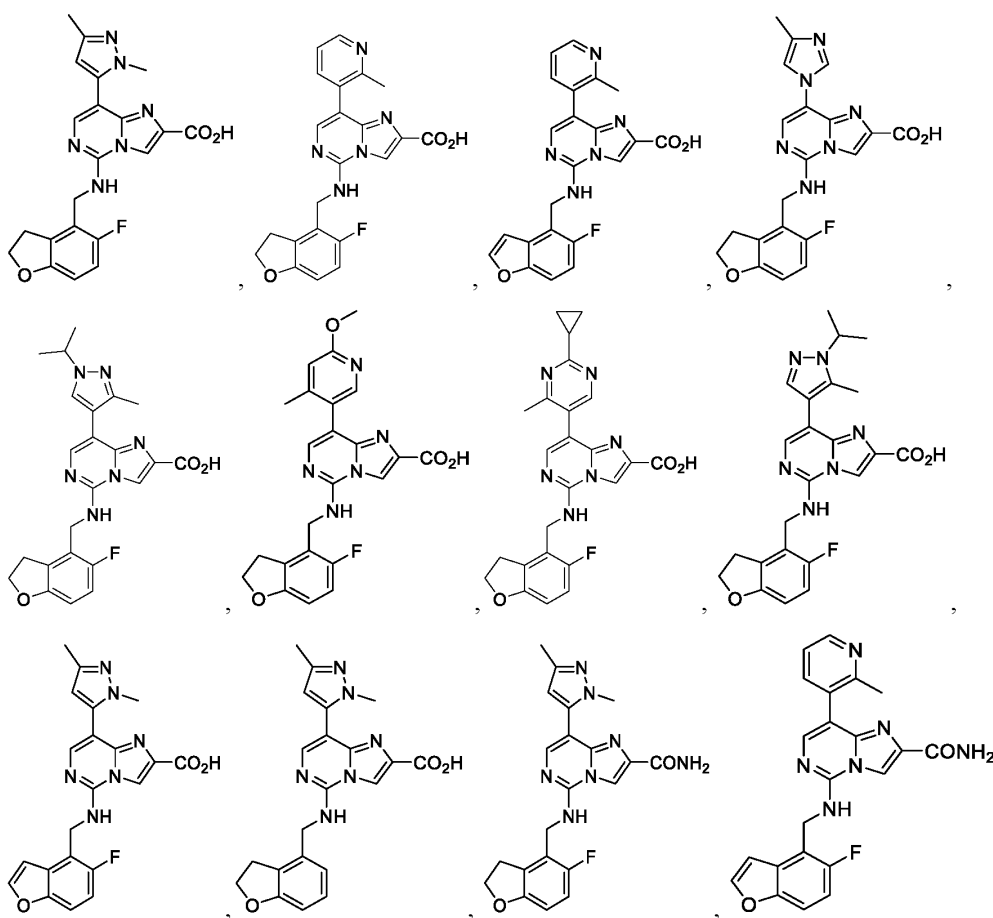
[00318] Embodiment 60: The method of embodiment 50, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^1 is pyridyl optionally substituted with one R^4 independently selected from cyano, halogen, alkoxy, hydroxyalkyl, heteroalkyl, haloalkyl, $-Y^1-C_1-C_6$ alkyl, $-L-N(R^5)_2$, $-Y^1-N(R^5)_2$, $-L$ -cycloalkyl, or $-L$ -heterocyclyl optionally substituted with one or more R^7 .

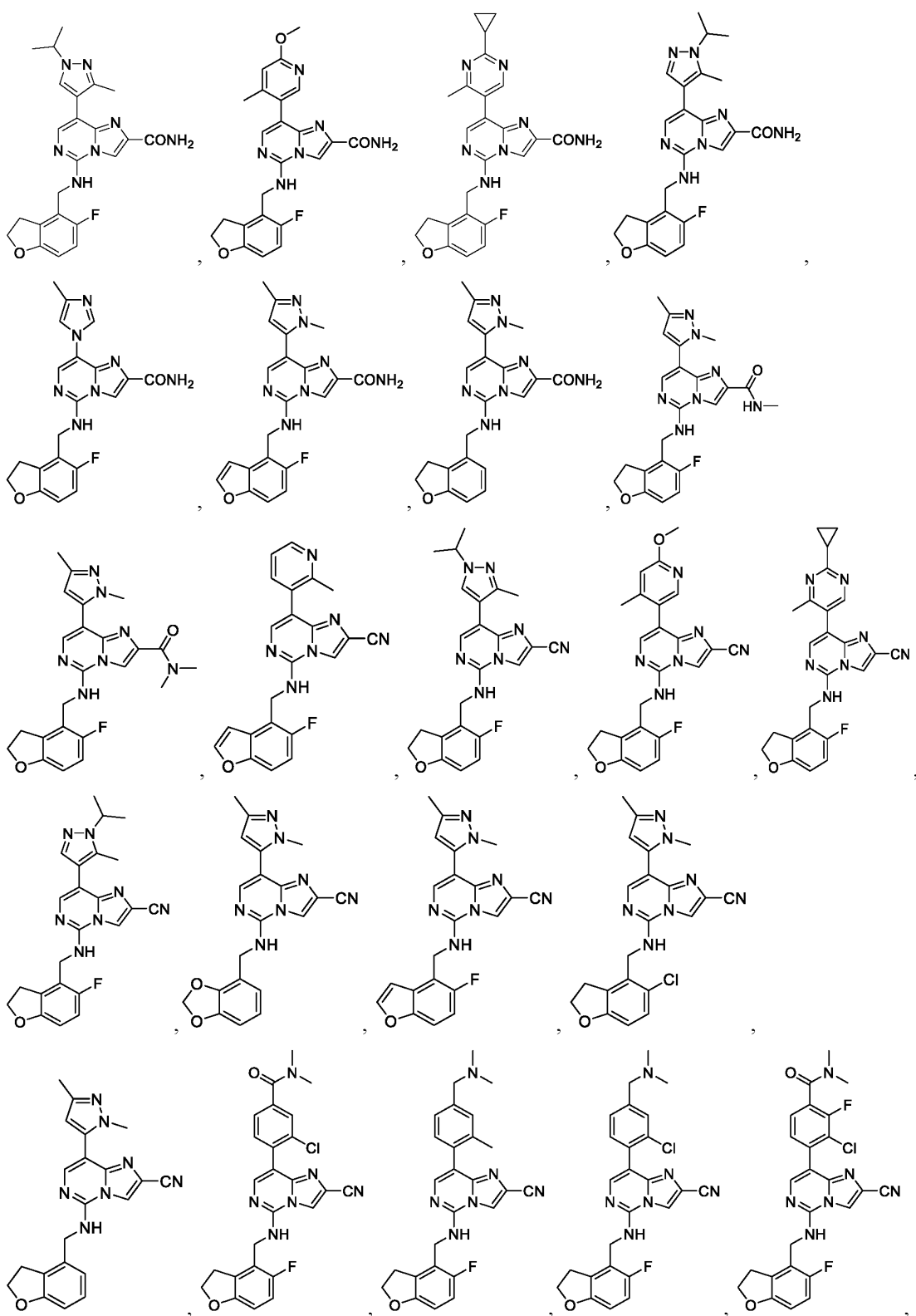
[00319] Embodiment 61: The method of any of embodiments 16 to 27, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^1 is -L-cycloalkyl optionally substituted with one or more R^4 .

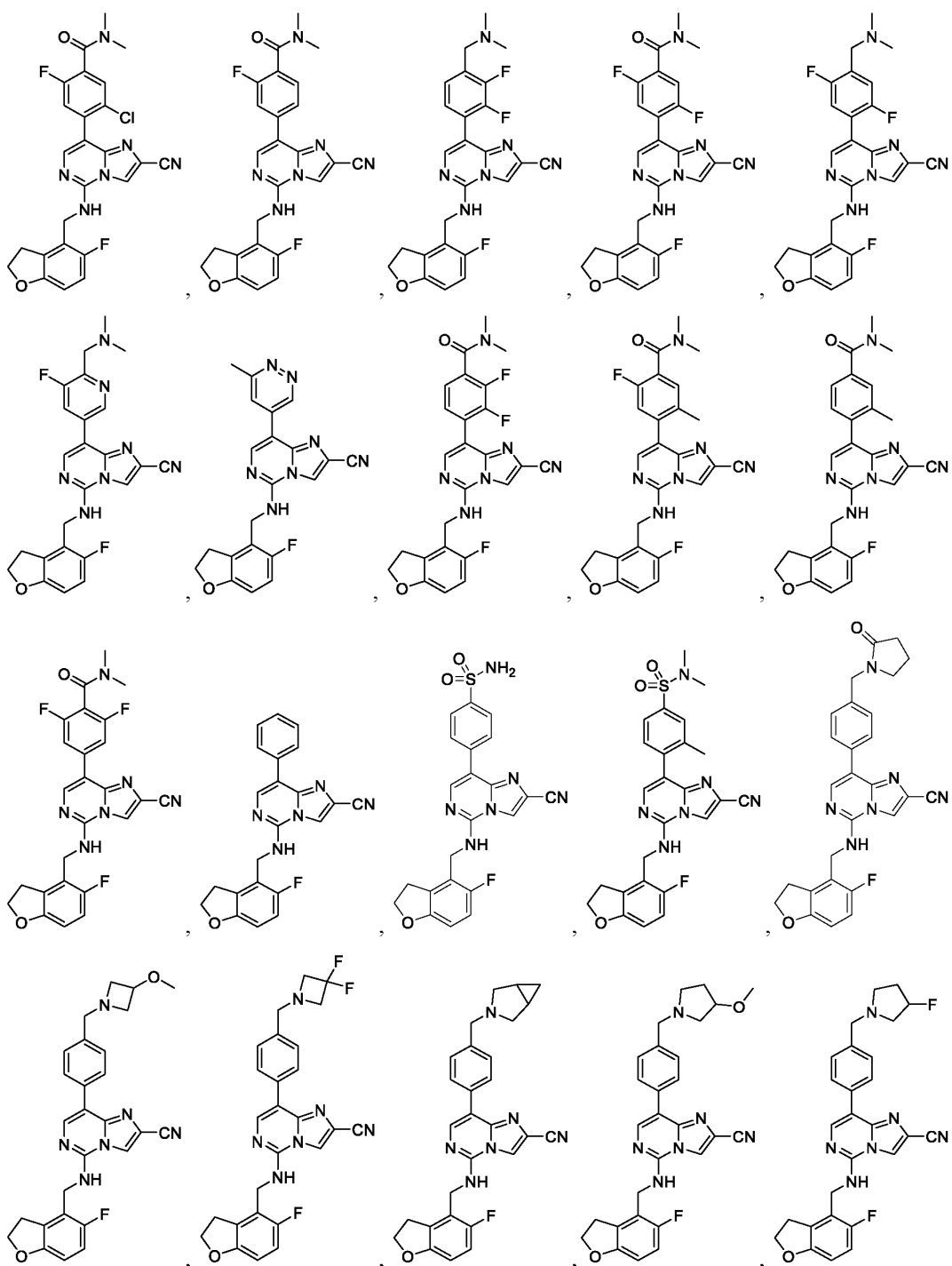
[00320] Embodiment 62: The method of any of embodiments 16 to 27, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, R^1 is -L-heterocyclyl optionally substituted with one or more R^4 .

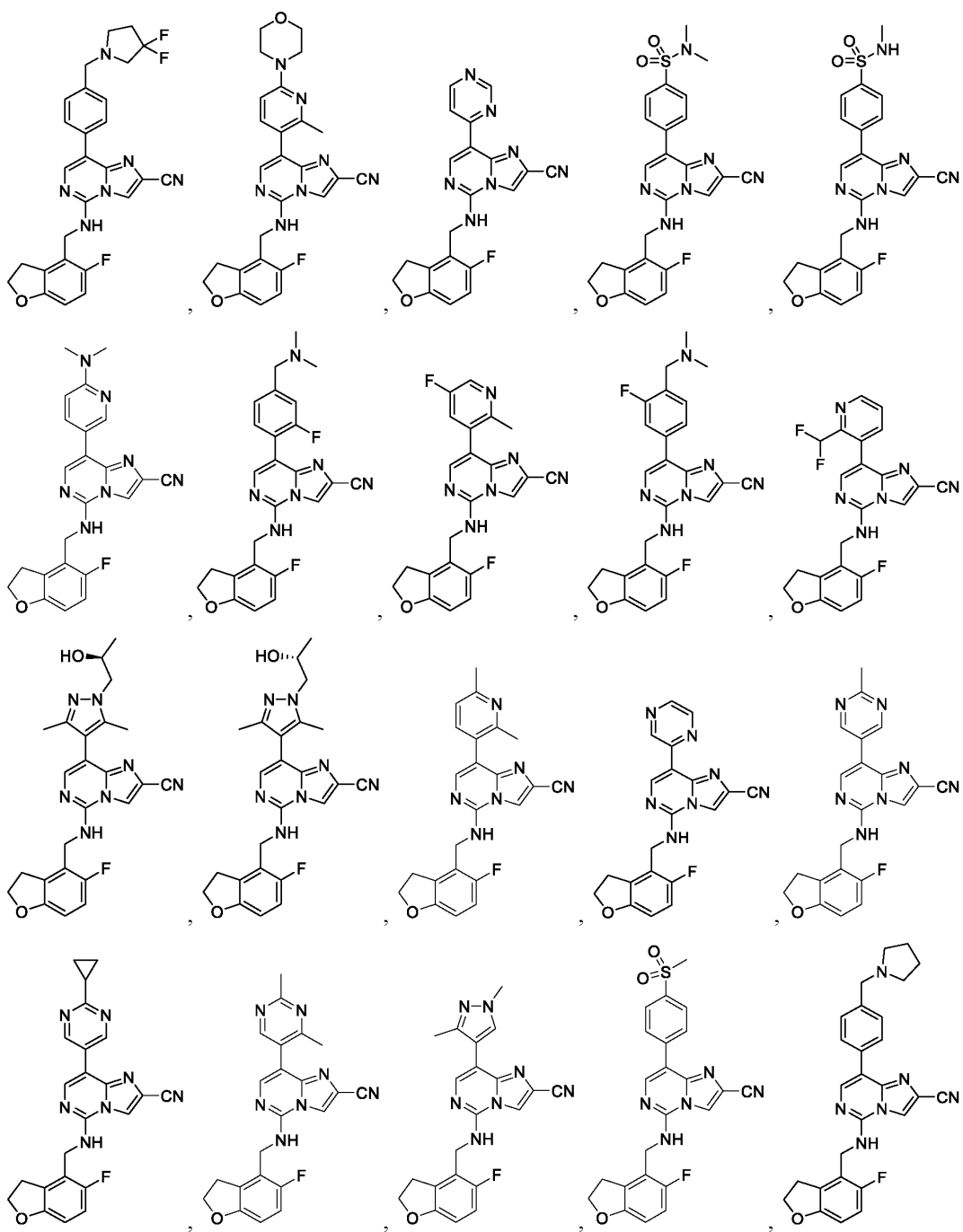
[00321] Embodiment 63: The method of embodiment 62, wherein in the compound of Formula (I), or a pharmaceutically acceptable salt thereof, L is a bond and the heterocyclyl is piperidinyl or tetrahydropyranyl.

