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(71) Applicant: **THE REGENTS OF THE UNIVERSITY OF COLORADO, A BODY CORPORATE** [US/US]; 1800 Grant Street, 8th Floor, Denver, Colorado 80203 (US).

(72) Inventor: **DOEBELE, Robert C.**; 752 S. Williams Street, Denver, Colorado 80209 (US).

(74) Agent: **SILVA, Domingos J.** et al.; Saul Ewing Arnstein & Lehr LLP, 1500 Market Street, 38th Floor, Philadelphia, Pennsylvania 19102 (US).

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— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*
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(54) Title: COMPOUNDS, COMPOSITIONS AND METHODS FOR TREATING OR PREVENTING HER-DRIVEN DRUG-RESISTANT CANCERS

(57) Abstract: The present invention includes methods of treating or preventing HER-driven drug-resistant cancers. In certain embodiments, the cancer comprises lung cancer.

WO 2019/051155 A1

TITLE OF THE INVENTION

Compounds, Compositions and Methods for Treating or Preventing HER-Driven Drug-Resistant Cancers

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Applications No. 62/556,121, filed September 8, 2017, and 62/712,531, filed July 31, 2018, all of which disclosures are hereby incorporated herein by reference in their entireties.

SEQUENCE LISTING

The present specification is being filed with a computer readable form (CRF) copy of the Sequence Listing. The CRF entitled 373330_7084WO1_ST25.txt, which was created on September 6, 2018 and is 451 KBytes in size, is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

The ErbB family of receptors is a subfamily of four closely related receptor tyrosine kinases: epidermal growth factor receptor or EGFR (ErbB-1; or HER1 in humans), HER2/c-neu (ErbB-2), HER3 (ErbB-3) and HER4 (ErbB-4).

EGFR is the cell-surface receptor for members of the epidermal growth factor family (EGF family) of extracellular protein ligands. Mutations that lead to EGFR overexpression (upregulation) or overactivity have been associated with a number of cancers, including squamous-cell carcinoma of the lung (80% of cases), anal cancers, glioblastoma (50% of cases), and epithelial tumors of the head and neck (80-100% of cases). These somatic mutations involving EGFR lead to its constant activation, which produces uncontrolled cell division. In a non-limiting example, in glioblastoma, a more or less specific mutation of EGFR, called EGFRvIII, is often observed. Mutations, amplifications, or misregulations of EGFR or family members are implicated in about 30% of all epithelial cancers.

The identification of EGFR as an oncogene has led to the development of anticancer therapeutics directed against EGFR (called "EGFR tyrosine kinase inhibitors"), including gefitinib, erlotinib, afatinib, osimertinib, and icotinib for lung cancer. Cetuximab, panitumumab, necitumumab, zalutumumab, nimotuzumab and matuzumab are examples of monoclonal

antibody EGFR inhibitors. Gefitinib, erlotinib, afatinib, dacomitinib, osimertinib, and lapatinib (mixed EGFR and ERBB2 inhibitor) are examples of small molecule EGFR kinase inhibitors.

Unfortunately, many patients develop resistance of the existent EGFR inhibitors. Non-limiting sources of resistance are the EGFR T790M Mutation, HER2 and MET oncogenes, transformation to small cell lung cancer (SCLC), epithelial to mesenchymal transition (EMT), nd fusions including those involving BRAF, NTRK1, RET, ALK, and/or ROS1. Options to combat resistance are limited, with only osimertinib being approved to treat EGFR T790M. While in frame deletions in exon 19 of EGFR and the L858R substitution in exon 21 of EGFR are sensitive to EGFR inhibitors (such as erlotinib, gefitinib, and afatinib), other mutations, such as in frame insertions in EGFR exon 20, demonstrate intrinsic resistance to these inhibitors. Other rare mutations in EGFR, such as G719X (exon 18) and L861Q (exon 21), have variable response to EGFR inhibitors. That said, the major reason why it is difficult to target these mutations is due to the undesirable on-target inhibition of WT EGFR in normal tissues.

There is thus a need in the art to identify compounds and methods that can be used to treat or prevent HER-driven drug-resistant cancers. In certain embodiments, the compounds and methods can be used to treat or prevent EGFR-driven, HER2-driven, and/or NGR1-driven drug-resistant cancers. The present application addresses and meets these needs.

BRIEF SUMMARY OF THE INVENTION

The application provides a method of treating or preventing a HER-driven drug-resistant cancer in a subject in need thereof. In certain embodiments, the method comprises providing tumor cells of the subject. In other embodiments, the method comprises detecting presence or absence of an EGFR exon 20 insertion mutation in the provided tumor cells. In yet other embodiments, the method comprises predicting the subject as being likely to be responsive to treatment by (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (Compound A) and/or (2E)-N-[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]-4-(dimethylamino)-2-butenamide) (Compound B), if the EGFR exon 20 insertion mutation is detected. In yet other embodiments, the method comprises administering a therapeutically effective amount of at least one compound selected from the group consisting of Compound A and Compound B, or a salt or a solvate thereof.

The application further provides a method of treating a HER-driven drug-resistant cancer in a subject with cancer, where an EGFR exon 20 insertion mutation is detected in tumor cells of the subject. In certain embodiments, the method comprises administering a therapeutically effective amount of at least one compound selected from the group consisting of Compound A and Compound B, or a salt or a solvate thereof.

The application further provides a method of predicting the responsiveness of a subject with a HER-driven drug-resistant cancer to treatment with at least one compound selected from the group consisting of Compound A and Compound B. In certain embodiments, the method comprises providing tumor cells of the subject. In other embodiments, the method comprises detecting presence or absence of an EGFR exon 20 insertion mutation in the provided tumor cells of the subject. In yet other embodiments, the method comprises predicting the subject as being likely to be responsive to a treatment with the at least one compound if the EGFR exon 20 insertion mutation is detected in the provided tumor cells of the subject.

The application further provides a method of predicting the responsiveness of a subject with a HER-driven drug-resistant cancer to treatment with at least one compound selected from the group consisting of Compound A and Compound B. In certain embodiments, the method comprises detecting presence or absence of an EGFR exon 20 insertion mutation in a sample of tumor cells from the subject. In other embodiments, the subject is likely to be responsive to the treatment with the at least one compound if the EGFR exon 20 insertion mutation is detected in the sample of tumor cells from the subject.

The application further provides a method of identifying a subject with cancer who is likely to be responsive to treatment with at least one compound selected from the group consisting of Compound A and Compound B. In certain embodiments, the method comprises providing tumor cells of the subject. In other embodiments, the method comprises detecting presence or absence of an EGFR exon 20 insertion mutation in the provided tumor cells. In yet other embodiments, the method comprises identifying the subject as being likely to be responsive to treatment with the at least one compound if the EGFR exon 20 insertion mutation is detected in the provided tumor cells.

In certain embodiments, the HER-driven cancer comprises an EGFR-driven cancer.

In certain embodiments, the EGFR-driven cancer comprises lung cancer.

In certain embodiments, the lung cancer comprises non-small cell lung cancer (NSCLC).

In certain embodiments, the mutation comprises at least one selected from the group consisting of A763_Y764insFQEA, A763_Y764insFQQA, A767_V769dupASV, D770_N771insGL, D770_N771insGT, D770_N771insNPG, D770_N771insSVD, E762Q_insFQEA, H773_V774insH, H773_V774insH, H773_V774insNPH, M766_A767insAI, M766_A767insASV, N771_H773dupNPH, P772_H773insYNP, P772_V774insPHV, S768_770dupSVD, V769_D770insASV, Y764_V765insHH, delD770insGY, and delL747_P753insS.

In certain embodiments, the cancer comprises at least one *ERBB2* gene fusion selected from the group consisting of *ZNF207-HER2*, *MDK-HER2*, *NOS2-HER2*, *ERBB2-GRB7*, *ERBB2-CTTN*, *ERBB2-PPP1R1B*, and *ERBB2-PSMB3*.

In certain embodiments, the cancer comprises at least one *ERBB2* mutation selected from the group consisting of G309A/E; S310F/Y; V659E/D; G660D; K753E; L755P/S; Del755-759; L768S; D769H/Y; V773L; A775_G776insYVMA; G776V/L, Cins; V777L; P780Ins; P780_Y781insGSP; V842I; L866M; and R896C.

In certain embodiments, the cancer comprises at least one *NRG1* gene fusion selected from the group consisting of *DOC4-NRG1*; *CD74-NRG1*; *SLC3A2-NRG1*; *RBPMS-NRG1*; *WRN-NRG1*; *SDC4-NRG1*; *RAB2IL1-NRG1*; *VAMP2-NRG1*; *KIF13B-NRG1*; *ATP1B1-NRG1*; *CDH6-NRG1*; *APP-NRG1*; *AKAP13-NRG1*; *THBS1-NRG1*; *PDE7A-NRG1*; *THAP7-NRG1*; *SMAD4-NRG1*; *RAB3IL1-NRG1*; *NRG1-PMEPA1*; and *NRG1-STMN2*.

In certain embodiments, the cancer comprises at least one *ERBB3* mutation selected from the group consisting of V104M; A232V; P262H; G284R; T389K; Q809R; S846I; and E928G.

In certain embodiments, the cancer comprises at least one *ERBB4* fusion selected from the group consisting of *EZR-ERBB4*; *IKZF2-ERBB4*; and *BGALT-ERBB4*.

In certain embodiments, the cancer is resistant to at least one agent selected from the group consisting of osimertinib, gefitinib, afatinib, and erlotinib.

In certain embodiments, the method further comprises administering at least one additional agent, or a salt or solvate thereof, that treats or prevents the cancer. In other embodiments, the at least one compound and the at least one additional agent are co-administered to the subject. In yet other embodiments, the at least one compound and the at least one additional agent are coformulated.

In certain embodiments, the at least one compound is administered by at least one route

selected from the group consisting of inhalational, oral, nasal, rectal, parenteral, sublingual, transdermal, transmucosal, intravesical, intrapulmonary, intraduodenal, intragastrical, intrathecal, epidural, intrapleural, intraperitoneal, intratracheal, otic, intraocular, subcutaneous, intramuscular, intradermal, intra-arterial, intravenous, intrabronchial, inhalation, and topical. In other embodiments, the at least one compound is administered orally. In yet other embodiments, the at least one compound is administered parenterally.

In certain embodiments, the subject is a mammal. In other embodiments, the mammal is a human. In yet other embodiments, the subject is in need of treatment thereof. In yet other embodiments, the subject is a human in need of treatment thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

The following detailed description of specific embodiments of the disclosure will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the disclosure, specific embodiments are shown in the drawings. It should be understood, however, that the disclosure is not limited to the precise arrangements and instrumentalities of the embodiments shown in the drawings.

FIG. 1 is a schematic illustration of the mechanism of activation of Compound A (tarloxotinib or TRLX, indicated as RN-4000) to Compound B (tarloxotinib-TKI or TRLX-TKI, indicated as RN-4000E), and subsequent irreversible inhibition of EGFR by Compound B.

FIG. 2 is a schematic illustration of oncogenic mutations observed in non-small cell lung cancer (NSCLC) patients.

FIGs. 3A-3B illustrate certain non-limiting EGFR domains. FIG. 3A is a schematic illustration to the EGFR kinase domain and certain oncogenic mutations thereof. exon 20_ins EGFR mutations are the third most common activating mutations in EGFR. Affected patients have poor response to first and second generation EGFR-TKIs (ORR~10%). FIG. 3B is a schematic representation of an EGFR protein domain, as well as localization of exon 20 insertions identified in patient derived cell lines generated in the present study. Three patient derived cell lines were isolated and propagated from pleural fluid; each of the cell lines presented a different EGFR exon 20 insertion mutation.

FIGs. 4A-4B comprise a series of graphs illustrating the finding that patient derived EGFR exon 20 insertion mutations cell lines depend on EGFR for proliferation. Cell lines were

transduced with one of two different shEGFR (1212014 or 10329) or with non-targeted shRNA (NTC). Proliferation was monitored by IncuCyte live-cell imagining system continuously for 80 h. Cell growth was expressed as an increase in percentage of confluence. EGFR exon 20 insertion cell lines include CUTO-14, CUTO-17, CUTO-18. EGFR mutant cell lines known to be EGFR dependent include PC9 (EGFR del 19), and H3255 (L858R).

FIGs. 5A-5C comprise a series of gel images illustrating the finding that the active metabolite of tarloxotinib, RN-4000E, inhibits EGFR phosphorylation and downstream signaling in cell lines harboring EGFR exon 20 insertion mutation cell lines. Cells were treated with the indicated doses of afatinib, gefitinib, and RN-4000E (active drug) for 2 hours, lysed and analyzed by immunoblot. Blots are representatives of three independent experiments.

FIGs. 6A-6B comprise a series of graphs illustrating dose response curves of cell viability of EGFR exon 20 insertion mutation patient derived cell lines (CUTO-14, CUTO-17, CUTO-18), mutated EGFR (PC9, H3255) and wild type EGFR (A549). Cells were treated with afatinib, gefitinib, RN-4000 (pro-drug) and RN-4000E (effector drug) for 72 hours under normoxic conditions and analyzed by MTS. Experiments were done by triplicate, mean and ±SEM is plotted. FIG. 6C comprises a table summarizing IC_{50} values of proliferation experiments. Values are expressed in nanomolar concentration. The data show that RN-4000E inhibits proliferation of cell lines harboring EGFR exon 20 insertion mutations. See also Table 5 for IC_{50} values of proliferation experiments.

FIGs. 7A-7B comprise graphs illustrating the finding that tarloxotinib reduces tumor burden in CUTO-14 murine xenografts. FIG. 7A: $1x10^6$ cells were injected in each flank of nude mice. Once tumors reached 200 mm^3, mice were randomly separated and treated with afatinib (6mg/Kg, daily, PO), cetuximab (40 mg/Kg, Q3d, IP), tarloxotinib (48 mg/Kg, Qwx4, IP) and vehicle for four weeks. Tumor volume was measured two times a week. FIG. 7B: Tarloxotinib is active in PDX models of EGFR exon 20 insertion that have been permitted to grow to a large size (average size of about 573 mm^3) before initiating treatment.

FIGs. 8A-8C comprise a series of gel images illustrating the finding that TRLX-TKI, also known as RN-4000E, has similar EGFR activity to afatinib in sensitizing EGFR mutation models.

FIGs. 9A-9C comprise a series of images and a table depicting data compiled from the cBioPortal (accessed June 14, 2018; Gao, *et al.*, 2013, Sci. Signal. 6(239):pl1). FIG. 9A

illustrates that *ERBB2* is mutated or amplified in NSCLC. FIG. 9B comprises a table illustrating presence of *ERBB2* gene amplification, missense mutations, indels, and gene fusions in NSCLC cohorts. Legend for genetic alteration: a, In-Frame Mutation (putative driver); b, Missense Mutation (putative driver); c, Missense Mutation (unknown significance); d, Truncating Mutation (unknown significance), e, Fusion; f, Amplification; g, Deep Deletion; h, No Alterations. Legend for study of origin: i, Genetic Characterization of NSCLC young adult patients (University of Turin, Lung Cancer 2016); j, Lung Adenocarcinoma (Broad, Cell 2012); k, Lung Adenocarcinoma (MSKCC, 2015); l, Lung Adenocarcinoma (TCGA, PanCancer Atlas); m, Lung Adenocarcinoma (TCGA, Provisional); n, MSK-IMPACT Clinical Sequencing Cohort for Non-Small Cell Cancer (MSK, Cancer Discovery 2017); o, Non-Small Cell Lung Cancer (MSK, JCO 2018); p, Pan-Lung Cancer (TCGA, Nat Genetics 2016). FIG. 9C illustrates a lollipop diagram highlighting recurrent mutations in NSCLC samples.

FIGs. 10A-10C comprise a series of gel images illustrating the finding that TRLX-TKI (RN-4000E) inhibits HER2 and HER3 phosphorylation and downstream signaling in AKT in cell lines harboring HER2 gene amplification (H2170 and Calu-3) or HER2 exon 20 insertion mutation (H1781). Cells were treated with the indicated doses of afatinib, gefitinib, osimertinib, TRLX-TKI (RN-4000E; active drug) for 2 hours under normoxic conditions, lysed and analyzed by immunoblot. Blots are representatives of three independent experiments.

FIG. 11 comprises a series of graphs illustrating dose response curves of cell viability of the patient derived cell lines harboring HER2 gene amplification (H2170 and CALU-3) or a HER2 exon 20 insertion mutation (H1781). Cells were treated with afatinib, gefitinib, osimertinib, TRLX (RN-4000; pro-drug) and TRLX-TKI (RN-4000E; effector drug) for 72 hours under normoxic conditions and analyzed by MTS. Experiments were done by triplicate, mean and ± SEM is plotted. See Table 3 for IC_{50} values of proliferation experiments. Values are expressed in nanomolar concentration. See Table 4 for fold difference in IC_{50} between the prodrug (TRLX) and the active metabolite (TRLX-TKI) under normoxic conditions.

FIG. 12 comprises a graph illustrating the finding that tarloxotinib reduces tumor burden in CUTO-17 murine xenografts. $1x10^6$ cells were injected in each flank of nude mice; once tumors reached 200 mm^3, mice were randomly separated and treated with cetuximab (40 mg/Kg, Q3d, IP), osimertinib (25 mg/kg, daily, PO), tarloxotinib (48 mg/Kg, Qwx4, IP) or vehicle for four weeks. Tumor volume was measured two times a week.

FIG. 13 comprises a graph illustrating dose response curves of cell viability of a patient derived cell line harboring *NRG1* gene amplification (HCC95). Cells were treated with afatinib, gefitinib, osimertinib, TRLX (RN-4000; pro-drug) and TRLX-TKI (RN-4000E; effector drug) for 72 hours under normoxic conditions and analyzed by MTS. As demonstrated herein, TRLX-TKI (RN-4000E) inhibits proliferation of this cell line.

FIG. 14 comprises a series of gel images illustrating the finding that TRLX-TKI (RN-4000E) inhibits HER3 phosphorylation and downstream signaling in the HCC95 cell line harboring *NRG1* gene amplification. Cells were treated with the indicated doses of afatinib, gefitinib, RN-4000E, and osimertinib (active drug) for 2 hours, lysed and analyzed by immunoblot. Blots are representatives of three independent experiments.

FIG. 15 comprises a graph illustrating the finding that TRLX-TKI (RN-4000E) inhibits proliferation of a breast cancer cell line harboring a *DOC4-NRG1* gene fusion (MDA-MB-157). Dose response curves of cell viability of these patient derived cell lines. Cells were treated with gefitinib, afatinib, TRLX-TKI (RN-4000E; effector drug), osimertinib, TRLX (RN-4000; pro-drug) or poziotinib for 72 hours under normoxic conditions and analyzed by MTS.

FIGs. 16A-16B comprise a series of graphs illustrating dose response curves of cell viability of EGFR exon 20 insertion mutation patient derived cell lines (CUTO-14, CUTO-17, CUTO-18). Cells were treated with TRLX-TKI, TRLX (pro-drug), gefitinib, afatinib, and osimertinib. As demonstrated herein, TRLX-TKI inhibits proliferation of cell lines harboring EGFR exon 20 insertion mutations.

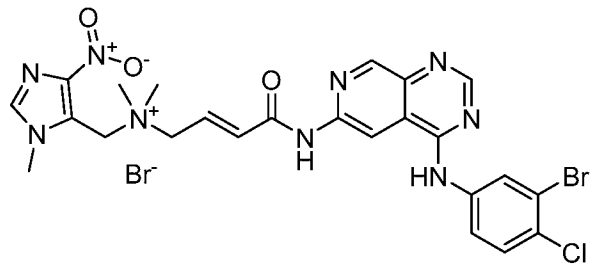
DETAILED DESCRIPTION OF THE INVENTION

The present disclosure relates in part to the discovery that certain nitromethylaryl quaternary ammonium salts (also referred to as NMQ prodrugs) can be used as small molecule EGFR inhibitors to treat or prevent certain HER-driven drug-resistant cancers. In certain embodiments, a small molecule EGFR inhibitor (RN-4000; also known as "(E)-4-((4-((3-bromo-4-chlorophenyl)amino)pyrido[3,4-d]pyrimidin-6-yl)amino)-N,N-dimethyl-N-((1-methyl-4-nitro-1H-imidazol-5-yl)methyl)-4-oxobut-2-en-1-aminium salt (bromide)"; also referred to herein as "(2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide"; also referred to herein as Compound A, RN-4000, TRLX, or tarloxotinib; FIG. 1) and/or its active

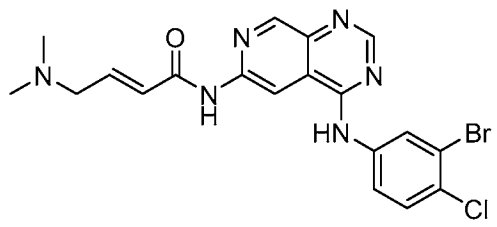
metabolite (RN-4000E; also known as "(E)-*N*-(4-((3-bromo-4-chlorophenyl)amino)pyrido[3,4-d]pyrimidin-6-yl)-4-(dimethylamino)but-2-enamide"; also referred to herein as "(2E)-N-[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]-4-(dimethylamino)-2-butenamide"); also referred to herein as Compound B; FIG. 1) are used to treat or prevent certain HER-driven drug-resistant cancers. In certain embodiments, the cancer is EGFR-driven.

It will be understood by one of ordinary skill in the art that recitation of Compound A herein also includes the corresponding cation ((E)-4-((4-((3-bromo-4-chlorophenyl)amino)pyrido[3,4-d]pyrimidin-6-yl)amino)-N,N-dimethyl-N-((1-methyl-4-nitro-1H-imidazol-5-yl)methyl)-4-oxobut-2-en-1-aminium), as well as any salt of such cation, such as but not limited to the bromide salt. It will be further understood by one of ordinary skill in the art that Compound A may exist as a cation or salt, for example, a bromide salt, as depicted below.

Structures of Compound A and Compound B are provided below:



Compound A, RN-4000, TH-4000, TRLX, or tarloxotinib

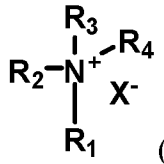


Compound B, RN-4000E, TH-4000E, or TRLX-TKI

The disclosure further contemplates the use of other NMQ prodrugs and/or small molecule EGFR inhibitors, including any other small molecule analogues of Compound A and/or Compound B, to treat or prevent certain HER-driven drug-resistant cancers. Such NMQ prodrugs and/or small molecule EGFR inhibitors include, but are not limited to those disclosed in WO2010104406, WO2011028135, US20120077811, and US20120202832, each of which is incorporated herein by reference in its entirety.

For example, the disclosure contemplates NMQ prodrugs of quaternary nitrogen salt

compounds of Formula I:



(I)

wherein in (I):

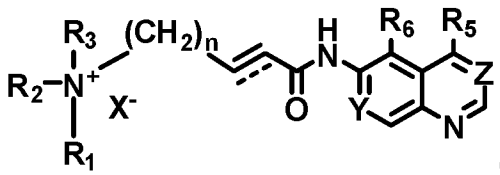
X is any negatively charged counterion (anion);

R_1 is a group of the formula $–(CH_2)_nTr$, where Tr is an aromatic nitroheterocycle or an aromatic nitrocarbocycle and $–(CH_2)_nTr$ acts as a reductively-activated fragmenting trigger ("reductive trigger"); and

n is an integer ranging from 0 to 6;

R_2, R_3 and R_4 are each independently an aliphatic or an aromatic group of a tertiary amine kinase inhibitor $(R_2)(R_3)(R_4)N$, or two of R_2, R_3, and R_4 may form an aliphatic or aromatic heterocyclic amine ring of a kinase inhibitor, or one of R_2, R_3 and R_4 may be absent and two of R_2, R_3 and R_4 form an aromatic heterocyclic amine ring of a kinase inhibitor.

In certain embodiments, the compounds are of Formula II:

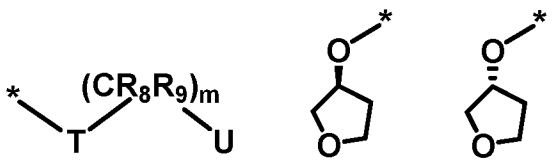


(II),

wherein:

X is any negatively charged counterion (anion);

Y is N or C-R_7, where R_7 is selected from the group consisting of H, C_1-C_6 alkyl, C_1-C_6 alkoxy, and a group of the one of the following Formulas IIIa, IIIb, and IIIc:



(IIIa) (IIIb) (IIIc) , wherein

* is the point of attachment;

T is selected from the group consisting of O, NH, N(C_1-C_6 alkyl), and a direct link (null);

m is an integer ranging from 0 to 6;

U is selected from the group consisting of OR_{10}, CF_3, OCF_3, CN, $NR_{11}R_{12}$, pyrrolidinyl,

piperidinyl, piperazinyl, N1-methylpiperazinyl, morpholinyl, $CON(R_{13})(R_{14})$, $SO_2N(R_{15})(R_{16})$, $N(R_{17})COR_{18}$, $N(R_{19})SO_2R_{20}$, COR_{21}, SOR_{22}, SO_2R_{23}, and $COOR_{24}$; and

R_8, R_9, R_{10}, R_{11}, R_{12}, R_{13}, R_{14} R_{15}, R_{16}, R_{17}, R_{18}, R_{19}, R_{20}, R_{21}, R_{22}, R_{23}, R_{24} are independently selected from the group consisting of H and C_1-C_6 alkyl;

Z is N or C-CN;

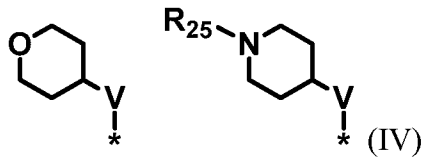
n is an integer ranging from 0 to 6;

R_1 is a group of the formula $(CH_2)_pTr$, wherein Tr is an aromatic nitroheterocycle or aromatic nitrocarbocycle and $-(CH_2)_pTr$ acts as a reductive trigger; and p is an integer ranging from from 0 to 6;

R_2 and R_3 are independently selected from the group consisting of C_1-C_6 alkyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, CH_2CH_2OH, and $CH_2CH_2O(C_1$-C_6 alkyl); or R_2 and R_3 may together form a non-aromatic carbocyclic ring or non-aromatic heterocyclic ring containing at least one heteroatom;

R_5 is selected from the group consisting of an aniline, an indole, an indoline, an amine, an aminoindole, and an aminoindazole, each of which may be optionally substituted with one or more substituents selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 alkoxy, F, Cl, Br, I, CN, CH_2F, CHF_2, CF_3, OH, NH_2, NO_2, $NH(C_1$-C_6 alkyl), $N(C_1$-C_6 alkyl$)_2$, $CONH_2$, $CO(C_1$-C_6 alkyl), SO_2NH_2, and $SO_2(C_1$-C_6 alkyl); and

R_6 is selected from the group consisting of H, C_1-C_6 alkyl, C_1-C_6 alkoxy, $NH(C_1$-C_6 alkyl), $N(C_1$-C_6 alkyl$)_2$, and a group of the following Formula IV:



(IV)

wherein:

* is the point of attachment;

V is selected from the group consisting of $(CH_2)_k$, O, NH, and $N(C_1$-C_6 alkyl);

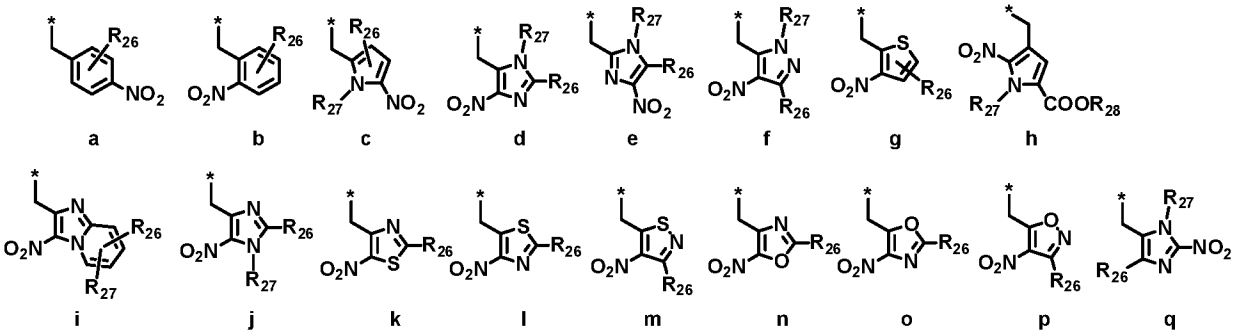
k is an integer ranging from 0 to 6, and

R_{25} is selected from the group consisting of H and C_1-C_6 alkyl.

In certain embodiments, X is selected from the group consisting of halide (*e.g.*, fluoride, chloride, bromide, iodide), methanesulfonate, trifluoromethanesulfonate, acetate, trifluoroacetate, tosylate, lactate, citrate, and formate. In certain embodiments, X is a halide. In

certain embodiments, X is selected from the group consisting of fluoride, chloride, bromide, and iodide.

In certain embodiments, R_1 is a group of one of the following Formulas Va-Vq:



wherein:

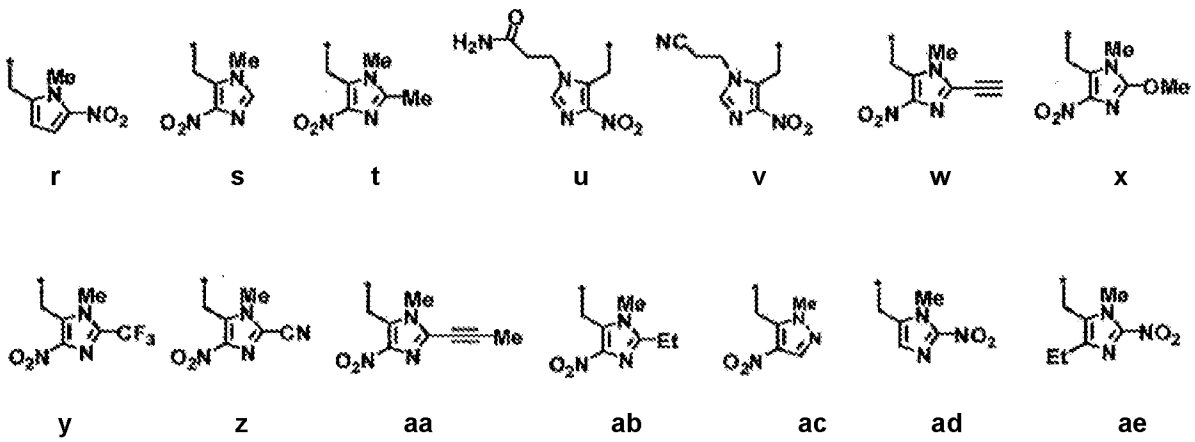
* is the point of attachment to the quaternary nitrogen of a compound of Formula II;

R_{26} is selected from the group consisting of H, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_2-C_6 alkenyl, C_2-C_6 alkynyl, CF_3, OCF_3, F, Cl, Br, I, NO_2, CN, COOH, COO(C1-C6 alkyl), $CONH_2$, $CONH(C_1$-C_6 alkyl), $CON(C_1$-C_6 alkyl)$_2$, $CO(C_1$-C_6 alkyl), SO_2NH_2, $SO_2NH(C_1$-C_6 alkyl), $SO_2N(C_1$-C_6 alkyl)$_2$, $SO_2(C_1$-C_6 alkyl), and a group of Formula IIIa, as defined above, wherein * is the point of attachment to a group of Formula V;

R_{27} is selected from the group consisting of H, C_1-C_6 alkyl, and a group of Formula IIIa, as defined above, wherein * is the point of attachment to a group of Formula V; and

R_{28} is selected from the group consisting of H and C_1-C_6 alkyl.

In certain embodiments, R_1 is a group of one of the following Formulas Vr-Vae:

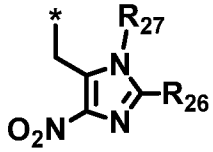


In certain embodiments, R_1 is a group of Formula Vc, wherein R_{26} is H and R_{27} is CH_3.

In certain embodiments, R_1 is a group of Formula Vd, where R_{26} is selected from the

group consisting of H, C_1-C_6 alkyl (*e.g.*, methyl), C_1-C_6 alkoxy (*e.g.*, OCH_3), C_2-C_6 alkynyl (*e.g.*, ethynyl), $CONH_2$, CONHMe, CF_3, OCF_3, Br, NO_2, and CN, and R_{27} is selected from the group consisting of CH_3, $CH_2CH_2CONH_2$, and CH_2CH_2CN.

In certain embodiments, R_1 is a group of Formula Vd,



(Vd)

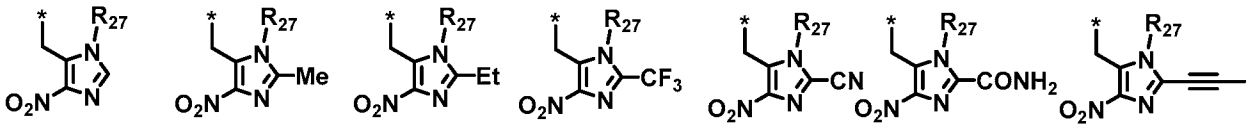
wherein * is a point of attachment, R_{26} is selected from the group consisting of H and C_1-C_3 alkyl, and R_{27} is selected from the group consisting of H and C_1-C_6 alkyl. In certain embodiments, R_{26} is selected from the group consisting of H, methyl, ethyl, trifluoromethyl, -CN, -$CONH_2$, and propyn-1-yl, and R_{27} is C_1-C_6 alkyl.