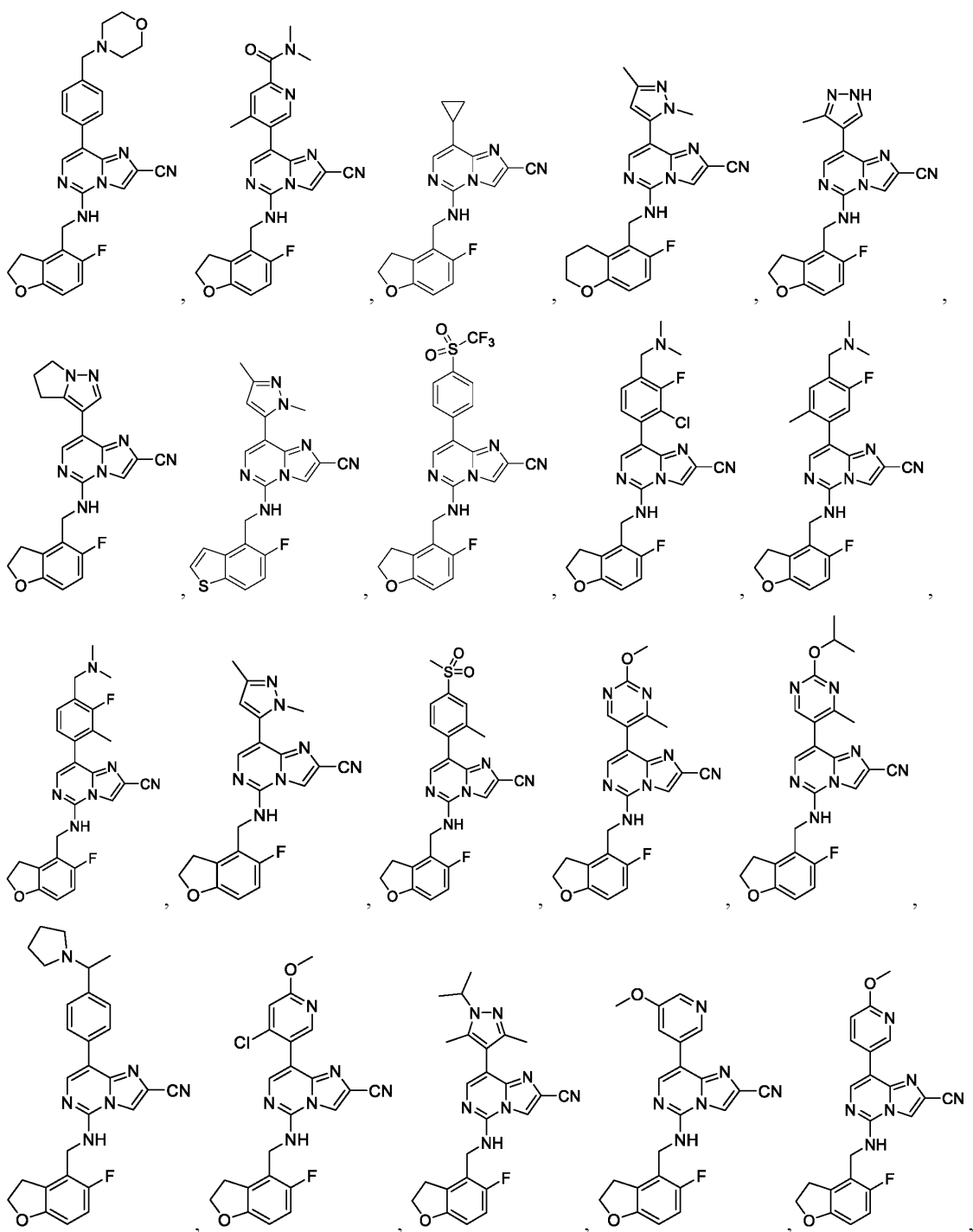
[00322] Embodiment 64: The method of any one of embodiments 1 to 13, wherein the embryonic ectoderm development (EED) inhibitor is a compound selected from the group consisting of:

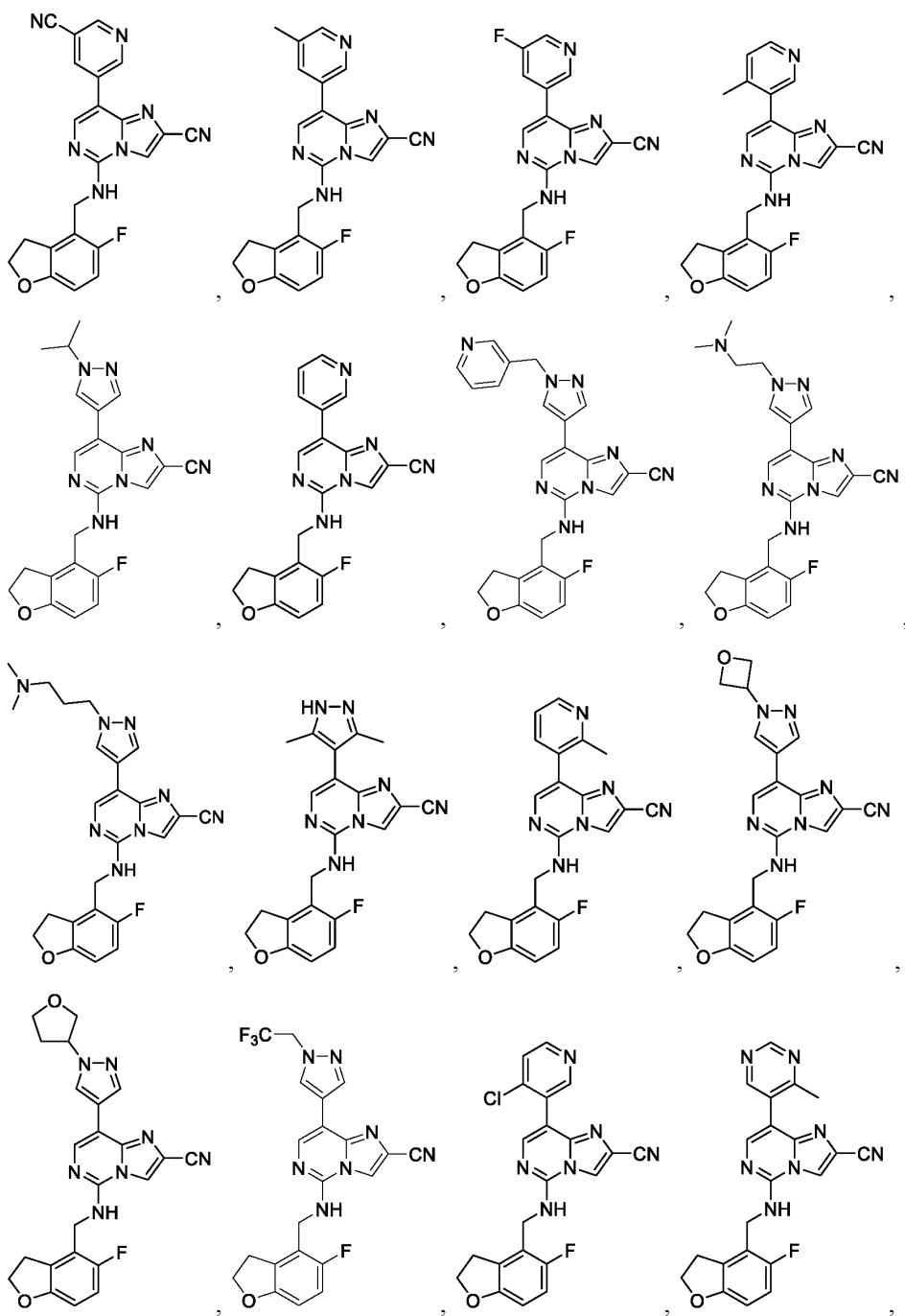


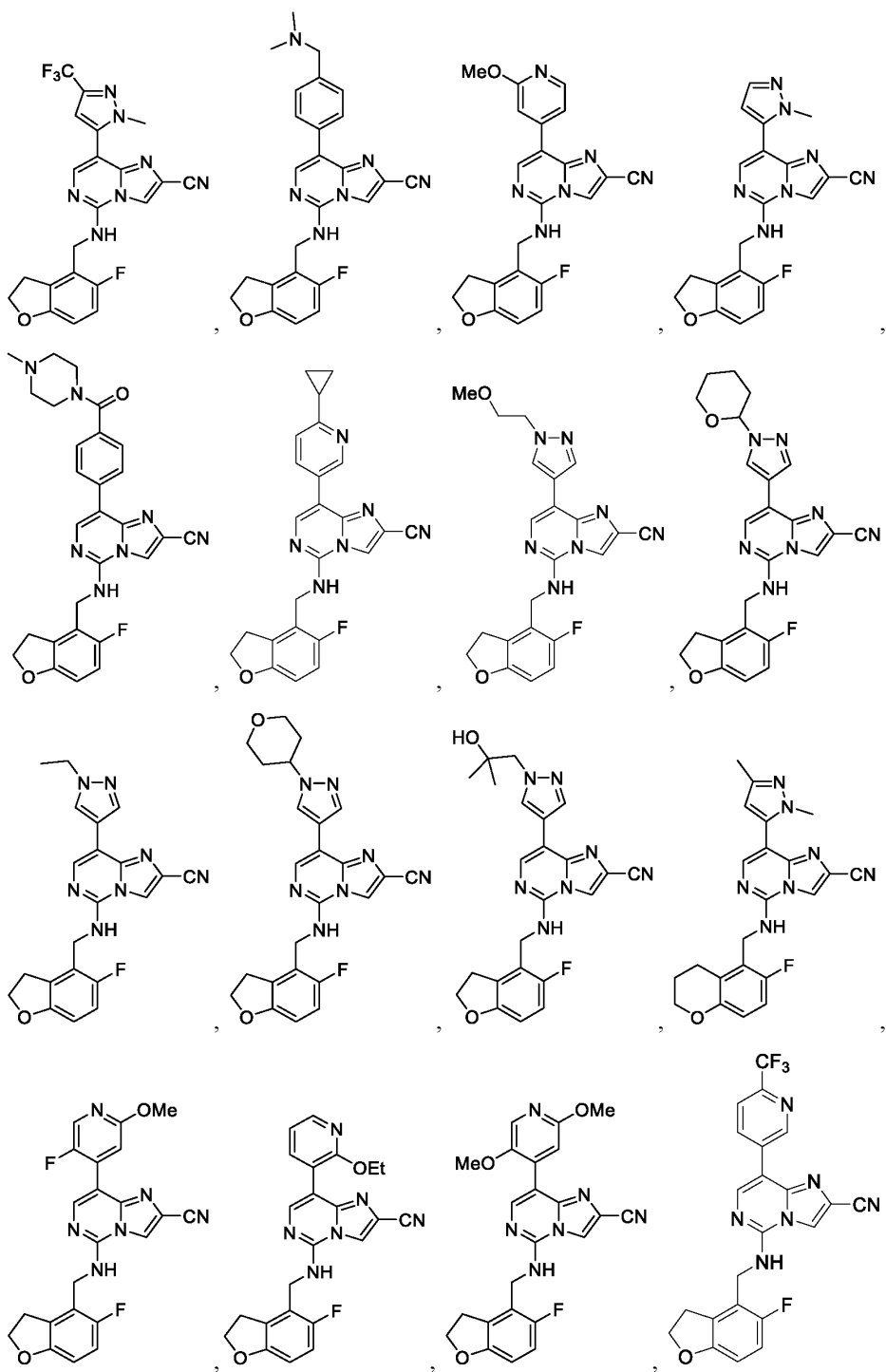


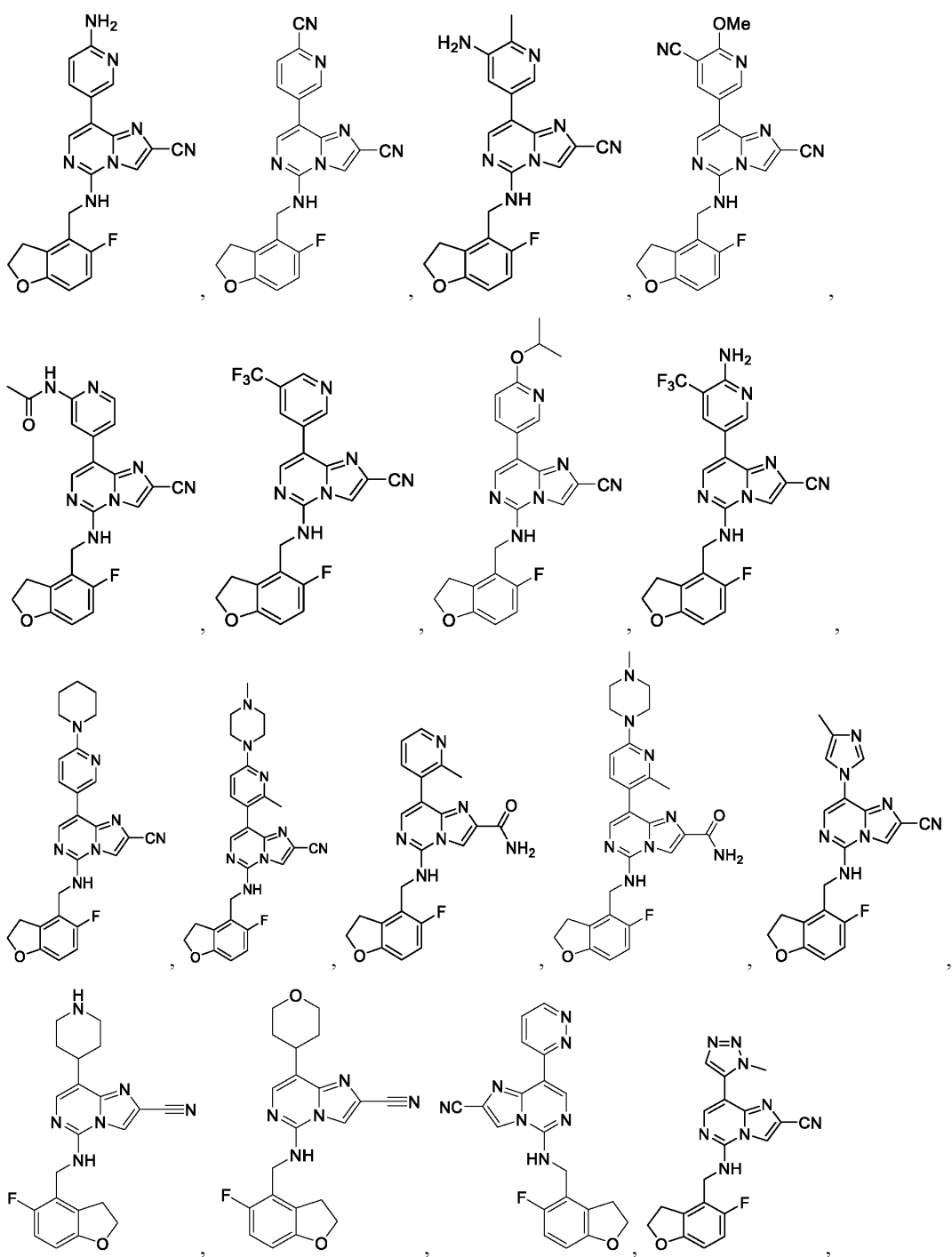


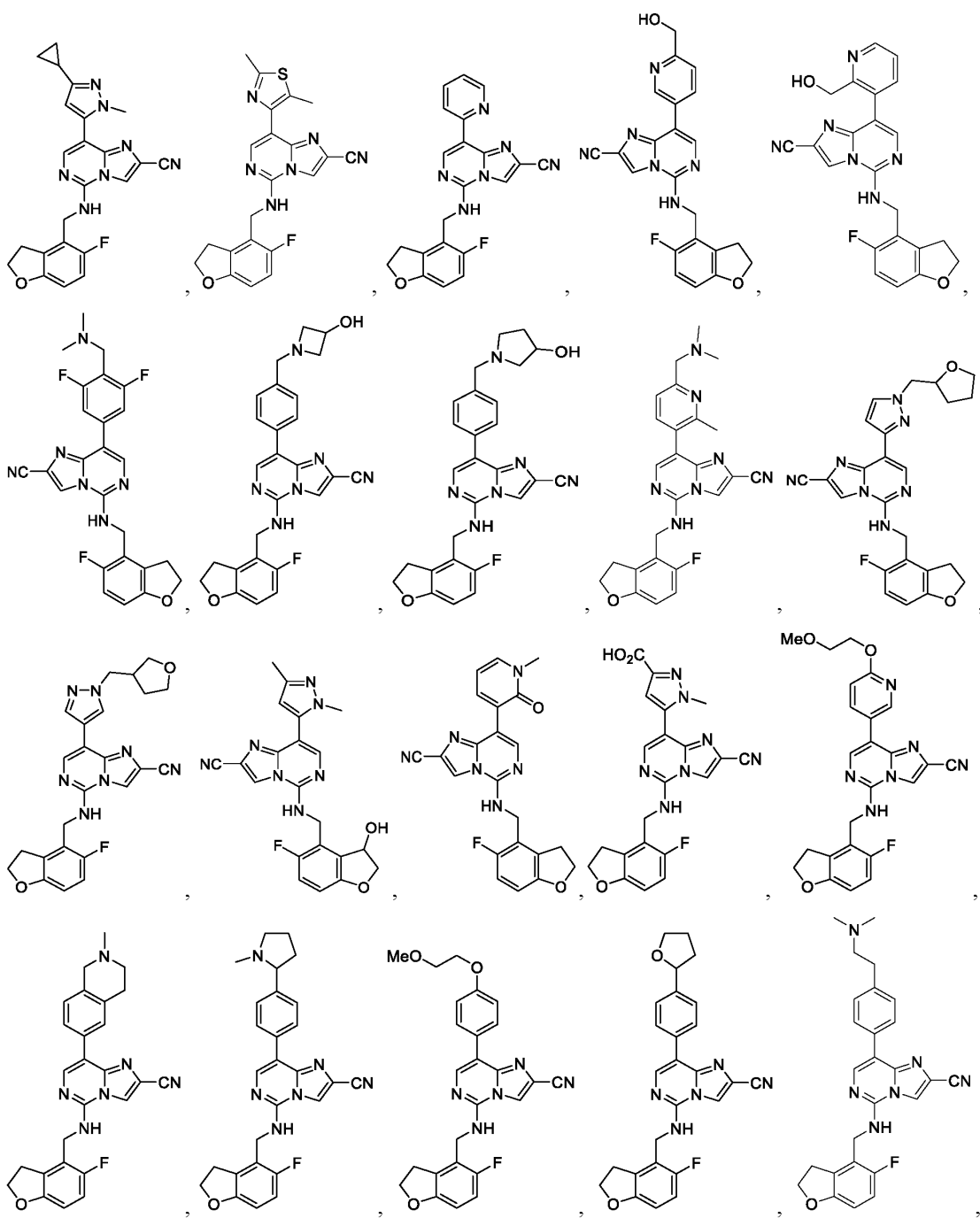




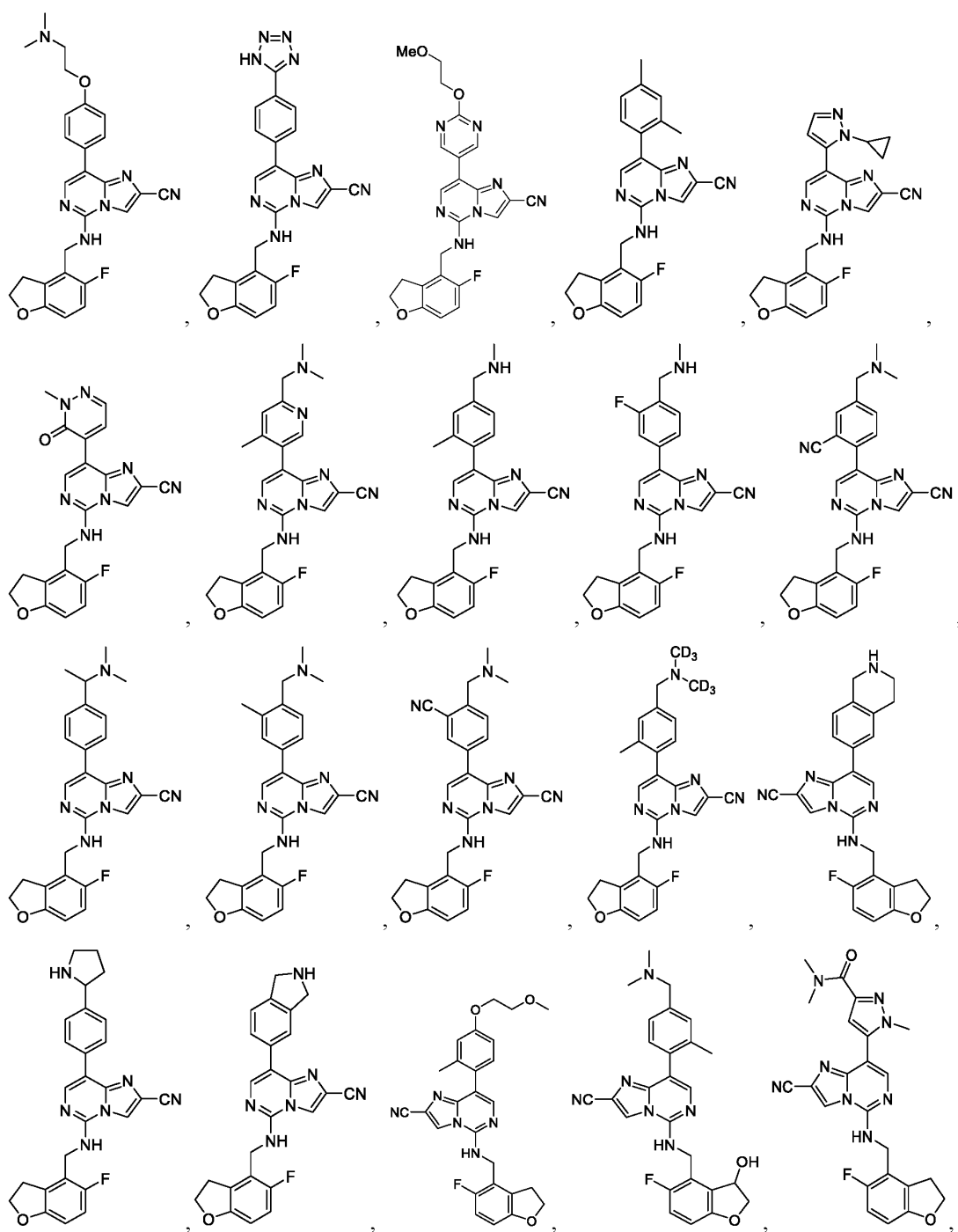


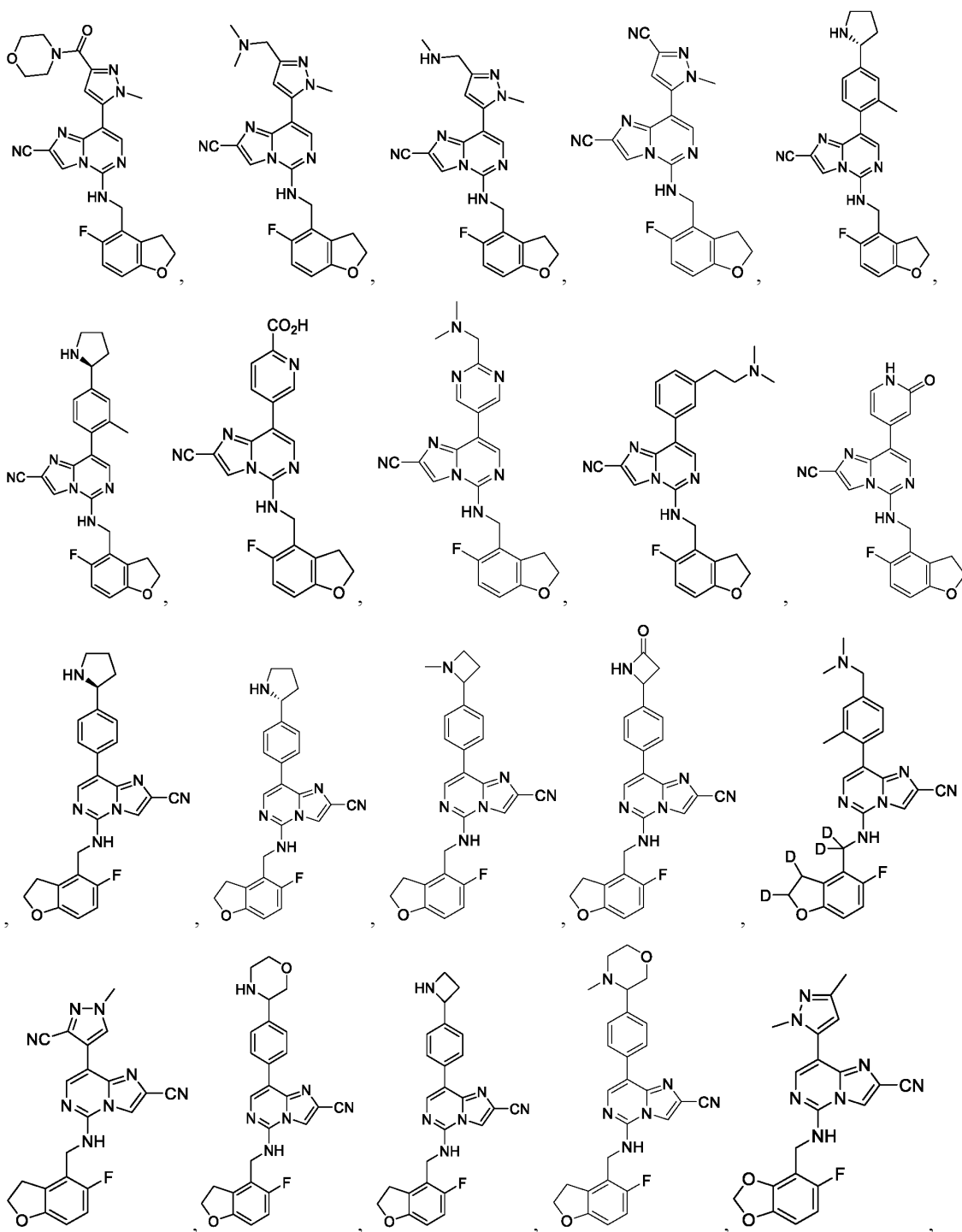


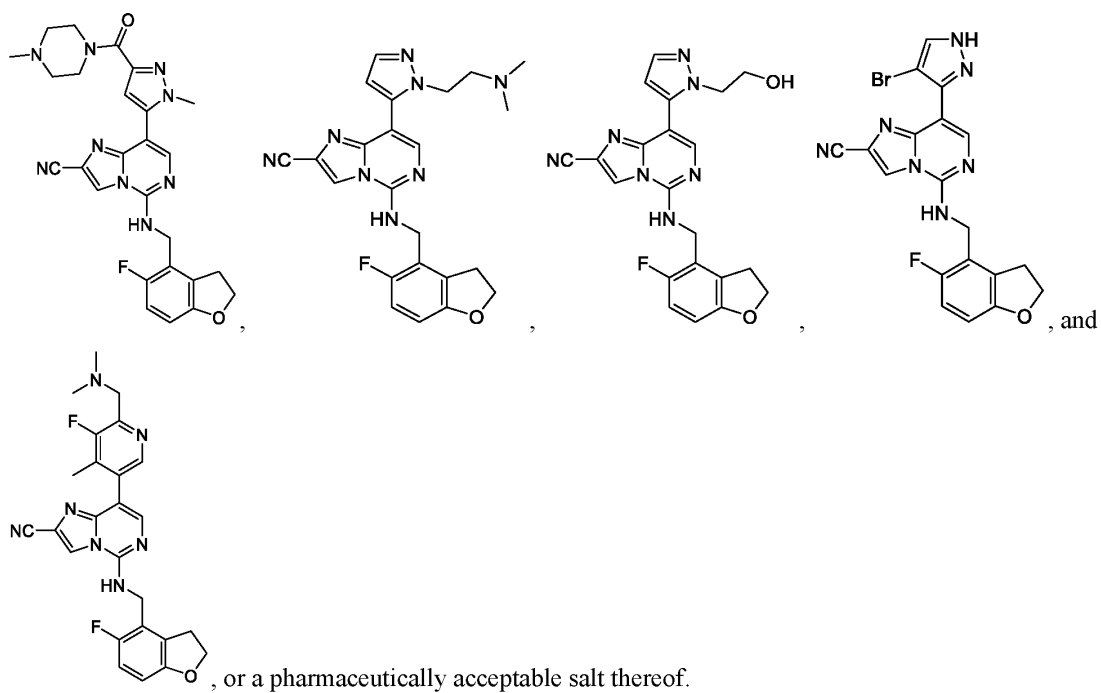






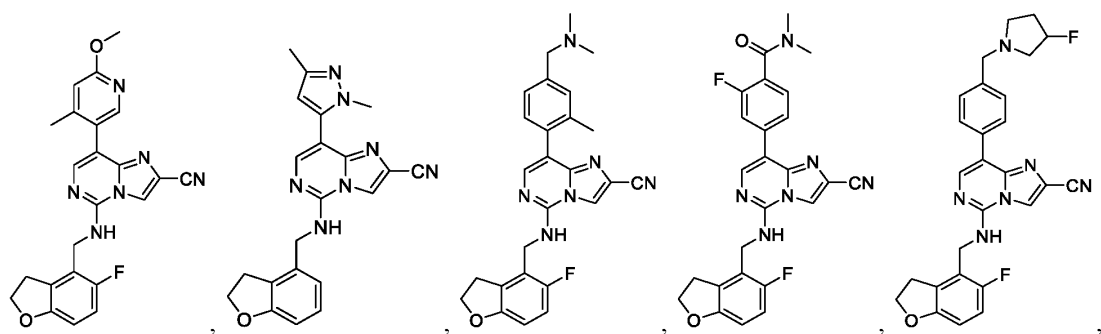


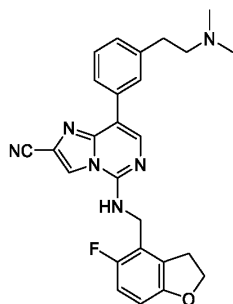
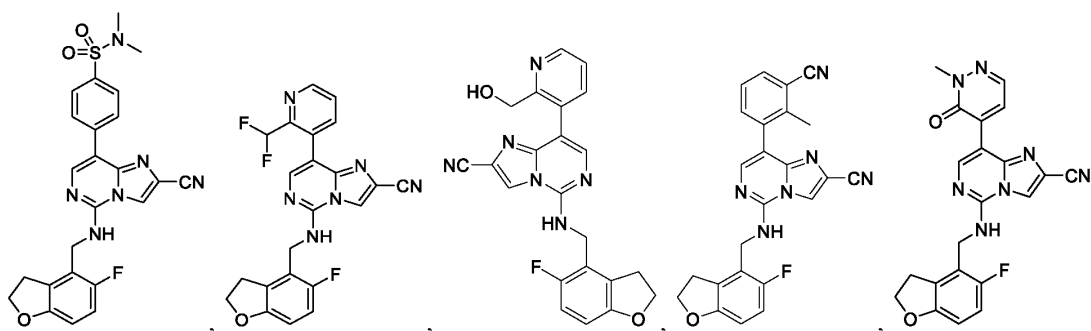




[00323] Embodiment 65: The method of any one of embodiments 1 to 13, wherein the embryonic ectoderm

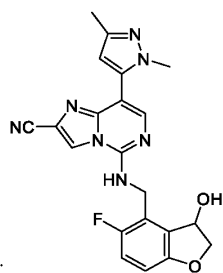
development (EED) inhibitor is a compound selected from the group consisting of:





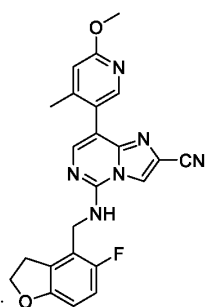
and , or a pharmaceutically acceptable salt thereof.

[00324] Embodiment 66: The method of embodiment 65, wherein the embryonic ectoderm development



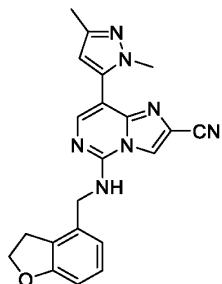
(EED) inhibitor is: (Compound 1), or a pharmaceutically acceptable salt thereof.

[00325] Embodiment 67: The method of embodiment 65, wherein the embryonic ectoderm development



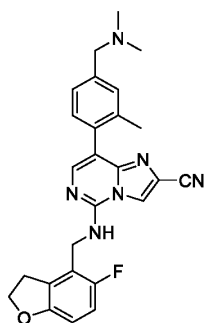
(EED) inhibitor is: (Compound 2), or a pharmaceutically acceptable salt thereof.

[00326] Embodiment 68: The method of embodiment 65, wherein the embryonic ectoderm development



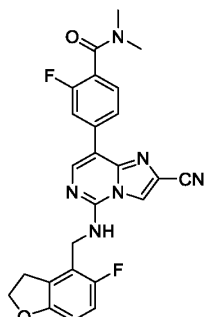
(EED) inhibitor is: (Compound 3), or a pharmaceutically acceptable salt thereof.

[00327] Embodiment 69: The method of embodiment 65, wherein the embryonic ectoderm development



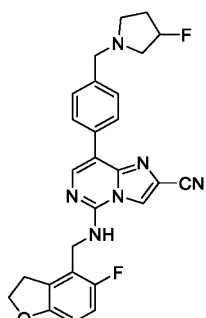
(EED) inhibitor is: (Compound 4), or a pharmaceutically acceptable salt thereof.

[00328] Embodiment 70: The method of embodiment 65, wherein the embryonic ectoderm development



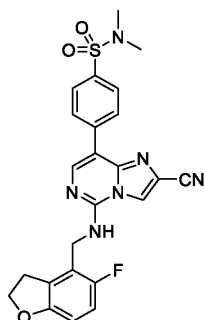
(EED) inhibitor is: (Compound 5), or a pharmaceutically acceptable salt thereof.

[00329] Embodiment 71: The method of embodiment 65, wherein the embryonic ectoderm development



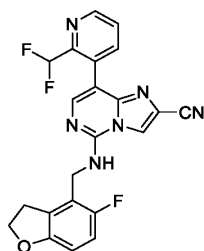
(EED) inhibitor is: (Compound 6), or a pharmaceutically acceptable salt thereof.

[00330] Embodiment 72: The method of embodiment 65, wherein the embryonic ectoderm development



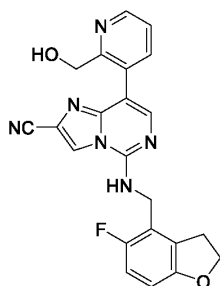
(EED) inhibitor is: (Compound 7), or a pharmaceutically acceptable salt thereof.

[00331] Embodiment 73: The method of embodiment 65, wherein the embryonic ectoderm development



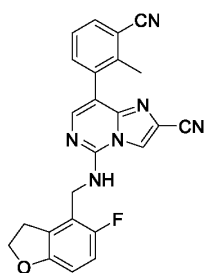
(EED) inhibitor is: (Compound 8), or a pharmaceutically acceptable salt thereof.

[00332] Embodiment 74: The method of embodiment 65, wherein the embryonic ectoderm development



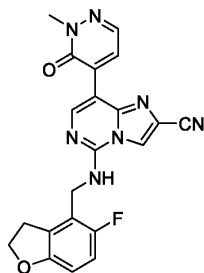
(EED) inhibitor is: (Compound 9), or a pharmaceutically acceptable salt thereof.

[00333] Embodiment 75: The method of embodiment 65, wherein the embryonic ectoderm development



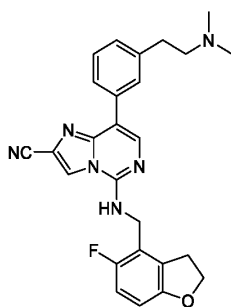
(EED) inhibitor is: (Compound 10), or a pharmaceutically acceptable salt thereof.

[00334] Embodiment 76: The method of embodiment 65, wherein the embryonic ectoderm development



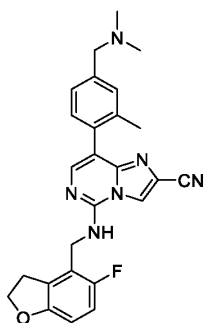
(EED) inhibitor is: (Compound 11), or a pharmaceutically acceptable salt thereof.

[00335] Embodiment 77: The method of embodiment 65, wherein the embryonic ectoderm development



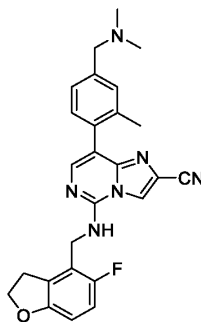
(EED) inhibitor is: (Compound 12), or a pharmaceutically acceptable salt thereof.

[00336] Embodiment 78: A method of treating prostate cancer in a subject, comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor of



formula (1), or a pharmaceutically acceptable salt thereof.

[00337] Embodiment 79: A method of treating prostate cancer in a subject, wherein the prostate cancer in the subject has been determined to be resistant to one or more first androgen receptor signaling inhibitors (ARSI), comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic



ectoderm development (EED) inhibitor of formula (2), or a pharmaceutically acceptable salt thereof.

[00338] Embodiment 80: The method of embodiment 78, wherein the one or more first androgen receptor signaling inhibitors (ARSI) is selected from CYP17 inhibitors and androgen receptor inhibitors.

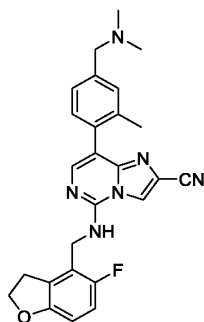
[00339] Embodiment 81: The method of embodiment 80, wherein the one or more first androgen receptor signaling inhibitors (ARSI) is selected from CYP17 inhibitors.

[00340] Embodiment 82: The method of embodiment 81, wherein the CYP17 inhibitor is abiraterone acetate.

[00341] Embodiment 83: The method of embodiment 80, wherein the one or more first androgen receptor signaling inhibitors (ARSI) is selected from one or more androgen receptor inhibitors.

[00342] Embodiment 84: The method of embodiment 81, wherein the one or more first androgen receptor inhibitors is selected from apalutamide, darolutamide, and enzalutamide.

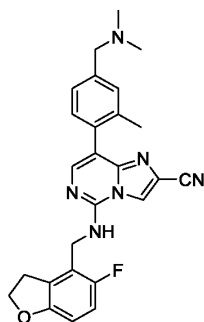
[00343] Embodiment 85: A method of treating prostate cancer in a subject, wherein the prostate cancer in the subject has been determined to be resistant to a CYP17 inhibitor, comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor of formula



, or a pharmaceutically acceptable salt thereof.

[00344] Embodiment 86: The method of embodiment 85, wherein the CYP17 inhibitor is abiraterone acetate.

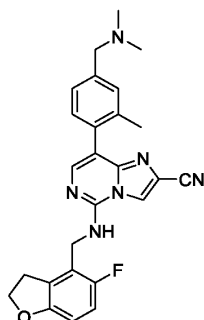
[00345] Embodiment 87: A method of treating prostate cancer in a subject, wherein the prostate cancer in the subject has been determined to be resistant to abiraterone, comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor of formula



, or a pharmaceutically acceptable salt thereof.