In certain embodiments, R_{26} is H and R_{27} is C_1-C_3 alkyl (*e.g.*, methyl).

In certain embodiments, R_1 is a group of Formula Vd, wherein R_{26} is 1-propynyl and R_{27} is CH_3.

In certain embodiments, R_1 is a group of Formula Vq, where R_{26} is selected from the group consisting of H, C_1-C_6 alkyl (*e.g.*, methyl or ethyl) and C_1-C_6 alkoxy (*e.g.*, OCH_3), and R_{27} is CH_3.

In certain embodiments, R_1 is a group of any one of Formulas $Vd^{(1)}$-$Vd^{(7)}$:

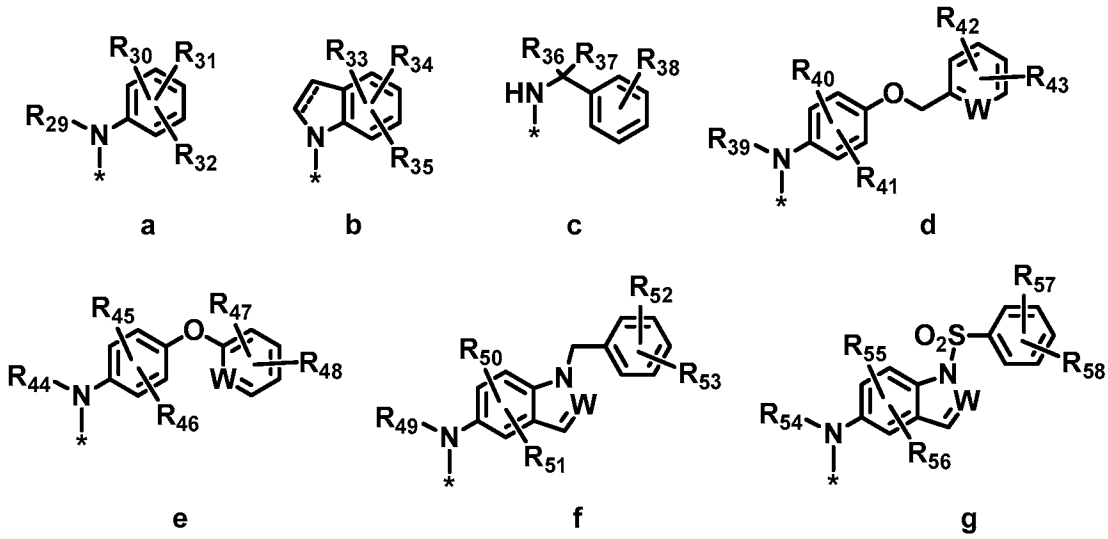


$Vd^{(1)}$ $Vd^{(2)}$ $Vd^{(3)}$ $Vd^{(4)}$ $Vd^{(5)}$ $Vd^{(6)}$ $Vd^{(7)}$

In certain embodiments, R_{27} is selected from the group consisting of methyl, ethyl and propyl. In certain embodiments R_{27} is methyl.

In certain embodiments, R_2 and R_3 form a ring selected from the group consisting of pyrrolidinium, piperidinium, piperazinium, N1-methylpiperazinium, and morpholinium.

In certain embodiments, R_5 is a group of one of the following Formulas VIa-VIg:



wherein:

* is the point of attachment;

R_{29}, R_{36}, R_{37}, R_{39}, R_{44}, R_{49} and R_{54}, are independently selected from the group consisting of H and C_1-C_6 alkyl;

R_{30}, R_{31}, R_{32}, R_{33}, R_{34}, R_{35}, R_{38}, R_{40}, R_{41}, R_{42}, R_{43}, R_{45}, R_{46}, R_{47}, R_{48}, R_{50}, R_{51}, R_{52}, R_{53}, R_{55}, R_{56}, R_{57} and R_{58} are independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 alkoxy, F, Cl, Br, I, CN, CH_2F, CHF_2, CF_3, OH, NH_2, NO_2, $NH(C_1$-C_6 alkyl), $N(C_1$-C_6 alkyl)$_2$, $CONH_2$, $CO(C_1$-C_6 alkyl), SO_2NH_2, and $SO_2(C_1$-C_6 alkyl); and

W is N or C-H.

In certain embodiments, Y is N, Z is N or C-CN; and R_1 is selected from the following:

(a) a group of Formula Vc, where R_{26} is H and R_{27} is CH_3;

(b) a group of Formula Vd, where (i) R_{26} is selected from the group consisting of H, C_1-C_6 alkyl (*e.g.*, methyl), C_1-C_6 alkoxy (*e.g.*, OCH_3), C_2-C_6 alkynyl (*e.g.*, ethynyl), CF_3, OCF_3, Br, NO_2, and CN, and R_{27} is selected from the group consisting of CH_3, $CH_2CH_2CONH_2$, and CH_2CH_2CN; or (ii) R_{26} is 1-propynyl and R_{27} is CH_3;

(c) a group of Formula Vf, where R_{26} is H and R_{27} is CH_3; and

(d) a group of Formula Vq, where R_{26} is selected from the group consisting of H, C_1-C_6 alkyl (*e.g.*, methyl and ethyl) and C_1-C_6 alkoxy (*e.g.*, OCH_3), and R_{27} is CH_3; wherein:

R_2 and R_3 are independently selected from the group consisting of C_1-C_6 alkyl, or together form a ring selected from the group consisting of pyrrolidinium, piperidinium, piperazinium, N1-

methylpiperazinium, and morpholinium; and

R_5 is selected from the following:

(a) a group of Formula VIa, where * is the point of attachment, R_{29} is H, and R_{30}, R_{31}, R_{32} are independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 alkoxy, F, Cl, Br, I, CN, CH_2F, CHF_2, CF_3, OH, NH_2, NO_2, NH(C_1-C_6 alkyl), and N(C_1-C_6 alkyl)$_2$;

(b) a group of Formula VId, where * is the point of attachment, R_{39} is H, and R_{40} and R_{41} are independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 alkoxy, F, Cl, Br, I, CN, CH_2F, CHF_2, CF_3, OH, NH_2, NO_2, NH(C_1-C_6 alkyl), and N(C_1-C_6 alkyl)$_2$; R_{42} and R_{43} are independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 alkoxy, F, Cl, Br, I, CN, CH_2F, CHF_2, CF_3, OH, NH_2, NO_2, NH(C_1-C_6 alkyl), and N(C_1-C_6 alkyl)$_2$; and W is N or C-H; and

(c) a group of Formula VIf, where * is the point of attachment, R_{49} is H, and R_{50} and R_{51} are independently selected from the group consisting of H and F; R_{52} and R_{53} are independently selected from the group consisting of H, C_1-C_6 alkyl, F, Cl, Br, I, CH_2F, CHF_2, and CF_3; W is N or C-H; R_6 is H; X is any negatively charged counterion; and n=1 or 2.

In certain embodiments, Y is C-H or C-(C_1-C_6 alkoxy), Z is N or C-CN; and R_1 is selected from the following:

(a) a group of Formula Vc, where R_{26} is H, and R_{27} is CH_3;

(b) a group of Formula Vd, where R_{26} is selected from the group consisting of H, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_2-C_6 alkynyl, CF_3, OCF_3, Br, NO_2, and CN, and R_{27} is selected from the group consisting of CH_3, $CH_2CH_2CONH_2$, and CH_2CH_2CN; or R_{26} is 1-propynyl and R_{27} is CH_3;

(c) a group of Formula Vf, where R_{26} is H and R_{27} is CH_3; and

(d) a group of Formula Vq, where R_{26} is selected from the group consisting of H, C_1-C_6 alkyl (*e.g.*, methyl and ethyl), and C_1-C_6 alkoxy (*e.g.*, OCH_3), and R_{27} is CH_3;

R_2 and R_3 are independently selected from the group consisting of C_1-C_6 alkyl, or together form a ring selected from pyrrolidinium, piperidinium, piperazinium, N1-methylpiperazinium, and morpholinium;

R_5 is selected from the following:

(a) a group of Formula VIa, where * is the point of attachment; R_{29} is H; and R_{30}, R_{31}, R_{32} are independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6

alkynyl, C_1-C_6 alkoxy, F, Cl, Br, I, CN, CH_2F, CHF_2, CF_3, OH, NH_2, NO_2, $NH(C_1$-C_6 alkyl), and $N(C_1$-C_6 alkyl)$_2$;

(b) a group of Formula VId, where * is the point of attachment; R_{39} is H; and R_{40} and R_{41} are independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 alkoxy, F, Cl, Br, I, CN, CH_2F, CHF_2, CF_3, OH, NH_2, NO_2, $NH(C_1$-C_6 alkyl), and $N(C_1$-C_6 alkyl)$_2$; R_{42} and R_{43} are independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 alkoxy, F, Cl, Br, I, CN, CH_2F, CHF_2, CF_3, OH, NH_2, NO_2, $NH(C_1$-C_6 alkyl), and $N(C_1$-C_6 alkyl)$_2$; and W is N or C-H; and

(c) a group of Formula VIf, where * is the point of attachment; R_{49} is H; and R_{50} and R_{51} are independently selected from the group consisting of H and F; R_{52} and R_{53} are independently selected from the group consisting of H, C_1-C_6 alkyl, F, Cl, Br, I, CH_2F, CHF_2, and CF_3; and W is N or C-H;

R_6 is H;

X is any negatively charged counterion (anion); and

n=1 or 2.

In certain embodiments, Y is C-R_7, where R_7 is a group of Formula IIIb; Z is N or C-CN; R_1 is selected from the following:

(a) a group of Formula Vc, where R_{26} is H, and R_{27} is CH_3;

(b) a group of Formula Vd, where R_{26} is selected from the group consisting of H, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_2-C_6 alkynyl, CF_3, OCF_3, Br, NO_2, and CN, and R_{27} is selected from the group consisting of CH_3, $CH_2CH_2CONH_2$, and CH_2CH_2CN; or R_{26} is 1-propynyl; and R_{27} is CH_3;

(c) a group of Formula Vf, where R_{26} is H and R_{27} is CH_3; and

(d) a group of Formula Vq, where R_{26} is selected from the group consisting of H, C_1-C_6 alkyl (*e.g.*, methyl and ethyl) and C_1-C_6 alkoxy (*e.g.*, OCH_3); and R_{27} is CH_3;

R_2 and R_3 are independently selected from C_1-C_6 alkyl, or together form a ring selected from the group consisting of pyrrolidinium, piperidinium, piperazinium, N1-methylpiperazinium, and morpholinium;

R_5 is selected from the following:

(a) a group of Formula VIa, where * is the point of attachment to a compound of Formula II; R_{29} is H; and R_{30}, R_{31}, R_{32} are independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 alkoxy, F, Cl, Br, I, CN, CH_2F, CHF_2, CF_3, OH, NH_2,

NO_2, $NH(C_1\text{-}C_6$ alkyl), and $N(C_1\text{-}C_6$ alkyl)$_2$;

(b) a group of Formula VId, where * is the point of attachment to a compound of Formula II; R_{39} is H; and R_{40} and R_{41} are independently selected from the group consisting of H, $C_1\text{-}C_6$ alkyl, $C_2\text{-}C_6$ alkenyl, $C_2\text{-}C_6$ alkynyl, $C_1\text{-}C_6$ alkoxy, F, Cl, Br, I, CN, CH_2F, CHF_2, CF_3, OH, NH_2, NO_2, $NH(C_1\text{-}C_6$ alkyl), and $N(C_1\text{-}C_6$ alkyl)$_2$; R_{42} and R_{43} are independently selected from the group consisting of H, $C_1\text{-}C_6$ alkyl, $C_2\text{-}C_6$ alkenyl, $C_2\text{-}C_6$ alkynyl, $C_1\text{-}C_6$ alkoxy, F, Cl, Br, I, CN, CH_2F, CHF_2, CF_3, OH, NH_2, NO_2, $NH(C_1\text{-}C_6$ alkyl), and $N(C_1\text{-}C_6$ alkyl)$_2$; and W is N or C-H; and

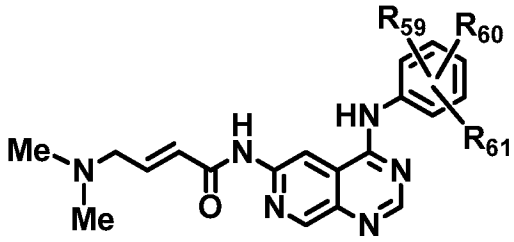
(c) a group of Formula VIf, where * is the point of attachment to a compound of Formula II; R_{49} is H; and R_{50} and R_{51} are independently selected from the group consisting of H or F; R_{52} and R_{53} are independently selected from the group consisting of H, $C_1\text{-}C_6$ alkyl, F, Cl, Br, I, CH_2F, CHF_2, and CF_3; and W is N or C-H;

R_6 is H;

X is any negatively charged counterion; and

n=1 or 2.

In certain embodiments, the compounds are of Formula VII:



(VII)

wherein either:

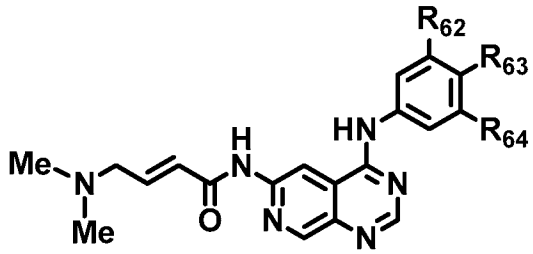
(1) R_{59} is H, and one of the following applies: (a) R_{60} is (3-chlorobenzyl)oxy- and R_{61} is chloro; (b) R_{60} and R_{61}, together with the carbon atoms to which they are attached, form 1-(3-fluorobenzyl)-1*H*-pyrazole; (c) R_{60} is 2-pyridinylmethoxy and R_{61} is chloro; (d) R_{60} and R_{61} are both chloro; (e) R_{60} is chloro and R_{61} is bromo; (f) R_{60} and R_{61} are both bromo; (g) R_{60} is fluoro and R_{61} is ethynyl; (h) R_{60} is chloro and R_{61} is ethynyl; (i) R_{60} is bromo and R_{61} is ethynyl; (j) other than when R_{60} is in the 3-position in combination with R_{61} in the 4-position, R_{60} is bromo and R_{61} is fluoro; (k) R_{60} is 2-pyridinylmethoxy and R_{61} is fluoro; or (l) R_{60} is 2-pyridinylmethoxy and R_{61} is bromo; or

(2) at least one of R_{59}, R_{60} and R_{61} is selected from the group consisting of benzyloxy, 3-chlorobenzyloxy, and 2-pyridinylmethoxy, and when at least one of R_{59}, R_{60} and R_{61} is not

benzyloxy, 3-chlorobenzyloxy or 2-pyridinylmethoxy, each of the others is independently selected from the group consisting of H, halogen, and C_2–C_4 alkynyl, with the proviso that when one of R_{59}, R_{60} and R_{61} is benzyloxy or 2-pyridinylmethoxy, the other two of R_{59}, R_{60} and R_{61} are not H; or

(3) two of R_{59}, R_{60} and R_{61}, together with the carbon atoms to which they are attached, form 1-(3-fluorobenzyl)-1*H*-pyrazole, and the other is selected from the group consisting of H, halogen, and C_2–C_4 alkynyl.

In certain embodiments, the compound of Formula VII is a compound according to Formula VIII:



(VIII)

wherein R_{62} is H, and either (a) R_{63} is (3-chlorobenzyl)oxy- and R_{64} is chloro; (b) R_{63} and R_{64}, together with the carbon atoms to which they are attached, form 1-(3-fluorobenzyl)-1*H*-pyrazole; (c) R_{63} is 2-pyridinylmethoxy and R_{64} is chloro; (d) R_{63} and R_{64} are both chloro; (e) R_{63} is chloro and R_{64} is bromo; (f) R_{63} is bromo and R_{64} is chloro; (g) R_{63} and R_{64} are both bromo; (h) R_{63} is fluoro and R_{64} is ethynyl; (i) R_{63} is chloro and R_{64} is ethynyl; (j) R_{63} is bromo and R_{64} is ethynyl; (k) R_{63} is bromo and R_{64} is fluoro; (l) R_{63} is 2-pyridinylmethoxy and R_{64} is fluoro; or (m) R_{63} is 2-pyridinylmethoxy and R_{64} is bromo.

In certain embodiments, the compound of Formula VII is selected from the group consisting of:

(2*E*)-*N*-(4-{3-chloro-4-[(3-chlorobenzyl)oxy]anilino}pyrido[3,4-*d*]pyrimidin-6-yl)-4-(dimethylamino)-2-butenamide (**1**),

(2*E*)-4-(dimethylamino)-*N*-(4-{[1-(3-fluorobenzyl)-1*H*-indazol-5-yl]amino}pyrido[3,4-*d*]pyrimidin-6-yl)-2-butenamide (**2**),

(2*E*)-*N*-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}-4-(dimethylamino)-2-butenamide (**3**),

(2*E*)-*N*-[4-(3,4-dichloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-4-(dimethylamino)-2-

butenamide (**4**),

(2*E*)-*N*-[4-(3-bromo-4-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-4-(dimethylamino)-2-butenamide (**5**),

(2*E*)-*N*-[4-(4-bromo-3-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-4-(dimethylamino)-2-butenamide (**6**),

(2*E*)-*N*-[4-(3,4-dibromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-4-(dimethylamino)-2-butenamide (**7**),

(2*E*)-4-(dimethylamino)-*N*-[4-(3-ethynyl-4-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-2-butenamide (**8**),

(2*E*)-*N*-[4-(4-chloro-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-4-(dimethylamino)-2-butenamide (**9**),

(2*E*)-*N*-[4-(4-bromo-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-4-(dimethylamino)-2-butenamide (**10**),

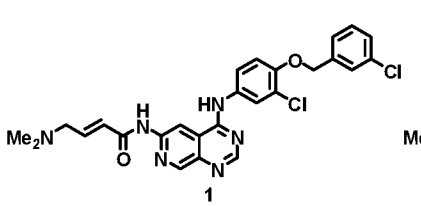
(2*E*)-*N*-[4-(4-bromo-3-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-4-(dimethylamino)-2-butenamide (**11**),

(2*E*)-4-(dimethylamino)-*N*-{4-[3-fluoro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}-2-butenamide (**89**) and

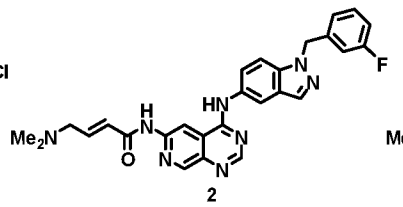
(2*E*)-*N*-{4-[3-bromo-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}-4-(dimethylamino)-2-butenamide (**90**);

or a salt, solvate, or a salt thereof

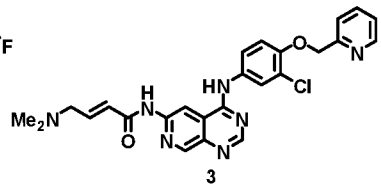
The structures of compounds provided in the list above are depicted below:



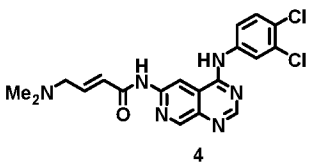
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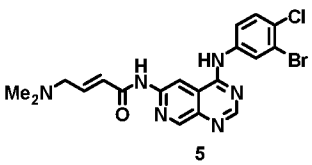
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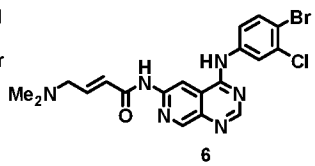
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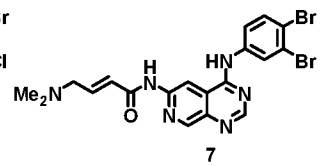
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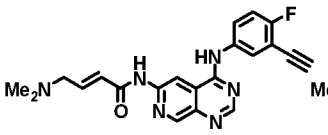
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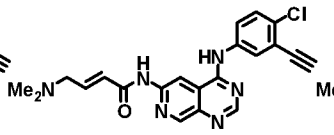


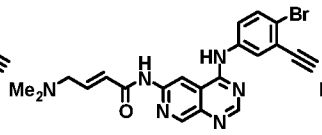
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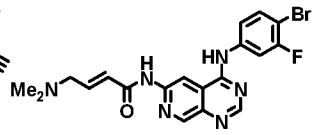


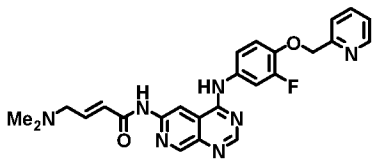
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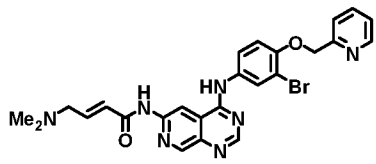

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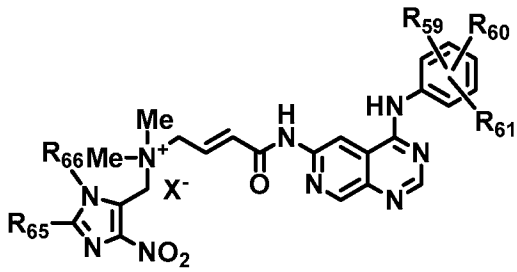

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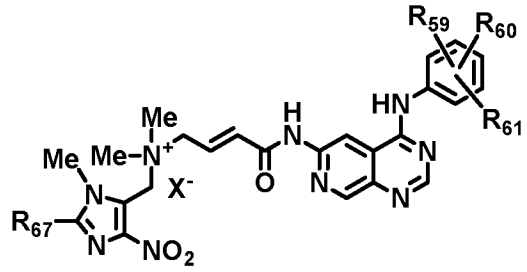

90

In certain embodiments, the compounds are of Formula IX:


(IX)

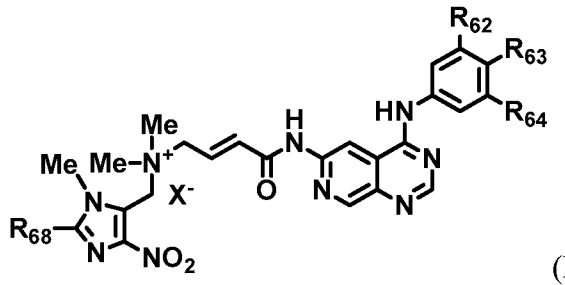
wherein X is any negatively charged counterion, R_{59}, R_{60} and R_{61} are as defined for Formula VII, R_{65} is selected from the group consisting of H, methyl, ethyl, trifluoromethyl, -CN, $-CONH_2$, and propyn-1-yl, and R_{66} is C_1-C_6 alkyl.

In certain embodiments, the compounds are of Formula X:


(X)

wherein X is any negatively charged counterion, R_{59}, R_{60} and R_{61} are as defined for Formula VII and R_{67} is selected from the group consisting of H, methyl, ethyl, trifluoromethyl, -CN, $-CONH_2$, and propyn-1-yl.

In certain embodiments, the compounds are of Formula XI:



(XI)

wherein X is any negatively charged counterion, R_{62}, R_{63} and R_{64} are as defined for Formula VIII and R_{68} is selected from the group consisting of H, methyl, ethyl, trifluoromethyl, -CN, -$CONH_2$, and propyn-1-yl.

In certain embodiments, X is selected from the group consisting of halide (*e.g.*, fluoride, chloride, bromide, iodide), methanesulfonate, trifluoromethanesulfonate, acetate, trifluoroacetate, tosylate, lactate, citrate and formate.

In certain embodiments, the compounds are selected from the group consisting of:

(2*E*)-4-[(4-{3-chloro-4-[(3-chlorobenzyl)oxy]anilino}pyrido[3,4-*d*]pyrimidin-6-yl)amino]-*N*,*N*-dimethyl-*N*-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (**12**),

(2*E*)-4-[(4-{[1-(3-fluorobenzyl)-1*H*-indazol-5-yl]amino}pyrido[3,4-*d*]pyrimidin-6-yl)amino]-*N*,*N*-dimethyl-*N*-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (**13**),

(2*E*)-*N*-[(1,2-dimethyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-[(4-{[1-(3-fluorobenzyl)-1*H*-indazol-5-yl]amino}pyrido[3,4-*d*]pyrimidin-6-yl)amino]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**14**),

(2*E*)-4-({4-[3-chloro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N*,*N*-dimethyl-*N*-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (**15**),

(2*E*)-4-{[4-(3,4-dichloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-*N*-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (**16**),

(2*E*)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-*N*-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (**17**),

(2*E*)-4-{[4-(4-bromo-3-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-*N*-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (**18**),

(2*E*)-4-{[4-(3,4-dibromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-*N*-[(1-

methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (**19**),

(2*E*)-4-{[4-(3-ethynyl-4-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-*N*-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (**20**),

(2*E*)-4-{[4-(4-chloro-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-*N*-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (**21**),

(2*E*)-4-[(4-{3-chloro-4-[(3-chlorobenzyl)oxy]anilino}pyrido[3,4-*d*]pyrimidin-6-yl)amino]-*N*-[(1,2-dimethyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**22**),

(2*E*)-4-[(4-{3-chloro-4-[(3-chlorobenzyl)oxy]anilino}pyrido[3,4-*d*]pyrimidin-6-yl)amino]-*N*-[(2-ethyl-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**23**),

(2*E*)-4-[(4-{3-chloro-4-[(3-chlorobenzyl)oxy]anilino}pyrido[3,4-*d*]pyrimidin-6-yl)amino]-*N*,*N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(trifluoromethyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**24**),

(2*E*)-4-[(4-{3-chloro-4-[(3-chlorobenzyl)oxy]anilino}pyrido[3,4-*d*]pyrimidin-6-yl)amino]-*N*-[(2-cyano-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**25**),

(2*E*)-*N*-{[2-(aminocarbonyl)-1-methyl-4-nitro-1*H*-imidazol-5-yl]methyl}-4-[(4-{3-chloro-4-[(3-chlorobenzyl)oxy]anilino}pyrido[3,4-*d*]pyrimidin-6-yl)amino]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**26**),

(2*E*)-4-[(4-{3-chloro-4-[(3-chlorobenzyl)oxy]anilino}pyrido[3,4-*d*]pyrimidin-6-yl)amino]-*N*,*N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(1-propynyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**27**),

(2*E*)-*N*-[(2-ethyl-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-[(4-{[1-(3-fluorobenzyl)-1*H*-indazol-5-yl]amino}pyrido[3,4-*d*]pyrimidin-6-yl)amino]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**28**),

(2*E*)-4-[(4-{[1-(3-fluorobenzyl)-1*H*-indazol-5-yl]amino}pyrido[3,4-*d*]pyrimidin-6-yl)amino]-*N*,*N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(trifluoromethyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**29**),

(2*E*)-*N*-[(2-cyano-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-[(4-{[1-(3-fluorobenzyl)-1*H*-indazol-5-yl]amino}pyrido[3,4-*d*]pyrimidin-6-yl)amino]-*N*,*N*-dimethyl-4-oxo-2-buten-1-

ammonium bromide (**30**),

(2*E*)-*N*-{[2-(aminocarbonyl)-1-methyl-4-nitro-1*H*-imidazol-5-yl]methyl}-4-[(4-{[1-(3-fluorobenzyl)-1*H*-indazol-5-yl]amino}pyrido[3,4-*d*]pyrimidin-6-yl)amino]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**31**),

(2*E*)-4-[(4-{[1-(3-fluorobenzyl)-1*H*-indazol-5-yl]amino}pyrido[3,4-*d*]pyrimidin-6-yl)amino]-*N*,*N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(1-propynyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**32**),

(2*E*)-4-({4-[3-chloro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N*-[(1,2-dimethyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**33**),

(2*E*)-4-({4-[3-chloro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N*-[(2-ethyl-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**34**),

(2*E*)-4-({4-[3-chloro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N*,*N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(trifluoromethyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**35**),

(2*E*)-4-({4-[3-chloro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N*-[(2-cyano-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**36**),

(2*E*)-*N*-{[2-(aminocarbonyl)-1-methyl-4-nitro-1*H*-imidazol-5-yl]methyl}-4-({4-[3-chloro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**37**),

(2*E*)-4-({4-[3-chloro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N*,*N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(1-propynyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**38**),

(2*E*)-4-{[4-(3,4-dichloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(1,2-dimethyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**39**),

(2*E*)-4-{[4-(3,4-dichloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(2-ethyl-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**40**),

(2*E*)-4-{[4-(3,4-dichloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(trifluoromethyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium

bromide (**41**),

(2*E*)-*N*-[(2-cyano-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-{[4-(3,4-dichloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**42**),

(2*E*)-*N*-{[2-(aminocarbonyl)-1-methyl-4-nitro-1*H*-imidazol-5-yl]methyl}-4-{[4-(3,4-dichloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**43**),

(2*E*)-4-{[4-(3,4-dichloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(1-propynyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**44**),

(2*E*)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(1,2-dimethyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**45**),

(2*E*)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(2-ethyl-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**46**),

(2*E*)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(trifluoromethyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**47**),

(2*E*)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(2-cyano-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**48**),

(2*E*)-*N*-{[2-(aminocarbonyl)-1-methyl-4-nitro-1*H*-imidazol-5-yl]methyl}-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**49**),

(2*E*)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(1-propynyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**50**),

(2*E*)-4-{[4-(4-bromo-3-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(1,2-dimethyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**51**),

(2*E*)-4-{[4-(4-bromo-3-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(2-ethyl-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide

(**52**),

(2*E*)-4-{[4-(4-bromo-3-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(trifluoromethyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**53**),

(2*E*)-4-{[4-(4-bromo-3-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(2-cyano-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**54**),

(2*E*)-*N*-{[2-(aminocarbonyl)-1-methyl-4-nitro-1*H*-imidazol-5-yl]methyl}-4-{[4-(4-bromo-3-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**55**),

(2*E*)-4-{[4-(4-bromo-3-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(1-propynyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**56**),

(2*E*)-4-{[4-(3,4-dibromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(1,2-dimethyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**57**),

(2*E*)-4-{[4-(3,4-dibromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(2-ethyl-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**58**),

(2*E*)-4-{[4-(3,4-dibromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(trifluoromethyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**59**),

(2*E*)-*N*-[(2-cyano-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-{[4-(3,4-dibromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**60**),

(2*E*)-*N*-{[2-(aminocarbonyl)-1-methyl-4-nitro-1*H*-imidazol-5-yl]methyl}-4-{[4-(3,4-dibromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**61**),

(2*E*)-4-{[4-(3,4-dibromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(1-propynyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**62**),

(2*E*)-*N*-[(1,2-dimethyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-{[4-(3-ethynyl-4-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium

bromide (**63**),

(2*E*)-*N*-[(2-ethyl-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-{[4-(3-ethynyl-4-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**64**),

(2*E*)-4-{[4-(3-ethynyl-4-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(trifluoromethyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**65**),

(2*E*)-*N*-[(2-cyano-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-{[4-(3-ethynyl-4-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**66**),

(2*E*)-*N*-{[2-(aminocarbonyl)-1-methyl-4-nitro-1*H*-imidazol-5-yl]methyl}-4-{[4-(3-ethynyl-4-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**67**),

(2*E*)-4-{[4-(3-ethynyl-4-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(1-propynyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**68**),

(2*E*)-4-{[4-(4-chloro-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(1,2-dimethyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**69**),

(2*E*)-4-{[4-(4-chloro-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(2-ethyl-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**70**),

(2*E*)-4-{[4-(4-chloro-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(trifluoromethyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**71**),

(2*E*)-4-{[4-(4-chloro-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(2-cyano-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**72**),

(2*E*)-*N*-{[2-(aminocarbonyl)-1-methyl-4-nitro-1*H*-imidazol-5-yl]methyl}-4-{[4-(4-chloro-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**73**),

(2*E*)-4-{[4-(4-chloro-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(1-propynyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**74**),

(2*E*)-4-{[4-(4-bromo-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-*N*-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (**75**),

(2*E*)-4-{[4-(4-bromo-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(1,2-dimethyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**76**),

(2*E*)-4-{[4-(4-bromo-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(2-ethyl-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**77**),

(2*E*)-4-{[4-(4-bromo-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(trifluoromethyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**78**),

(2*E*)-4-{[4-(4-bromo-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(2-cyano-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**79**),

(2*E*)-*N*-{[2-(aminocarbonyl)-1-methyl-4-nitro-1*H*-imidazol-5-yl]methyl}-4-{[4-(4-bromo-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**80**),

(2*E*)-4-{[4-(4-bromo-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(1-propynyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**81**),

(2*E*)-4-{[4-(4-bromo-3-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-*N*-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (**82**),

(2*E*)-4-{[4-(4-bromo-3-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(1,2-dimethyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**83**),

(2*E*)-4-{[4-(4-bromo-3-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(2-ethyl-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**84**),

(2*E*)-4-{[4-(4-bromo-3-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-*N*-

{[1-methyl-4-nitro-2-(trifluoromethyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**85**),

(2*E*)-4-{[4-(4-bromo-3-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(2-cyano-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**86**),

(2*E*)-*N*-{[2-(aminocarbonyl)-1-methyl-4-nitro-1*H*-imidazol-5-yl]methyl}-4-{[4-(4-bromo-3-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**87**),

(2*E*)-4-{[4-(4-bromo-3-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*,*N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(1-propynyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**88**),

(2*E*)-4-({4-[3-fluoro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N*,*N*-dimethyl-*N*-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (**91**),

(2*E*)-*N*-[(1,2-dimethyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-({4-[3-fluoro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**92**),

(2*E*)-*N*-[(2-ethyl-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-({4-[3-fluoro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**93**),

(2*E*)-4-({4-[3-fluoro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N*,*N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(trifluoromethyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**94**),

(2*E*)-*N*-[(2-cyano-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-({4-[3-fluoro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**95**),

(2*E*)-*N*-{[2-(aminocarbonyl)-1-methyl-4-nitro-1*H*-imidazol-5-yl]methyl}-4-({4-[3-fluoro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**96**),

(2*E*)-4-({4-[3-fluoro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N*,*N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(1-propynyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-

ammonium bromide (**97**),

(2*E*)-4-({4-[3-bromo-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N*,*N*-dimethyl-*N*-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (**98**),

(2*E*)-4-({4-[3-bromo-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N*-[(1,2-dimethyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**99**),

(2*E*)-4-({4-[3-bromo-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N*-[(2-ethyl-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**100**),

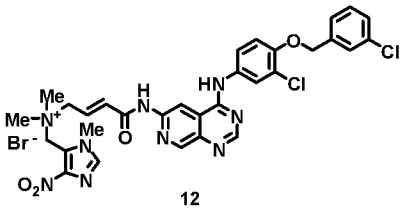
(2*E*)-4-({4-[3-bromo-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N*,*N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(trifluoromethyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**101**),

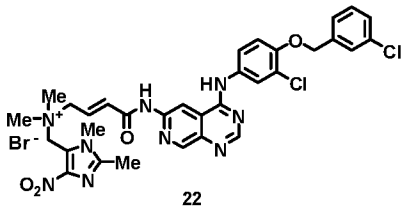
(2*E*)-4-({4-[3-bromo-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N*-[(2-cyano-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**102**),

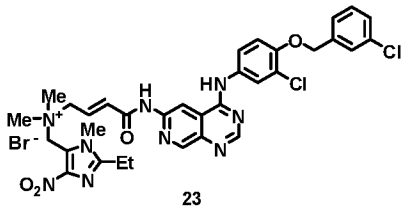
(2*E*)-*N*-{[2-(aminocarbonyl)-1-methyl-4-nitro-1*H*-imidazol-5-yl]methyl}-4-({4-[3-bromo-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N*,*N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**103**) and

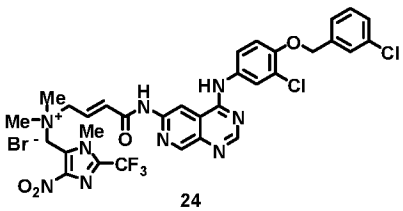
(2*E*)-4-({4-[3-bromo-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N*,*N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(1-propynyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**104**); or any other salt thereof (*e.g.*, the listed counterion is exchanged for any other counterion, which in certain embodiments is a pharmaceutically acceptable counterion, of same polarity − negative or positive), or any solvate thereof.