[00346] Embodiment 88: A method of treating prostate cancer in a subject, wherein the subject has received prior administration of one or more CYP17 inhibitors, comprising administering to the subject (a) an

androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor of formula



, or a pharmaceutically acceptable salt thereof.

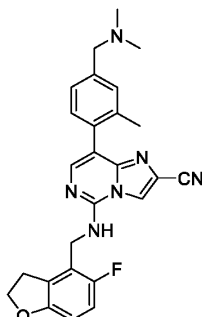
[00347] Embodiment 89: The method of any one of embodiments 1 to 88, wherein the androgen receptor inhibitor is selected from apalutamide, darolutamide, and enzalutamide

[00348] Embodiment 90: The method of embodiment 89, wherein the androgen receptor inhibitor is apalutamide.

[00349] Embodiment 91: The method of embodiment 89, wherein the androgen receptor inhibitor is darolutamide.

[00350] Embodiment 92: The method of embodiment 89, wherein the androgen receptor inhibitor is enzalutamide.

[00351] Embodiment 93: A method of treating prostate cancer in a subject, wherein the subject (i) has received prior administration of one or more CYP17 inhibitors, and (ii) is androgen receptor inhibitor naïve, comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm



development (EED) inhibitor of formula , or a pharmaceutically acceptable salt thereof.

[00352] Embodiment 94: The method of any one of embodiments 1 to 93, wherein the subject has received one or more prior chemotherapeutic treatments prior the administration of the androgen receptor inhibitor, and the embryonic ectoderm development (EED) inhibitor.

[00353] Embodiment 95: The method of any one of embodiments 1 to 93, wherein the subject has received up to one prior chemotherapeutic treatments prior the administration of the androgen receptor inhibitor, and the embryonic ectoderm development (EED) inhibitor.

[00354] Embodiment 96: The method of any one of embodiments 1 to 95, wherein the subject is CYP17 inhibitor-naïve prior to the administration to the subject of the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor.

[00355] Embodiment 97: The method of any one of embodiments 1 to 96, wherein the subject is androgen receptor inhibitor naïve prior to the administration to the subject of the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor

[00356] Embodiment 98: The method of any one of embodiments 1 to 97, wherein the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor are administered to the subject sequentially or simultaneously.

[00357] Embodiment 99: The method of embodiment 98, wherein the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor are administered to the subject sequentially.

[00358] Embodiment 100: The method of any one of embodiments 1 to 99, wherein the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor are administered to the subject on the same day.

[00359] Embodiment 101: The method of any one of embodiments 1 to 100, wherein the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor are administered to the subject within a 24-hour period.

[00360] Embodiment 102: The method of embodiment 98, wherein the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor are administered to the subject simultaneously.

[00361] Embodiment 103: The method of any one of embodiments 1 to 102, wherein the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor are administered to the subject once per day or twice per day.

[00362] Embodiment 104: The method of embodiment 103, wherein the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor are administered to the subject once per day.

[00363] Embodiment 105: The method of embodiment 103, wherein the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor are administered to the subject twice per day.

[00364] Embodiment 106: The method of any one of embodiments 1 to 102, wherein the androgen receptor inhibitor is administered to the subject twice per day and the embryonic ectoderm development (EED) inhibitor is administered to the subject once per day.

[00365] Embodiment 107: The method of any one of embodiments 1 to 102, wherein the androgen receptor inhibitor is administered to the subject once per day and the embryonic ectoderm development (EED) inhibitor is administered to the subject twice per day.

[00366] Embodiment 108: The method of any one of embodiments 1 to 107, wherein the androgen receptor inhibitor is administered to the subject with food or without food.

[00367] Embodiment 109: The method of embodiment 108, wherein the androgen receptor inhibitor is administered to the subject with food.

[00368] Embodiment 110: The method of embodiment 108, wherein the androgen receptor inhibitor is administered to the subject without food.

[00369] Embodiment 111: The method of any one of embodiments 1 to 110, wherein the subject has been administered one or more prior androgen deprivation therapies prior to the administration to the subject of the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor.

[00370] Embodiment 112: The method of any one of embodiments 1 to 110, wherein the subject has been administered a gonadotropin-releasing hormone (GnRH) analog prior to the administration to the subject of the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor.

[00371] Embodiment 113: The method of any one of embodiments 1 to 110, wherein the subject receives a gonadotropin-releasing hormone (GnRH) analog concurrently with the administration to the subject of the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor.

[00372] Embodiment 114: The method of any one of embodiments 1 to 110, wherein the subject had a bilateral orchiectomy prior to the administration to the subject of the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor.

[00373] Embodiment 115: The method of any one of embodiments 1 to 114, wherein the subject is not administered a compound that is a substrate of CYP3A4, CYP2C19, CYP2C8, CYP2C9, UGT, P-gp, BCRP, or OATP1B1 during the time period in which the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor are administered to the subject.

[00374] Embodiment 116: The method of any one of embodiments 1 to 114, wherein the subject is not administered a compound that is a substrate of CYP3A4, CYP2C9, UGT, P-gp, BCRP, or OATP1B1 during the time period in which the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor are administered to the subject.

[00375] Embodiment 117: The method of any one of embodiments 1 to 114, wherein the subject is not administered a compound that is (a) a CYP2C8 inhibitor, (b) a CYP3A4 inducer, or (c) a substrate of CYP3A4, CYP2C9, or CYP2C19 during the time period in which the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor are administered to the subject.

[00376] Embodiment 118: The method of any one of embodiments 1 to 114, wherein the subject is not administered a compound that is (a) a CYP3A4 inducer, (b) a PG-p inhibitor, (c) a CYP3A4 inhibitor, (d) a BCRP substrate, (e) an OATP1B1 substrate, or (f) a OATP1B3 substrate during the time period in which the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor are administered to the subject.

[00377] Embodiment 119: The method of any one of embodiments 1 to 118, wherein the prostate cancer in the subject is selected from metastatic prostate cancer, non-metastatic prostate cancer, metastatic castration-resistant prostate cancer, metastatic castration-sensitive prostate cancer, localized high risk prostate cancer, recurrent prostate cancer, non-metastatic castration-resistant prostate cancer, non-metastatic castration-sensitive prostate cancer, androgen receptor inhibitor-sensitive prostate cancer, androgen receptor inhibitor-resistant prostate cancer, androgen receptor-dependent prostate cancer, androgen receptor-independent prostate cancer, neuroendocrine prostate cancer (NEPC), metastatic neuroendocrine prostate cancer (NEPC),

prostate cancer with small cell features, metastatic prostate cancer with small cell features, and aggressive-variant prostate cancer.

[00378] Embodiment 120: The method of embodiment 119, wherein the prostate cancer in the subject is metastatic prostate cancer.

[00379] Embodiment 121: The method of embodiment 119, wherein the prostate cancer in the subject is non-metastatic prostate cancer.

[00380] Embodiment 122: The method of embodiment 119, wherein the prostate cancer in the subject is metastatic castration-resistant prostate cancer.

[00381] Embodiment 123: The method of embodiment 119, wherein the prostate cancer in the subject is metastatic castration-sensitive prostate cancer.

[00382] Embodiment 124: The method of embodiment 119, wherein the prostate cancer in the subject is localized high risk prostate cancer.

[00383] Embodiment 125: The method of embodiment 119, wherein the prostate cancer in the subject is recurrent prostate cancer.

[00384] Embodiment 126: The method of embodiment 119, wherein the prostate cancer in the subject is non-metastatic castration-resistant prostate cancer.

[00385] Embodiment 127: The method of embodiment 119, wherein the prostate cancer in the subject is non-metastatic castration-sensitive prostate cancer.

[00386] Embodiment 128: The method of embodiment 119, wherein the prostate cancer in the subject is androgen receptor inhibitor-sensitive prostate cancer.

[00387] Embodiment 129: The method of embodiment 119, wherein the prostate cancer in the subject is androgen receptor inhibitor-resistant prostate cancer.

[00388] Embodiment 130: The method of embodiment 119, wherein the prostate cancer in the subject is androgen receptor-dependent prostate cancer.

[00389] Embodiment 131: The method of embodiment 119, wherein the prostate cancer in the subject is androgen receptor-independent prostate cancer.

[00390] Embodiment 132: The method of embodiment 119, wherein the prostate cancer in the subject is neuroendocrine prostate cancer (NEPC).

[00391] Embodiment 133: The method of embodiment 119, wherein the prostate cancer in the subject is metastatic neuroendocrine prostate cancer (NEPC).

[00392] Embodiment 134: The method of embodiment 119, wherein the prostate cancer in the subject is prostate cancer with small cell features.

[00393] Embodiment 135: The method of embodiment 119, wherein the prostate cancer in the subject is metastatic prostate cancer with small cell features.

[00394] Embodiment 136: The method of embodiment 119, wherein the prostate cancer in the subject is aggressive-variant prostate cancer.

EXAMPLES**Example 1: Preparation of crystalline Form 1 of Compound 4**

[00395] 150 μ L of methanol was added to 50 mg of the free base of Compound 4 and the resulting slurry was stirred at room temperature for one day. The resulting solids were vacuum filtered and dried under ambient condition overnight to afford Form 1 of Compound 4.

Example 2A: Preparation of crystalline Form 2 of Compound 4

[00396] 400 mg of the free base of Compound 4 was dissolved in 1.5 mL of 2-methyltetrahydrofuran at 50 °C, to which was added 1.5 mL n-heptane at about 47 °C, and the resulting mixture was cooled 10 °C. The resulting solids were vacuum filtered and allowed to air dry overnight under ambient conditions to afford Form 2 of Compound 4.

Example 2B: Preparation of crystalline Form 2 of Compound 4

[00397] A quantity of the free base of Compound 4 was dissolved in 2-methyltetrahydrofuran (10 volumes) and then distilled to 3 volumes. The temperature of the solution was adjusted to about 25 °C and the resulting slurry was stirred for greater than 30 minutes. To the slurry was added n-heptane (7 volumes) over 2 hours and the resulting mixture was stirred for greater than 4 hours. The resulting solid was filtered, the filter cake was washed with 30% 2-methyltetrahydrofuran /heptane (2 volumes) and dried in a vacuum oven to provide Form 2 of Compound 4.

Example 3A: X-ray powder diffraction (XRPD) analysis of Form 1 and Form 2 of Compound 4

[00398] XRPD analyses of crystalline polymorphic forms of Compound 4 were performed using Panalytical X'pert³ X-ray powder diffractometer. Samples were spread on the middle of a zero-background Si holder. The 2-theta position was calibrated against a Panalytical Si reference standard disc. The parameters used for the analyses are set forth in Table 1.

Table 1

Parameter	Reflection Mode
X-ray wavelength	Cu, $\text{K}\alpha$ $\text{K}\alpha 1$ (angstroms): 1.540598; $\text{K}\alpha 2$ (angstroms): 1.544426 $\text{K}\alpha 2/\text{K}\alpha 1$ intensity ratio: 0.50
X-ray tube setting	45 kV, 40 mA
Divergence slit	1/8°
Scan mode	Continuous
Scan range (° 2-theta)	3° to 40°
Scan step time (seconds)	18.87
Step size (° 2-theta)	0.0131
Test time	About 4 minutes, 15 seconds

[00399] Polymorphic Form 1 of Compound 4 was analyzed by XRPD as set forth above and exhibited the peaks set forth in Table 2. The error associated with each $^{\circ}2$ -theta position was determined to be $\pm 0.2^{\circ}$ theta.

Table 2

Position ($^{\circ}2$ -theta)	Relative intensity (%)
5.7	23.83
8.1	100
9.6	32.45
9.8	17.88
12.9	18.43
15.2	18.62
17.7	18.68
19.7	22.92
22.0	22.15

[00400] Polymorphic Form 2 of Compound 4 was analyzed by XRPD as set forth above and exhibited the peaks set forth in Table 3. The error associated with each $^{\circ}2$ -theta position was determined to be $\pm 0.2^{\circ}$ theta.

Table 3

Position ($^{\circ}2$ -theta)	Relative intensity (%)
5.5	70.17
7.7	100
8.6	62.56
10.6	55.03
11.0	41.46
13.7	85.08
15.4	47.42
15.9	60.49
19.2	78.68
19.9	60.93
21.0	50.16
24.1	68.95
25.3	38.36
26.3	57.53

Example 3B: Thermal gravimetric analyses and differential scanning calorimetry analyses of Form 1 and Form 2 of Compound 4

[00401] Thermal gravimetric analysis (TGA) data were collected using TA Discovery TGA 550 TGA from TA Instruments, and differential scanning calorimetry (DSC) analyses were performed using a TA Q2000 DSC from TA Instruments using the parameters set forth in Table 4.

Table 4

Parameter	TGA	DSC
Method	Ramp	Ramp
Sample pan	Platinum, open	Aluminum, crimped
Temperature range	Room temperature to about 380 °C	Room temperature to about 380 °C
Heating rate	10 °C/minute	10 °C/minute
Purge gas	Nitrogen	Nitrogen

[00402] A thermal gravimetric analysis (TGA) of a sample of Form 1 of Compound 4, when conducted under the conditions set forth in Table 4, exhibited a weight loss of about 1% upon heating the sample from room temperature to about the onset of melting (about 207 °C). A differential scanning calorimetry (DSC) analysis of Form 1 of Compound 4, when conducted under the conditions set forth in Table 4, exhibited peaks at between about 170 °C and 172 °C, and between about 207 °C and 208 °C.

[00403] A thermal gravimetric analysis (TGA) of a sample of Form 2 of Compound 4, when conducted under the conditions set forth in Table 4, exhibited a weight loss of about 2% upon heating the sample from room temperature to about the onset of melting (about 204 °C). A differential scanning calorimetry (DSC) analysis of Form 2 of Compound 4, when conducted under the conditions set forth in Table 4, exhibited a peak between about 203 °C and 204 °C.

Example 3C: Preparation of crystalline Form 2 of Compound 4

[00404] A reactor was evacuated and charged with nitrogen to atmospheric pressure. The reactor was then charged with a solution of Compound 4 (approximately 2.41 kg as determined by solution assay using HPLC) in 2-methyltetrahydrofuran (2-MeTHF, 36 kg, 15 volumes) and the batch was concentrated to a batch volume of about 5 L (about 2 volumes) via distillation under reduced pressure. The resulting solution was adjusted to about 25 °C and then n-heptane (0.4 kg, 0.2 volumes) was added in portions over a period of about 3 hours. The resulting solution was then seeded with Compound 4 Form 2 (9 g, 0.4 wt%), the resulting mixture was stirred for about 1.3 hours, and then additional n-heptane (24 kg, 10 volumes) was added over about 6 hours. The resulting slurry was stirred for about 4.25 hours at 25 °C and then filtered. The reactor was then rinsed with n-heptane (5.8 kg, 2.5 V), and this mixture was rinsed forward to the filter cake, which was deliquored and the solids were dried under reduced pressure at 40 °C and 50 °C for 19 hours to provide 2.48 kg of Form 2 of Compound 4.

Example 4: Study of Compound 4 and darolutamide in 22Rv1 in intact BALB/c nude mice (low hormone)

[00405] Compound 4, alone and in combination with darolutamide, was evaluated in 22Rv1, an enzalutamide-resistant prostate carcinoma cell line xenograft model, in intact male mice with low hormone

levels. Intact male BALB/c nude mice were purchased from the Beijing Vital River Laboratory Animal Technology Co., Ltd. Animals were housed in individually ventilated cages under specific pathogen free (SPF) environment of vivarium facility and acclimated to their new environment for at least 7 days prior to initiation of any experiment. Mice were approximately 6-8 weeks of age at the time of tumor cell inoculation. The human prostate carcinoma cell line 22Rv1 was purchased from the American Type Culture Collection (ATCC® CRL-2505™). 22Rv1 cells were cultured in RPMI 1640 medium with L-glutamine and 10% of fetal bovine serum (FBS) at 37 °C in an atmosphere of 5% CO₂ in air. The medium was changed every 2 to 3 days. Cells were sub-cultured at a confluence of 80-90% by trypsin-EDTA. The cells growing in an exponential growth phase were harvested and counted for inoculation in mice. The dosing vehicle for Compound 4 was prepared using Vehicle 2, which consisted of 0.5% methyl cellulose (MC) in 50 mM phosphate buffer at pH 6.8, and was stored at 4 °C. Preparation of Vehicle 2: equal volumes of 50 mM Na₂HPO₄ (Sigma Cat#S5136) and 50 mM NaH₂PO₄ (Sigma Cat#5011) were mixed and the pH of the resulting mixture was adjusted to pH 6.8 using 50 mM H₃PO₄ (Sigma Cat#79622) solution. Methyl cellulose (MC; Sigma Cat#M0262) powder was added to make 0.5% MC 50 mM phosphate buffer, and the final pH of the 20 mg/mL dosing solution of Compound 4 was about pH 6.8. A 20 mg/mL suspension of Compound 4 was prepared by adding 2 mL of Vehicle (0.5% Methyl Cellulose in 50mM Phosphate Buffer, pH 6.8) to 40 mg of Compound 4 and the resulting mixture was placed in heated water bath (~50-60 °C) and sonicator and sonicated for 15 minutes to provide a suspension of Compound 4 that was found to be stable for 7 days when stored at 4 °C between uses. When used for dosing, the dosing vials were removed from storage at 4 °C around 15 to 20 minutes prior to use and the suspension was mixed between loading syringes to ensure mixing.

[00406] The dosing mixture comprising 10 mg/mL darolutamide was prepared using Vehicle 1, which consisted of 50% PEG400/30% propylene glycol (PG)/20% D5W. The dosing mixture was prepared by dissolving 100 mg of darolutamide in 5 mL of PEG400 with vortexing and sonification, add 3 mL of PG with vortexing and sonification, adding 2 mL of D5W with vortexing and sonification to obtain a suspension. The formulation was stored at 4 °C and protected from light between uses.

[00407] The dosing mixture comprising 3 mg/mL enzalutamide was prepared using Vehicle 3, which consisted of 5% DMSO, 0.25% Carboxymethyl cellulose (CMC), 0.2% Tween80 in water for injection (WFI), and was stored at 4 °C. The dosing mixture comprising enzalutamide was prepared by adding 99 mg enzalutamide into an appropriate glass vial, adding 1.65 mL DMSO (equal to 5% of total final volume), and vortexing and sonicating 10 minutes to obtain a clear solution. 31.35 mL of diluent (0.25% CMC/0.2% Tween 80) was then added and the resulting mixture was vortexed to afford a mixture having a total volume of 33 mL, which was sonicated for 2 hours in a water bath and was allowed to continue to stir until what appeared to be a homogeneous suspension was formed. The dosing formulation was prepared one day before dosing and prepared once per week and was stored at room temperature and protected from light prior to usage.

[00408] The design of the study is set forth below in Table 5 (wherein, n/a = not applicable; PO = oral gavage; QD = once daily dosing starting on day 0; BID, twice daily dosing starting on day 0).

Table 5

Group	No. animals per group	Test Article	Route of Admin.	Dose (mg/kg)	Volume (mL/kg)	Dosing Regimen (frequency x days)
1	8	Vehicle 1 + Vehicle 2	PO	n/a	5	BID x 21 QD x 21
2	8	Compound 4	PO	100	5	QD x 21
3	8	Darolutamide	PO	50	5	BID x 21
4	8	Compound 4 + Darolutamide	PO	100 50	5	QD x 21 BID x 21
5	8	Enzalutamide	PO	30	10	QD x 21

[00409] 22Rv1 tumor cells were inoculated into right flanks of intact male BALB/c nude mice. Each mouse received 4×10^6 cells mixed 1:1 with Matrigel at a total volume of 100 μ L. Tumor growth was monitored and measured when tumors were palpable. When tumors sizes reached near 100-175 mm³, 40 tumor-bearing mice were randomly divided into 5 groups with 8 mice in each group for treatment as described in Table 5. Treatment started on the day of randomization. The treatment start day was denoted as treatment day 0. Mice were dosed by oral administration of vehicle solutions, Compound 4 at 100 mg/kg QD, darolutamide at 50 mg/kg BID, enzalutamide at 30 mg/kg QD for 21 days (Day 0 to Day 20) in accordance with groupings listed in Table 5. BID dosing was performed 7-8 hours apart. The dosing volume was 5 mL/kg for each formulation based on the most recent body weight, except Group 5, which was dosed at 10 mL/kg. Subcutaneous tumor volumes were measured twice a week by caliper with following formula: Tumor volume (TV) = (length $\times$ width²)/2. Mouse body weights were measured daily with a weighing scale. Conditions of animal health and clinical signs of side effects were monitored by daily observation of gross morphology and necropsy of euthanized animals at study endpoint. The study was terminated following 21 days of treatment as defined in the study protocol. Tumor Growth Inhibition (TGI) and Regression Calculations were calculated using following formula: $TGI = [1 - (TV_{tf} - TV_{t0}) / (TV_{cf} - TV_{c0})] \times 100\%$, wherein: TV_{tf} was the tumor volume (TV) mean of treatment group at final or last treatment day; TV_{t0} was the TV mean of treatment group at treatment day 0; TV_{cf} was the TV mean of control group at final or last treatment day; and TV_{c0} was the TV mean of control group at treatment day 0. Group tumor volume means, SD and SEM were calculated using standard statistical tools (GraphPad PRISM 9.5). Comparison of tumor volume differences in groups at the end of study (EOS) was analyzed using the One-Way Analysis of Variance (One-Way ANOVA) method, followed by Dunnett's multiple comparisons test of treated vs. vehicle tumor volume in overall tumor growth, and Tukey's multiple comparisons test of treated end of study tumor volumes across all treatment groups. A p value less than 0.05 was considered statistically significant.

[00410] Results: Compound 4 demonstrated tumor growth inhibition as a monotherapy and in combination with darolutamide as shown in Table 6. Compared to vehicle control, 21-day daily repeated oral administration of Compound 4 at 100 mg/kg QD and in combination with darolutamide at 50 mg/kg twice daily (BID) inhibited tumor growth by 46% and 62% respectively (FIG. 1, Table 6). At end of study (EOS), both the Compound 4 monotherapy as well as Compound 4 and darolutamide combination treated groups had tumor volumes significantly smaller compared to the vehicle group (p-value < 0.005). No statistical differences were observed with monotherapy darolutamide at 50 mg/kg BID or monotherapy enzalutamide at 30 mg/kg QD. In summary, Compound 4 demonstrated tumor growth inhibition and significant reduction of EOS tumor volumes as a monotherapy and further improved tumor growth inhibition in combination with darolutamide in the 22Rv1 prostate cancer xenograft model when established in low hormone setting.

Table 6

Treatment Group	Dose level (mg/kg)	Dosing Regimen (frequency x days)	Animal No	% TGI (EOS)	EOS Tumor Volume (mm ³)	p-value (vs. vehicle)
Vehicle 1 + Vehicle 2	n/a	BID x 21 QD x 21	8	n/a	1525 ± 368	-
Compound 4	100	QD x 21	8	46.1	878 ± 216	0.0003
Darolutamide	50	BID x 21	8	9.1	1397 ± 182	0.87
Compound 4 + Darolutamide	100 50	QD x 21 BID x 21	8	62.2	653 ± 194	<0.0001
Enzalutamide	30	QD x 21	8	23.5	1196 ± 329	0.13

Example 5: Study of Compound 4 and darolutamide in a darolutamide acquired resistance 22Rv1 model using intact BALB/c nude mice

[00411] Compound 4, alone and in combination with darolutamide, was evaluated in a darolutamide acquired resistance 22Rv1 model using intact BALB/c nude mice. Intact male BALB/c nude mice were purchased from the Beijing Vital River Laboratory Animal Technology Co., Ltd. Animals were housed in individually ventilated cages under specific pathogen free (SPF) environment of vivarium facility and acclimated to their new environment for at least 7 days prior to initiation of any experiment. Mice were approximately 6-8 weeks of age at the time of tumor cell inoculation. 22Rv1 cells were cultured in RPMI 1640 medium with L-glutamine and 10% of fetal bovine serum (FBS) at 37 °C in an atmosphere of 5% CO₂ in air. The medium was changed every 2 to 3 days. Cells were sub-cultured at a confluence of 80-90% by trypsin-EDTA. The cells growing in an exponential growth phase were harvested and counted for inoculation in mice. The dosing vehicle for Compound 4 was prepared using Vehicle 2, which consisted of 0.5% methyl cellulose (MC) in 50 mM phosphate buffer at pH 6.8, and was stored at 4 °C. Preparation of Vehicle 2: equal volumes of 50 mM Na₂HPO₄ (Sigma Cat#S5136) and 50 mM NaH₂PO₄ (Sigma Cat#5011) were mixed and the pH of the resulting mixture was adjusted to pH 6.8 using 50 mM H₃PO₄ (Sigma Cat#79622) solution. Methyl cellulose (MC; Sigma Cat#M0262) powder was added to make 0.5% MC 50 mM phosphate buffer, and the final pH of the 20 mg/mL dosing solution of Compound 4 was about pH 6.8. A 20 mg/mL suspension of Compound 4 was prepared by adding 2 mL of Vehicle (0.5% Methyl Cellulose in 50mM Phosphate Buffer,

pH 6.8) to 40 mg of Compound 4 and the resulting mixture was placed in heated water bath (~50-60 °C) and sonicator and sonicated for 15 minutes to provide a suspension of Compound 4 that was found to be stable for 7 days when stored at 4 °C between uses. When used for dosing, the dosing vials were removed from storage at 4 °C around 15 to 20 minutes prior to use and the suspension was mixed between loading syringes to ensure mixing.

[00412] The dosing mixture comprising 10 mg/mL darolutamide was prepared using Vehicle 1, which consisted of 50% PEG400/30% propylene glycol (PG)/20% D5W. The dosing mixture was prepared by dissolving 100 mg of darolutamide in 5 mL of PEG400 with vortexing and sonification, add 3 mL of PG with vortexing and sonification, adding 2 mL of D5W with vortexing and sonification to obtain a suspension. The formulation was stored at 4 °C and protected from light between uses.

[00413] The design of the study (Stage I and Stage II) is set forth below in Tables 7 and 8 (wherein, n/a = not applicable; PO = oral gavage; QD = once daily dosing starting on day 0; BID, twice daily dosing starting on day 0).