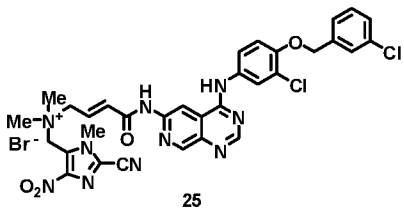
The structures of the compounds provided in the list above are depicted below:

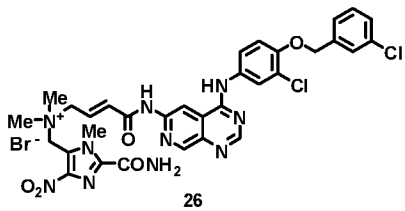

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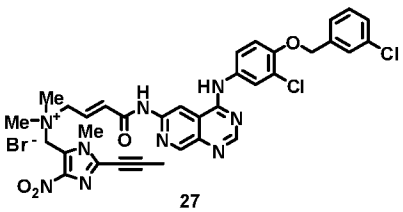

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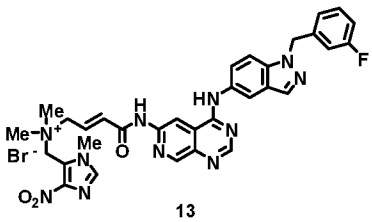

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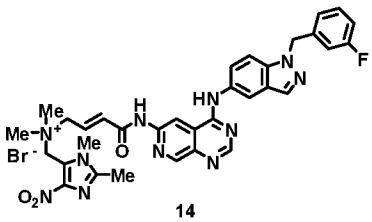

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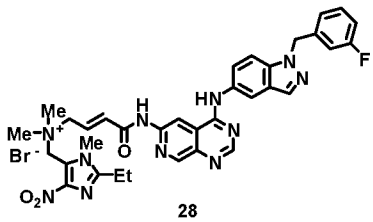

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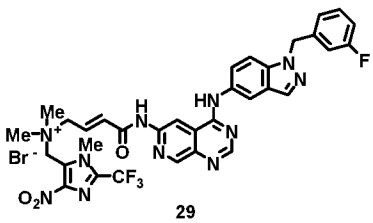

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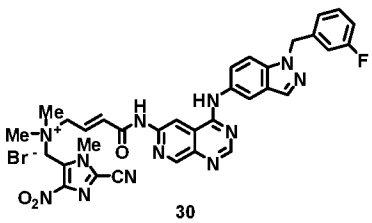

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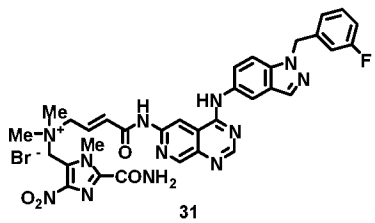

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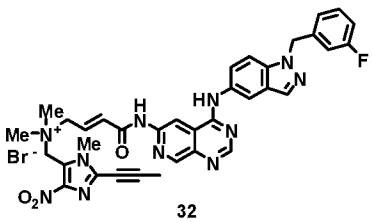

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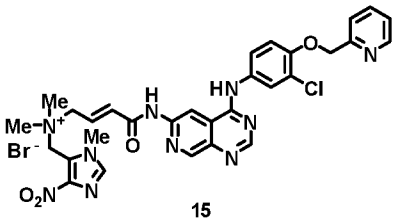

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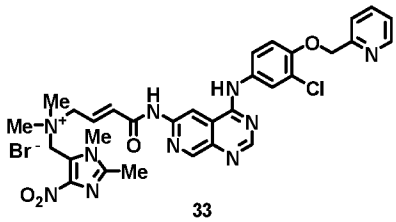

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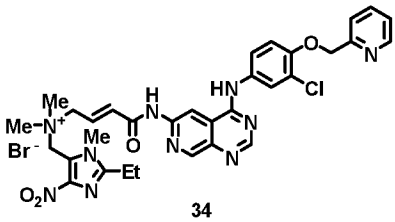

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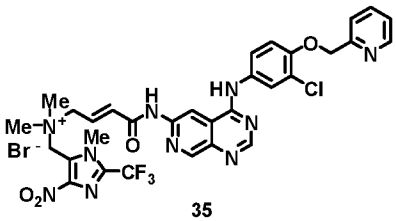
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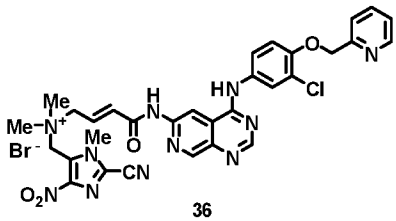
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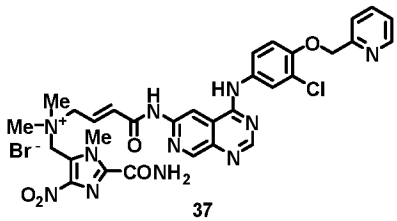
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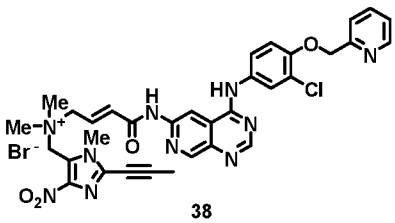
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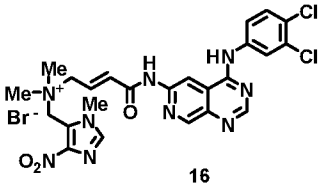
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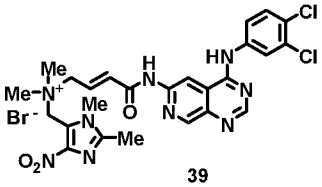
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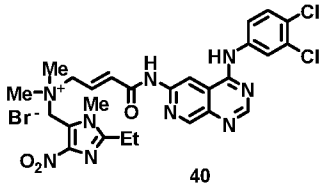
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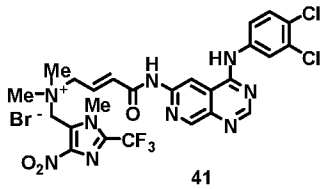
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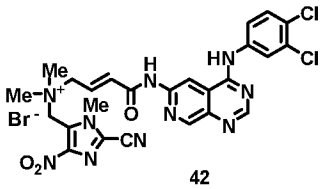
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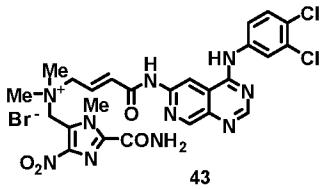
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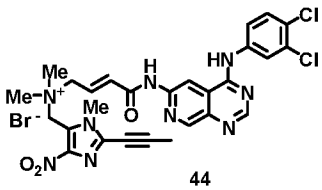
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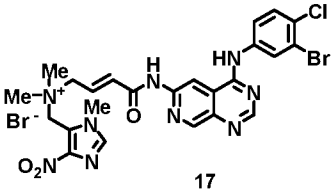
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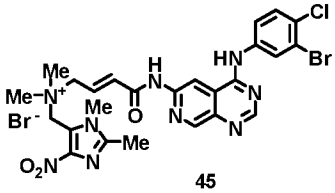


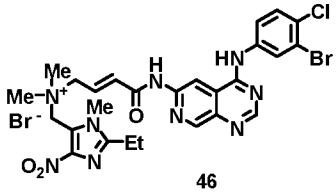
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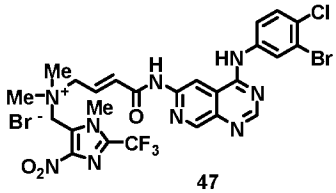


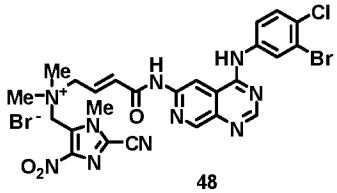
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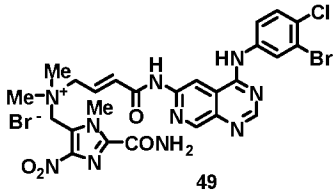

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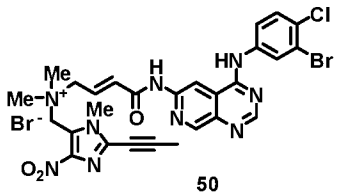

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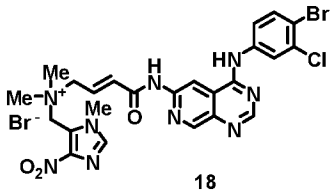

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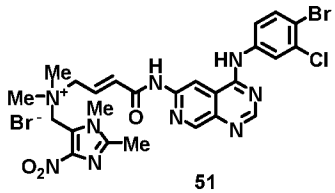

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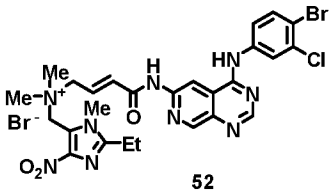

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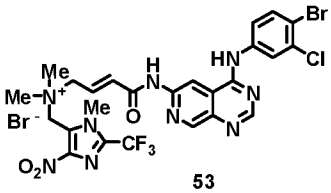

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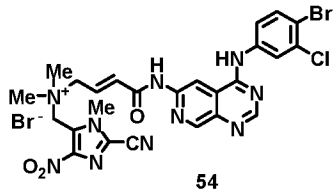

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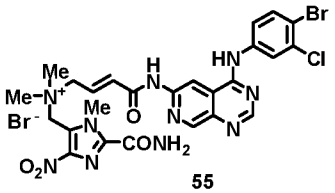

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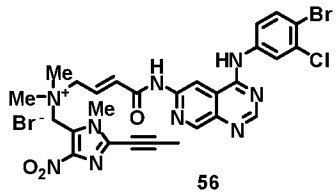

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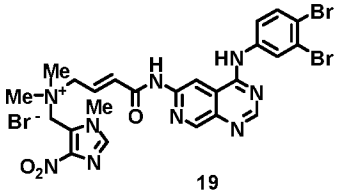

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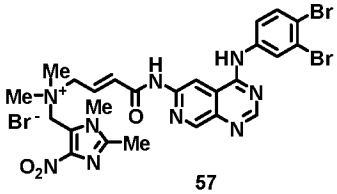

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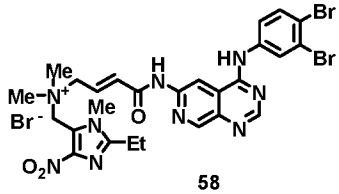

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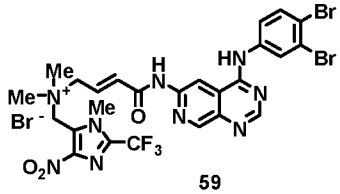

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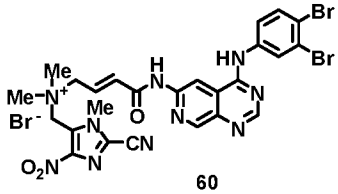

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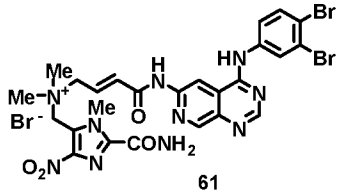

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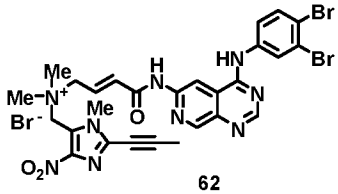

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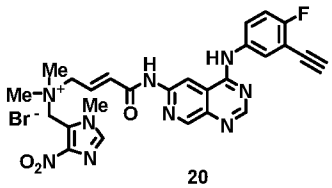

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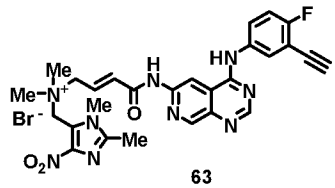

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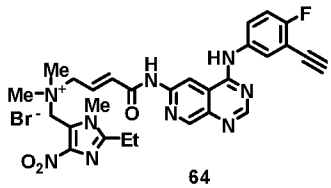

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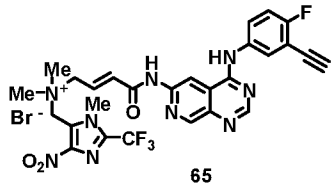

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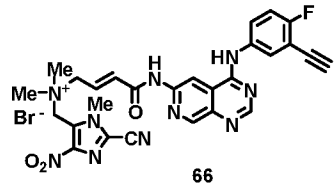

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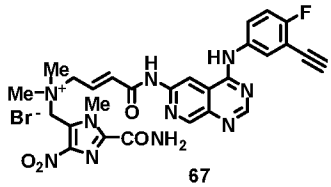

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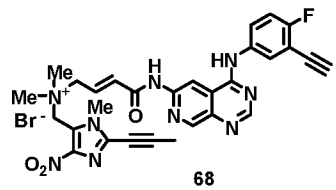

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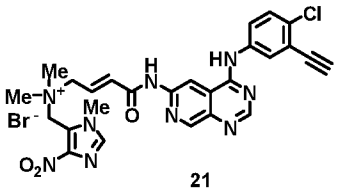

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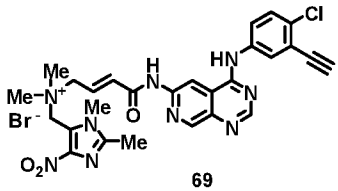

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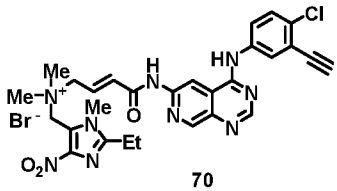

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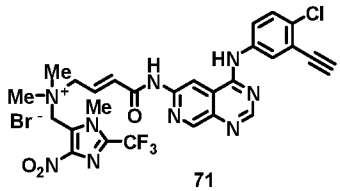
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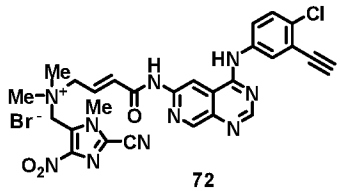
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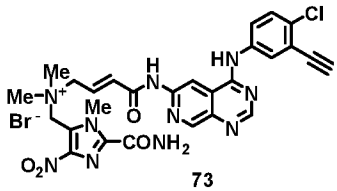
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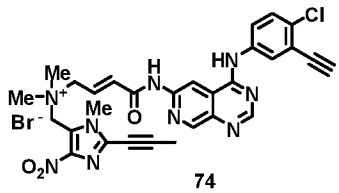
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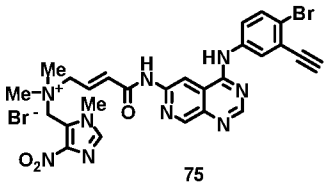
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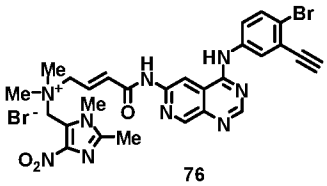
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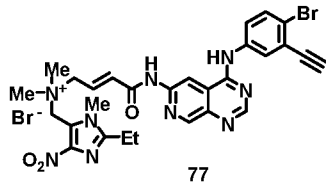
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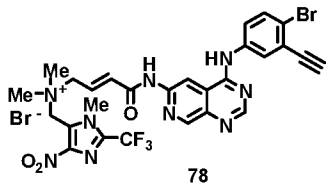
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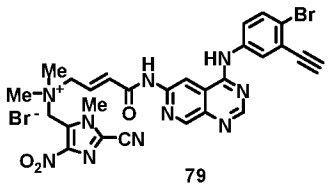
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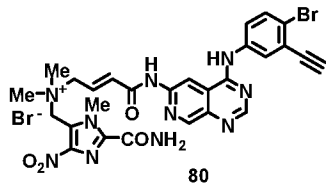
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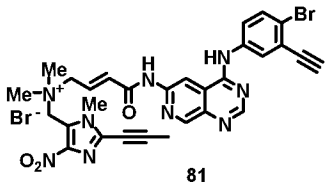
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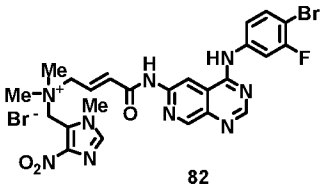
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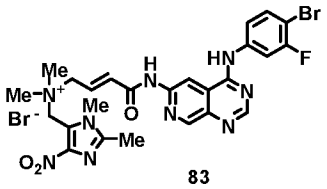
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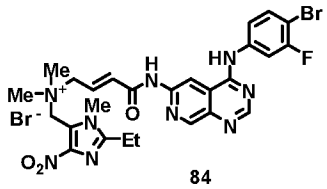
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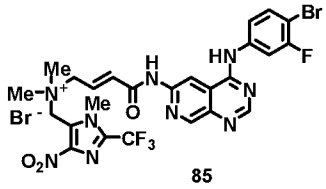
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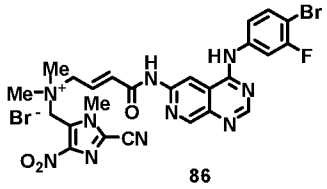
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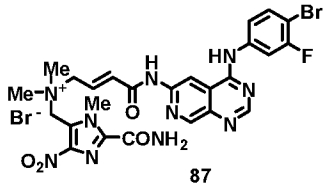
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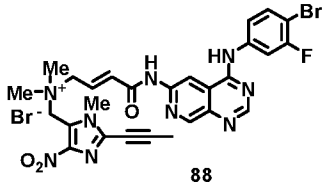
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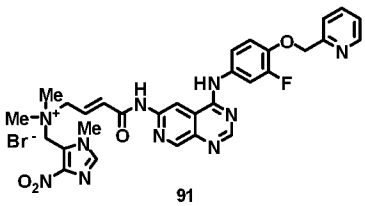
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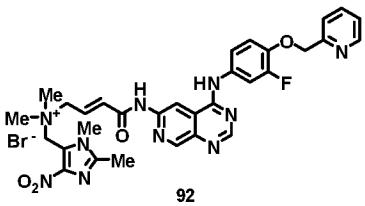
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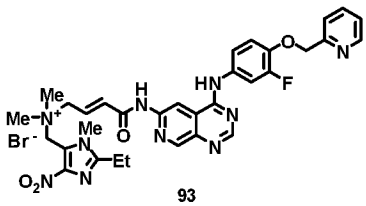
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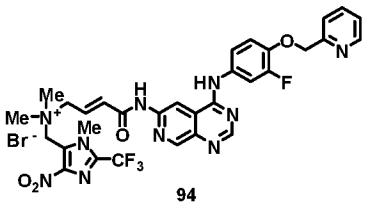
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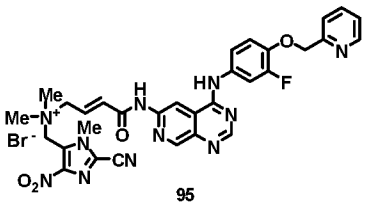
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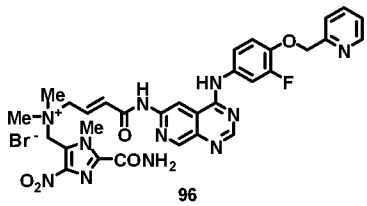
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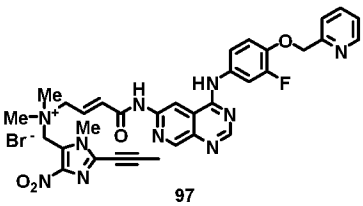
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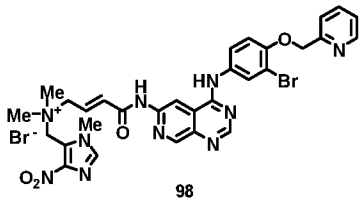
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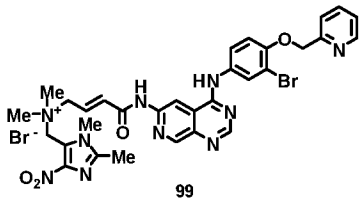


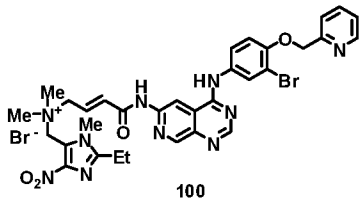
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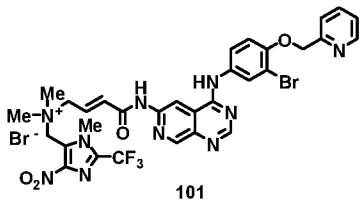


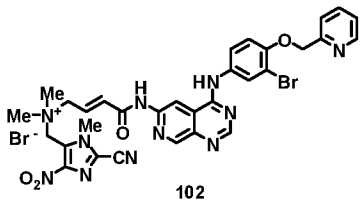
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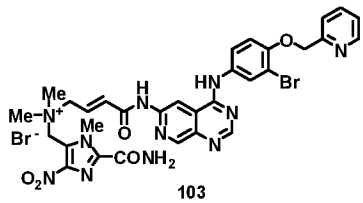

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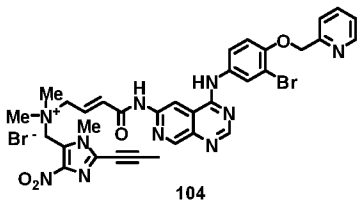

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104

In certain embodiments, the compounds are selected from the group consisting of.

(2E)-4-{[4-(3-bromoanilino)-6-quinazolinyl]amino}-N,N-dimethyl-N-(4-nitro-benzyl)-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-bromoanilino)-6-quinazolinyl]amino}-N,N-dimethyl-N-(2-nitro- benzyl)-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-bromoanilino)-6-quinazolinyl]amino}-N,N-dimethyl-N-[(1-methyl-5-nitro-1H-pyrrol-2-yl)methyl]-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-bromoanilino)-6-quinazolinyl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-bromoanilino)-6-quinazolinyl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-2-yl)methyl]-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-bromoanilino)-6-quinazolinyl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-pyrazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-bromoanilino)-6-quinazolinyl]amino}-N,N-dimethyl-N-[(3-nitroimidazo[1,2-a]pyridin-2-yl)methyl]-4-oxo-2-buten-1-ammonium bromide;

1-((2E)-4-{[4-(3-bromoanilino)-6-quinazolinyl]amino}-4-oxo-2-butenyl)-1-[-(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]piperidinium bromide;

4-((2E)-4-{[4-(3-bromoanilino)-6-quinazolinyl]amino}-4-oxo-2-butenyl)-4-[-(1-methyl-4-

nitro-1H-imidazol-5-yl)methyl]morpholin-4-ium formate

(2E)-4-{[4-(3-chloro-4-fluoroanilino)-7-methoxy-6-quinazolinyl]amino}-N,N- -dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-bromo-4-fluoroanilino)-6-quinazolinyl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(4-fluoro-3-methoxyanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl) methyl]-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-bromo-4-fluoroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,-N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-bromo-4-fluoroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N-[(1,2-dimethyl-4-nitro-1H-imidazol-5-yl)methyl]N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-bromo-4-fluoroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N-[(2-methoxy-1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-bromo-4-fluoroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N-[(2-ethynyl-1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-bromo-4-fluoroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,-N-dimethyl-N-{[1-methyl-4-nitro-2-(trifluoromethyl)-1H-imidazol-5-yl]methy-l}-4-oxo-2-buten-1-ammonium bromide;

(2E)-N-{[1-(3-amino-3-oxopropyl)-4-nitro-1H-imidazol-5-yl]methyl}-4-{[4-(-3-bromo-4-fluoroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-bromo-4-fluoroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N-[(2-cyano-1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-bromo-4-fluoroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N-[(2-cyano-1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-N,N-dimethyl-4-oxo-2-buten-1-ammonium trifluoroacetate;

(2E)-4-{[4-(3-bromo-4-fluoroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N-{[1-(2-cyanoethyl)-4-nitro-1H-imidazol-5-yl]methyl}-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-({4-[4-fluoro-3-(trifluoromethyl)anilino]pyrido[3,4-d]pyrimidin-6-yl}amino)-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide;

(2E)-N-[(1,2-dimethyl-4-nitro-1H-imidazol-5-yl)methyl]-4-({4-[4-fluoro-3-(trifluoromethyl)anilino]pyrido[3,4-d]pyrimidin-6-yl}amino)-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-({4-[4-fluoro-3-(trifluoromethyl)anilino]pyrido[3,4-d]pyrimidin-6-yl}amino)-N-[(2-methoxy-1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-N-[(2-ethynyl-1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-({4-[4-fluoro-3-(trifluoromethyl)anilino]pyrido[3,4-d]pyrimidin-6-yl}amino)-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-({4-[4-fluoro-3-(trifluoromethyl)anilino]pyrido[3,4-d]pyrimidin-6-yl}amino)-N,N-dimethyl-N-{[1-methyl-4-nitro-2-(trifluoromethyl)-1H-imidazo- 1-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide;

(2E)-N-{[1-(3-amino-3-oxopropyl)-4-nitro-1H-imidazol-5-yl]methyl}-4-({4-[-4-fluoro-3-(trifluoromethyl)anilino]pyrido[3,4-d]pyrimidin-6-yl}amino)-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-N-[(2-cyano-1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-({4-[4-fluoro-3-(trifluoromethyl)anilino]pyrido[3,4-d]pyrimidin-6-yl}amino)-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-N-{[1-(2-cyanoethyl)-4-nitro-1H-imidazol-5-yl]methyl}-4-({4-[4-fluoro-3-(trifluoromethyl)anilino]pyrido[3,4-d]pyrimidin-6-yl}amino)-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-ethynylanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimet- hyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide;

(2E)-N-[(1,2-dimethyl-4-nitro-1H-imidazol-5-yl)methyl]-4-{[4-(3-ethynylanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-ethynylanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N-[(2-methoxy-1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-ethynylanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N-[(2-ethynyl-1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-ethynylanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-{[1-methyl-4-nitro-2-(trifluoromethyl)-1H-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium

bromide;

(2E)-N-{[1-(3-amino-3-oxopropyl)-4-nitro-1H-imidazol-5-yl]methyl}-4-{[4-(-3-ethynylanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-N-[(2-cyano-1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-{[4-(3-ethynylanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-4-oxo-2-buten-1- ammonium bromide;

(2E)-N-{[1-(2-cyanoethyl)-4-nitro-1H-imidazol-5-yl]methyl}-4-{[4-(3-ethyn-ylanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-({4-(3-chloro-4-fluoroanilino)-7-[(3S)-tetrahydro-3-furanyloxy]-6-quinazolinyl}amino)-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium trifluoroacetate;

(2E)-4-({4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}amino)-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium trifluoroacetate;

(2E)-4-{[4-(3-chloro-4-fluoroanilino)-3-cyano-7-ethoxy-6-quinolinyl]amino-}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten- -1-ammonium bromide;

2-(4-{[6-(2,6-dichlorophenyl)-8-methyl-7-oxo-7,8-dihydropyrido[2,3-d]pyrimidin-2-yl]amino}phenoxy)-N,N-diethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]ethanammonium bromide;

2-(4-{[6-(2,6-dichlorophenyl)-8-methyl-7-oxo-7,8-dihydropyrido[2,3-d]pyrimidin-2-yl]amino}phenoxy)-N-[(1,2-dimethyl-4-nitro-1H-imidazol-5-yl)methyl-]N,N-diethylethanammonium bromide;

4-{[6-(2,6-dichlorophenyl)-8-methyl-7-oxo-7,8-dihydropyrido[2,3-d]pyrimidin-2-yl]amino}-1-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]pyridinium bromide;

1-[2-(4-{[6-(2,6-dichlorophenyl)-8-methyl-7-oxo-7,8-dihydropyrido[2,3-d]pyrimidin-2-yl]amino}phenoxy)ethyl]-1-[(1-methyl-4-nitro-1H-imidazol-5-yl) methyl]piperidinium bromide;

N,N-diethyl-2-[({5-[(Z)-(5-fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrol-3-yl}carbonyl)amino]-N-[(1-methyl-4-nitro-1H-imidazol-5-

yl)methyl]ethanammonium trifluoroacetate;

N-[(1,2-dimethyl-4-nitro-1H-imidazol-5-yl)methyl]-N,N-diethyl-2-[({5-[(Z)-(5-fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)methyl]2,4-dimethyl-1H-pyrrol-3-yl}carbonyl)amino]ethanammonium bromide;

4-({[4-(4-bromo-2-fluoroanilino)-6-methoxy-7-quinazolinyl]oxy}methyl)-1-m- ethyl-1-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]piperidinium trifluoroacetate;

(2E)-4-{[4-(3-bromo-4-fluoroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N-[(2-ethyl-1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-bromo-4-fluoroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,-N-dimethyl-N-{[1-methyl-4-nitro-2-(1-propynyl)-1H-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-bromo-4-fluoroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,-N-dimethyl-N-[(1-methyl-2-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-bromo-4-fluoroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N-[(4-ethyl-1-methyl-2-nitro-1H-imidazol-5-yl)methyl]-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide; and

(2E)-N-[(2-ethyl-1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-{[4-(3-ethynylanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-4-oxo-2-buten-1 ammonium bromide.

In certain embodiments, the cancer comprises lung cancer. In other embodiments, the lung cancer comprises non-small cell lung cancer. In yet other embodiments, the cancer comprises gastric cancer. In yet other embodiments, the cancer comprises breast cancer. In yet other embodiments, the cancer comprises head and neck squamous cell carcinoma (HNSCC). In yet other embodiments, the cancer comprises gastric/gastroesophageal (GE) junction cancer. In yet other embodiments, the cancer comprises esophageal cancer. In yet other embodiments, the cancer comprises salivary cancer. In yet other embodiments, the cancer comprises ovarian cancer. In yet other embodiments, the cancer comprises endometrial cancer. In yet other embodiments, the cancer comprises uterine cancer. In yet other embodiments, the cancer comprises pancreatic cancer.

In certain embodiments, the cancer is selected from the group of lung cancer, gastric cancer, breast cancer, HNSCC, GE junction cancer, esophageal cancer, salivary cancer, ovarian

cancer, endometrial cancer, uterine cancer, prostate cancer, pancreatic cancer, colon cancer, glioblastoma, mesothelioma, and adenocarcinoma.

In certain embodiments, the cancer is non-small cell lung cancer.

In certain embodiments, the cancer comprises an EGFR exon insertion mutation.

In certain embodiments, the cancer comprises an EGFR exon 20 insertion mutation. In certain embodiments, the EGFR exon 20 insertion mutation includes a mutation such as but not limited to any of the mutations described in Yasuda, *et al.*, 2013, Sci. Transl. Med. 5(216):216ra177; doi:10.1126/scitranslmed.3007205, and Arcila, *et al.*, 2013, Mol. Cancer Ther. 12:220; each of which is incorporated herein in its entirety by reference.

In certain embodiments, the EGFR exon 20 insertion mutation comprises A763_Y764insFQEA. In other embodiments, the EGFR exon 20 insertion mutation comprises A763_Y764insFQQA. In yet other embodiments, the EGFR exon 20 insertion mutation comprises A767_V769dupASV. In yet other embodiments, the EGFR exon 20 insertion mutation comprises D770_N771insGL. In yet other embodiments, the EGFR exon 20 insertion mutation comprises D770_N771insGT. In yet other embodiments, the EGFR exon 20 insertion mutation comprises D770_N771insNPG. In yet other embodiments, the EGFR exon 20 insertion mutation comprises D770_N771insSVD. In yet other embodiments, the EGFR exon 20 insertion mutation comprises E762Q_insFQEA. In yet other embodiments, the EGFR exon 20 insertion mutation comprises H773_V774insH. In yet other embodiments, the EGFR exon 20 insertion mutation comprises H773_V774insH. In yet other embodiments, the EGFR exon 20 insertion mutation comprises H773_V774insNPH. In yet other embodiments, the EGFR exon 20 insertion mutation comprises M766_A767insAI. In yet other embodiments, the EGFR exon 20 insertion mutation comprises M766_A767insASV. In yet other embodiments, the EGFR exon 20 insertion mutation comprises N771_H773dupNPH. In yet other embodiments, the EGFR exon 20 insertion mutation comprises P772_H773insYNP. In yet other embodiments, the EGFR exon 20 insertion mutation comprises P772_V774insPHV. In yet other embodiments, the EGFR exon 20 insertion mutation comprises S768_770dupSVD. In yet other embodiments, the EGFR exon 20 insertion mutation comprises V769_D770insASV. In yet other embodiments, the EGFR exon 20 insertion mutation comprises Y764_V765insHH. In yet other embodiments, the EGFR exon 20 insertion mutation comprises delD770insGY. In yet other embodiments, the EGFR exon 20 insertion mutation comprises delL747_P753insS.

In certain embodiments, the EGFR exon 20 insertion mutation comprises A767_V769dupASV (also referred to as A767_V769insASV). In other embodiments, the EGFR exon 20 insertion mutation comprises A763_Y764insFHEA, V769_D770insASV. In yet other embodiments, the EGFR exon 20 insertion mutation comprises D770_N771insSVD. In other embodiments, the EGFR exon 20 insertion mutation comprises A767_V769dupASV. In other embodiments, the EGFR exon 20 insertion mutation comprises N771_H773dupNPH. In yet other embodiments, the EGFR exon 20 insertion mutation comprises S768_770dupSVD.

In certain embodiments, the cancer comprises an *ERBB2* gene fusion. In other embodiments, the *ERBB2* gene fusion comprises *ZNF207-HER2*. In yet other embodiments, the *ERBB2* gene fusion comprises *MDK-HER2*. In yet other embodiments, the *ERBB2* gene fusion comprises *NOS2-HER2*. In yet other embodiments, the *ERBB2* gene fusion comprises *ERBB2-GRB7*. In yet other embodiments, the *ERBB2* gene fusion comprises *ERBB2-CTTN*. In yet other embodiments, the *ERBB2* gene fusion comprises *ERBB2-PPP1R1B*. In yet other embodiments, the *ERBB2* gene fusion comprises *ERBB2-PSMB3*.

In certain embodiments, the cancer comprises a *ERBB2* mutation. In other embodiments, the *ERBB2* mutation comprises G309A/E. In yet other embodiments, the *ERBB2* mutation comprises S310F/Y. In yet other embodiments, the *ERBB2* mutation comprises V659E/D. In yet other embodiments, the *ERBB2* mutation comprises G660D. In yet other embodiments, the *ERBB2* mutation comprises K753E. In yet other embodiments, the *ERBB2* mutation comprises L755P/S. In yet other embodiments, the *ERBB2* mutation comprises Del755-759. In yet other embodiments, the *ERBB2* mutation comprises L768S. In yet other embodiments, the *ERBB2* mutation comprises D769H/Y. In yet other embodiments, the *ERBB2* mutation comprises V773L. In yet other embodiments, the *ERBB2* mutation comprises A775_G776insYVMA. In yet other embodiments, the *ERBB2* mutation comprises G776V/L, Cins. In yet other embodiments, the *ERBB2* mutation comprises V777L. In yet other embodiments, the *ERBB2* mutation comprises P780Ins. In yet other embodiments, the *ERBB2* mutation comprises P780_Y781insGSP. In yet other embodiments, the *ERBB2* mutation comprises V842I. In yet other embodiments, the *ERBB2* mutation comprises L866M. In yet other embodiments, the *ERBB2* mutation comprises R896C.

In certain embodiments, the cancer comprises a *NRG1* gene fusion. In other embodiments, the *NRG1* gene fusion comprises *DOC4-NRG1*. In yet other embodiments, the

NRG1 gene fusion comprises *CD74-NRG1*. In yet other embodiments, the *NRG1* gene fusion comprises *SLC3A2-NRG1*. In yet other embodiments, the *NRG1* gene fusion comprises *RBPMS-NRG1*. In yet other embodiments, the *NRG1* gene fusion comprises *WRN-NRG1*. In yet other embodiments, the *NRG1* gene fusion comprises *SDC4-NRG1*. In yet other embodiments, the *NRG1* gene fusion comprises *RAB2IL1-NRG1*. In yet other embodiments, the *NRG1* gene fusion comprises *VAMP2-NRG1*. In yet other embodiments, the *NRG1* gene fusion comprises *KIF13B-NRG1*. In yet other embodiments, the *NRG1* gene fusion comprises *ATP1B1-NRG1*. In yet other embodiments, the *NRG1* gene fusion comprises *CDH6-NRG1*. In yet other embodiments, the *NRG1* gene fusion comprises *APP-NRG1*. In yet other embodiments, the *NRG1* gene fusion comprises *AKAP13-NRG1*. In yet other embodiments, the *NRG1* gene fusion comprises *THBS1-NRG1*. In yet other embodiments, the *NRG1* gene fusion comprises *PDE7A-NRG1*. In yet other embodiments, the *NRG1* gene fusion comprises *THAP7-NRG1*. In yet other embodiments, the *NRG1* gene fusion comprises *SMAD4-NRG1*. In yet other embodiments, the *NRG1* gene fusion comprises *RAB3IL1-NRG1*. In yet other embodiments, the *NRG1* gene fusion comprises *NRG1-PMEPA1*. In yet other embodiments, the *NRG1* gene fusion comprises *NRG1-STMN2*.

In certain embodiments, the cancer comprises an ERBB3 mutation. In other embodiments, the ERBB3 mutation comprises V104M. In yet other embodiments, the ERBB3 mutation comprises A232V. In yet other embodiments, the ERBB3 mutation comprises P262H. In yet other embodiments, the ERBB3 mutation comprises G284R. In yet other embodiments, the ERBB3 mutation comprises T389K. In yet other embodiments, the ERBB3 mutation comprises Q809R. In yet other embodiments, the ERBB3 mutation comprises S846I. In yet other embodiments, the ERBB3 mutation comprises E928G.

In certain embodiments, the cancer comprises an *ERBB4* fusion. In other embodiments, the ERBB4 fusion comprises *EZR-ERBB4*. In yet other embodiments, the ERBB4 fusion comprises *IKZF2-ERBB4*. In yet other embodiments, the ERBB4 fusion comprises *BGALT-ERBB4*.

In certain embodiments, the cancer is resistant to at least one selected from the group consisting of osimertinib, gefitinib, afatinib, and erlotinib. In other embodiments, the cancer is resistant to osimertinib. In yet other embodiments, the cancer is resistant to gefitinib. In yet other embodiments, the cancer is resistant to afatinib. In yet other embodiments, the cancer is resistant to erlotinib.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present application, illustrative methods and materials are described.

As used herein, each of the following terms has the meaning associated with it in this section.

As used herein, the articles "a" and "an" are used to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

As used herein, "about," when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of ±20% or ±10%, more preferably ±5%, even more preferably ±1%, and still more preferably ±0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

As used herein, the term "afatinib" refers to N-[4-[(3-Chloro-4-fluorophenyl)amino]-7-[[(3S)-tetrahydro-3-furanyl]oxy]-6-quinazolinyl]-4(dimethylamino)-2-butenamide, or a salt or solvate thereof.

A disease or disorder is "alleviated" if the severity of a symptom of the disease or disorder, the frequency with which such a symptom is experienced by a patient, or both, is reduced.

As used herein, the terms "alkyl", "alkenyl", "alkynyl" and "alkoxy" include both straight chain and branched chain groups, and unsubstituted and substituted groups. The optional substituents may include, without limitation, halogen, C_1-C_6 alkoxy, CN, OH, NH_2, NO_2, $NH(C_1$-C_6 alkyl), $N(C_1$-C_6 alkyl)$_2$, $CONH_2$, $CO(C_1$-C_6 alkyl), SO_2NH_2 and $SO_2(C_1$-C_6 alkyl).

As used herein, the term "aromatic nitroheterocycle" means an aromatic heterocyclic moiety substituted at any ring position by one or more nitro (NO_2) groups. The aromatic heterocyclic moiety may be a monocyclic or bicyclic ring containing 4 to 12 atoms of which at least one atom is chosen from the group consisting of nitrogen, sulphur or oxygen. The aromatic

heterocyclic moiety may be carbon or nitrogen linked. The aromatic heterocyclic moiety may additionally be substituted by one or more additional substituents at any available ring carbon or heteroatom. The substituents may include, but are not limited to the groups as defined for R_{26} in Formula V.

As used herein, the term "aromatic nitrocarbocycle" means a benzene moiety substituted at any position by one or more nitro (NO_2) groups. In addition, two adjacent ring carbon atoms may optionally be linked to form a fused carbocyclic or heterocyclic ring. The benzene moiety (and optional fused ring) may additionally be substituted by one or more additional substituents at any available carbon or heteroatom. The substituents may include, but are not limited to, the groups as defined for R_{26} in Formula V.

In one aspect, the terms "co-administered" and "co-administration" as relating to a subject refer to administering to the subject a compound contemplated herein or salt thereof along with a compound that may also treat the disorders or diseases contemplated herein. In one embodiment, the co-administered compounds are administered separately, or in any kind of combination as part of a single therapeutic approach. The co-administered compound may be formulated in any kind of combinations as mixtures of solids and liquids under a variety of solid, gel, and liquid formulations, and as a solution.

As used herein, the term "composition" or "pharmaceutical composition" refers to a mixture of at least one compound contemplated herein with a pharmaceutically acceptable carrier. The pharmaceutical composition facilitates administration of the compound to a patient or subject. Multiple techniques of administering a compound exist in the art including, but not limited to, intravenous, oral, aerosol, parenteral, ophthalmic, nasal, pulmonary and topical administration.

The term "container" includes any receptacle for holding the pharmaceutical composition or to add protection to manage stability and or water-uptake. For example, in one embodiment, the container is the packaging that contains the pharmaceutical composition such as liquid (solution and suspension), semisolid, lyophilized solid, solution and powder or lyophilized formulation present in dual chambers. In other embodiments, the container is not the packaging that contains the pharmaceutical composition, *i.e.*, the container is a receptacle, such as a box or vial that contains the packaged pharmaceutical composition or unpackaged pharmaceutical composition and the instructions for use of the pharmaceutical composition. Moreover,

packaging techniques are well known in the art. It should be understood that the instructions for use of the pharmaceutical composition may be contained on the packaging containing the pharmaceutical composition, and as such the instructions form an increased functional relationship to the packaged product. However, it should be understood that the instructions may contain information pertaining to the compound's ability to perform its intended function, *e.g.*, treating, preventing, or reducing a breathing disorder in a patient.

The term "determining" as used herein generally refers to any form of measurement, and includes detecting the presence of a mutation, including, for example, an EGFR exon 20 insertion mutation in the tumor cells, as disclosed herein. The term "determining" includes both quantitative and/or qualitative determination. The mutation (e.g., EGFR exon 20 insertion mutation) may be determined by any suitable method known to those skilled in the art, including those as further disclosed herein.

A "disease" as used herein is a state of health of an animal wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal's health continues to deteriorate.

A "disorder" as used herein in an animal is a state of health in which the animal is able to maintain homeostasis, but in which the animal's state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the animal's state of health.

As used herein, the terms "effective amount," "pharmaceutically effective amount" and "therapeutically effective amount" refer to a nontoxic but sufficient amount of a compound or agent to provide the desired biological result. That result may be reduction and/or alleviation of the signs, symptoms, or causes of a disease, or any other desired alteration of a biological system. An appropriate therapeutic amount in any individual case may be determined by one of ordinary skill in the art using routine experimentation. A therapeutic benefit or improvement need not be complete ablation of any one, most or all symptoms, complications, consequences or underlying causes associated with the disorder or disease. Thus, in certain embodiments, a satisfactory endpoint is achieved when there is a transient, medium or long term, incremental improvement in a subject's condition, or a partial reduction in the occurrence, frequency, severity, progression, or duration, or inhibition or reversal, of one or more associated adverse symptoms or complications or consequences or underlying causes, worsening or progression

(*e.g.*, stabilizing one or more symptoms or complications of the condition, disorder or disease), of the disorder or disease, over a duration of time (hours, days, weeks, months, and so forth).

As used herein, the term "erlotinib" refers to N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)quinazolin-4-amine, or a salt or solvate thereof.

As used herein, the term "HER-driven cancer" refers to a cancer that is caused or promoted by a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and/or a *ERBB4* fusion.

The HER-driven cancer may be indicated by the presence of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and/or a *ERBB4* fusion. The HER-driven cancer may be resistant to osimertinib, gefitinib, afatinib, and/or erlotinib, as described herein. The HER-driven cancer may also have an EGFR exon insertion mutation (*e.g.*, an EGFR exon 20 insertion mutation), where the EGFR exon insertion mutation is indicated phenotypically, for example, by histopathology, imaging, tumor growth, DNA analysis, RNA analysis or other diagnostic means, as described herein.

"Instructional material," as that term is used herein, includes a publication, a recording, a diagram, or any other medium of expression that can be used to communicate the usefulness of a composition and/or compound contemplated herein in a kit. The instructional material of the kit may, for example, be affixed to a container that contains the compound and/or composition contemplated herein or be shipped together with a container that contains the compound and/or composition. Alternatively, the instructional material may be shipped separately from the container with the intention that the recipient uses the instructional material and the compound cooperatively. Delivery of the instructional material may be, for example, by physical delivery of the publication or other medium of expression communicating the usefulness of the kit, or may alternatively be achieved by electronic transmission, for example by means of a computer, such as by electronic mail, or download from a website.

As used herein, "likelihood", "likely to", and similar generally refers to an increase in the probability of an event. Thus, "likelihood", "likely to", and similar, when used in reference to responsiveness to cancer therapy, generally contemplates an increased probability that the individual will exhibit a reduction in the severity of cancer or the symptoms of cancer or the retardation or slowing of the cancer progression. The term "likelihood", "likely to", and similar,

when used in reference to responsiveness to cancer therapy, can also generally mean the increase of indicators that may evidence an increase in cancer treatment.

As used herein, the term "osimertinib" refers to N-(2-{2-dimethylaminoethyl-methylamino}-4-methoxy-5-{[4-(1-methylindol-3-yl)pyrimidin-2-yl]amino}phenyl)prop-2-enamide, or a salt or solvate thereof.

The terms "patient," "subject" or "individual" are used interchangeably herein, and refer to any animal, or cells thereof whether *in vitro* or *in situ*, amenable to the methods described herein. In non-limiting embodiments, the patient is a human. In certain embodiments, the subject is a subject in need of treatment thereof. In other embodiments, the subject has a cancer comprising an EGFR mutation, for example, an EGFR insertion mutation. In yet other embodiments, the subject has EGFR exon 20 insertion mutation.

As used herein, the term "pharmaceutically acceptable" refers to a material, such as a carrier or diluent, which does not abrogate the biological activity or properties of the compound, and is relatively non-toxic, *i.e.*, the material may be administered to an individual without causing undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained.

As used herein, the term "pharmaceutically acceptable carrier" means a pharmaceutically acceptable material, composition or carrier, such as a liquid or solid filler, stabilizer, dispersing agent, suspending agent, diluent, excipient, thickening agent, solvent or encapsulating material, involved in carrying or transporting a compound disclosed herein or to the patient such that it may perform its intended function. Typically, such constructs are carried or transported from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation, including compounds disclosed herein, and not injurious to the patient. Some examples of materials that may serve as pharmaceutically acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as

magnesium hydroxide and aluminum hydroxide; surface active agents; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations.

As used herein, "pharmaceutically acceptable carrier" also includes any and all coatings, antibacterial and antifungal agents, and absorption delaying agents, and the like that are compatible with the activity of compounds disclosed herein, and are physiologically acceptable to the patient. Supplementary active compounds may also be incorporated into the compositions.

The "pharmaceutically acceptable carrier" may further include a pharmaceutically acceptable salt of the compounds disclosed herein. Other additional ingredients that may be included in the pharmaceutical compositions disclosed herein are known in the art and described, for example in Remington's Pharmaceutical Sciences (Genaro, Ed., Mack Publishing Co., 1985, Easton, PA), which is incorporated herein by reference.

As used herein, the language "pharmaceutically acceptable salt" refers to a salt of the administered compounds prepared from pharmaceutically acceptable non-toxic acids, including inorganic acids, organic acids, solvates, hydrates, or clathrates thereof.

As used herein, the term "predict" can mean to determine or tell in advance. When used to "predict" the responsiveness to a treatment for example, the term "predict" can mean that the likelihood of the outcome of the cancer treatment can be determined at the outset, before the treatment has begun, or before the treatment period has progressed substantially. A predictive method may also be described as a prognostic method.

The term "prevent," "preventing" or "prevention," as used herein, means avoiding or delaying the onset of symptoms associated with a disease or condition in a subject that has not developed such symptoms at the time the administering of an agent or compound commences.

As used herein, the term "prodrug" refers to a compound that, after administration, is metabolized or otherwise converted to a biologically active or more active compound (or drug) with respect to at least one property. A prodrug, relative to the drug, is modified chemically in a manner that renders it, relative to the drug, less active or inactive, but the chemical modification is such that the corresponding drug is generated by metabolic or other biological processes after the prodrug is administered. A prodrug can have, relative to the active drug, altered metabolic stability or transport characteristics, fewer side effects or lower toxicity, or improved flavour (for example, see the reference Nogrady, 1985, Medicinal Chemistry A Biochemical Approach,

Oxford University Press, New York, pages 388-392, incorporated herein by reference). A prodrug may be synthesized using reactants other than the corresponding drug.

As used herein, the phrase "providing tumor cells" refers to the step of obtaining cells of the individual (*e.g.* by way of biopsy or otherwise), and/or refers to the step of receiving a sample of tumor cells that has previously been obtained from the individual.

A "therapeutic" treatment is a treatment administered to a subject who exhibits signs of pathology, for the purpose of diminishing or eliminating those signs.

The term "responsiveness" or "responsive," when used in reference to a treatment, refers to the degree of effectiveness of the treatment in lessening or decreasing the symptoms of a disease, disorder, or condition being treated. For example, the term "increased responsiveness," when used in reference to a treatment of a cell or a subject, refers to an increase in the effectiveness in lessening or decreasing the symptoms of the disease when measured using any methods known in the art. In certain embodiments, the increase in the effectiveness is at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, or at least about 50%.

In certain embodiments, tumor cells comprise a "sample." In other embodiments, the sample comprises a biological sample and can be, for instance, a cell, a cell culture, a tissue, and/or a biological fluid. Suitably, the biological sample can comprise a tumor cell biopsy, a plurality of samples from a clinical trial, or the like. The sample can be a crude sample, or can be purified to various degrees prior to storage, processing, or measurement.

As used herein, the term "treatment" or "treating" is defined as the application or administration of a therapeutic agent, *i.e.*, a compound disclosed herein (alone or in combination with another pharmaceutical agent), to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient (*e.g.*, for diagnosis or *ex vivo* applications), who has a condition contemplated herein, a symptom of a condition contemplated herein or the potential to develop a condition contemplated herein, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect a condition contemplated herein, the symptoms of a condition contemplated herein or the potential to develop a condition contemplated herein. Such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics.

In certain embodiments, the term "treatment" or "treating" refers to an action that occurs

while an individual is suffering from the specified cancer, which reduces the severity of the cancer or the symptoms of the cancer, and/or retards or slows the progression of the cancer. For instance, in certain embodiments, "treatment" or "treat" refers to a 5%, 10%, 25%, 50%, or 100% decrease in the rate of progress of a tumor. In other embodiments, "treatment" refers to a 5%, 10%, 25%, 50%, or 100% decrease in determined tumor burden (*i.e.*, number of cancerous cells present in the individual, and/or the size of the tumor). In yet other embodiments, "treatment" refers to a 5%, 10%, 25%, 50%, or 100% decrease in any physical symptom(s) of a cancer. In yet other embodiments, "treatment" refers to a 5%, 10%, 25%, 50%, or 100% increase in the general health of the individual, as determined by any suitable means, such as cell counts, assay results, or other suitable means. As used herein, the cancer can be any cancer, including those contemplated herein, including, for example, a HER-driven drug-resistant cancer.

As used herein, the term "EGFR" or "ErbB1" or "ErbB-1" or "HER1" refers to epidermal growth factor receptor. The amino acid sequence for the human EGFR (isoform 1; canonical – UniProt ID P00533-1) is recited as SEQ ID NO:1.

The following non-limiting alternative isoforms of EGFR (as relating to the canonical isoform) are also contemplated: Isoform 2 (UniProt ID P00533-2), SEQ ID NO:2; Isoform 3 (UniProt ID P00533-3), SEQ ID NO:3; and Isoform 4 (UniProt ID P00533-4); SEQ ID NO:4.

The nucleotide sequence for the EGFR gene, complete cds, alternatively spliced, is recited as SEQ ID NO:5.

The nucleotide sequence for the EGFR exon 18 is recited as SEQ ID NO:6.

The nucleotide sequence for the EGFR exon 19 is recited as SEQ ID NO:7.

The nucleotide sequence for the EGFR exon 20 is recited as SEQ ID NO:8.

The nucleotide sequence for the EGFR exon 21 is recited as SEQ ID NO:9.

As used herein, the term "ErbB2" or "ErbB-2" or "HER2" or "HER-2" refers to receptor tyrosine-protein kinase erbB-2. The amino acid sequence for the human ErbB2 (isoform 1; canonical – UniProt ID P04626-1) is recited as SEQ ID NO:10.

The following non-limiting alternative isoforms of ErbB2 (as relating to the canonical isoform) are also contemplated: Isoform 2 (UniProt ID P04626-2), SEQ ID NO:11; Isoform 3 (UniProt ID P04626-3), SEQ ID NO:12; Isoform 4 (UniProt ID P04626-4), SEQ ID NO:13; Isoform 5 (UniProt ID P04626-5), SEQ ID NO:14; and Isoform 6 (UniProt ID P04626-6), SEQ

ID NO:15.

As used herein, the term "ErbB3" or "ErbB-3" or "HER3" or "HER-3" refers to receptor tyrosine-protein kinase erbB-3. The amino acid sequence for the human ErbB3 (isoform 1; canonical − (UniProt ID P21860-1) is recited as SEQ ID NO:16.

The following non-limiting alternative isoforms of ErbB3 (as relating to the canonical isoform) are also contemplated: Isoform 2 (UniProt ID P21860-2), SEQ ID NO:17; Isoform 3 (UniProt ID P21860-3), SEQ ID NO:18; Isoform 4 (UniProt ID P21860-4), SEQ ID NO:19; and Isoform 5 (UniProt ID P21860-5), SEQ ID NO:20.

As used herein, the term "ErbB4" or "ErbB-4" or "HER4" or "HER-4" refers to receptor tyrosine-protein kinase erbB-4. The amino acid sequence for the human ErbB4 (isoform JM-A CYT-1; canonical − UniProt ID Q15303-1) is recited as SEQ ID NO:21.

The following non-limiting alternative isoforms of ErbB4 (as relating to the canonical isoform) are also contemplated: Isoform JM-B CYT-1 (UniProt ID Q15303-2), SEQ ID NO:22; Isoform JM-A CYT-2 (UniProt ID Q15303-3), SEQ ID NO:23; and Isoform JM-B CYT-2 (UniProt ID Q15303-4), SEQ ID NO:24.

As used herein, the term "NRG1" refers to pro-neuregulin-1. The amino acid sequence for the human NRG1 (isoform 1; canonical − UniProt ID Q02297-1) is recited as SEQ ID NO:25.

The following non-limiting alternative isoforms of NRG1 (as relating to the canonical isoform) are also contemplated: Isoform 2 (UniProt ID Q02297-2), SEQ ID NO:26; Isoform 3 (UniProt ID Q02297-3), SEQ ID NO:27; Isoform 4 (UniProt ID Q02297-4), SEQ ID NO:28; Isoform 6 (UniProt ID Q02297-6), SEQ ID NO:29; Isoform 7 (UniProt ID Q02297-7), SEQ ID NO:30; Isoform 8 (UniProt ID Q02297-8), SEQ ID NO:31; Isoform 9 (UniProt ID Q02297-9), SEQ ID NO:32; Isoform 10 (UniProt ID Q02297-10), SEQ ID NO:33; Isoform 11 (UniProt ID Q02297-11), SEQ ID NO:34; and Isoform 12 (UniProt ID Q02297-12), SEQ ID NO:35.

Throughout this disclosure, various aspects of the disclosure can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the disclosure herein. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example,

description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.1, 5.3, 5.5, and 6. Thus, for example, reference to a range of 90-100% includes 91-99%, 92-98%, 93-95%, 91-98%, 91-97%, 91-96%, 91-95%, 91-94%, 91-93%, and so forth. Reference to a range of 90-100% also includes 91%, 92%, 93%, 94%, 95%, 96%, 97%, etc., as well as 91.1%, 91.2%, 91.3%, 91.4%, 91.5%, etc., 92.1%, 92.2%, 92.3%, 92.4%, 92.5%, and so forth. A series of ranges are disclosed throughout this document. The use of a series of ranges includes combinations of the upper and lower ranges to provide another range. This construction applies regardless of the breadth of the range and in all contexts throughout this patent document. Thus, for example, reference to a series of ranges such as 5-10, 10-20, 20-30, 30-40, 40-50, 50-75, 75-100, 100-150, includes ranges such as 5-20, 5-30, 5-40, 5-50, 5-75, 5-100, 5-150, and 10-30, 10-40, 10-50, 10-75, 10-100, 10-150, and 20-40, 20-50, 20-75, 20-100, 20-150, and so forth. This applies regardless of the breadth of the range.

Compositions and Methods

Provided herein are compositions and methods using compound of Formula I, II, VII, VIII, IX, X and/or XI, as disclosed herein.

In certain embodiments, the compound is RN-4000 ["(E)-4-((4-((3-bromo-4-chlorophenyl)amino)pyrido[3,4-d]pyrimidin-6-yl)amino)-N,N-dimethyl-N-((1-methyl-4-nitro-1H-imidazol-5-yl)methyl)-4-oxobut-2-en-1-aminium salt (bromide)"; also referred to herein as "(2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide" Compound A], and/or RN-4000E ["(2*E*)-*N*-[4-(3-bromo-4-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-4-(dimethylamino)-2-butenamide"; also referred to herein "(E)-N-(4-((3-bromo-4-chlorophenyl)amino)pyrido[3,4-d]pyrimidin-6-yl)-4-(dimethylamino)but-2-enamide"; Compound B].

In certain embodiments, the compounds of Formula I, II, VII, VIII, IX, X and/or XI, as disclosed herein, are part of a pharmaceutical composition, which optionally further comprises at least one additional agent that treats or prevents a HER-driven (such as, in a non-limiting example, an EGFR-driven) drug-resistant cancer.

In certain embodiments, Compound A and/or Compound B is part of a pharmaceutical composition, which optionally further comprises at least one additional agent that treats or prevents a HER-driven (such as, in a non-limiting example, an EGFR-driven) drug-resistant cancer.

Also provided herein is a method of treating or preventing HER-driven drug-resistant cancer in a subject. Further provided herein is a method of treating or preventing EGFR-driven drug-resistant cancer in a subject. In certain embodiments, the method comprises administering to the subject in need thereof a therapeutically effective amount of at least one compound contemplated herein, for example, a compound of Formula I, II, VII, VIII, IX, X and/or XI, as disclosed herein, including Compound A and/or Compound B, or a salt or solvate thereof. In other embodiments, the method comprises administering to the subject in need thereof a therapeutically effective amount of Compound A and/or Compound B, or a salt or solvate thereof

HER-driven cancers include, but are limited to, cancers caused by a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and/or a *ERBB4* fusion.

The oncogenic alterations involving a *ERBB2* (HER2) proto-oncogene are illustrated in Table 1, and each of those alterations is contemplated herein.

Table 1.

***ERBB2* gene amplification**	***ERBB2* gene fusions**	***ERBB2* mutations**
Breast[1]	*ZNF207-HER2*[2]	G309A/E[3,4]
Lung[5,6]	*MDK-HER2*[2]	S310F/Y[4,7]
Gastric/GE Junction[8,9]	*NOS2-HER2*[2]	V659E/D[10]
Esophageal[11]	*ERBB2-GRB7*[12-14]	G660D[10]
Salivary[15,16]	*ERBB2-CTTN*[17]	K753E[18,19]
Ovarian[20]	*ERBB2-PPP1R1B*[17]	L755P/S[3,7,18,19,21-24]
Endometrial[25]	*ERBB2-PSMB3*[17]	Del755-759[3]
Uterine[26]		L768S[18,19]
Pancreatic[27]		D769H/Y[3]
		V773L[18,19]
		A775_G776insYVMA[24,28,29]
		G776V/L, Cins[28]
		V777L[3,7,21]
		P780Ins[3]
		P780_Y781insGSP[28]

		V842I[3,7,22]
		L866M[7]
		R896C[3]

References relating to Table 1:

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2. Yu, *et al.*, 2015, J Transl Med 13:116, 2015.
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4. Greulich, *et al.*, 2012, Proc Natl Acad Sci U S A 109:14476-81.
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9. Das, *et al.*, 2014, Cancer Lett 353:167-75.
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14. Chmielecki, *et al.*, 2015, Oncologist 20:7-12.
15. Nardi, *et al.*, 2013, Clin Cancer Res 19:480-90.
16. Williams, *et al.*, 2010, Clin Cancer Res 16:2266-74.
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20. Tuefferd, *et al.*, 2007, PLoS One 2:e1138.
21. Bellmunt, *et al.*, 2015, Cancer Med 4:844-52.
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26. Slomovitz, *et al.*, 2004, J Clin Oncol 22:3126-32.
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29. Wang, *et al.*, 2006, Cancer Cell 10:25-38.

Neuregulin 1 (*NRG1*) encodes a growth factor ligand that binds to the human epidermal growth factor receptor 3 (HER3) encoded by the erb-b2 receptor tyrosine kinase 3 (*ERBB3*) gene and human epidermal growth factor receptor 4 (HER4) encoded by the erb-b2 receptor tyrosine kinase 4 (*ERBB4*). Binding of NRG1 to HER3 and HER4 can induce dimerization with other HER family members, including EGFR and HER3. Unlike EGFR, HER2, and HER4, the HER3 kinase domain is catalytically inactive but can activate EGFR or HER2 and thus can initiate oncogene signaling via its heterodimerization partner.

Gene fusions that contain sequences from *NRG1* were initially discovered in a breast cancer cell line, MDA-MB-175 (Wang, *et al.*, 1999, Oncogene 18:5718-21). Later *CD74-NRG1* fusions were discovered in lung adenocarcinoma (Fernandez-Cuesta L, *et al.*, 2014, Cancer Discov 4:415-22). *NRG1* fusions have been identified in breast, NSCLC, cholangiocarcinoma, pancreatic cancer, and ovarian cancer (Wang, *et al.*, 1999, Oncogene 18:5718-21; Fernandez-Cuesta L, *et al.*, 2014, Cancer Discov 4:415-22; Dhanasekaran, *et al.*, 2014, Nat Commun 5:5893; Heining, *et al.*, 2018, Cancer Discov). *NRG1* gene fusions have been demonstrated to be oncogenic by inducing overexpression the ligand Neuregulin 1 which induces heterodimerization of HER3 with HER2. Similarly, NRG1 overexpression by gene amplification or other mechanisms are be predicted to activate HER3:HER2 dimers. Tarloxotinib is a potent inhibitor of HER2 and thus inhibits proliferation of cancer cells that utilize HER3/HER2 signaling. Gene fusions involving the *ERBB4* gene, similar to other fusions involving receptor tyrosine kinases, induce constitutive activation of HER4 and consequently activation of MAPK (Nakaoku, *et al.*, 2014, Clin Cancer Res 20:3087-93). In certain embodiments, tarloxotinib inhibits HER4 oncogene-driven cancers. Currently there are no U.S. FDA approved (or other regulatory agencies) drugs for *NRG1* fusions, *NRG1* gene amplification, *NRG1* overexpression, *ERBB3* activation mutations, or *ERBB4* fusions.

The oncogenic alterations involving a *NRG1*, *ERBB3* (HER3) and *ERBB4* (HER4) proto-oncogene are illustrated in Table 2, and each of those alterations is contemplated herein.

Table 2.