Table 7: Stage I Treatment Before Randomization

Group	No. animals	Test Article	Route of Admin.	Dose (mg/kg)	Volume (mL/kg)	Dosing Regimen (frequency x days)
1	64	darolutamide	PO	50	5	BID x 28

Table 8: Stage II Treatment After Randomization

Group	Mouse	Animal Number	Test Article	Route of Administration	Dose (mg/kg)	Volume (mL/kg)	Dosing Regimen (frequency x days)
1	Darolutamide treated intact mice	8	Vehicle 1 + Vehicle 2	PO	n/a	5	BID x 28 QD x 28
2		8	Compound 4	PO	100	5	QD x 28
3		8	darolutamide	PO	50	5	BID x 28
4		8	Compound 4 + darolutamide	PO	100 50	5	QD x 28 BID x 28

[00414] During Stage I, 64 intact male BALB/c nude mice were treated with darolutamide at 50 mg/kg BID for 28 days as described in Table 7. On Day 14 of darolutamide treatment in Stage I, 22Rv1 tumor cells were inoculated into right flanks of intact male BALB/c nude mice. Each mouse received 4×10^6 cells mixed 1:1 with Matrigel at a total volume of 100 μ L. Tumor growth was monitored and measured when tumors were palpable. After another 2 weeks of darolutamide treatment in Stage I, when tumors sizes reached near 130-140 mm³, 32 tumor-bearing mice were randomly divided into 4 groups with 8 mice in each group for treatment as described in Table 8. Stage II treatment started on the day of randomization. The Stage II treatment start day was denoted as treatment day 0. Mice were dosed by oral administration of vehicle solutions, Compound 4 at 100 mg/kg QD and darolutamide at 50 mg/kg BID for 28 days (Day 0 to Day 27)

in accordance with groupings listed in Table 8. BID dosing was performed 7-8 hours apart. The dosing volume was 5 mL/kg for each formulation based on the most recent body weight. Subcutaneous tumor volumes were measured twice a week by caliper with following formula: Tumor volume (TV) = (length × width²)/2. Mouse body weights were measured daily with a weighing scale. Conditions of animal health and clinical signs of side effects were monitored by daily observation of gross morphology and necropsy of euthanized animals at study endpoint. The study was terminated following 28 days of treatment as defined in the study protocol. Tumor growth inhibition (TGI) was calculated using following formula: $TGI = [1 - (TV_{tf} - TV_{t0}) / (TV_{cf} - TV_{c0})] \times 100\%$, wherein TV_{tf} was the tumor volume (TV) mean of treatment group at final or last treatment day, TV_{t0} was the TV mean of treatment group at treatment day 0, TV_{cf} was the TV mean of control group at final or last treatment day, and TV_{c0} was the TV mean of control group at treatment day 0. Group tumor volume means, SD and SEM were calculated using standard statistical tools (GraphPad PRISM 9.5). Comparison of tumor volume differences in groups at the end of study (EOS) was analyzed using the One-Way Analysis of Variance (One-Way ANOVA) method, followed by Dunnett's multiple comparisons test of treated vs. vehicle tumor volume in overall tumor growth, and Tukey's multiple comparisons test of treated end of study tumor volumes across all treatment groups. A p value less than 0.05 was considered statistically significant.

[00415] Results: Compared to vehicle control, 28-day daily repeated oral administration of Compound 4 at 100 mg/kg QD and in combination with darolutamide at 50 mg/kg BID inhibited tumor growth by 22% and 59% respectively, while monotherapy darolutamide resulted in 23% tumor growth inhibition. At end of study (EOS), the tumor volume in the Compound 4 and darolutamide combination group was significantly smaller compared to the vehicle group (p-value 0.04) (Table 9 and FIG. 2). However, no statistical differences were observed among the remaining monotherapy treated groups.

Table 9

Treatment Group	Dose Level (mg/kg)	Dosing Regimen (frequency x days)	Animal No	% TGI (EOS)	EOS Tumor Volume (mm ³)	p-value (vs. vehicle)
Vehicle 1 + Vehicle 2	n/a	BID x 28 QD x 28	8	n/a	1637 ± 1026	-
Compound 4	100	QD x 28	8	22.1	1306 ± 507	0.72
Darolutamide	50	BID x 28	8	23.1	1291 ± 429	0.69
Compound 4 + Darolutamide	100 50	QD x 28 BID x 28	8	59.4	749 ± 269	0.04

Example 6: Study of Compound 4, PF-06821497, and darolutamide in subcutaneous C4-2 castrated prostate cancer xenograft model in male NPG mice

[00416] Compound 4 and PF-06821497 (an inhibitor of EZH2), alone and in combination with darolutamide, were evaluated in a subcutaneous C4-2 castrated prostate cancer xenograft model in male NPG mice. The dosing vehicle for Compound 4 was prepared using Vehicle 2, which consisted of 0.5% methyl cellulose

(MC) in 50 mM phosphate buffer at pH 6.8 was prepared and stored 4 °C. Preparation of Vehicle 2: equal volumes of 50 mM Na₂HPO₄ (Sigma Cat#S5136) and 50 mM NaH₂PO₄ (Sigma Cat#5011) were mixed and the pH of the resulting mixture was adjusted to pH 6.8 using 50 mM H₃PO₄ (Sigma Cat#79622) solution. Methyl cellulose (MC; Sigma Cat#M0262) powder was added to make 0.5% MC 50 mM phosphate buffer, and the final pH of the 20 mg/mL dosing solution of Compound 4 was about pH 6.8. A 20 mg/mL suspension of Compound 4 was prepared by adding 2 mL of Vehicle (0.5% Methyl Cellulose in 50mM Phosphate Buffer, pH 6.8) to 40 mg of Compound 4 and the resulting mixture was placed in heated water bath (~50-60 °C) and the resulting mixture was placed into a sonicator and sonicated for 15 minutes to provide a suspension of Compound 4 that was found to be stable for 7 days when stored at 4 °C between uses. When used for dosing, the dosing vials were removed from storage at 4 °C around 15 to 20 minutes prior to use and the suspension was mixed between loading syringes to ensure mixing.

[00417] The dosing mixture comprising 10 mg/mL darolutamide was prepared using Vehicle 1, which consisted of 50% PEG400/30% propylene glycol (PG)/20% D5W. The dosing mixture was prepared by dissolving 100 mg of darolutamide in 5 mL of PEG400 with vortexing and sonification, add 3 mL of PG with vortexing and sonification, adding 2 mL of D5W with vortexing and sonification to obtain a suspension. The formulation was stored at 4 °C and protected from light between uses.

[00418] The dosing mixture comprising 20 mg/mL of PF-06821497 was prepared in a mixture of 0.5% NaCMC and 0.1% Tween 80, at a final pH of 4.5.

[00419] The C4-2 prostate cancer cells were maintained in vitro with DMEM/Ham's F12K

(4:1)+Insulin+T3+Transferrin+d-Biotin+Adenine medium supplemented with 10% fetal bovine serum at 37 °C in an atmosphere of 5% CO₂ in the air. The cells in exponential growth phase were harvested and quantitated by cell counter before tumor inoculation. Each mouse was inoculated subcutaneously at the right upper flank region with C4-2 tumor cells (5 x 10⁶) in 0.1 ml of PBS mixed with Matrigel (1:1) for tumor development. Castration surgery was done to all mice when mean tumor size reached ~200 mm³. The randomization was started when the mean tumor size reached approximately 239 mm³. 60 mice were enrolled in the study. All animals were randomly allocated to 6 study groups as set forth in Table 10, with 10 mice in each group. Randomization was performed based on “Matched distribution” method (StudyDirector™ software, version 3.1.399.19).

Table 10

Group	Dose (mg/kg)	Dosing Regimen (frequency x days)	Animal No.	% TGI (Day 27) based on QD x 28 days	Day 27 Tumor Volume (mm ³)	p-value (vs. vehicle)	Progression Free Survival (days) based on QD x 31 days
Vehicle 1 + Vehicle 2	n/a	QD x 28 BID x 28	10	n/a	2288.97 ± 198.46	n/a	11
Darolutamide	50	BID x 28	10	68.12	893.04 ± 143.54	≤0.0001	12.5
Compound 4	100	QD x 28	10	69.35	867.7 ± 58.28	≤0.0001	27

Group	Dose (mg/kg)	Dosing Regimen (frequency x days)	Animal No.	% TGI (Day 27) based on QD x 28 days	Day 27 Tumor Volume (mm ³)	p-value (vs. vehicle)	Progression Free Survival (days) based on QD x 31 days
Compound 4 + Darolutamide	100 50	QD × 28 BID × 28	10	83.03	587.52 ± 84.31	≤0.0001	Not reached
PF-06821497	100	BID × 28	10	62.39	1010.42 ± 72.36	≤0.0001	15.5
PF-06821497+ Darolutamide	100 50	BID × 28 BID × 28	10	67.92	897.25 ± 248.54	≤0.0001	24.5

[00420] Results: The TGI data was based on dosing each group for 28 days, which was the number of days for which there were still viable animals in the vehicle group. In this model darolutamide (50 mg/kg twice daily (BID)) provided significant antitumor efficacy relative to vehicle control resulting in 68% TGI (Table 10, FIG. 3). Compound 4 (100 mg/kg, QD) as a single agent provided tumor growth inhibition of 69% with even more robust inhibition in combination with darolutamide achieving 83% TGI. PF-06821497 also provided antitumor growth inhibition both as a single agent (100 mg/kg, BID) and in combination with darolutamide, TGI of 62% and 68%, respectively. All treatment groups resulted in significantly smaller EOS tumors relative to the vehicle treatment group with no differences between groups. Comparing the darolutamide combination groups revealed that Compound 4 combination had the most robust antitumor effect in the C4-2 castrated model relative to that of the PF-06821497 combination with 83% and 68% TGI, respectively.

[00421] Assessment of treatment groups based on progression free survival (PFS), defined as either tumor volumes exceeding 800mm³ or found dead, resulted in increased PFS in all groups relative to vehicle. The PFS data was based on dosing the mice in each group, other than the vehicle group, QD for 31 days. Darolutamide extended median PFS by 1.5 days relative to vehicle treatment. PF-06821497 also provided enhanced PFS both as a single agent or in combination with darolutamide resulting in 15.5 days and 24.5 days median PFS, respectively. Compound 4 treatment provided the most benefit to median PFS extension either as a single agent or in combination with darolutamide, reaching 27 days for single agent cohort and not reaching median PFS by study end (Table 10, FIG 9).

Example 7: Study of Compound 4, PF-06821497, and darolutamide in subcutaneous C4-2 prostate cancer xenograft model in intact male NPG mice

[00422] Compound 4 and PF-06821497 (an inhibitor of EZH2), alone and in combination with darolutamide, were evaluated in a subcutaneous C4-2 prostate cancer xenograft model in intact male NPG mice. The study was conducted as generally described in Example 6, using doses and dosing frequencies for each of Compound 4, PF-06821497 and darolutamide as set forth in Table 11. In this model, darolutamide (50 mg/kg twice daily (BID)) provided a TGI of negative 10.33 percent (Table 11, FIG. 4). Compound 4 (100 mg/kg

QD) when used as a single agent provided tumor growth inhibition of about 31 percent, while the combination of Compound 4 (100 mg/kg QD) and darolutamide (50 mg/kg BID) provided a TGI of about 72 percent (Table 11). PF-06821497 when used as a single agent (100 mg/kg BID) provided a TGI of about 55 percent, while use of the combination of PF-06821497 (100 mg/kg BID) and darolutamide (50 mg/kg BID) provided a TGI of about 58% (Table 11).

[00423] Assessment of treatment groups based on progression free survival (PFS), defined as either tumor volumes exceeding 800mm³ or found dead, resulted in increased PFS in all groups relative to vehicle. Darolutamide extended median PFS by 4 days relative to vehicle treatment. PF-06821497 also provided enhanced PFS both as a single agent or in combination with darolutamide resulting in 15.5 days and 17 days median PFS, respectively. Compound 4 treatment provided benefit to median PFS as a single agent extending to 14 days. Compound 4 in combination with darolutamide provided the greater extension of median PFS reaching 24 days by study end (Table 11, FIG. 10).

Table 11

Group	Dose (mg/kg)	Dosing Regimen (frequency x days)	Animal No.	% TGI (Day 24)	Day 24 Tumor Volume (mm ³)	p-value (vs. vehicle)	Progression Free Survival (days)
Vehicle 1 + Vehicle 2	n/a	QD × 25 BID × 25	10	n/a	2647.09 ± 179.8	n/a	7
Darolutamide	50	BID × 25	10	-10.33	2898.35 ± 432.32	not significant	11
Compound 4	100	QD × 25	10	31.1	1891.22 ± 190.1	not significant	14
Compound 4 + Darolutamide	100 50	QD × 25 BID × 25	10	71.65	905.49 ± 69.61	≤0.0001	24
PF-06821497	100	BID × 25	10	55.25	1304.34 ± 196.51	≤0.001	15.5
PF-06821497+ Darolutamide	100 50	BID × 25 BID × 25	10	58.17	1233.21 ± 167.17	≤0.001	17

Example 8: In vitro study of Compound 4 in combination with androgen receptor signaling inhibitors in C4-2 and LNCaP prostate cancer cell lines.

[00424] Cell-based studies in C4-2 and LNCaP prostate cancer cell lines were performed to evaluate the in vitro drug combination effects of Compound 4 and androgen receptor signaling inhibitors. Cell viability was assessed by quantitatively measuring ATP, a measure of metabolically active cells, after treatment with Compound 4, androgen receptor signaling inhibitors, and combinations of Compound 4 and androgen receptor signaling inhibitors. Analysis and visualization of the combination response data revealed synergy between Compound 4 and androgen receptor signaling inhibitors in multiple prostate cancer cell lines, utilizing quantitative methods to assess synergy (Bliss, Loewe and HSA).

[00425] The test articles were as follows: (a) Compound 4, (b) PF-06821497 (an EZH2 inhibitor), (c) enzalutamide; and (d) darolutamide. Each test article was dissolved in DMSO to generate a 10 mM stock solution. Dosing solutions of each test article were prepared by serial dilution in DMSO followed by dilution

in complete media to result in a 0.4% DMSO in the assay. Stock formulations were stored at -80 °C and dosing solutions were prepared for each experiment immediately prior to use. The effects of test articles were measured based on viability of C4-2 and LNCaP cells by CellTiter-Glo® (CTG) assays 14 days following treatment of the cells with the test articles.

[00426] The cells, reagents and working solutions used in the studies were sourced and prepared as follows. The samples of C4-2 and LNCaP cell lines were obtained from American Type Culture Collection (ATCC). The culture media for both cells lines was: RPMI plus 10% fetal bovine serum (FBS) plus 2 mM L-Glut plus 0.5 µg/m Penicillin-Streptomycin; RPMI1640 with phenol red (Corning, Cat#: 15-040-CV); FBS (Omega Scientific, Cat#: FB-11); L-glutamine (Corning, Cat#: 25-005-CI); Penicillin-Streptomycin (Gibco, Cat#: 15140122); assay media was the same as culture medium; CellTiter-Glo® 2.0 Cell Viability Assay (Promega, Cat#: G9243); and DMSO (Sigma, Cat#: D2660). The following materials and equipment were used to perform the studies: Bravo Liquid Handler (Agilent, Cat#: G5523BA); 96-well non-sterile polypropylene V-bottom plates (Corning, Cat#: 3363); 384-well sterile white with flat clear bottom plates (Greiner, Cat#: 781098); and TopSeal-A Plus plate seal (PerkinElmer, Cat#: 6050185).