***NRG1* gene amplification/ overexpression**	***NRG1* gene fusions**	***ERBB3 mutations***	***ERBB4 fusion***
NSCLC[6,9]	*DOC4-NRG1*[4,9]	V104M[10]	EZR-ERBB4[8]
Gastric cancer[11]	*CD74-NRG1*[5,8,9]	A232V[10]	*IKZF2-ERBB4*[9]
Breast cancer[12]	*SLC3A2-NRG1*[8]	P262H[10]	*BGALT-ERBB4*[13]
HNSCC[12]	*RBPMS-NRG1*[6]	G284R[10]	
	WRN-NRG1[6]	T389K[10]	
	SDC4-NRG1[6,9]	Q809R[10]	
	RAB2IL1-NRG1[6]	S846I[10]	
	VAMP2-NRG1[14]	E928G[10]	
	KIF13B-NRG1[15]		
	ATP1B1-NRG1[7,9,16]		
	CDH6-NRG1[7]		
	APP-NRG1[7]		
	AKAP13-NRG1[9]		
	THBS1-NRG1[9]		
	PDE7A-NRG1[9]		
	THAP7-NRG1[9]		
	SMAD4-NRG1[9]		
	RAB3IL1-NRG1[9]		
	NRG1-PMEPA1[9]		
	NRG1-STMN2[9]		

References relating to Table 2:

4. Wang, *et al.*, 1999, Oncogene 18:5718-21.
5. Fernandez-Cuesta, *et al.*, 2014, Cancer Discov 4:415-22.
6. Dhanasekaran, *et al.*, 2014, Nat Commun 5:5893.
7. Heining, *et al.*, 2018, Cancer Discov.
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9. Drilon, *et al.*, 2018, Cancer Discov 8:686-695.
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14. Jung, *et al.*, 2015, J Thorac Oncol 10:1107-11.
15. Xia, *et al.*, 2017, Int J Surg Pathol 25:238-240.
16. Jones, *et al.*, 2017, Ann Oncol 28:3092-3097.

The present application contemplates methods of treating a subject with cancer with the compounds contemplated herein, where an EGFR exon insertion mutation is present in the tumor cells of the subject. The present application also contemplates related uses of such methods.

In certain embodiments, the EGFR exon insertion mutation is an EGFR exon 20 insertion mutation. Cancers with an EGFR insertion mutation exhibit certain characteristics which indicate the presence of the mutation. For example, cancers with EGFR Exon 20 insertion mutations exhibit resistance and/or poor response to EGFR-TKIs such as osimertinib, gefitinib, afatinib, and erlotinib (*see*, *e.g.*, Takeda *et al.*, 2018, Oncotarget 9(30): 21132, incorporated herein by reference in its entirety). Accordingly, the present application further contemplates methods of treating a subject with cancer with the compounds contemplated herein, wherein the presence of an EGFR insertion mutation in the tumor cells of a subject is indicated by resistance and/or poor response to an oncology agent, such as an EGFR-TKI. An EGFR insertion mutation may also be indicated from a particular phenotype characteristic of the cancer, for example, histopathology, imaging, tumor growth, DNA analysis, RNA analysis, or other diagnostic means, and/or survival rate of the patient (*see*, *e.g.*, Naidoo *et al.*, 2015, Cancer 121(18): 3212, incorporated herein by reference in its entirety).

The present application further contemplates methods of treating a subject with cancer with the compounds contemplated herein, wherein the treatment is part of a maintenance therapy for subjects with recurring or refractory cancer. For example, the present application contemplates a method of treating a resistant or refractory cancer in a subject with the compounds disclosed herein. In certain embodiments, the treatment leads to a full response, remission, and/or complete cure in the subject with recurring or refractory cancer. In certain embodiments, the treatment maintains a stable disease, leads to a partial response (*e.g.*, some tumor regression), or prevents the return of tumors that have fully regressed. In certain embodiments, the cancer has an EGFR exon insertion mutations. In certain embodiments, the EGFR exon insertion mutation is an EGFR exon 20 insertion mutation. The present application further contemplates methods of treating a subject with cancer with the compounds contemplated herein, where at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion is present in the tumor

cells of the subject. The present application also contemplates related uses of such methods.

The present application further contemplates methods of treating or preventing a HER-driven drug-resistant cancer in a subject, the method comprising administering to the subject in need thereof a therapeutically effective amount of a compound contemplated herein, wherein at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion, is present in the tumor cells of the subject. The present application also contemplates related uses of such methods.

In certain embodiments, the compound of the methods and related uses disclosed herein is of a compound of Formula I, II, VII, VIII, IX, X, and/or XI. In certain embodiments, the compound is of Formula I, II, IX, X, and/or XI. In certain embodiments, the compound is of Formula VII and/or VIII. In certain embodiments, the compound is one of Compounds 12-88 and 91-104. In certain embodiments, the compound is one of Compounds 1-11, 89 and 90. In certain embodiments, the compound is Compound 17. In certain embodiments, the compound is Compound 5. In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In one aspect, the present application provides a method of treating or preventing a HER-driven drug-resistant cancer in a subject, the method comprising administering to the subject in need thereof a therapeutically effective amount of at least one compound selected from the group consisting of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (Compound A), and (2*E*)-*N*-[4-(3-bromo-4-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-4-(dimethylamino)-2-butenamide (Compound B), or a salt or solvate thereof.

In another aspect, the present application provides a method of treating a HER-driven drug-resistant cancer in a subject with cancer, wherein an EGFR exon 20 insertion mutation is detected in tumor cells of the subject, wherein the method comprises administering a therapeutically effective amount of at least one compound selected from the group consisting of Compound A and Compound B, or a salt or a solvate thereof

In another aspect, the present application provides a method of treating cancer in a subject with cancer. In other embodiments, the method comprises:

(a) providing tumor cells of the subject;

(b) detecting presence or absence of an EGFR exon 20 insertion mutation in provided tumor cells of the subject;

(c) predicting the subject as being likely to be responsive to treatment by a compound contemplated herein if the EGFR exon 20 insertion mutation is detected;

(d) administering a therapeutically effective amount of a compound contemplated herein to the subject.

In certain embodiments, the cancer is a HER-driven drug-resistant cancer.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In another aspect, the present application provides a method of treating cancer in a subject with cancer. In other embodiments, the method comprises:

(a) detecting presence or absence of an EGFR exon 20 insertion mutation in tumor cells of the subject;

(b) predicting the subject as being likely to be responsive to treatment by a compound contemplated herein if the EGFR exon 20 insertion mutation is detected;

(c) administering a therapeutically effective amount of a compound contemplated herein to the subject.

In certain embodiments, the cancer is a HER-driven drug-resistant cancer.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In yet another aspect, the present application provides a method of treating cancer in a subject with cancer, where an EGFR exon 20 insertion mutation is detected in tumor cells of the subject. In other embodiments, the method comprises administering a therapeutically effective amount of a compound contemplated herein to the subject. In yet other embodiments, the cancer is a HER-driven drug-resistant cancer. In yet other embodiments, the compound is Compound A or Compound B. In yet another aspect, the present application provides the use of a compound contemplated herein in the manufacture of a composition for the treatment of cancer in a subject with cancer, where an EGFR exon 20 insertion mutation is detected in the tumor cells of the subject. In other embodiments, the present application provides the use of a compound contemplated herein in the manufacture of a composition for the treatment of cancer in a subject with cancer, where an EGFR exon 20 insertion mutation is detected in a sample of tumor cells

from the subject. In yet other embodiments, the cancer is a HER-driven drug-resistant cancer. In yet other embodiments, the compound is Compound A or Compound B.

In yet another aspect, the present application provides the use of a compound contemplated herein in the treatment of cancer in a subject with cancer, where an EGFR exon 20 insertion mutation is detected in tumor cells of the subject. In other embodiments, the present application provides the use of a compound contemplated herein in the treatment of cancer in a subject with cancer, where an EGFR exon 20 insertion mutation is detected in a sample of tumor cells of the subject. In yet other embodiments, the cancer is a HER-driven drug-resistant cancer. In yet other embodiments, the compound is Compound A or Compound B.

In yet another aspect, the present application provides a compound contemplated herein for use in the treatment of cancer in a subject with cancer, where an EGFR exon 20 insertion mutation is detected in tumor cells of the subject. In other embodiments, the present application provides a compound contemplated herein for use in the treatment of cancer in a subject with cancer, where an EGFR exon 20 insertion mutation is detected in a sample of tumor cells of the subject. In yet other embodiments, the cancer is a HER-driven drug-resistant cancer. In yet other embodiments, the compound is Compound A or Compound B.

In yet another aspect, the present application provides a compound contemplated herein for use in the treatment of cancer in a subject with cancer, comprising:

(a) providing tumor cells of the subject;

(b) detecting presence or absence of an EGFR exon 20 insertion mutation in the provided tumor cells of the subject; and

(c) administering a therapeutically effective amount of a compound contemplated herein to the subject if the EGFR exon 20 insertion mutation is detected.

In certain embodiments, the cancer is a HER-driven drug-resistant cancer.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In yet another aspect, the present application provides a compound contemplated herein for use in the treatment of cancer in a subject with cancer. In certain embodiments, the method comprises:

(a) detecting presence or absence of an EGFR exon 20 insertion mutation in tumor cells of the subject; and

(b) administering a therapeutically effective amount of a compound contemplated herein to the subject if the EGFR exon 20 insertion mutation is detected in the provided tumor cells of the subject.

In certain embodiments, the cancer is a HER-driven drug-resistant cancer.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In yet another aspect, the present application provides a method of treating cancer in a subject with cancer. In other embodiments, the method comprises:

(a) providing tumor cells of the subject;

(b) detecting presence or absence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in provided tumor cells of the subject;

(c) predicting the subject as being likely to be responsive to treatment by a compound contemplated herein if the at least one fusion, duplication, or mutation is detected;

(d) administering a therapeutically effective amount of a compound contemplated herein to the subject.

In certain embodiments, the cancer is a HER-driven drug-resistant cancer.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In another aspect, the present application provides a method of treating cancer in a subject with cancer. In other embodiments, the method comprises:

(a) detecting the presence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in tumor cells of the subject;

(b) predicting the subject as being likely to be responsive to treatment by a compound contemplated herein if the at least one fusion, duplication, or mutation is detected;

(c) administering a therapeutically effective amount of a compound contemplated herein to the subject.

In certain embodiments, the cancer is a HER-driven drug-resistant cancer.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In yet another aspect, the present application provides a method of treating cancer in a subject with cancer, where at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion is detected in tumor cells of the subject. In other embodiments, the method comprises administering a therapeutically effective amount of a compound contemplated herein to the subject. In yet other embodiments, the cancer is a HER-driven drug-resistant cancer. In yet other embodiments, the compound is Compound A or Compound B.

In yet another aspect, the present application provides the use of a compound contemplated herein in the manufacture of a composition for the treatment of cancer in a subject with cancer, where at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion is detected in the tumor cells of the subject. In other embodiments, the present application provides the use of a compound contemplated herein in the manufacture of a composition for the treatment of cancer in a subject with cancer, where at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion is detected in a sample of tumor cells from the subject. In yet other embodiments, the cancer is a HER-driven drug-resistant cancer. In yet other embodiments, the compound is Compound A or Compound B.

In yet another aspect, the present application provides the use of a compound contemplated herein in the treatment of cancer in a subject with cancer, where at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion is detected in tumor cells of the subject. In other embodiments, the present application provides the use of a compound contemplated herein in the treatment of cancer in a subject with cancer, where at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a

ERBB2 gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion is detected in a sample of tumor cells of the subject. In yet other embodiments, the cancer is a HER-driven drug-resistant cancer. In yet other embodiments, the compound is Compound A or Compound B.

In yet another aspect, the present application provides a compound contemplated herein for use in the treatment of cancer in a subject with cancer, where at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion is detected in tumor cells of the subject. In other embodiments, the present application provides a compound contemplated herein for use in the treatment of cancer in a subject with cancer, where at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion is detected in a sample of tumor cells of the subject. In yet other embodiments, the cancer is a HER-driven drug-resistant cancer. In yet other embodiments, the compound is Compound A or Compound B.

In yet another aspect, the present application provides a compound contemplated herein for use in the treatment of cancer in a subject with cancer, comprising:

(a) providing tumor cells of the subject;

(b) detecting presence or absence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in the provided tumor cells of the subject; and

(c) administering a therapeutically effective amount of a compound contemplated herein to the subject if the at least one fusion, duplication, or mutation is detected.

In certain embodiments, the cancer is a HER-driven drug-resistant cancer.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In yet another aspect, the present application provides a compound contemplated herein for use in the treatment of cancer in a subject with cancer. In certain embodiments, the method comprises:

(a) detecting the presence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in tumor cells of the subject; and

(b) administering a therapeutically effective amount of a compound contemplated herein to the subject if the at least one fusion, duplication, or mutation is detected in the provided tumor cells of the subject.

In certain embodiments, the cancer is a HER-driven drug-resistant cancer.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In certain embodiments, the subject is further administered at least one additional agent, or a salt or solvate thereof, that treats or prevents the drug-resistant cancer. Non-limiting examples of additional anti-proliferative agents contemplated include, but are not limited to, compounds listed on the cancer chemotherapy drug regimens in the 14th Edition of the Merck Index (2006), which is hereby incorporated by reference, such as asparaginase, bleomycin, carboplatin, carmustine, chlorambucil, cisplatin, colaspase, cyclophosphamide, cytarabine, dacarbazine, dactinomycin, daunorubicin, doxorubicin (adriamycine), epirubicin, etoposide, 5-fluorouracil, hexamethylmelamine, hydroxyurea, ifosfamide, irinotecan, leucovorin, lomustine, mechlorethamine, 6-mercaptopurine, mesna, methotrexate, mitomycin C, mitoxantrone, prednisolone, prednisone, procarbazine, raloxifen, streptozocin, tamoxifen, thioguanine, topotecan, vinblastine, vincristine, and vindesine. Additional anti-proliferative agents include other molecular targeted agents that modulate parallel pathways such as MEK 1/2 inhibitors, AKT inhibitors and mTOR inhibitors, monoclonal antibodies (such as Cetuximab), oxaliplatin, gemcitabine, gefinitib, taxotere, ara A, ara C, herceptin, BCNU, CCNU, DTIC, and actinomycin D. Still further anti-proliferative agents include but are not limited to those compounds acknowledged to be used in the treatment of neoplastic diseases in Goodman and Gilman's The Pharmacological Basis of Therapeutics (Eleventh Edition), editor Molinoff et al., publ. by McGraw-Hill, pages 1225-1287 (2006), which is hereby incorporated by reference, such as aminoglutethimide, L-asparaginase, azathioprine, 5-azacytidine cladribine, busulfan, diethylstilbestrol, 2′,2′-difluorodeoxycytidine, docetaxel, erythrohydroxynonyladenine, ethinyl estradiol, 5-fluorodeoxyuridine, 5-fluorodeoxyuridine monophosphate, fludarabine phosphate,

fluoxymesterone, flutamide, hydroxyprogesterone caproate, idarubicin, interferon, medroxyprogesterone acetate, megestrol acetate, melphalan, mitotane, paclitaxel, pentostatin, N-phosphonoacetyl-L-aspartate (PALA), plicamycin, semustine, tenipdside, testosterone propionate, thiotepa, trimethylmelamine, uridine, and vinorelbine.

In certain embodiments, a compound of any one of Formulas I, II, VII, VIII, IX, X and/or XI, as disclosed herein, and at least one additional agent, are co-administered to the subject. In other embodiments, a compound of any one of Formulas I, II, VII, VIII, IX, X and/or XI, as disclosed herein, and at least one additional agent, are co-formulated.

In certain embodiments, Compound A or Compound B, and at least one additional agent, are co-administered to the subject. In other embodiments, Compound A or Compound B, and at least one additional agent, are co-formulated.

In certain embodiments, a compound of any one of Formulas I, II, VII, VIII, IX, X and/or XI, as disclosed herein, is administered by at least one route selected from the group consisting of inhalational, oral, nasal, rectal, parenteral, sublingual, transdermal, transmucosal, intravesical, intrapulmonary, intraduodenal, intragastrical, intrathecal, epidural, intrapleural, intraperitoneal, intratracheal, otic, intraocular, subcutaneous, intramuscular, intradermal, intra-arterial, intravenous, intrabronchial, inhalation, and topical. In other embodiments, the subject is a mammal. In yet other embodiments, the mammal is a human. In certain embodiments, the subject is a human in need of treatment thereof.

In certain embodiments, Compound A or Compound B is administered by at least one route selected from the group consisting of inhalational, oral, nasal, rectal, parenteral, sublingual, transdermal, transmucosal, intravesical, intrapulmonary, intraduodenal, intragastrical, intrathecal, epidural, intrapleural, intraperitoneal, intratracheal, otic, intraocular, subcutaneous, intramuscular, intradermal, intra-arterial, intravenous, intrabronchial, inhalation, and topical. In other embodiments, the subject is a mammal. In yet other embodiments, the mammal is a human. In certain embodiments, the subject is a human in need of treatment thereof.

Also provided herein is a kit comprising a compound of any one of Formulas I, II, VII, VIII, IX, X and/or XI, as disclosed herein, an applicator and instructional material for use thereof, wherein the instructional material comprises instructions for preventing or treating HER-driven drug-resistant cancers.

Further provided herein is a kit comprising Compound A or Compound B, an applicator

and instructional material for use thereof, wherein the instructional material comprises instructions for preventing or treating HER-driven drug-resistant cancers.

Salts

The compounds described herein may form salts with acids, and such salts are included in the present application. In one embodiment, the salts are pharmaceutically acceptable salts. The term "salts" embraces addition salts of free acids that are useful within the methods disclosed herein. The term "pharmaceutically acceptable salt" refers to salts that possess toxicity profiles within a range that affords utility in pharmaceutical applications. Pharmaceutically unacceptable salts may nonetheless possess properties such as high crystallinity, which have utility in the practice of the present application, such as for example utility in process of synthesis, purification or formulation of compounds useful within the methods disclosed herein.

Suitable pharmaceutically acceptable acid addition salts may be prepared from an inorganic acid or from an organic acid. Examples of inorganic acids include sulfate, hydrogen sulfate, hydrochloric, hydrobromic, hydriodic, nitric, carbonic, sulfuric, and phosphoric acids (including hydrogen phosphate and dihydrogen phosphate). Appropriate organic acids may be selected from aliphatic, cycloaliphatic, aromatic, araliphatic, heterocyclic, carboxylic and sulfonic classes of organic acids, examples of which include formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, ascorbic, glucuronic, maleic, fumaric, pyruvic, aspartic, glutamic, benzoic, anthranilic, 4-hydroxybenzoic, phenylacetic, mandelic, embonic (pamoic), methanesulfonic, ethanesulfonic, benzenesulfonic, pantothenic, trifluoromethanesulfonic, 2-hydroxyethanesulfonic, p-toluenesulfonic, sulfanilic, cyclohexylaminosulfonic, stearic, alginic, β-hydroxybutyric, salicylic, galactaric, galacturonic acid, glycerophosphonic acids and saccharin (*e.g.*, saccharinate, saccharate). Salts may be comprised of a fraction of one, one or more than one molar equivalent of acid or base with respect to any compound contemplated herein.

Suitable pharmaceutically acceptable base addition salts of compounds contemplated herein include, for example, metallic salts including alkali metal, alkaline earth metal and transition metal salts such as, for example, calcium, magnesium, potassium, sodium and zinc salts. Pharmaceutically acceptable base addition salts also include organic salts made from basic amines such as, for example, N,N'-dibenzylethylene-diamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine. All of these

salts may be prepared from the corresponding compound by reacting, for example, the appropriate acid or base with the compound.

Predictive / Identification Methods

The present application also contemplates methods of predicting responsiveness of a subject with cancer to treatment with a compound contemplated herein.

In certain embodiments, the compound is of a compound of Formula I, II, VII, VIII, IX, X and/or XI. In certain embodiments, the compound is of Formula I, II, IX, X and/or XI. In certain embodiments, the compound is of Formula VII and/or VIII. In certain embodiments, the compound is one of Compounds 12-88 and 91-104. In certain embodiments, the compound is one of Compounds 1-11, 89 and 90. In certain embodiments, the compound is Compound 17. In certain embodiments, the compound is Compound 5. In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In one aspect, the present application provides a method of predicting the responsiveness of a subject with cancer to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) providing tumor cells of the subject;

(b) detecting presence or absence of an EGFR exon 20 insertion mutation in the provided tumor cells of the subject;

(c) predicting the subject as being likely to be responsive to a treatment with a compound contemplated herein if the EGFR exon 20 insertion mutation is detected in the provided tumor cells of the subject.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In another aspect, the present application provides a method of predicting the responsiveness of a subject with cancer to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) detecting presence or absence of an EGFR exon 20 insertion mutation in a sample from the subject, wherein the sample comprises tumor cells;

(b) predicting the subject as being likely to be responsive to a treatment with a compound contemplated herein if the EGFR exon 20 insertion mutation is detected in the sample from the

subject.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In yet another aspect, the present application provides a method of predicting the responsiveness of a subject with cancer to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) detecting presence or absence of an EGFR exon 20 insertion mutation in tumor cells of the subject; and

(b) predicting the subject as being likely to be responsive to treatment with a compound contemplated herein if an EGFR exon 20 insertion mutation is detected in the tumor cells of the subject.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In yet another aspect, the present application provides a method of predicting the responsiveness of a subject with cancer to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) detecting presence or absence of an EGFR exon 20 insertion mutation in a sample of tumor cells from the subject; and

(b) predicting the subject as being likely to be responsive to treatment with a compound contemplated herein if the EGFR exon 20 insertion mutation is detected in the sample of tumor cells from the subject.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In yet another aspect, the present application provides a method of predicting the responsiveness of a subject with cancer to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) providing tumor cells of the subject;

(b) detecting presence or absence of an EGFR exon 20 insertion mutation in the provided tumor cells of the subject;

wherein the subject is likely to be responsive to the treatment with a compound contemplated herein if the EGFR exon 20 insertion mutation is detected in the provided tumor cells of the

subject.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In yet another aspect, the present application provides a method of predicting the responsiveness of a subject with cancer to treatment with a compound contemplated herein. In certain embodiments, the method comprises detecting presence or absence of an EGFR exon 20 insertion mutation in tumor cells of the subject;

wherein the subject is likely to be responsive to the treatment with a compound contemplated herein if the EGFR exon 20 insertion mutation is detected in the tumor cells of the subject.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In yet another aspect, the present application provides a method of predicting the responsiveness of a subject with cancer to treatment with a compound contemplated herein. In certain embodiments, the method comprises detecting presence or absence of an EGFR exon 20 insertion mutation in a sample of tumor cells from the subject;

wherein the subject is likely to be responsive to the treatment with a compound contemplated herein if the EGFR exon 20 insertion mutation is detected in the sample of tumor cells from the subject.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In yet another aspect, the present application provides a method of predicting the responsiveness of a subject with cancer to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) providing tumor cells of the subject; and

(b) detecting presence or absence of an EGFR exon 20 insertion mutation in the provided tumor cells of the subject;

wherein the presence of an EGFR exon 20 insertion mutation in the provided tumor cells of the subject correlates with an increased likelihood of responsiveness or an increased responsiveness of the subject to the treatment.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In certain embodiments of the various methods provided herein, the prediction of the responsiveness of the subject with cancer to treatment by a compound contemplated herein is made by detecting the presence of an EGFR exon 20 insertion mutation in the tumor cells. In other embodiments, the method further comprises administration of a compound contemplated herein to the subject. In yet other embodiments, the method further comprises administration of a compound contemplated herein to the subject if the subject is predicted to be likely to be responsive to the treatment. In yet other embodiments, a compound contemplated herein is administered in an therapeutically effective amount.

The present application also contemplates methods of predicting whether a subject with cancer is likely to be responsive to treatment with a compound contemplated herein.

In one aspect, the present application provides a method of predicting whether a subject with cancer is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) providing tumor cells of the subject;

(b) detecting presence or absence of an EGFR exon 20 insertion mutation in the provided tumor cells of the subject;

(c) predicting the subject as being likely to be responsive to a treatment with a compound contemplated herein if the EGFR exon 20 insertion mutation is detected in the provided tumor cells of the subject.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In another aspect, the present application provides a method of predicting whether a subject with cancer is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) detecting presence or absence of an EGFR exon 20 insertion mutation in a sample from the subject, wherein the sample comprises tumor cells;

(b) predicting the subject as being likely to be responsive to a treatment with a compound contemplated herein if the EGFR exon 20 insertion mutation is detected in the sample from the subject.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In yet another aspect, the present application provides a method of predicting whether a subject with cancer is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) detecting presence or absence of an EGFR exon 20 insertion mutation in tumor cells of the subject; and

(b) predicting the subject as being likely to be responsive to treatment with a compound contemplated herein if an EGFR exon 20 insertion mutation is detected in the tumor cells of the subject.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In yet another aspect, the present application provides a method of predicting whether a subject with cancer is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) detecting presence or absence of an EGFR exon 20 insertion mutation in a sample of tumor cells from the subject; and

(b) predicting the subject as being likely to be responsive to treatment with a compound contemplated herein if the EGFR exon 20 insertion mutation is detected in the sample of tumor cells from the subject.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In yet another aspect, the present application provides a method of predicting whether a subject with cancer is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) providing tumor cells of the subject;

(b) detecting presence or absence of an EGFR exon 20 insertion mutation in the provided tumor cells;

wherein the subject is likely to be responsive to the treatment with a compound contemplated herein if the EGFR exon 20 insertion mutation is detected in the provided cells.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In yet another aspect, the present application provides a method of predicting whether a

subject with cancer is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises detecting presence or absence of an EGFR exon 20 insertion mutation in tumor cells of the subject;

wherein the subject is likely to be responsive to the treatment with a compound contemplated herein if the EGFR exon 20 insertion mutation is detected.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In another aspect, the present application provides a method of predicting whether a subject with cancer is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises detecting presence or absence of an EGFR exon 20 insertion mutation in a sample of tumor cells from the subject;

wherein the subject is likely to be responsive to the treatment with a compound contemplated herein if the EGFR exon 20 insertion mutation is detected.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In another aspect, the present application provides a method of predicting whether a subject with cancer is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) providing tumor cells of the subject; and

(b) detecting presence or absence of an EGFR exon 20 insertion mutation in the tumor cells;

wherein the presence of the EGFR exon 20 insertion mutation correlates with an increased likelihood of responsiveness or an increased responsiveness of the subject to the treatment.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In certain embodiments of the various methods provided herein, the prediction of whether a subject with cancer is likely to be responsive to treatment by a compound contemplated herein is made by detecting the presence of an EGFR exon 20 insertion mutation in the tumor cells. In other embodiments, the method further comprises administration of a compound contemplated herein to the subject. In yet other embodiments, the method further comprises administration of a compound contemplated herein to the subject if the subject is predicted to be likely to be responsive to the treatment. In yet other embodiments, a compound contemplated herein is

administered in a therapeutically effective amount.

The present application also contemplates methods of identifying a subject with cancer who is likely to be responsive to treatment with a compound contemplated herein.

In one aspect, the present application provides a method of identifying a subject with cancer who is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) providing tumor cells of the subject;

(b) detecting presence or absence of an EGFR exon 20 insertion mutation in the provided tumor cells;

(c) identifying the subject as being likely to be responsive to treatment with a compound contemplated herein if the EGFR exon 20 insertion mutation is detected in the provided tumor cells.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In another aspect, the present application provides a method of identifying a subject with cancer who is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) detecting presence or absence of an EGFR exon 20 insertion mutation in a sample from the subject, wherein the sample comprises tumor cells;

(b) identifying the subject as being likely to be responsive to a treatment with a compound contemplated herein if the EGFR exon 20 insertion mutation is detected in the sample from the subject.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In yet another aspect, the present application provides a method of identifying a subject with cancer who is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) detecting presence or absence of an EGFR exon 20 insertion mutation in tumor cells of the subject; and

(b) identifying the subject as being likely to be responsive to a treatment with a compound contemplated herein if the EGFR exon 20 insertion mutation is detected in the tumor cells of the

subject.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In another aspect, the present application provides a method of identifying a subject with cancer who is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) detecting presence or absence of an EGFR exon 20 insertion mutation in a sample of tumor cells from the subject; and

(b) identifying the subject as being likely to be responsive to a treatment with a compound contemplated herein if the EGFR exon 20 insertion mutation is detected in the sample.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In another aspect, the present application provides a method of identifying a subject with cancer who is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) providing tumor cells of the subject;

(b) detecting presence or absence of an EGFR exon 20 insertion mutation in the provided tumor cells;

wherein the subject is identified as likely to be responsive to the treatment with a compound contemplated herein if the EGFR exon 20 insertion mutation is detected in the provided tumor cells.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In another aspect, the present application provides a method of identifying a subject with cancer who is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises detecting presence or absence of an EGFR exon 20 insertion mutation in tumor cells of the subject;

wherein the subject is identified as likely to be responsive to the treatment with a compound contemplated herein if the EGFR exon 20 insertion mutation is detected in the tumor cells.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In another aspect, the present application provides a method of identifying a subject with cancer who is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises detecting presence or absence of an EGFR exon 20 insertion mutation in a sample of tumor cells from the subject;

wherein the subject is identified as likely to be responsive to the treatment with a compound contemplated herein if the EGFR exon 20 insertion mutation is detected in the sample.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In another aspect, the present application provides a method of identifying a subject with cancer who is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) providing tumor cells of the subject; and

(b) detecting presence or absence of an EGFR exon 20 insertion mutation in the provided tumor cells;

wherein the presence of an EGFR exon 20 insertion mutation identifies the subject as likely to be responsive to the treatment with a compound contemplated herein.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In certain embodiments of the various methods provided herein, the identification of a subject with cancer who is likely to be responsive to treatment by a compound contemplated herein is made by detecting the presence of an EGFR exon 20 insertion mutation in the tumor cells. In other embodiments, the method further comprises administration of a compound contemplated herein to the subject. In yet other embodiments, the method further comprises administration of a compound contemplated herein to the subject that is identified to be likely to be responsive to the treatment. In yet other embodiments, a compound contemplated herein is administered in a therapeutically effective amount.

In yet another aspect, provided herein is a method for determining whether a subject with cancer is sensitive to a treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) providing tumor cells of the subject;

(b) detecting presence or absence of an EGFR exon 20 insertion mutation in the provided

tumor cells;

(c) diagnosing the subject as being sensitive to the treatment with a compound contemplated herein if the EGFR exon 20 insertion mutation is detected in the provided tumor cells.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In certain embodiments, the method further comprises administration of a compound contemplated herein to the subject. In other embodiments, the method further comprises administration of a compound contemplated herein to the subject if the subject is determined to be sensitive to the treatment. In yet other embodiments, a compound contemplated herein is administered in a therapeutically effective amount.

In yet another aspect, the present application provides a method of determining whether a subject with cancer is sensitive to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) providing tumor cells of the subject;

(b) detecting presence or absence of an EGFR exon 20 insertion mutation in the provided tumor cells;

(c) diagnosing the subject as being sensitive to treatment to the treatment with a compound contemplated herein if the EGFR exon 20 insertion mutation is detected in the provided tumor cells.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In certain embodiments, the method further comprises administration of a compound contemplated herein to the subject. In other embodiments, the method further comprises administration of a compound contemplated herein to the subject if the subject is determined to be sensitive to the treatment. In yet other embodiments, a compound contemplated herein is administered in a therapeutically effective amount.

In yet another aspect, the present application provides a method of determining whether a subject with cancer is sensitive to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) detecting presence or absence of an EGFR exon 20 insertion mutation in a sample from the subject, wherein the sample comprises tumor cells;

(b) diagnosing the subject as being sensitive to treatment to the treatment with a compound contemplated herein if the EGFR exon 20 insertion mutation is detected in the sample.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In one aspect, the present application provides a method of predicting the responsiveness of a subject with cancer to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) providing tumor cells of the subject;

(b) detecting presence or absence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion mutation in the provided tumor cells of the subject;

(c) predicting the subject as being likely to be responsive to a treatment with a compound contemplated herein if the at least one fusion, duplication, or mutation is detected in the provided tumor cells of the subject.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In another aspect, the present application provides a method of predicting the responsiveness of a subject with cancer to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) detecting presence or absence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion mutation in a sample from the subject, wherein the sample comprises tumor cells;

(b) predicting the subject as being likely to be responsive to a treatment with a compound contemplated herein if the at least one fusion, duplication, or mutation is detected in the sample from the subject.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In yet another aspect, the present application provides a method of predicting the responsiveness of a subject with cancer to treatment with a compound contemplated herein. In

certain embodiments, the method comprises:

(a) detecting presence or absence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in tumor cells of the subject; and

(b) predicting the subject as being likely to be responsive to treatment with a compound contemplated herein if the at least one fusion, duplication, or mutation is detected in the tumor cells of the subject.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In yet another aspect, the present application provides a method of predicting the responsiveness of a subject with cancer to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) detecting presence or absence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in a sample of tumor cells from the subject; and

(b) predicting the subject as being likely to be responsive to treatment with a compound contemplated herein if the at least one fusion, duplication, or mutation is detected in the sample of tumor cells from the subject.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In yet another aspect, the present application provides a method of predicting the responsiveness of a subject with cancer to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) providing tumor cells of the subject;

(b) detecting presence or absence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in the provided tumor cells of the subject;

wherein the subject is likely to be responsive to the treatment with a compound contemplated

herein if the at least one fusion, duplication, or mutation is detected in the provided tumor cells of the subject.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In yet another aspect, the present application provides a method of predicting the responsiveness of a subject with cancer to treatment with a compound contemplated herein. In certain embodiments, the method comprises detecting presence or absence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in tumor cells of the subject;

wherein the subject is likely to be responsive to the treatment with a compound contemplated herein if the at least one fusion, duplication, or mutation is detected in the tumor cells of the subject.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In yet another aspect, the present application provides a method of predicting the responsiveness of a subject with cancer to treatment with a compound contemplated herein. In certain embodiments, the method comprises detecting presence or absence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in a sample of tumor cells from the subject;

wherein the subject is likely to be responsive to the treatment with a compound contemplated herein if at least one fusion, duplication, or mutation is detected in the sample of tumor cells from the subject.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In yet another aspect, the present application provides a method of predicting the responsiveness of a subject with cancer to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) providing tumor cells of the subject; and

(b) detecting presence or absence of at least one fusion, duplication, or mutation selected

from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in the provided tumor cells of the subject;

wherein the presence of the at least one fusion, duplication, or mutation in the provided tumor cells of the subject correlates with an increased likelihood of responsiveness or an increased responsiveness of the subject to the treatment.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In certain embodiments of the various methods provided herein, the prediction of the responsiveness of the subject with cancer to treatment by a compound contemplated herein is made by detecting the presence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in the tumor cells. In other embodiments, the method further comprises administration of a compound contemplated herein to the subject. In yet other embodiments, the method further comprises administration of a compound contemplated herein to the subject if the subject is predicted to be likely to be responsive to the treatment. In yet other embodiments, a compound contemplated herein is administered in an therapeutically effective amount.

The present application also contemplates methods of predicting whether a subject with cancer is likely to be responsive to treatment with a compound contemplated herein.

In one aspect, the present application provides a method of predicting whether a subject with cancer is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) providing tumor cells of the subject;

(b) detecting presence or absence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in the provided tumor cells of the subject;

(c) predicting the subject as being likely to be responsive to a treatment with a compound contemplated herein if the at least one fusion, duplication, or mutation is detected in the provided tumor cells of the subject.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In another aspect, the present application provides a method of predicting whether a subject with cancer is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) detecting presence or absence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in a sample from the subject, wherein the sample comprises tumor cells;

(b) predicting the subject as being likely to be responsive to a treatment with a compound contemplated herein if the at least one fusion, duplication, or mutation is detected in the sample from the subject.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In yet another aspect, the present application provides a method of predicting whether a subject with cancer is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) detecting presence or absence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in tumor cells of the subject; and

(b) predicting the subject as being likely to be responsive to treatment with a compound contemplated herein if the at least one fusion, duplication, or mutation is detected in the tumor cells of the subject.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In yet another aspect, the present application provides a method of predicting whether a subject with cancer is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) detecting presence or absence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2*

gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in a sample of tumor cells from the subject; and

(b) predicting the subject as being likely to be responsive to treatment with a compound contemplated herein if the at least one fusion, duplication, or mutation is detected in the sample of tumor cells from the subject.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In yet another aspect, the present application provides a method of predicting whether a subject with cancer is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) providing tumor cells of the subject;

(b) detecting presence or absence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in the provided tumor cells;

wherein the subject is likely to be responsive to the treatment with a compound contemplated herein if the at least one fusion, duplication, or mutation is detected in the provided cells.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In yet another aspect, the present application provides a method of predicting whether a subject with cancer is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises detecting presence or absence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in tumor cells of the subject;

wherein the subject is likely to be responsive to the treatment with a compound contemplated herein if the at least one fusion, duplication, or mutation is detected.

In certain embodiments, the compound is of a compound of Formula I, II, VII, VIII, IX, X and/or XI. In certain embodiments, the compound is Compound A and/or Compound B.

In another aspect, the present application provides a method of predicting whether a subject with cancer is likely to be responsive to treatment with a compound contemplated herein.

In certain embodiments, the method comprises detecting presence or absence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in a sample of tumor cells from the subject;

wherein the subject is likely to be responsive to the treatment with a compound contemplated herein if the at least one fusion, duplication, or mutation is detected.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In another aspect, the present application provides a method of predicting whether a subject with cancer is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) providing tumor cells of the subject; and

(b) detecting presence or absence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in the tumor cells;

wherein the presence of the at least one fusion, duplication, or mutation correlates with an increased likelihood of responsiveness or an increased responsiveness of the subject to the treatment.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In certain embodiments of the various methods provided herein, the prediction of whether a subject with cancer is likely to be responsive to treatment by a compound contemplated herein is made by detecting the presence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in the tumor cells. In other embodiments, the method further comprises administration of a compound contemplated herein to the subject. In yet other embodiments, the method further comprises administration of a compound contemplated herein to the subject if the subject is predicted to be likely to be responsive to the treatment. In yet other embodiments, a compound contemplated herein is administered in a therapeutically effective amount.

The present application also contemplates methods of identifying a subject with cancer who is likely to be responsive to treatment with a compound contemplated herein.

In one aspect, the present application provides a method of identifying a subject with cancer who is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) providing tumor cells of the subject;

(b) detecting presence or absence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in the provided tumor cells;

(c) identifying the subject as being likely to be responsive to treatment with a compound contemplated herein if the at least one fusion, duplication, or mutation is detected in the provided tumor cells.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In another aspect, the present application provides a method of identifying a subject with cancer who is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) detecting presence or absence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in a sample from the subject, wherein the sample comprises tumor cells;

(b) identifying the subject as being likely to be responsive to a treatment with a compound contemplated herein if the at least one fusion, duplication, or mutation is detected in the sample from the subject.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In yet another aspect, the present application provides a method of identifying a subject with cancer who is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) detecting presence or absence of at least one fusion, duplication, or mutation selected

from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in tumor cells of the subject; and

(b) identifying the subject as being likely to be responsive to a treatment with a compound contemplated herein if the at least one fusion, duplication, or mutation is detected in the tumor cells of the subject.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In another aspect, the present application provides a method of identifying a subject with cancer who is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) detecting presence or absence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in a sample of tumor cells from the subject; and

(b) identifying the subject as being likely to be responsive to a treatment with a compound contemplated herein if the at least one fusion, duplication, or mutation is detected in the sample.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In another aspect, the present application provides a method of identifying a subject with cancer who is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) providing tumor cells of the subject;

(b) detecting presence or absence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in the provided tumor cells;

wherein the subject is identified as likely to be responsive to the treatment with a compound contemplated herein if the at least one fusion, duplication, or mutation is detected in the provided tumor cells.

In certain embodiments, the compound is Compound A. In certain embodiments, the

compound is Compound B.

In another aspect, the present application provides a method of identifying a subject with cancer who is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises detecting presence or absence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in tumor cells of the subject;

wherein the subject is identified as likely to be responsive to the treatment with a compound contemplated herein if the at least one fusion, duplication, or mutation is detected in the tumor cells.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In another aspect, the present application provides a method of identifying a subject with cancer who is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises detecting presence or absence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in a sample of tumor cells from the subject;

wherein the subject is identified as likely to be responsive to the treatment with a compound contemplated herein if the at least one fusion, duplication, or mutation is detected in the sample.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In another aspect, the present application provides a method of identifying a subject with cancer who is likely to be responsive to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) providing tumor cells of the subject; and

(b) detecting presence or absence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in the provided tumor cells;

wherein the presence of the at least one fusion, duplication, or mutation identifies the subject

as likely to be responsive to the treatment with a compound contemplated herein.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In certain embodiments of the various methods provided herein, the identification of a subject with cancer who is likely to be responsive to treatment by a compound contemplated herein is made by detecting the presence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in the tumor cells. In other embodiments, the method further comprises administration of a compound contemplated herein to the subject. In yet other embodiments, the method further comprises administration of a compound contemplated herein to the subject that is identified to be likely to be responsive to the treatment. In yet other embodiments, a compound contemplated herein is administered in a therapeutically effective amount.

In yet another aspect, provided herein is a method for determining whether a subject with cancer is sensitive to a treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) providing tumor cells of the subject;

(b) detecting presence or absence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in the provided tumor cells;

(c) diagnosing the subject as being sensitive to the treatment with a compound contemplated herein if the at least one fusion, duplication, or mutation is detected in the provided tumor cells.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In certain embodiments, the method further comprises administration of a compound contemplated herein to the subject. In other embodiments, the method further comprises administration of a compound contemplated herein to the subject if the subject is determined to be sensitive to the treatment. In yet other embodiments, a compound contemplated herein is administered in a therapeutically effective amount.

In yet another aspect, the present application provides a method of determining whether a

subject with cancer is sensitive to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) providing tumor cells of the subject;

(b) detecting presence or absence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in the provided tumor cells;

(c) diagnosing the subject as being sensitive to treatment to the treatment with a compound contemplated herein if the at least one fusion, duplication, or mutation is detected in the provided tumor cells.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In certain embodiments, the method further comprises administration of a compound contemplated herein to the subject. In other embodiments, the method further comprises administration of a compound contemplated herein to the subject if the subject is determined to be sensitive to the treatment. In yet other embodiments, a compound contemplated herein is administered in a therapeutically effective amount.

In yet another aspect, the present application provides a method of determining whether a subject with cancer is sensitive to treatment with a compound contemplated herein. In certain embodiments, the method comprises:

(a) detecting presence or absence of at least one fusion, duplication, or mutation selected from the group consisting of a *EGFR* gene fusion, a *EGFR* kinase domain duplication, a *ERBB2* gene fusion, a *ERBB2* mutation, a *NRG1* gene fusion, a *ERBB3* mutation, and a *ERBB4* fusion in a sample from the subject, wherein the sample comprises tumor cells;

b) diagnosing the subject as being sensitive to treatment to the treatment with a compound contemplated herein if the at least one fusion, duplication, or mutation is detected in the sample.

In certain embodiments, the compound is Compound A. In certain embodiments, the compound is Compound B.

In certain embodiments, the method further comprises administration of a compound contemplated herein to the subject. In other embodiments, the method further comprises administration of a compound contemplated herein to the subject if the subject is determined to

be sensitive to the treatment. In yet other embodiments, a compound contemplated herein is administered in a therapeutically effective amount.

Combination and Concurrent Therapies

In one embodiment, the compounds contemplated herein are useful in the methods disclosed herein when used concurrently with at least one additional compound useful for preventing and/or treating diseases and/or disorders contemplated herein.

In one embodiment, the compounds contemplated herein are useful in the methods of present application in combination with at least one additional compound useful for preventing and/or treating diseases and/or disorders contemplated herein.

These additional compounds may comprise compounds of the present application or other compounds, such as commercially available compounds, known to treat, prevent, or reduce the symptoms of diseases and/or disorders contemplated herein. In certain embodiments, the combination of at least one compound contemplated herein or a salt thereof, and at least one additional compound useful for preventing and/or treating diseases and/or disorders contemplated herein, has additive, complementary or synergistic effects in the prevention and/or treatment of diseases and/or disorders contemplated herein.

In another non-limiting example, the compounds contemplated herein, or a salt or solvate thereof, can be used concurrently or in combination with one or more agents known to be useful in treating or preventing HER-driven (such as an EGFR-driven) drug-resistant cancer.

As used herein, combination of two or more compounds may refer to a composition wherein the individual compounds are physically mixed or wherein the individual compounds are physically separated. A combination therapy encompasses administering the components separately to produce the desired additive, complementary or synergistic effects.

In one embodiment, the compound and the agent are physically mixed in the composition. In another embodiment, the compound and the agent are physically separated in the composition.

A synergistic effect may be calculated, for example, using suitable methods such as, for example, the Sigmoid-E_{max} equation (Holford & Scheiner, 19981, Clin. Pharmacokinet. 6: 429-453), the equation of Loewe additivity (Loewe & Muischnek, 1926, Arch. Exp. Pathol Pharmacol. 114: 313-326), the median-effect equation (Chou & Talalay, 1984, Adv. Enzyme

Regul. 22: 27-55), and through the use of isobolograms (Tallarida & Raffa, 1996, Life Sci. 58: 23-28). Each equation referred to above may be applied to experimental data to generate a corresponding graph to aid in assessing the effects of the drug combination. The corresponding graphs associated with the equations referred to above are the concentration-effect curve, isobologram curve and combination index curve, respectively.

Administration/Dosage/Formulations

The regimen of administration may affect what constitutes an effective amount. The therapeutic formulations may be administered to the subject either prior to or after the onset of a disease or disorder contemplated herein. Further, several divided dosages, as well as staggered dosages may be administered daily or sequentially, or the dose may be continuously infused, or may be a bolus injection. Further, the dosages of the therapeutic formulations may be proportionally increased or decreased as indicated by the exigencies of the therapeutic or prophylactic situation.

Administration of the compositions contemplated herein to a patient, preferably a mammal, more preferably a human, may be carried out using known procedures, at dosages and for periods of time effective to treat a disease or disorder contemplated herein. An effective amount of the therapeutic compound necessary to achieve a therapeutic effect may vary according to factors such as the state of the disease or disorder in the patient; the age, sex, and weight of the patient; and the ability of the therapeutic compound to treat a disease or disorder contemplated herein. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. A non-limiting example of an effective dose range for a therapeutic compound contemplated herein is from about 1 and 5,000 mg/kg of body weight/per day. One of ordinary skill in the art would be able to study the relevant factors and make the determination regarding the effective amount of the therapeutic compound without undue experimentation.

Actual dosage levels of the active ingredients in the pharmaceutical compositions disclosed herein may be varied so as to obtain an amount of the active ingredient that is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

The therapeutically effective amount or dose of a compound contemplated herein n depends on the age, sex and weight of the patient, the current medical condition of the patient and the progression of a disease or disorder contemplated herein.

A medical doctor, *e.g.*, physician or veterinarian, having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds contemplated herein employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

A suitable dose of a compound contemplated herein may be in the range of from about 0.01 mg to about 5,000 mg per day, such as from about 0.1 mg to about 1,000 mg, for example, from about 1 mg to about 500 mg, such as about 5 mg to about 250 mg per day. The dose may be administered in a single dosage or in multiple dosages, for example from 1 to 4 or more times per day. When multiple dosages are used, the amount of each dosage may be the same or different. For example, a dose of 1 mg per day may be administered as two 0.5 mg doses, with about a 12-hour interval between doses.

Compounds contemplated herein for administration may be in the range of from about 1 μg to about 10,000 mg, about 20 μg to about 9,500 mg, about 40 μg to about 9,000 mg, about 75 μg to about 8,500 mg, about 150 μg to about 7,500 mg, about 200 μg to about 7,000 mg, about 3050 μg to about 6,000 mg, about 500 μg to about 5,000 mg, about 750 μg to about 4,000 mg, about 1 mg to about 3,000 mg, about 10 mg to about 2,500 mg, about 20 mg to about 2,000 mg, about 25 mg to about 1,500 mg, about 30 mg to about 1,000 mg, about 40 mg to about 900 mg, about 50 mg to about 800 mg, about 60 mg to about 750 mg, about 70 mg to about 600 mg, about 80 mg to about 500 mg, and any and all whole or partial increments there between.

In some embodiments, the dose of a compound contemplated herein is from about 1 mg and about 2,500 mg. In some embodiments, a dose of a compound contemplated herein used in compositions described herein is less than about 10,000 mg, or less than about 8,000 mg, or less than about 6,000 mg, or less than about 5,000 mg, or less than about 3,000 mg, or less than about 2,000 mg, or less than about 1,000 mg, or less than about 500 mg, or less than about 200 mg, or less than about 50 mg. Similarly, in some embodiments, a dose of a second compound as described herein is less than about 1,000 mg, or less than about 800 mg, or less than about 600

mg, or less than about 500 mg, or less than about 400 mg, or less than about 300 mg, or less than about 200 mg, or less than about 100 mg, or less than about 50 mg, or less than about 40 mg, or less than about 30 mg, or less than about 25 mg, or less than about 20 mg, or less than about 15 mg, or less than about 10 mg, or less than about 5 mg, or less than about 2 mg, or less than about 1 mg, or less than about 0.5 mg, and any and all whole or partial increments thereof.

In one embodiment, the compositions contemplated herein are administered to the patient in dosages that range from one to five times per day or more. In another embodiment, the compositions contemplated herein are administered to the patient in range of dosages that include, but are not limited to, once every day, every two, days, every three days to once a week, and once every two weeks. It is readily apparent to one skilled in the art that the frequency of administration of the various combination compositions contemplated herein varies from individual to individual depending on many factors including, but not limited to, age, disease or disorder to be treated, gender, overall health, and other factors. Thus, the present application should not be construed to be limited to any particular dosage regime and the precise dosage and composition to be administered to any patient is determined by the attending physical taking all other factors about the patient into account.

It is understood that the amount of compound dosed per day may be administered, in non-limiting examples, every day, every other day, every 2 days, every 3 days, every 4 days, or every 5 days. For example, with every other day administration, a 5 mg per day dose may be initiated on Monday with a first subsequent 5 mg per day dose administered on Wednesday, a second subsequent 5 mg per day dose administered on Friday, and so on.

In the case wherein the patient's status does improve, upon the doctor's discretion the administration of the compound contemplated herein is optionally given continuously; alternatively, the dose of drug being administered is temporarily reduced or temporarily suspended for a certain length of time (*i.e.*, a "drug holiday"). The length of the drug holiday optionally varies between 2 days and 1 year, including by way of example only, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 12 days, 15 days, 20 days, 28 days, 35 days, 50 days, 70 days, 100 days, 120 days, 150 days, 180 days, 200 days, 250 days, 280 days, 300 days, 320 days, 350 days, or 365 days. The dose reduction during a drug holiday includes from 10%-100%, including, by way of example only, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%.

Once improvement of the patient's conditions has occurred, a maintenance dose is administered if necessary. Subsequently, the dosage or the frequency of administration, or both, is reduced, as a function of the disease or disorder, to a level at which the improved disease is retained. In one embodiment, patients require intermittent treatment on a long-term basis upon any recurrence of symptoms and/or infection.

The compounds for use in the method disclosed herein may be formulated in unit dosage form. The term "unit dosage form" refers to physically discrete units suitable as unitary dosage for patients undergoing treatment, with each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, optionally in association with a suitable pharmaceutical carrier. The unit dosage form may be for a single daily dose or one of multiple daily doses (*e.g.*, about 1 to 4 or more times per day). When multiple daily doses are used, the unit dosage form may be the same or different for each dose.

Toxicity and therapeutic efficacy of such therapeutic regimens are optionally determined in cell cultures or experimental animals, including, but not limited to, the determination of the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between the toxic and therapeutic effects is the therapeutic index, which is expressed as the ratio between LD_{50} and ED_{50}. The data obtained from cell culture assays and animal studies are optionally used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with minimal toxicity. The dosage optionally varies within this range depending upon the dosage form employed and the route of administration utilized.

In one embodiment, the compositions contemplated herein are formulated using one or more pharmaceutically acceptable excipients or carriers. In one embodiment, the pharmaceutical compositions contemplated herein comprise a therapeutically effective amount of a compound contemplated herein and a pharmaceutically acceptable carrier.

The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity may be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms may be achieved by various antibacterial and antifungal agents, for example,

parabens, chlorobutanol, phenol, ascorbic acid, thimerosal and the like. In many cases, it is preferable to include isotonic agents, for example, sugars, sodium chloride, or polyalcohols such as mannitol and sorbitol, in the composition.

In one embodiment, the present application is directed to a packaged pharmaceutical composition comprising a container holding a therapeutically effective amount of a compound contemplated herein, alone or in combination with a second pharmaceutical agent; and instructions for using the compound to treat, prevent, or reduce one or more symptoms of a disease or disorder contemplated herein.

Formulations may be employed in admixtures with conventional excipients, *i.e.*, pharmaceutically acceptable organic or inorganic carrier substances suitable for any suitable mode of administration, known to the art. The pharmaceutical preparations may be sterilized and if desired mixed with auxiliary agents, *e.g.*, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure buffers, coloring, flavoring and/or aromatic substances and the like. They may also be combined where desired with other active agents, *e.g.*, analgesic agents.

Routes of administration of any of the compositions contemplated herein include inhalational, oral, nasal, rectal, parenteral, sublingual, transdermal, transmucosal (*e.g.*, sublingual, lingual, (trans)buccal, (trans)urethral, vaginal (*e.g.*, trans- and perivaginally), (intra)nasal, and (trans)rectal), intravesical, intrapulmonary, intraduodenal, intragastrical, intrathecal, epidural, intrapleural, intraperitoneal, intratracheal, otic, intraocular, subcutaneous, intramuscular, intradermal, intra-arterial, intravenous, intrabronchial, inhalation, and topical administration.

Additional Administration Forms

Additional dosage forms contemplated hereininclude dosage forms as described in U.S. Patents Nos. 6,340,475; 6,488,962; 6,451,808; 5,972,389; 5,582,837; and 5,007,790. Additional dosage forms contemplated herein also include dosage forms as described in U.S. Patent Applications Nos. 20030147952; 20030104062; 20030104053; 20030044466; 20030039688; and 20020051820. Further dosage forms contemplated herein include dosage forms as described in PCT Applications Nos. WO 03/35041; WO 03/35040; WO 03/35029; WO 03/35177; WO 03/35039; WO 02/96404; WO 02/32416; WO 01/97783; WO 01/56544; WO 01/32217; WO

98/55107; WO 98/11879; WO 97/47285; WO 93/18755; and WO 90/11757.

Controlled Release Formulations and Drug Delivery Systems

In one embodiment, the formulations contemplated herein may be, but are not limited to, short-term, rapid-offset, as well as controlled, for example, sustained release, delayed release and pulsatile release formulations.

The term sustained release is used in its conventional sense to refer to a drug formulation that provides for gradual release of a drug over an extended period of time, and that may, although not necessarily, result in substantially constant blood levels of a drug over an extended time period. The period of time may be as long as a month or more and should be a release which is longer that the same amount of agent administered in bolus form.

For sustained release, the compounds may be formulated with a suitable polymer or hydrophobic material that provides sustained release properties to the compounds. As such, the compounds for use in the methods disclosed herein may be administered in the form of microparticles, for example, by injection or in the form of wafers or discs by implantation.

In one embodiment, the compounds contemplated herein are administered to a patient, alone or in combination with another pharmaceutical agent, using a sustained release formulation.

The term delayed release is used herein in its conventional sense to refer to a drug formulation that provides for an initial release of the drug after some delay following drug administration and that may, although not necessarily, includes a delay of from about 10 minutes up to about 12 hours.

The term pulsatile release is used herein in its conventional sense to refer to a drug formulation that provides release of the drug in such a way as to produce pulsed plasma profiles of the drug after drug administration.

The term immediate release is used in its conventional sense to refer to a drug formulation that provides for release of the drug immediately after drug administration.

As used herein, short-term refers to any period of time up to and including about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4 hours, about 3 hours, about 2 hours, about 1 hour, about 40 minutes, about 20 minutes, or about 10 minutes and any or all whole or partial increments thereof after drug administration after drug administration.

As used herein, rapid-offset refers to any period of time up to and including about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4 hours, about 3 hours, about 2 hours, about 1 hour, about 40 minutes, about 20 minutes, or about 10 minutes, and any and all whole or partial increments thereof after drug administration.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures, embodiments, claims, and examples described herein. Such equivalents were considered to be within the scope of the present disclosure and covered by the claims appended hereto. For example, it should be understood, that modifications in reaction conditions, including but not limited to reaction times, reaction size/volume, and experimental reagents, such as solvents, catalysts, pressures, atmospheric conditions, *e.g.*, nitrogen atmosphere, and reducing/oxidizing agents, with art-recognized alternatives and using no more than routine experimentation, are within the scope of the present application.

It is to be understood that wherever values and ranges are provided herein, all values and ranges encompassed by these values and ranges, are meant to be encompassed within the scope of the present disclosure. Moreover, all values that fall within these ranges, as well as the upper or lower limits of a range of values, are also contemplated by the present application.

The following examples further illustrate aspects of the present disclosure. However, they are in no way a limitation of the teachings or disclosure of the present disclosure as set forth herein.

EXAMPLES

Certain aspects of the application are now described with reference to the following Examples. These Examples are provided for the purpose of illustration only and the application should in no way be construed as being limited to these Examples, but rather should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

Example 1:

Tarloxotinib (TRLX or Compound A) is a hypoxia-activated EGFR/HER2/HER4 TKI prodrug that releases an irreversible EGFR/HER2 tyrosine kinase inhibitor (TRLX-TKI; in this

case, Compound B) under pathophysiologically hypoxic conditions (FIG. 1). Non-small cell lung cancer (NSCLC) has been characterized as a hypoxic disease and approximately 15% of lung adenocarcinomas harbor EGFR mutations (FIG. 2). While most EGFR mutations predict for response to several FDA-approved tyrosine kinase inhibitors, in-frame insertions in exon 20 of EGFR are activating mutations in the tyrosine kinase domain that have significantly decreased sensitivity to EGFR inhibitors and currently have no approved targeted therapies (FIGs. 3A-3B).

Three human lung adenocarcinoma cell lines with different EGFR exon20 insertions were derived and characterized, in order to accelerate development of targeted therapies for this mutation class (FIGs. 4A-4B). Cell lines were transduced with one of two different shEGFR (1212014 or 10329) or with non-targeted shRNA (NTC). Proliferation was monitored by IncuCyte live-cell imagining system continuously for 80 h. Cell growth was expressed as an increase in % confluence. EGFR exon 20 insertion cell lines: CUTO-14, CUTO-17, CUTO-18. (FIG. 4A) EGFR mutant cell lines known to be EGFR dependent: PC9 (EGFR del 19; FIG. 4A), and H3255 (L858R; FIG. 4B). Using these novel cell lines, tarloxotinib was evaluated as a therapeutic agent for tumors harboring this type of mutations. The three patient derived cell lines — CUTO14 (p.A767_V769dupASV), CUTO17 (p.N771_H773dupNPH), and CUTO18 (p.S768_770dupSVD) — were found to be dependent on EGFR for cell proliferation, using shRNA mediated knockdown. Thus, successful pharmacologic inhibition of EGFR in cancer cells harboring EGFR exon 20 insertion mutations has been proven to result in growth inhibition.

The active metabolite of tarloxotinib, RN-4000E, inhibits EGFR phosphorylation and downstream signaling in cell lines harboring EGFR exon 20 insertion mutation cell lines, as demonstrated in FIGs. 5A-5C. Cells were treated with the indicated doses (nM) of afatinib, gefitinib, and RN-4000E (TH4000E, active drug) for 2 hours, lysed and analyzed by immunoblot for phosphorylated EGFR (Y1068), total EGFR, phosphorylated AKT (S473), total AKT, phosphorylated ERK (T202/Y204) , total ERK and GAPDH (loading control). Results demonstrate that RN-4000E inhibits pEGFR as well as downstream pAKT and pERK at ~10-100nM. These data demonstrate the RN-4000E inhibits its intended target in these cancer cell lines harboring EGFR exon 20 insertions and further that inhibition of EGFR blocks critical downstream signaling pathways including MAPK and AKT. Blots are representatives of three independent experiments. Further evidence that that active metabolite of tarloxotinib, RN-4000E, has similar EGFR activity to afatinib in sensitizing EGFR mutation models (using PC9,

H3255) but no effect in a KRAS mutation cell line that does not harbor an EGFR mutation (A549 cells) is provided in FIGs. 8A-8C. Cells were treated with the indicated doses (nM) of afatinib, gefitinib, and RN-4000E (TH4000E, active drug) for 2 hours, lysed and analyzed by immunoblot for phosphorylated EGFR (Y1068), total EGFR, phosphorylated AKT (S473), total AKT, phosphorylated ERK (T202/Y204), total ERK and GAPDH (loading control). Results demonstrate that RN-4000E inhibits pEGFR as well as downstream pAKT and pERK at ~0.1-10nM in cancer cells harboring EGFR sensitizing mutations (PC9 and H3255). These data demonstrate the RN-4000E inhibits its intended target in these cancer cell lines harboring EGFR exon 20 insertions, and further that inhibition of EGFR blocks critical downstream signaling pathways including MAPK and AKT. pEGFR is not detectable in A549 cells and RN-4000E does not inhibit MAPK or AKT signaling even at does of 1000nM, consistent with a lack of EGFR activation and dependence in this KRAS mutant cell line. Blots are representatives of three independent experiments.

The results showed that EGFR exon 20 insertion cell lines are resistant to gefitinib. However, treatment with afatinib or TRLX-TKI reduces cell proliferation and signaling in a similar manner.

Further studies were performed to demonstrate that the active metabolite of tarloxotinib, RN-4000E, inhibits proliferation of cell lines harboring EGFR exon 20 insertion mutations (FIGs. 6A-6B). FIGs. 6A-6B illustrate dose response curves of cell viability of EGFR exon 20 insertion mutation patient derived cell lines (CUTO-14, CUTO-17, CUTO-18), mutated EGFR (PC9, H3255) and wild type EGFR (A549). Cells were treated with afatinib, gefitinib, RN-4000 (pro-drug) and RN-4000E (effector drug) for 72 hours under normoxic conditions and analyzed by MTS. Experiments were done by triplicate, mean and ±SEM is plotted. FIG. 6C comprises a table summarizing IC_{50} values of proliferation experiments. Values are expressed in nanomolar concentration. The IC_{50} values for the three cell lines were 203nM, 89nM and 709 nM for afatinib, and 208nM, 33nM and 345nM for TRLX-TKI, respectively. The prodrug form of tarloxotinib has minimal effect on cell proliferation in these models, consistent with the necessity for hypoxia-induced activation (to TRLX-TKI).

The effect of tarloxotinib was evaluated *in vivo* using murine xenograft models of CUTO14 (FIGs. 7A-7B). The data illustrated in FIG. 7A was obtained by injecting $1x10^6$ cells in each flank of nude mice; once tumors reached 200 mm^3, mice were randomly separated and

treated with afatinib (6mg/Kg, daily, PO), cetuximab (40 mg/Kg, Q3d, IP), tarloxotinib (48 mg/Kg, Qwx4, IP) and vehicle for four weeks. Tumor volume was measured two times a week. After four weeks of treatment, afatinib did not alter tumor growth compared to untreated tumors, whereas treatment with tarloxotinib induced significant tumor regression. As demonstrated in FIG. 7B, tarloxotinib was active in PDX models of EGFR exon 20 insertion that were permitted to grow to a large size (average size of about 573 mm^3) before initiating treatment.

The effect of tarloxotinib was further evaluated *in vivo* using murine xenograft models of CUTO17 (FIG. 12). The data illustrated in FIG. 12 was obtained by injecting $1x10^6$ cells in each flank of nude mice; once tumors reached 200 mm^3, mice were randomly separated and treated with cetuximab (40 mg/Kg, Q3d, IP), osimertinib (25 mg/kg, daily, PO), tarloxotinib (48 mg/Kg, Qwx4, IP) or vehicle for four weeks. Tumor volume was measure two times a week

The *in vivo* data suggest that the activated TKI of tarloxotinib is accumulating to biologically active concentrations in tumors following cleavage of tarloxotinib under hypoxic conditions.

In summary, the EGFR exon 20 insertions cell lines represent novel models for the investigation of therapeutic strategies for this mutation class. These cell lines have the ability to develop tumors *in vivo* and show reduced sensitivity to current EGFR TKIs, mimicking the lack of response in patients with these mutations. As demonstrated herein, tarloxotinib can overcome intrinsic EGFR exon 20 mutation resistance to standard EGFR TKIs.

Example 2: Anti-tumor activity of tarloxotinib in HER2 driven cell lines

Lung adenocarcinoma is characterized by distinct subsets that can be classified by oncogene status. *ERBB2* (HER2) gene amplification is present in ~3% and *ERBB2* activating mutations in ~3% of the patients (FIGs. 9A-9C). FIGs. 9A-9B shows the type and frequence of ERBB2 alterations in the indicated lung cancer cohorts (data derived from www *dot* cbioportal *dot* .org). FIG. 9C demonstrates the distribution of ERBB2 mutations identified in lung cancer across the ERBB2 gene (with key protein domains depicted by boxes) and also demonstrate the number of alterations identified at each corresponding amino acid position (www *dot* cbioportal *dot* org). Although HER2-directed therapies are available for breast and gastric cancer, the use of HER2-directed monoclonal antibodies and tyrosine kinase inhibitors (TKIs) have been disappointing in lung cancer.

As illustrated in FIGs. 10A-10B, using MTS, proliferation of three HER2-driven cell lines (Calu-3 and H2071 with *ERBB2* amplification; H1781 with *ERBB2* p.G776>VC insertion mutation) treated with TRLX-TKI or TRLX or 1st, 2nd or 3rd generation EGFR/HER2 TKIs were evaluated. Cells were treated with the indicated doses of afatinib, gefitinib, osimertinib, TRLX-TKI (RN-4000E; active drug) for 2 hours under normoxic conditions, lysed, and analyzed by immunoblot. On-target and signaling effects elicited by TRLX-TKI were evaluated via immunoblots of pHER2, pHER3, pERK and pAKT. Using a nude mice xenograft model, effects of the TRLX prodrug with other TKIs on tumor growth were compared.

Further, dose response curves of cell viability of these patient derived cell lines, which harbor HER2 gene amplification (H2170 and CALU-3) or a HER2 exon 20 insertion mutation (H1781), were obtained (FIG. 11). Cells were treated with afatinib, gefitinib, osimertinib, TRLX (RN-4000; pro-drug) and TRLX-TKI (RN-4000E; effector drug) for 72 hours under normoxic conditions and analyzed by MTS. Experiments were done by triplicate. Table 3 further comprises a table summarizing IC_{50} values of proliferation experiments. Values are expressed in nanomolar concentration. These data demonstrate that TRLX-TKI is the most potent inhibitor of cell proliferation in comparison to the other tyrosine kinase inhibitors tested for each of the 3 cancer cell lines bearing ERBB2 alterations. Table 4 comprises the fold difference in IC_{50} between the prodrug (TRLX) and the active metabolite (TRLX-TKI) under normoxic conditions. The large fold difference in activity between RN-4000 (pro-drug) and RN-4000E (effector drug) demonstrates a key differentiating factor of RN-4000 compared to other tyrosine kinase inhibitors. RN-4000 has low activity against HER2, but the RN-4000E effector drug (produced in high concentrations at the tumor site by hypoxia) is potent against HER2.