[00427] C4-2 and LNCaP cells were cultured in complete culture media at exponential growth phase for at least one week prior to performance of each assay. The different cell suspensions were counted and seeded into 384-well sterile white with flat clear bottom plates using a Bravo liquid handler at a concentration of 500 cells in 56 µL of media per well. To prepare the compound dilutions from 10 mM stock solutions, dilution plates were prepared using the Bravo liquid handler by performing 10-point 1/3-fold serial dilutions in DMSO (for Compound 4 and PF-06821497) or 6-point one-third (1/3)-fold serial dilutions in DMSO (for darolutamide and enzalutamide) to a final concentration of 500-fold the initial concentration in the final assay plate. Compound dilutions in media were added to cells by dispensing 2 µL into cells plated the same day in 56 µL of culture media. Plates were placed in a 37 °C, 5% CO₂ incubator. Following 7 days of incubation the cell culture media and inhibitors were replenished and incubated for an additional 7 days. Prior to performing the viability assay, plates were allowed to equilibrate at room temperature before the addition of 1:1 ratio of CTG 2.0 using the Bravo liquid handler. The plates were sealed with a PerkinElmer TopSeal-A Plus plate seal and incubated at room temperature for 10 minutes before reading the luminescence on a plate reader (Tecan SPARK). The measured luminescence was a direct readout of the presence of ATP in the cells for measure of cell viability. Cell viability was measured with CTG in quadruplicates.

[00428] Data analysis: Data from a Tecan SPARK plate reader was transferred to a Microsoft Excel file and entered into GraphPad Prism. Curves were fitted with a four-parameter model and percent inhibition was calculated by GraphPad Prism. DMSO wells were used to define the upper limit (zero percent inhibition). The degree of combination synergy, neutrality, or antagonism, was quantified by comparing the observed drug combination response against the expected response, calculated using a reference model that assumes no interaction between drugs using the SynergyFinder website (Ianevski 2022). These models quantify the degree of synergy with different algorithms, either as the multiplicative effect of single drugs as if they acted

independently (Bliss), expected response corresponding to an additive effect as if the single drugs were the same compound (Loewe), or excess over the maximum single drug response (HSA).

[00429] Results: Compound 4, PF-06821497, darolutamide and enzalutamide when each was used as a single agent (not in combination) demonstrated nanomolar cell potency against C4-2 and LNCaP prostate cancer cells grown in complete media with full FBS and no additional hormones. The combinations of (a) Compound 4 and enzalutamide; and (b) Compound 4 and darolutamide each demonstrated a synergistic effect in the reduction in cell viability and a shift in the cell growth inhibition curves in both the C4-2 and LNCaP cell lines as demonstrated by the data in Table 12. As also shown in Table 12, synergistic effects were obtained in the C4-2 cell line with the combination of PF-06821497 and enzalutamide.

Table 12

Cell Line	Combination	Bliss	Loewe	HSA
C4-2	Compound 4 plus enzalutamide	10.6	15.8	16.7
C4-2	Compound 4 plus darolutamide	10.1	5.6	15.5
C4-2	PF-06821497 plus enzalutamide	9.9	14.0	15.4
LNCaP	Compound 4 plus enzalutamide	5.2	11.6	13.6

Example 9: Clinical evaluation of Compound 4 in human subjects having cancer

[00430] A clinical study was conducted using Compound 4 in human subjects having cancer.. Compound 4 was orally administered to human subjects in the form of a pharmaceutical composition. Subjects enrolled in the study were assigned to a cohort in which they were administered Compound 4 in an amount of 100 mg once-per-day (QD), 200 mg QD, 400 mg QD, 600 mg QD, 700 mg QD, 800 mg QD, or 900 mg QD for 28-day cycles. The number of subjects enrolled and administered Compound 4 at each dose level was as follows: 100 mg QD (subjects), 200 mg QD (4 subjects), 400 mg QD (3 subjects), 600 mg (3 subjects), and 900 mg (6 subjects).

[00431] The concentrations over time of Compound 4 in the plasma of subjects on cycle 2, day 1 (C2D1) that were orally administered Compound 4 in the form of a pharmaceutical composition, and for which C2D1 data was available (100 mg QD (2 subjects), 200 mg QD (3 subjects), 400 mg QD (3 subjects), 600 mg QD (3 subjects), and 900 mg QD (3 subjects)), were determined and were plotted as shown in FIG. 5. The concentration of Compound 4 in the plasma of subjects on C2D1 generally exhibited increased exposure with increasing dose level, low intra-patient variability, low intra-cohort variability, and a half-life ($t_{1/2}$) of greater than 12 hours.

[00432] Peripheral blood mononuclear cells (PBMCs) and plasma samples were collected from subjects on Cycle 1 Day 1 (C1D1) prior to the administration of Compound 4, and on Cycle 1 Day 15 (C1D15) and Cycle 2 Day 1 (C2D1) following the oral administration of Compound 4 to the subjects in the form of a pharmaceutical composition, for the assessment of target engagement in monocytes and cell-free nucleosomes, respectively. H3K27me3 and total histone H3 levels were measured in monocytes to evaluate the on-treatment change in H3K27me3 intensity normalized to H3. Dying tumor cells release nucleosomes to

circulation, so changes in H3K27me3 levels in cell-free nucleosomes are expected to reflect target engagement in the tumor niche. Pharmacodynamic changes were therefore also captured by quantifying treatment-induced changes in H3K27me3 levels in cell-free nucleosomes, normalized to levels of histone variant H3.1 as shown.

[00433] The percent change from baseline of H3K27me3/H3 in monocytes (mean $\pm$ SEM) at cycle 1, day 15 was determined in subjects that were orally administered Compound 4 at doses of 200 mg QD, 400 mg QD, 600 mg QD, 700 mg QD, 800 mg QD, or 900 mg QD in the form of a pharmaceutical composition were determined and were plotted as shown in FIG. 6. As is shown in FIG. 6, there was a greater than or equal to 75% decrease in H3K27me3 in monocytes of the subjects at each dose level.

[00434] The change from baseline of the ratio of H3K27me3 to histone 3.1 (H3.1) in cell-free nucleosomes from subjects at cycle 1, day 15 (C1D15) that were orally administered Compound 4 at doses of 100 mg once per day (QD), 200 mg QD, 400 mg QD, 600 mg QD, 700 mg QD, or 900 mg QD in the form of a pharmaceutical composition was determined as depicted in FIG. 7.

[00435] The change from baseline of the ratio of H3K27me3 to histone 3.1 (H3.1) in cell-free nucleosomes from subjects at cycle 2, day 1 (C2D1) that were orally administered Compound 4 at doses of 100 mg once per day (QD), 200 mg QD, 400 mg QD, 600 mg QD, 700 mg QD, or 900 mg QD in the form of a pharmaceutical composition was determined as depicted in FIG. 8.

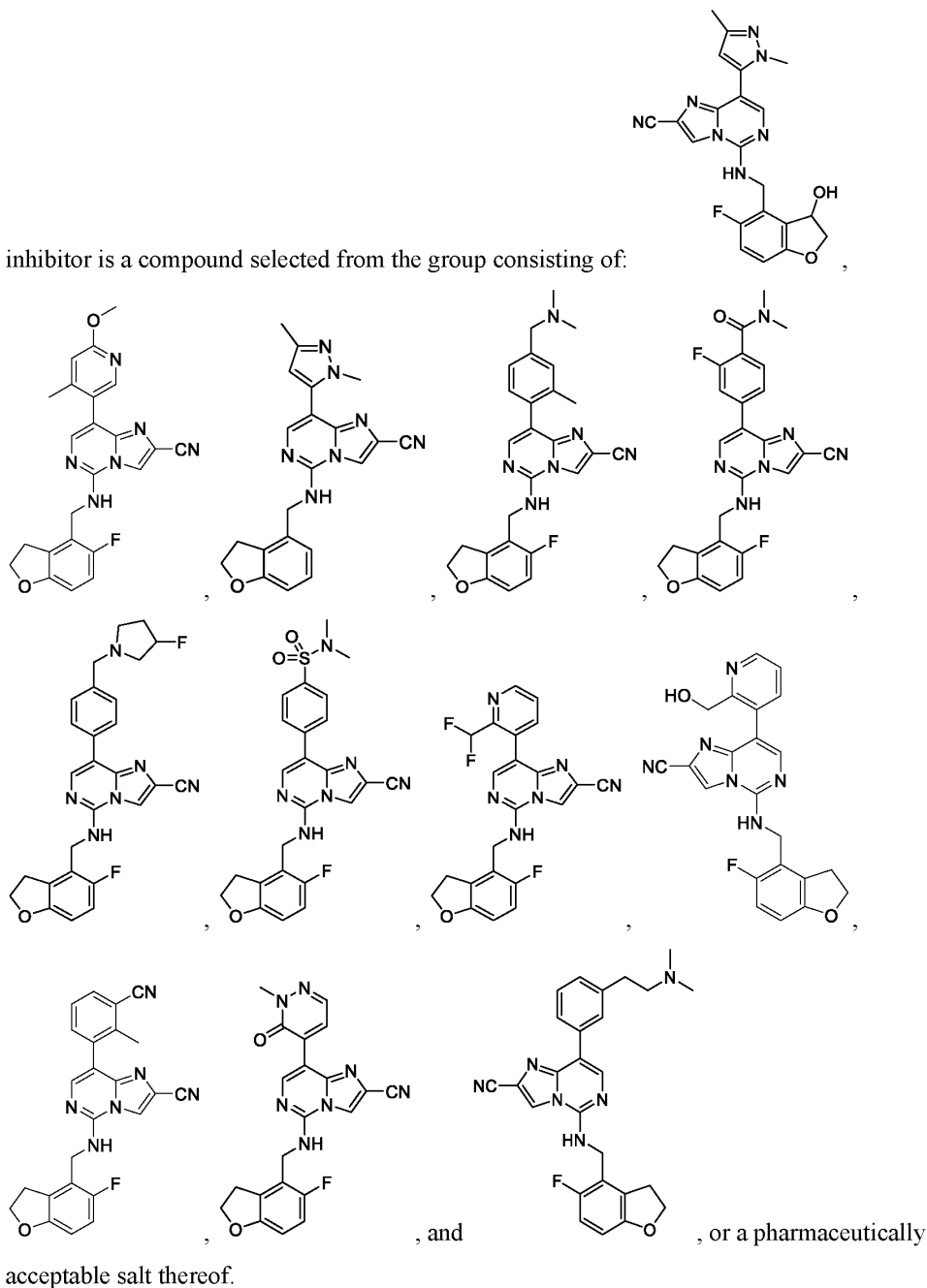
CLAIMS

WHAT IS CLAIMED is:

1. A method of treating prostate cancer in a subject, comprising administering to the subject (a) an androgen receptor inhibitor, and (b) an embryonic ectoderm development (EED) inhibitor.
2. The method of claim 1, wherein the subject has received prior administration of one or more CYP17 inhibitors.
3. The method of claim 2, wherein the subject has received prior administration of abiraterone or abiraterone acetate.
4. The method of any one of claims 1 to 3, wherein the prostate cancer in the subject has been determined to be resistant to abiraterone.
5. The method of claim 1, wherein the subject is CYP17 inhibitor-naïve prior to the administration to the subject of the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor.
6. The method of any one of claims 1 to 5, wherein the subject is androgen receptor inhibitor naïve prior to the administration to the subject of the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor.
7. The method of any one of claims 1 to 6, wherein the subject has been administered one or more prior androgen deprivation therapies prior to the administration to the subject of the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor.
8. The method of any one of claims 1 to 6, wherein the subject has been administered a gonadotropin-releasing hormone (GnRH) analog prior to the administration to the subject of the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor.
9. The method of any one of claims 1 to 8, wherein the androgen receptor inhibitor and the embryonic ectoderm development (EED) inhibitor are administered to the subject sequentially or simultaneously.
10. The method of any one of claims 1 to 9, wherein the androgen receptor inhibitor is selected from apalutamide, darolutamide, and enzalutamide.
11. The method of any one of claims 1 to 10, wherein the embryonic ectoderm development (EED) inhibitor is selected from EED226, A-395, APG-5918, BR-001, BR-002, EEDi-5285, EEDi-1056, FTX-6058, HJM-353, and MAK683.

12. The method of any one of claims 1 to 10, wherein the embryonic ectoderm development (EED)

inhibitor is a compound selected from the group consisting of:



13. The method of any one of claims 1 to 12, wherein the prostate cancer in the subject is selected from metastatic prostate cancer, non-metastatic prostate cancer, metastatic castration-resistant prostate cancer, metastatic castration-sensitive prostate cancer, localized high risk prostate cancer, recurrent prostate cancer, non-metastatic castration-resistant prostate cancer, non-metastatic castration-sensitive prostate cancer, androgen receptor inhibitor-sensitive prostate cancer, androgen receptor inhibitor-resistant prostate cancer, androgen receptor-dependent prostate cancer, androgen receptor-

independent prostate cancer, neuroendocrine prostate cancer (NEPC), metastatic neuroendocrine prostate cancer (NEPC), prostate cancer with small cell features, metastatic prostate cancer with small cell features, and aggressive-variant prostate cancer.

14. The method of claim 13, wherein the prostate cancer in the subject is metastatic prostate cancer.
15. The method of claim 14, wherein the prostate cancer in the subject is metastatic castration-resistant prostate cancer.