Table 3. IC_{50} of HER2 driven cell lines treated with EGFR/HER2 TKIs.

Drug	H2170	Calu-3	H1781
TRLX-TKI	11.4	2	15.4
TRLX	588.8	325	816.5
gefitinib	1156.1	1324	4168
afatinib	11.4	31.1	66.2
osimertinib	112.7	187.9	406.4

Table 4. Fold difference in IC_{50} between TRLX and TRLX-TKI under normoxic conditions.

	H2170	Calu-3	H1781
TRLX:TRLX-TKI ratio	51.6	162.5	53.0

The results demonstrate that HER2-driven lung cancer cell lines show the highest sensitivity to TRLX-TKI whereas the TRLX prodrug is >50-fold less potent under normoxic conditions, consistent with the required mechanism of hypoxia for activation. Afatinib, which has poor activity in HER2 mutation positive NSCLC, was the next most potent drug *in vitro*, followed by osimertinib and gefitinib (Table 1). In immunoblot analyses, TRLX-TKI inhibits HER2 phosphorylation between 10-100 nM whereas afatinib inhibits pHER2 at 100 nM, consistent with the cell proliferation data. Notably, a concurrent reduction in pHER3 phosphorylation with TKI treatment was also observed. Analysis of downstream signaling pathways demonstrated that pERK was not inhibited by any of the TKIs, whereas AKT signaling was inhibited at similar doses to that of upstream HER2.

TRLX-TKI is a potent HER2 inhibitor *in vitro* that can inhibit HER2 (and HER3) phosphorylation at low nanomolar doses and with greater potency than currently approved TKIs for NSCLC. This activity was observed in cell lines harboring both amplified or mutant ERBB2. HER2-driven cells depend on the AKT pathway for survival whereas MAPK inhibition was not necessary to inhibit cell proliferation. Tarloxotinib represents a new therapeutic approach for NSCLC patients harboring *ERBB2* gene alterations.

Example 3: Anti-tumor activity of tarloxotinib in cell lines harboring *NRG1* gene amplification

NRG1 overexpression by gene amplification or other mechanisms are be predicted to activate HER3:HER2 dimers. Currently there are no U.S. FDA approved (or other regulatory agencies) drugs for *NRG1* gene amplification.

As demonstrated herein (FIGs. 13-14), the active metabolite of tarloxotinib, TRLX-TKI (RN-4000E), inhibits proliferation of a cell line harboring *NRG1* gene amplification (in a non-limiting example, HCC95). FIG. 13 illustrates dose response curves of cell viability of this patient derived cell line. Cells were treated with afatinib, gefitinib, osimertinib, TRLX (RN-4000; pro-drug) and TRLX-TKI (RN-4000E; effector drug) for 72 hours under normoxic

conditions and analyzed by MTS. The figure recites IC_{50} values (in nanomolar) determined in the proliferation experiments. FIG. 14 comprises a series of gel images illustrating that RN-4000E inhibits HER3 phosphorylation and downstream signaling in the HCC95 cell line harboring *NRG1* gene amplification. Cells were treated with the indicated doses of afatinib, gefitinib, RN-4000E, and osimertinib (active drug) for 2 hours, lysed and analyzed by immunoblot. Blots are representatives of three independent experiments.

Example 4: Anti-tumor activity of tarloxotinib in cell lines harboring *NRG1* gene fusion

Gene fusions that contain sequences from *NRG1* have been identified in breast, lung adenocarcinoma, NSCLC, cholangiocarcinoma, pancreatic cancer, and ovarian cancer. *NRG1* gene fusions have been demonstrated to be oncogenic by inducing overexpression the ligand Neuregulin 1 which induces heterodimerization of HER3 with HER2. Currently there are no U.S. FDA approved (or other regulatory agencies) drugs for *NRG1* fusions.

As demonstrated herein (FIG. 15), the active metabolite of tarloxotinib, TRLX-TKI (RN-4000E), inhibits proliferation of a breast cancer cell line harboring a *DOC4-NRG1* gene fusion (in a non-limiting example, MDA-MB-157). FIG. 15 illustrates dose response curves of cell viability of this cell line. Cells were treated with gefitinib, afatinib, TRLX-TKI (RN-4000E; effector drug), osimertinib, TRLX (RN-4000; pro-drug) or poziotinib for 72 hours under normoxic conditions and analyzed by MTS. The figure recites IC_{50} values (in nanomolar) determined in the proliferation experiments.

Example 5: Anti-tumor activity of tarloxotinib in cell lines harboring EGFR exon 20 insertion mutations

Cancers with EGFR Exon 20 insertion mutations exhibit resistance and/or poor response to EGFR-TKIs such as osimertinib, gefitinib, afatinib, and erlotinib. Thus, there is great need to identify compounds that effectively treat and/or prevent such cancers.

As demonstrated herein (FIGs. 16A-16B), the active metabolite of tarloxotinib, TRLX-TKI (RN-4000E), inhibits proliferation of cell lines harboring EGFR exon 20 insertion mutations. FIGs. 16A-16B illustrate dose response curves of cell viability of of EGFR exon 20 insertion mutation patient derived cell lines (CUTO-14, CUTO-17, CUTO-18). Cells were treated with TRLX-TKI, TRLX (pro-drug), gefitinib, afatinib, or osimertinib for 72 hours under

normoxic conditions and analyzed by MTS.. Table 5 recites IC_{50} values (in nanomolar) determined in the proliferation experiments illustrated in FIGs. 16A-16B. Table 6 comprises the fold difference in IC_{50} between the prodrug (TRLX) and the active metabolite (TRLX-TKI) under normoxic conditions. The large fold difference in activity between RN-4000 (pro-drug) and RN-4000E (effector drug) demonstrates a key differentiating factor of RN-4000 compared to other tyrosine kinase inhibitors. RN-4000 has low activity against EGFR, but the RN-4000E effector drug (produced in high concentrations at the tumor site by hypoxia) is potent against EGFR.

Table 5. IC_{50} (nM) of EGFR exon 20 driven cell lines treated with EGFR/HER2 TKIs

Drug	CUTO-14	CUTO-17	CUTO-18
TRLX-TKI	72.2	48.1	158
TRLX	4645	3090	>10000
Gefitinib	3741	4197	>10000
Afatinib	111	220	841
Osimertinib	303	426	647

Table 6. Fold difference in IC_{50} between TRLX and TRLX-TKI under normoxic conditions in EGFR exon 20 cell lines

	CUTO-14	CUTO-17	CUTO-18
TRLX:TRLX-TKI ratio	64.3	64.2	>60

The disclosures of each and every patent, patent application, and publication, as well as other references, GenBan citations and ATCC citations, cited herein are hereby incorporated herein by reference in their entirety. While the present disclosure has been made with reference to specific embodiments, it is apparent that other embodiments and variations of these specific embodiments may be devised by others skilled in the art without departing from the true spirit and scope of the present disclosure. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

CLAIMS

What is claimed:

1. A method of treating or preventing a HER-driven drug-resistant cancer in a subject in need thereof, the method comprising:

(a) providing tumor cells of the subject;

(b) detecting presence or absence of an EGFR exon 20 insertion mutation in the provided tumor cells;

(c) predicting the subject as being likely to be responsive to treatment by (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (Compound A) and/or (2E)-N-[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]-4-(dimethylamino)-2-butenamide) (Compound B), if the EGFR exon 20 insertion mutation is detected; and

(d) administering a therapeutically effective amount of at least one compound selected from the group consisting of Compound A and Compound B, or a salt or a solvate thereof.

2. A method of treating a HER-driven drug-resistant cancer in a subject with cancer, where an EGFR exon 20 insertion mutation is detected in tumor cells of the subject, wherein the method comprises administering a therapeutically effective amount of at least one compound selected from the group consisting of Compound A and Compound B, or a salt or a solvate thereof.

3. A method of predicting the responsiveness of a subject with a HER-driven drug-resistant cancer to treatment with at least one compound selected from the group consisting of Compound A and Compound B, wherein the method comprises:

(a) providing tumor cells of the subject;

(b) detecting presence or absence of an EGFR exon 20 insertion mutation in the provided tumor cells of the subject;

(c) predicting the subject as being likely to be responsive to a treatment with the at least one compound if the EGFR exon 20 insertion mutation is detected in the provided tumor cells of the subject.

4. A method of predicting the responsiveness of a subject with a HER-driven drug-resistant cancer to treatment with at least one compound selected from the group consisting of Compound A and Compound B, wherein the method comprises detecting presence or absence of an EGFR exon 20 insertion mutation in a sample of tumor cells from the subject;

wherein the subject is likely to be responsive to the treatment with the at least one compound if the EGFR exon 20 insertion mutation is detected in the sample of tumor cells from the subject.

5. A method of identifying a subject with cancer who is likely to be responsive to treatment with at least one compound selected from the group consisting of Compound A and Compound B, wherein the method comprises:

(a) providing tumor cells of the subject;

(b) detecting presence or absence of an EGFR exon 20 insertion mutation in the provided tumor cells;

(c) identifying the subject as being likely to be responsive to treatment with the at least one compound if the EGFR exon 20 insertion mutation is detected in the provided tumor cells.

6. The method of any one of claims 1-5, wherein the HER-driven cancer comprises an EGFR-driven cancer.

7. The method of claim 6, wherein the EGFR-driven cancer comprises lung cancer.

8. The method of claim 7, wherein the lung cancer comprises non-small cell lung cancer (NSCLC).

9. The method of any one of claims 1-8, wherein the mutation comprises at least one selected from the group consisting of A763_Y764insFQEA, A763_Y764insFQQA, A767_V769dupASV, D770_N771insGL, D770_N771insGT, D770_N771insNPG, D770_N771insSVD, E762Q_insFQEA, H773_V774insH, H773_V774insH, H773_V774insNPH, M766_A767insAI, M766_A767insASV, N771_H773dupNPH, P772_H773insYNP, P772_V774insPHV, S768_770dupSVD, V769_D770insASV,

Y764_V765insHH, delD770insGY, and delL747_P753insS.

10. The method of any one of claims 1-9, wherein the cancer comprises at least one *ERBB2* gene fusion selected from the group consisting of *ZNF207-HER2*, *MDK-HER2*, *NOS2-HER2*, *ERBB2-GRB7*, *ERBB2-CTTN*, *ERBB2-PPP1R1B*, and *ERBB2-PSMB3*.

11. The method of any one of claims 1-10, wherein the cancer comprises at least one *ERBB2* mutation selected from the group consisting of G309A/E; S310F/Y; V659E/D; G660D; K753E; L755P/S; Del755-759; L768S; D769H/Y; V773L; A775_G776insYVMA; G776V/L, Cins; V777L; P780Ins; P780_Y781insGSP; V842I; L866M; and R896C.

12. The method of any one of claims 1-11, wherein the cancer comprises at least one *NRG1* gene fusion selected from the group consisting of *DOC4-NRG1*; *CD74-NRG1*; *SLC3A2-NRG1*; *RBPMS-NRG1*; *WRN-NRG1*; *SDC4-NRG1*; *RAB2IL1-NRG1*; *VAMP2-NRG1*; *KIF13B-NRG1*; *ATP1B1-NRG1*; *CDH6-NRG1*; *APP-NRG1*; *AKAP13-NRG1*; *THBS1-NRG1*; *PDE7A-NRG1*; *THAP7-NRG1*; *SMAD4-NRG1*; *RAB3IL1-NRG1*; *NRG1-PMEPA1*; and *NRG1-STMN2*.

13. The method of any one of claims 1-12, wherein the cancer comprises at least one *ERBB3* mutation selected from the group consisting of V104M; A232V; P262H; G284R; T389K; Q809R; S846I; and E928G.

14. The method of any one of claims 1-13, wherein the cancer comprises at least one *ERBB4* fusion selected from the group consisting of *EZR-ERBB4*; *IKZF2-ERBB4*; and *BGALT-ERBB4*.

15. The method of any one of claims 1-14, wherein the cancer is resistant to at least one agent selected from the group consisting of osimertinib, gefitinib, afatinib, and erlotinib.

16. The method of any one of claims 1-15, further comprising administering at least one additional agent, or a salt or solvate thereof, that treats or prevents the cancer.

17. The method of claim 16, wherein the at least one compound and the at least one additional agent are co-administered to the subject.

18. The method of any one of claims 16-17, wherein the at least one compound and the at least one additional agent are coformulated.

19. The method of any one of claims 1-18, wherein the at least one compound is administered by at least one route selected from the group consisting of inhalational, oral, nasal, rectal, parenteral, sublingual, transdermal, transmucosal, intravesical, intrapulmonary, intraduodenal, intragastrical, intrathecal, epidural, intrapleural, intraperitoneal, intratracheal, otic, intraocular, subcutaneous, intramuscular, intradermal, intra-arterial, intravenous, intrabronchial, inhalation, and topical.

20. The method of any one of claims 1-19, wherein the subject is a mammal.

21. The method of any one of claims 1-20, wherein the mammal is a human.

22. The method of any one of claims 1-21, wherein the subject is a human in need of treatment thereof.

FIG. 1

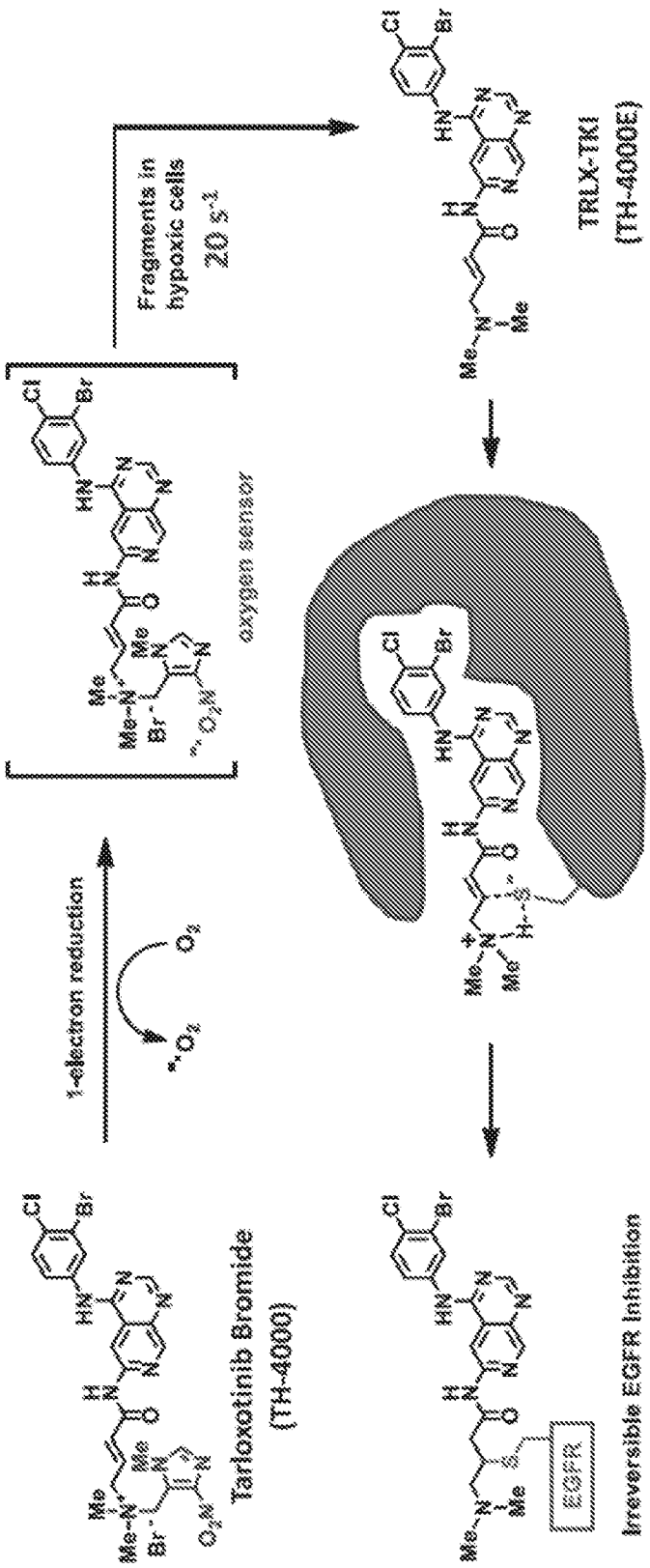

Tarloxotinib Bromide
(TH-4000)
1-electron reduction
oxygen sensor
Fragments in
hypoxic cells
20 s-1
TRLX-TKI
(TH-4000E)
EGFR
Irreversible EGFR Inhibition

FIG. 2

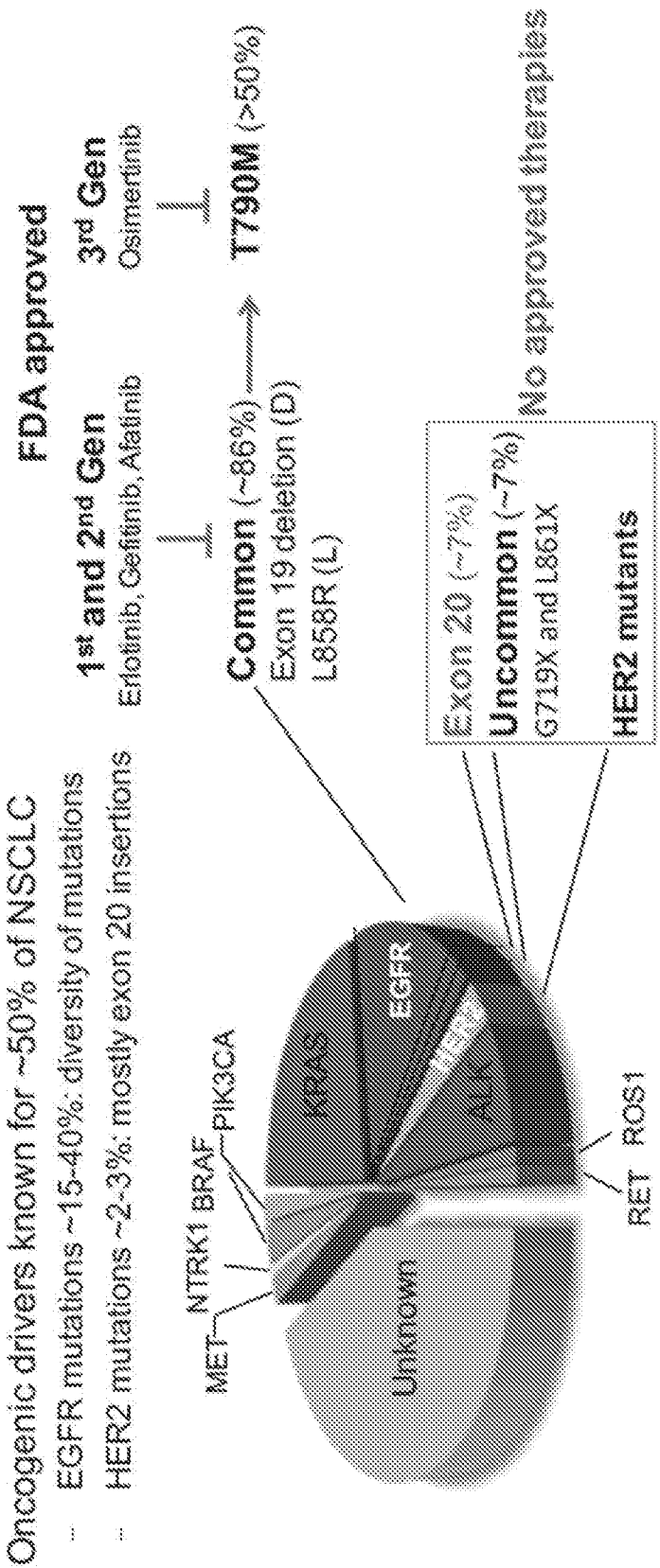

Oncogenic drivers known for ~50% of NSCLC
EGFR mutations ~15-40%: diversity of mutations
HER2 mutations ~2-3%: mostly exon 20 insertions
FDA approved
1st and 2nd Gen
Erlotinib, Gefitinib, Afatinib
3rd Gen
Osimertinib
Common (~86%)
Exon 19 deletion (D)
L858R (L)
T790M (>50%)
Exon 20 (~7%)
Uncommon (~7%)
G719X and L861X
HER2 mutants
No approved therapies
MET
NTRK1
BRAF
PIK3CA
KRAS
EGFR
ALK
RET
ROS1
Unknown

FIG. 3A

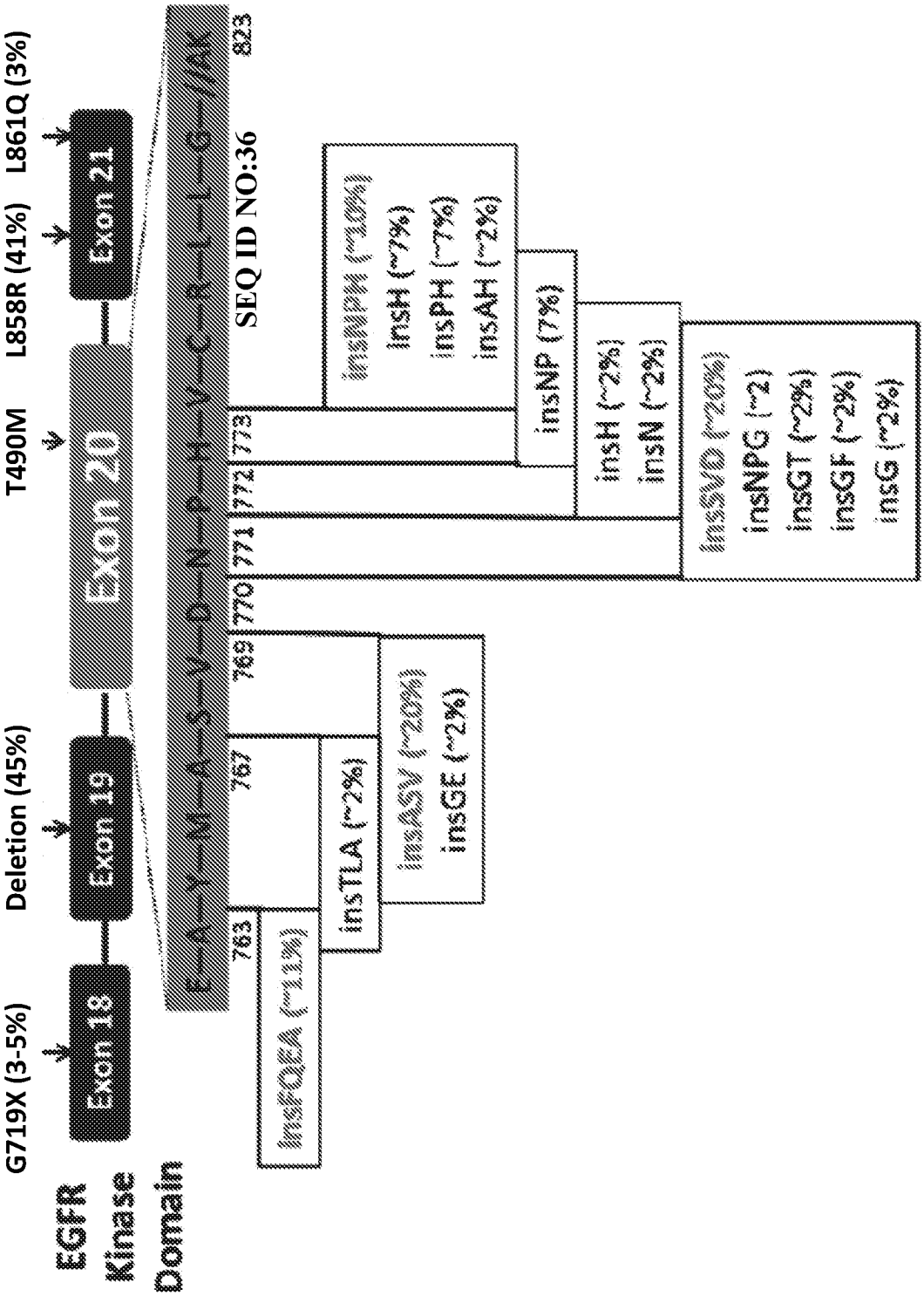

G719X (3-5%)
Deletion (45%)
T490M
L858R (41%)
L861Q (3%)
EGFR Kinase Domain
Exon 18
Exon 19
Exon 20
Exon 21
763
767
769
770
771
772
773
823
SEQ ID NO:36
insFQEA (~11%)
insTLA (~2%)
insASV (~20%)
insGE (~2%)
insNPH (~1%)
insH (~7%)
insPH (~7%)
insAH (~2%)
insNP (7%)
insH (~2%)
insN (~2%)
insSVD (~20%)
insNPG (~2)
insGT (~2%)
insGF (~2%)
insG (~2%)

FIG. 3B

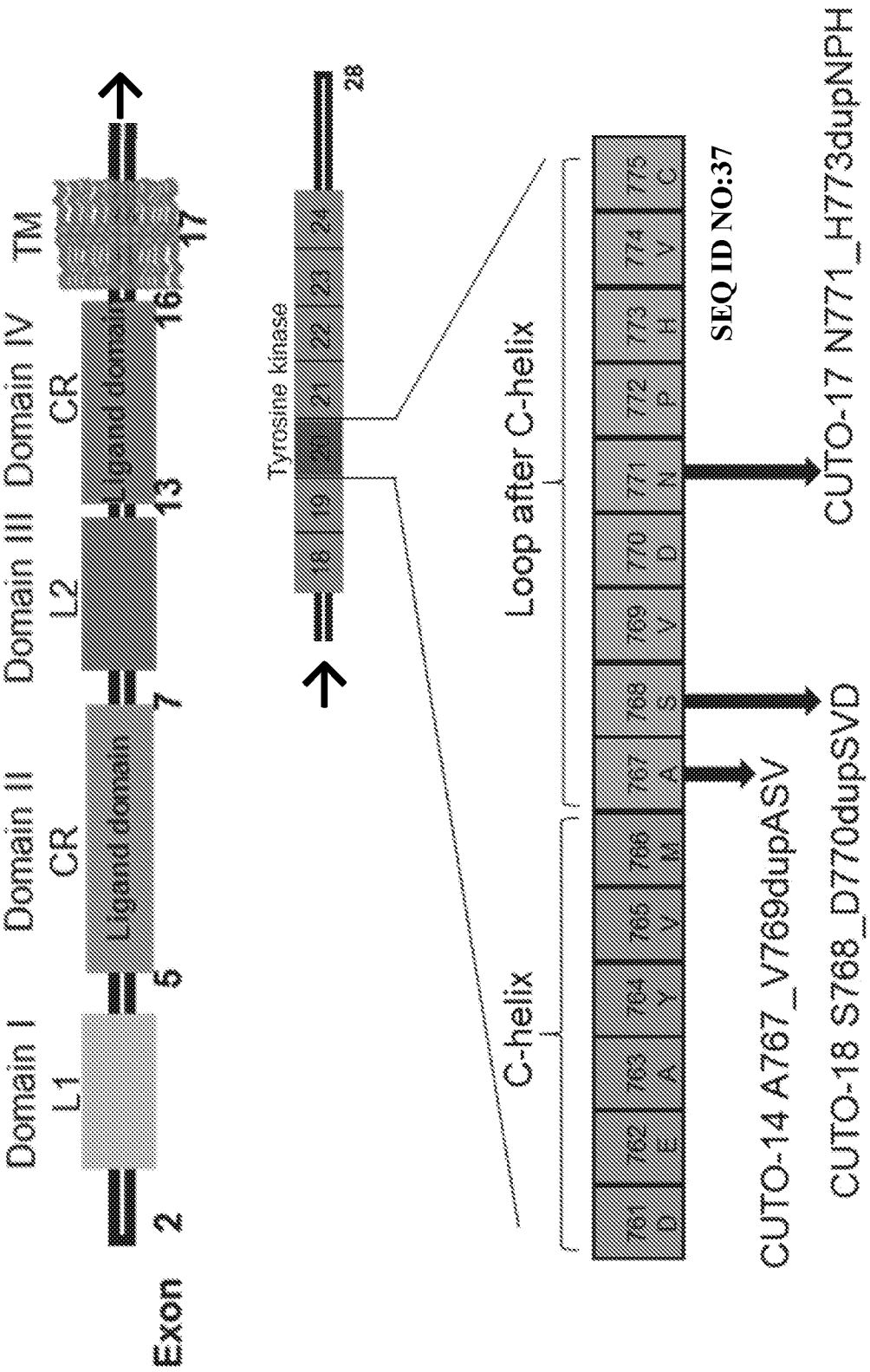

Exon
Domain I
L1
Domain II
CR
Ligand domain
Domain III
L2
Domain IV
CR
Ligand domain
TM
Tyrosine kinase
C-helix
Loop after C-helix
SEQ ID NO:37
CUTO-14 A767_V769dupASV
CUTO-18 S768_D770dupSVD
CUTO-17 N771_H773dupNPH

FIG. 4A

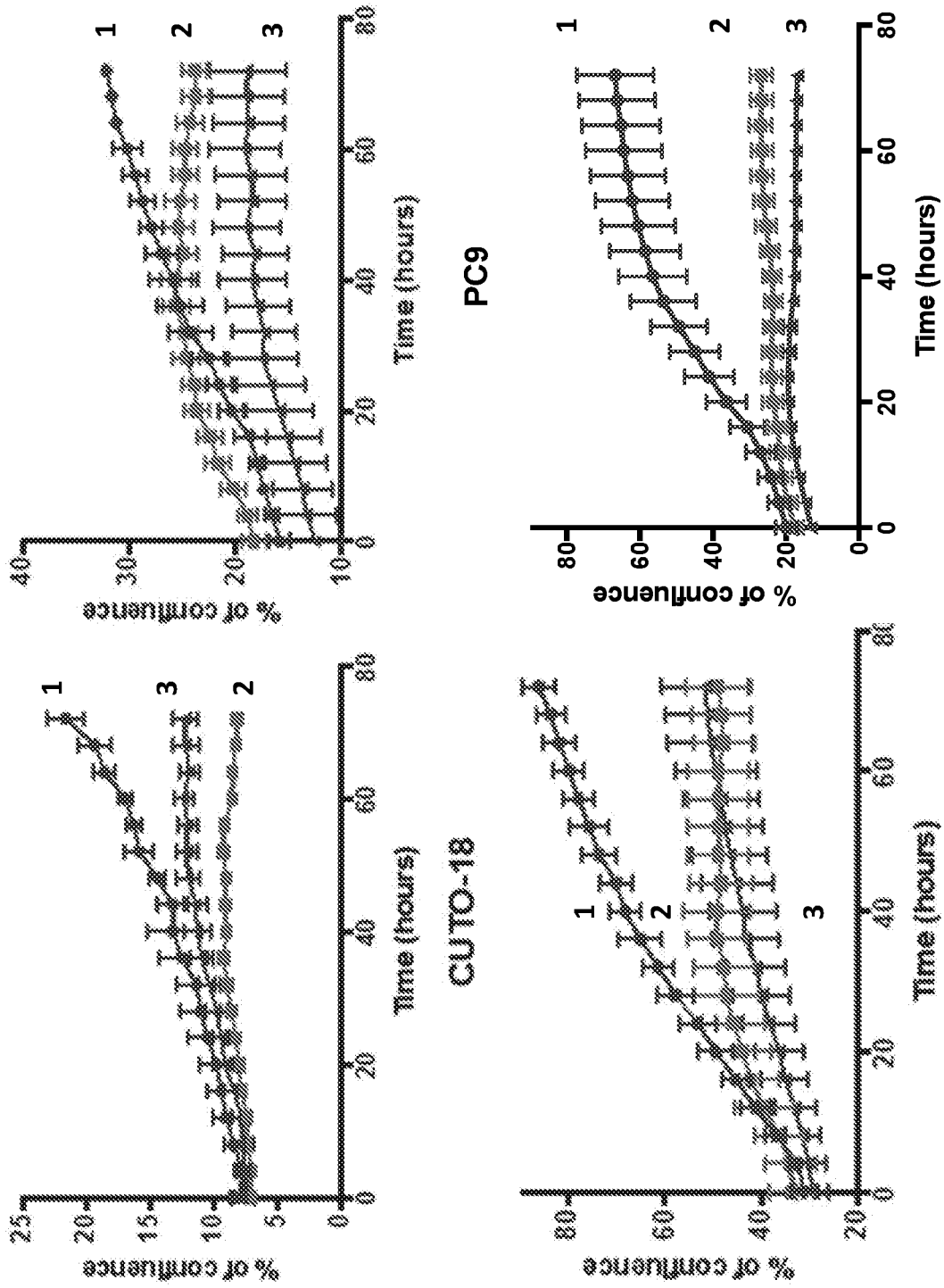


FIG. 4B

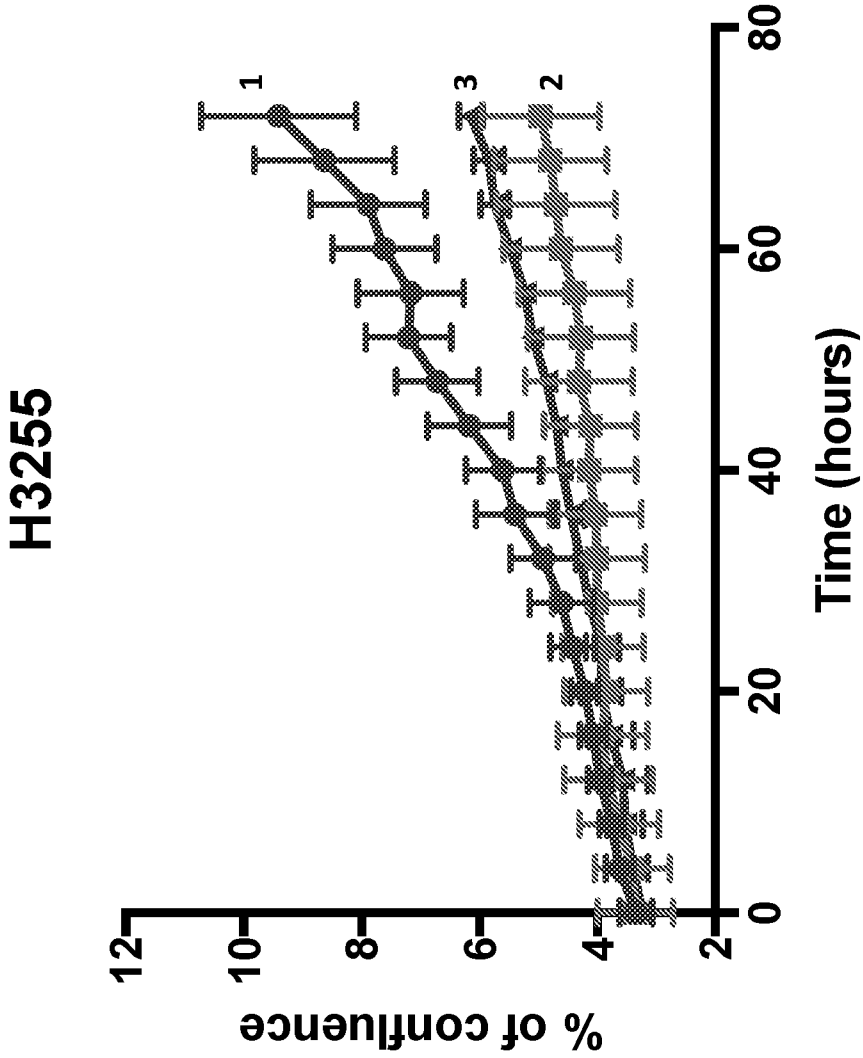

H3255
1 NTC
2 121204
3 10329
% of confluence
Time (hours)
2
4
6
8
10
12
0
20
40
60
80

FIG. 5A

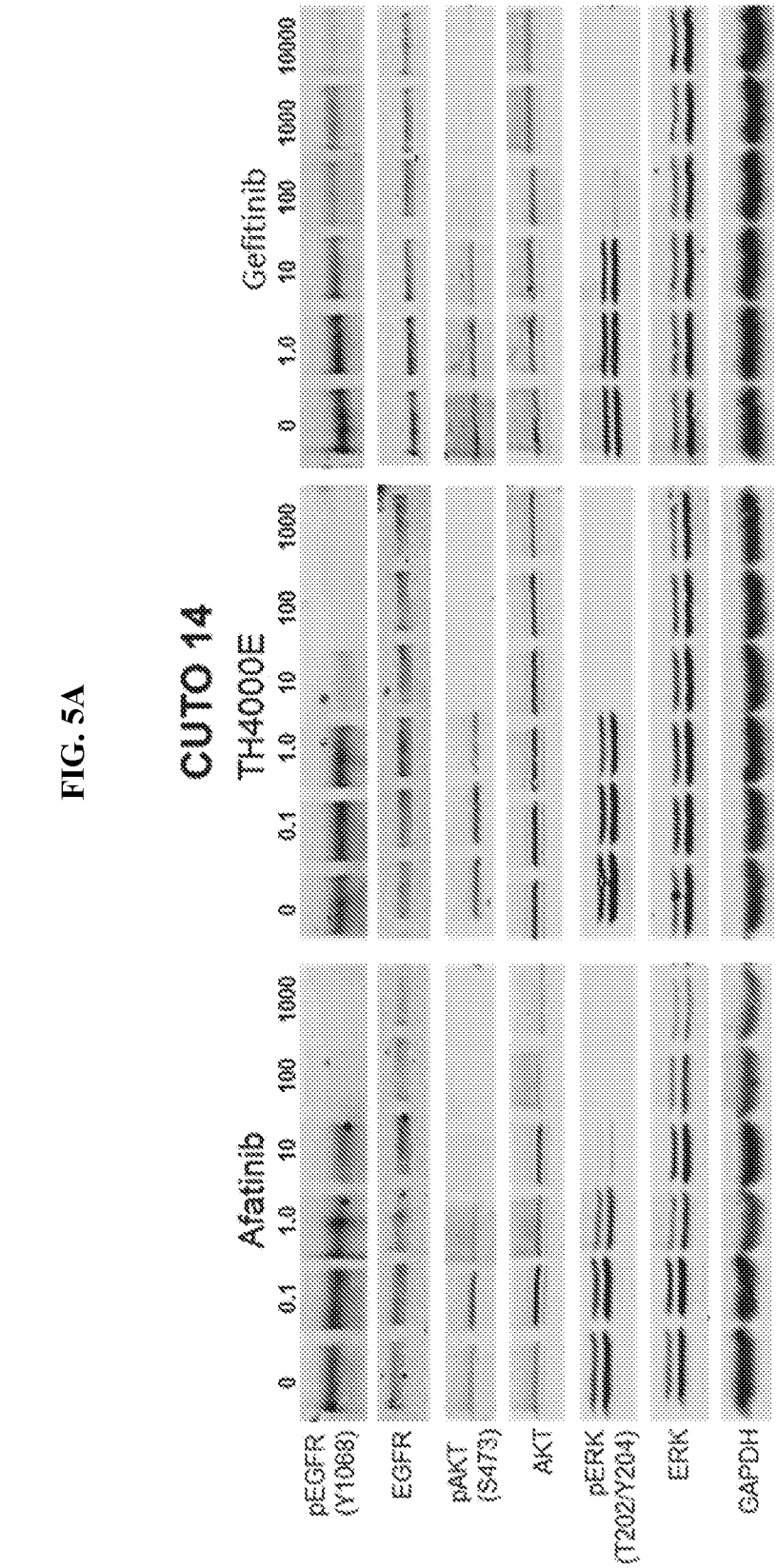

CUTO 14
TH4000E
Afatinib
Gefitinib
0
0.1
1.0
10
100
1000
10000
pEGFR (Y1068)
EGFR
pAKT (S473)
AKT
pERK (T202/Y204)
ERK
GAPDH

FIG. 5B

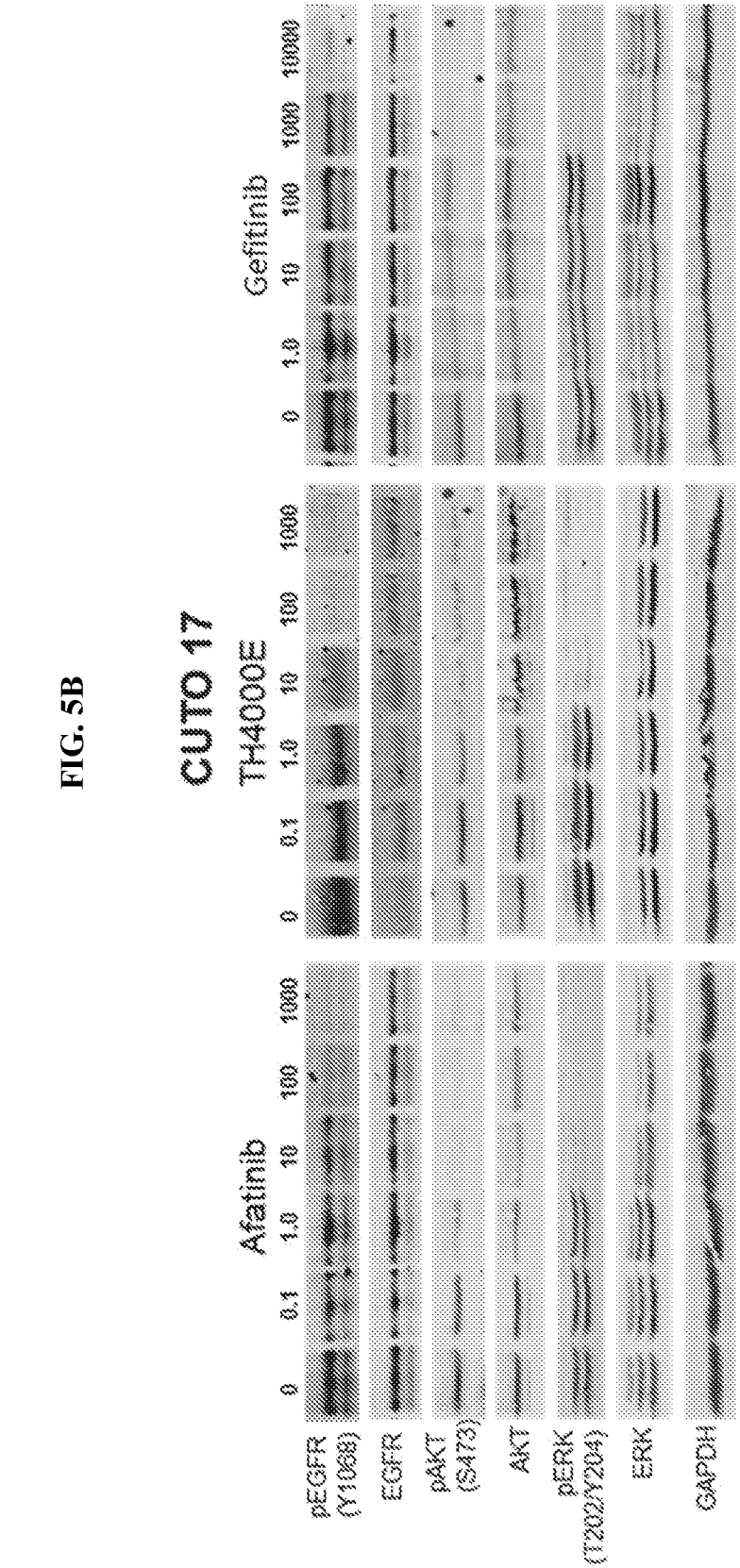

CUTO 17
Afatinib
TH4000E
Gefitinib
0
0.1
1.0
10
100
1000
10000
pEGFR (Y1068)
EGFR
pAKT (S473)
AKT
pERK (T202/Y204)
ERK
GAPDH

FIG. 5C

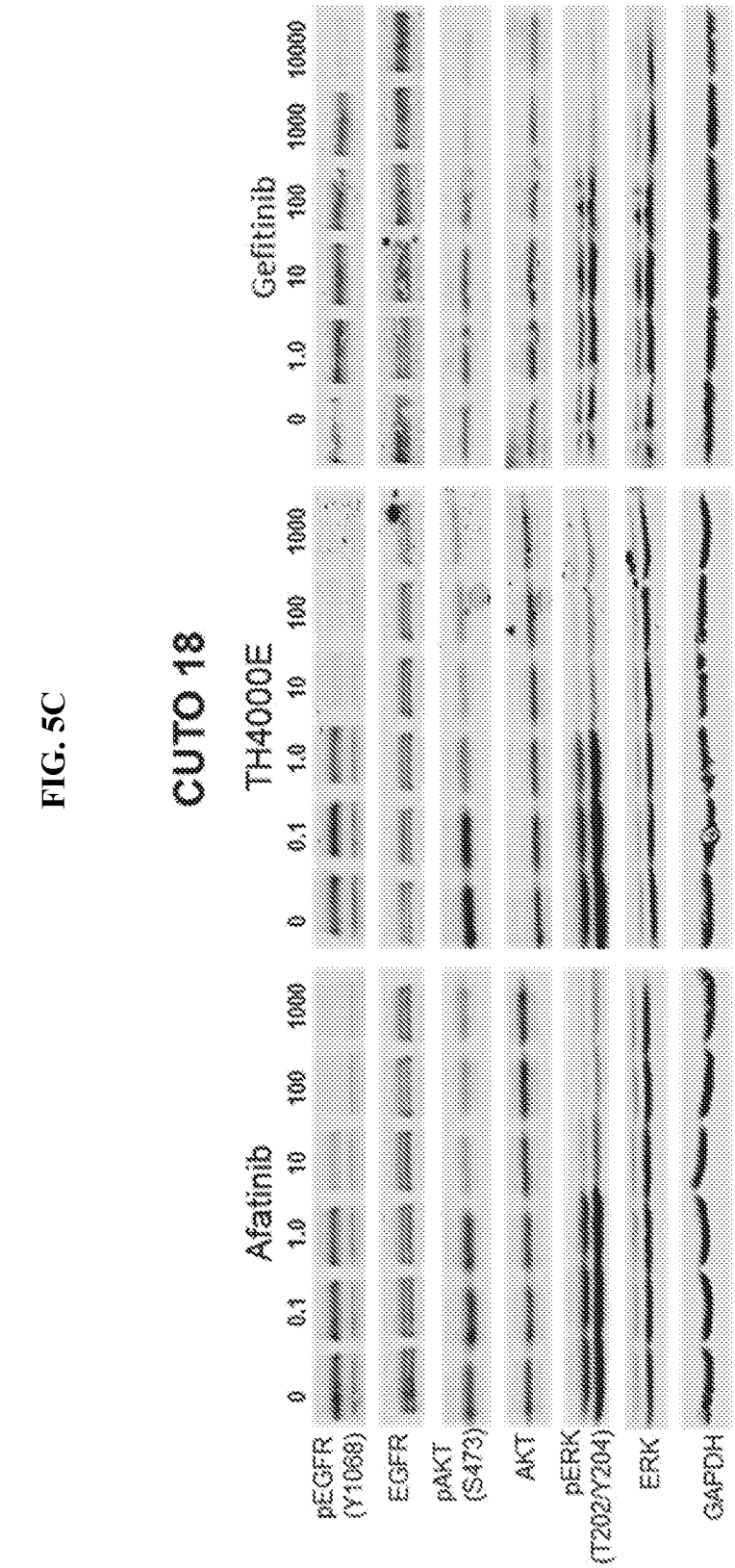

CUTO 18
Afatinib
TH4000E
Gefitinib
0
0.1
1.0
10
100
1000
0
0.1
1.0
10
100
1000
0
1.0
10
100
1000
10000
pEGFR (Y1068)
EGFR
pAKT (S473)
AKT
pERK (T202/Y204)
ERK
GAPDH

FIG. 6A

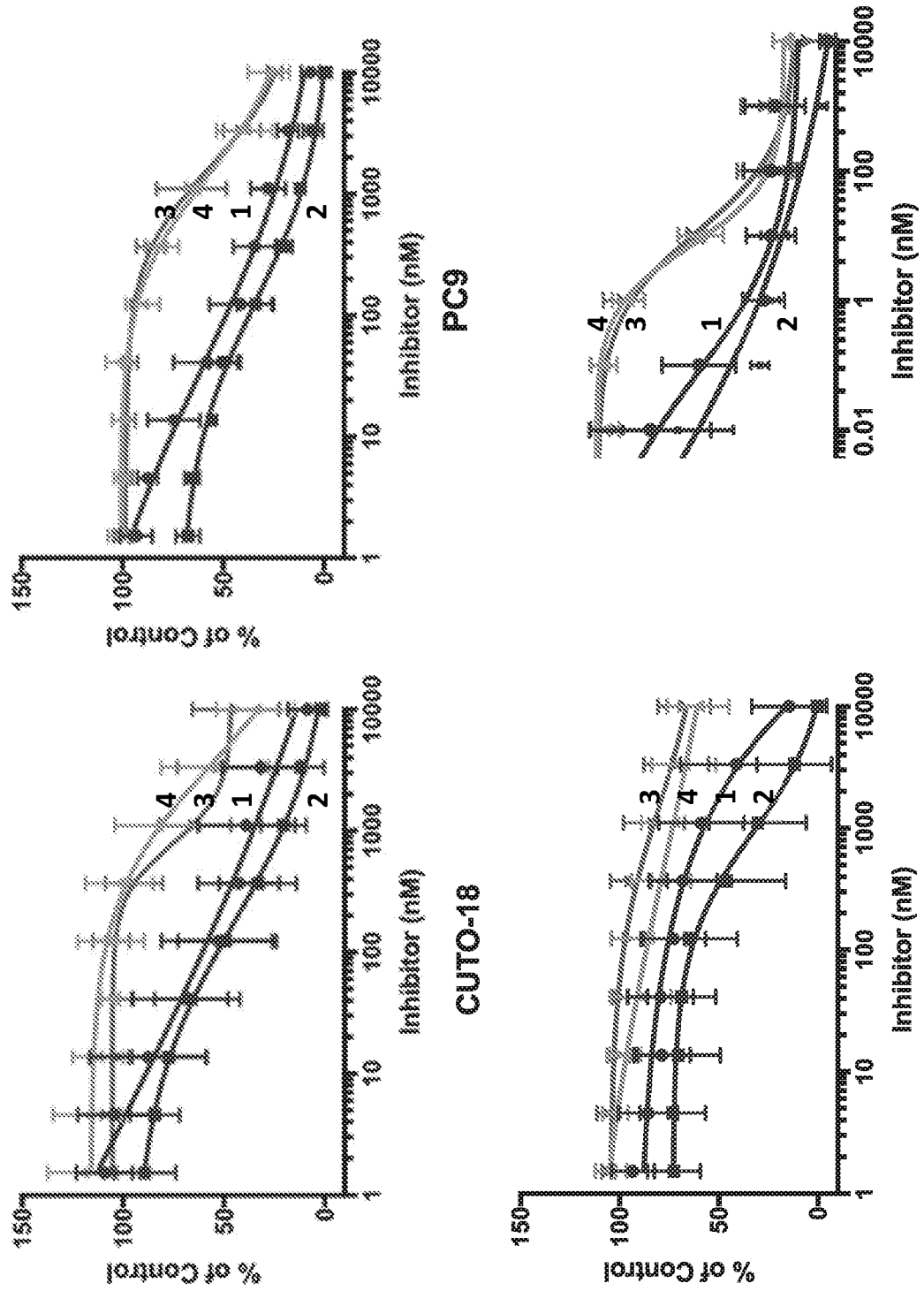

CUTO-17
PC9
CUTO-14
CUTO-18
% of Control
Inhibitor (nM)
0
50
100
150
1
10
100
1000
10000
0.01
1
2
3
4

FIG. 6B

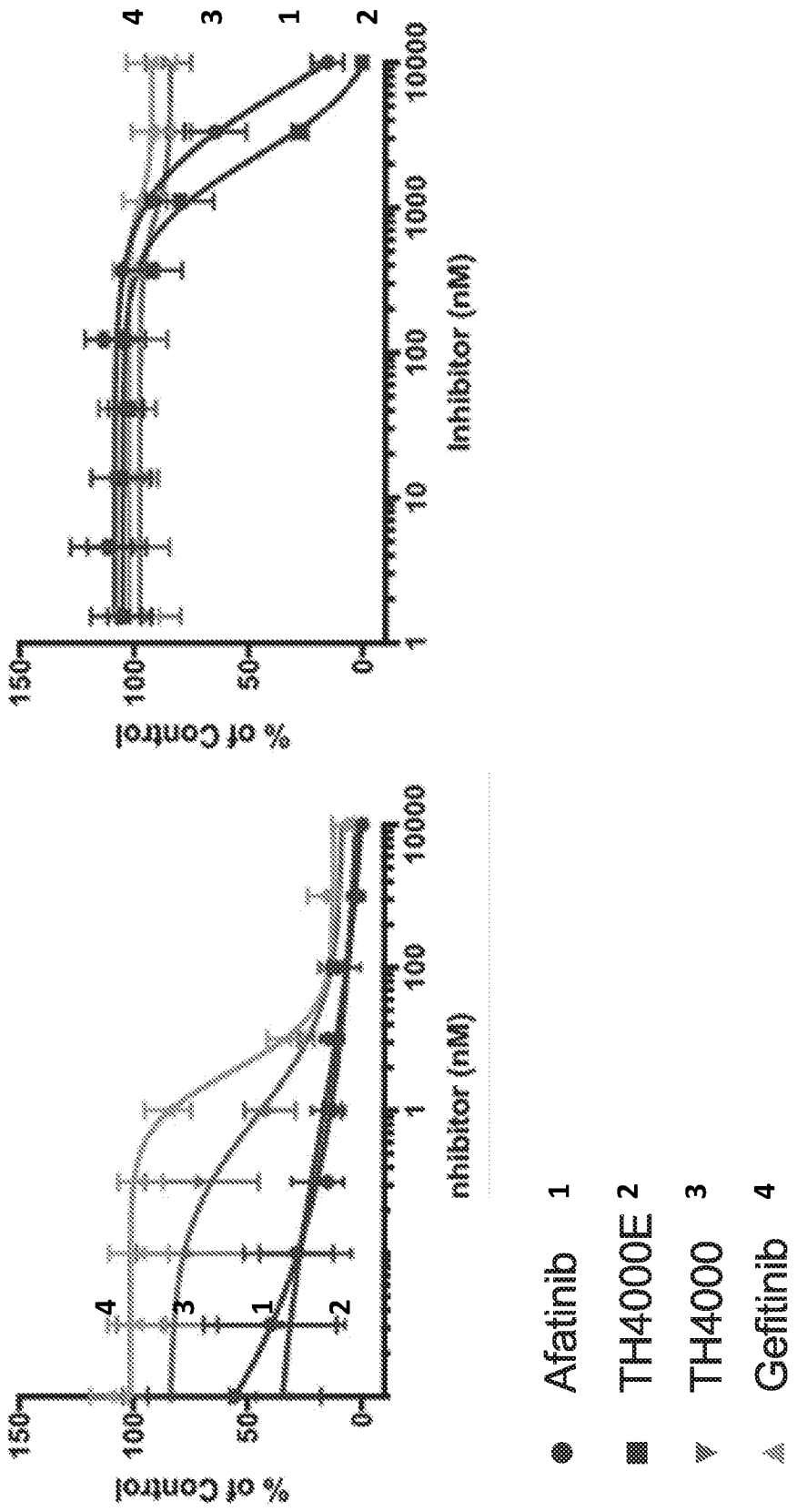

A549
H3255
% of Control
Inhibitor (nM)
0
50
100
150
1
10
100
1000
10000
1
2
3
4
Afatinib
TH4000E
TH4000
Gefitinib

FIG. 6C

	PC9	H3255	A549
afatinib	0.336	0.0002	4,545
TH4000E	0.08	0.4637	2,098
TH4000	18	5.6	>10,000
gefitinib	30	0.562	>10,000

FIG. 7A

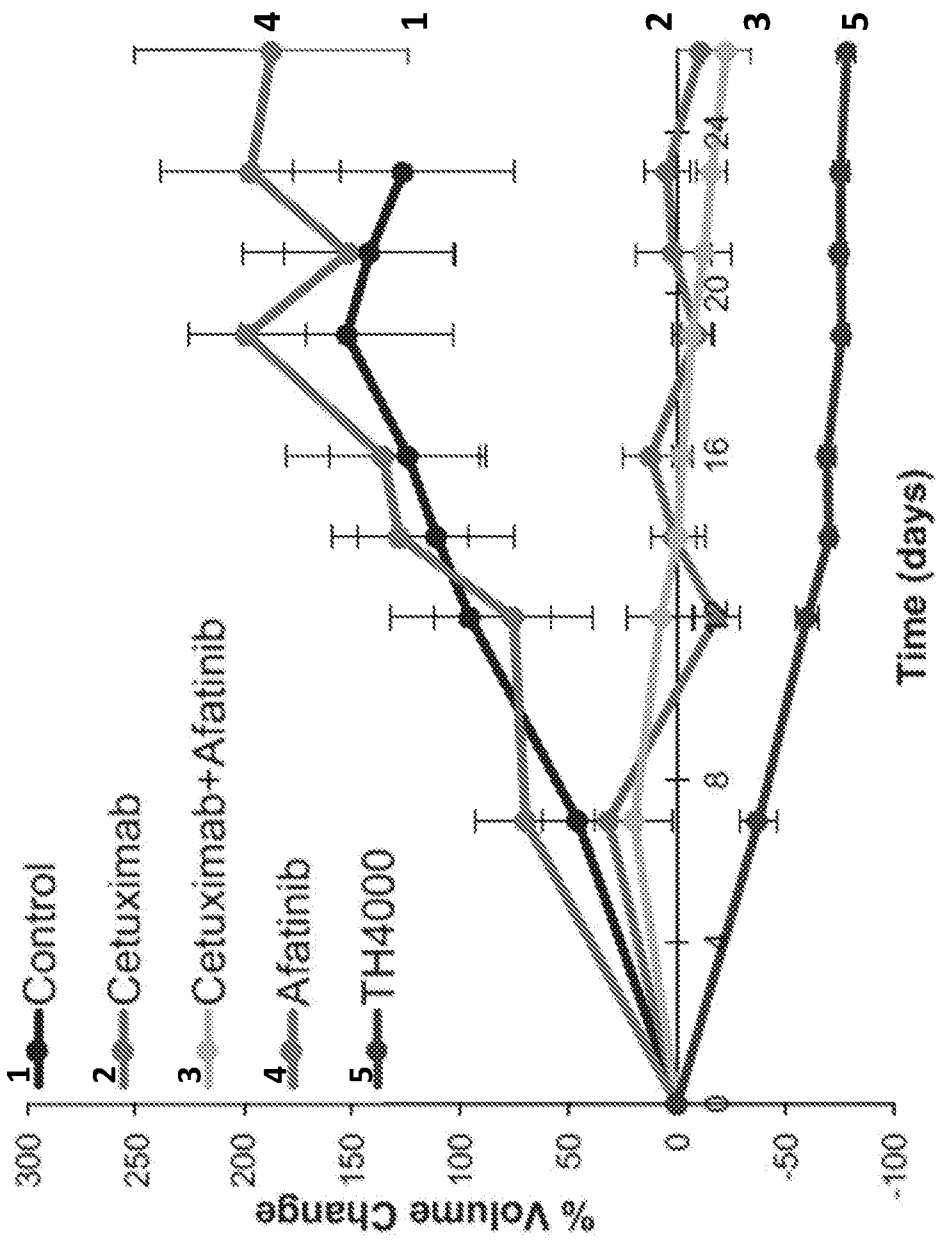

CUTO-14
1 Control
2 Cetuximab
3 Cetuximab+Afatinib
4 Afatinib
5 TH4000
% Volume Change
300
250
200
150
100
50
0
-50
-100
0
4
8
12
16
20
24
Time (days)

FIG. 7B

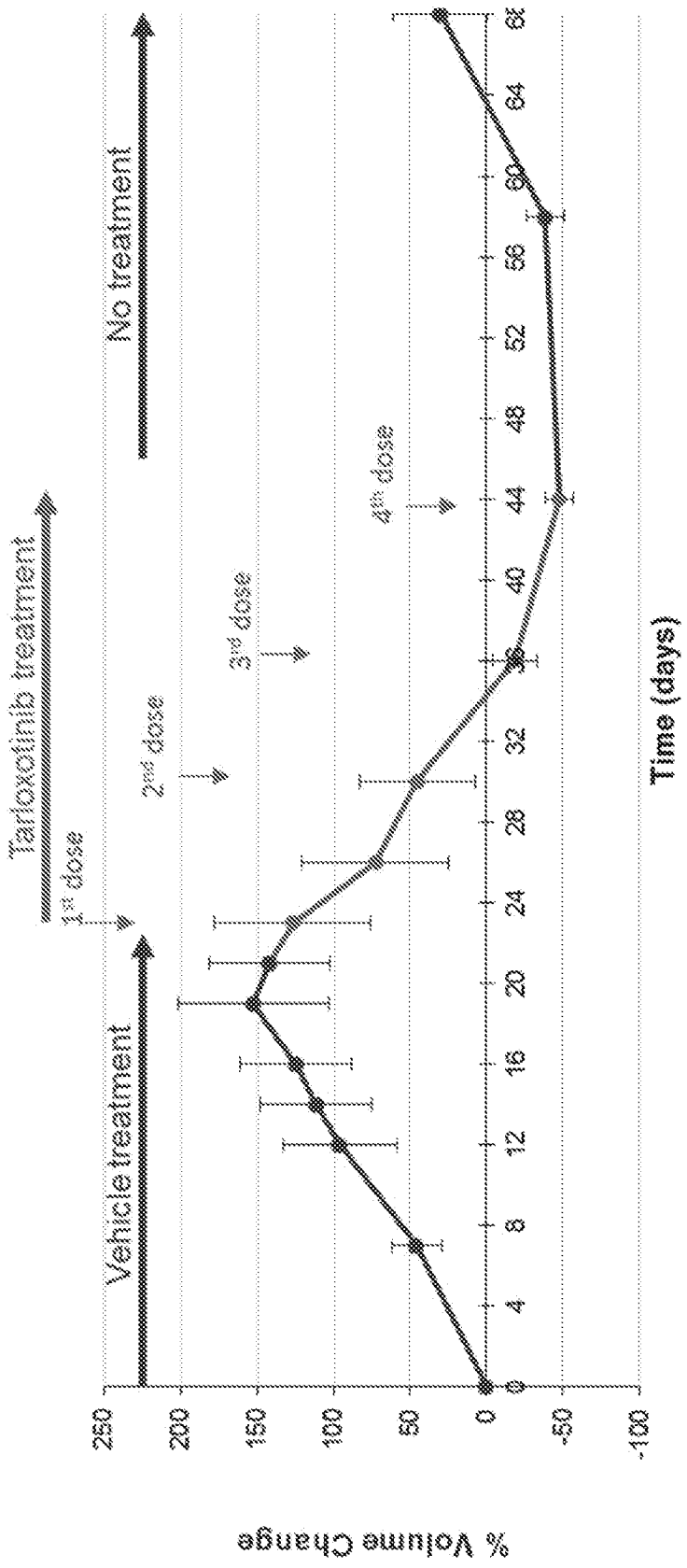

Vehicle treatment
Tarloxotinib treatment
No treatment
1st dose
2nd dose
3rd dose
4th dose
% Volume Change
Time (days)
250
200
150
100
50
0
-50
-100
0
4
8
12
16
20
24
28
32
36
40
44
48
52
56
60
64
68

FIG. 8A

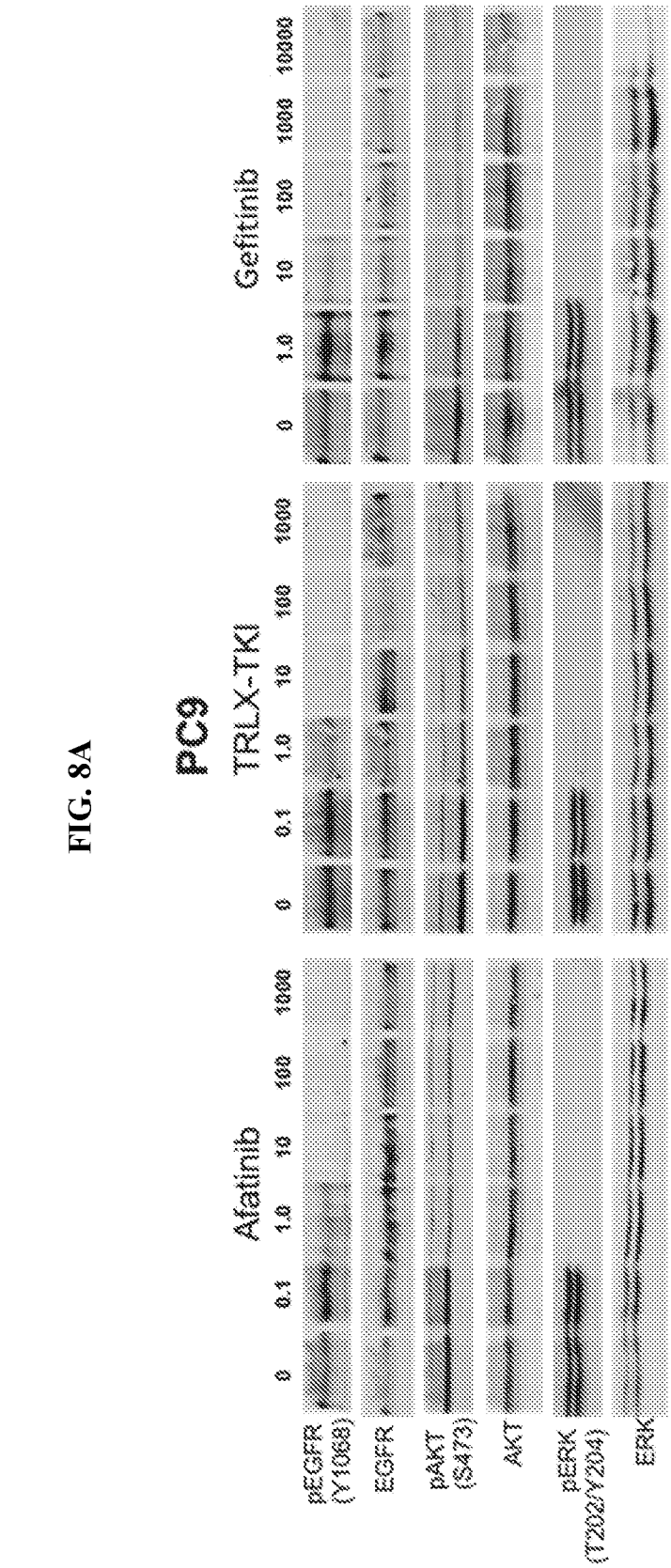

PC9
Afatinib
TRLX-TKI
Gefitinib
0
0.1
1.0
10
100
1000
10000
pEGFR (Y1068)
EGFR
pAKT (S473)
AKT
pERK (T202/Y204)
ERK
GAPDH

FIG. 8B

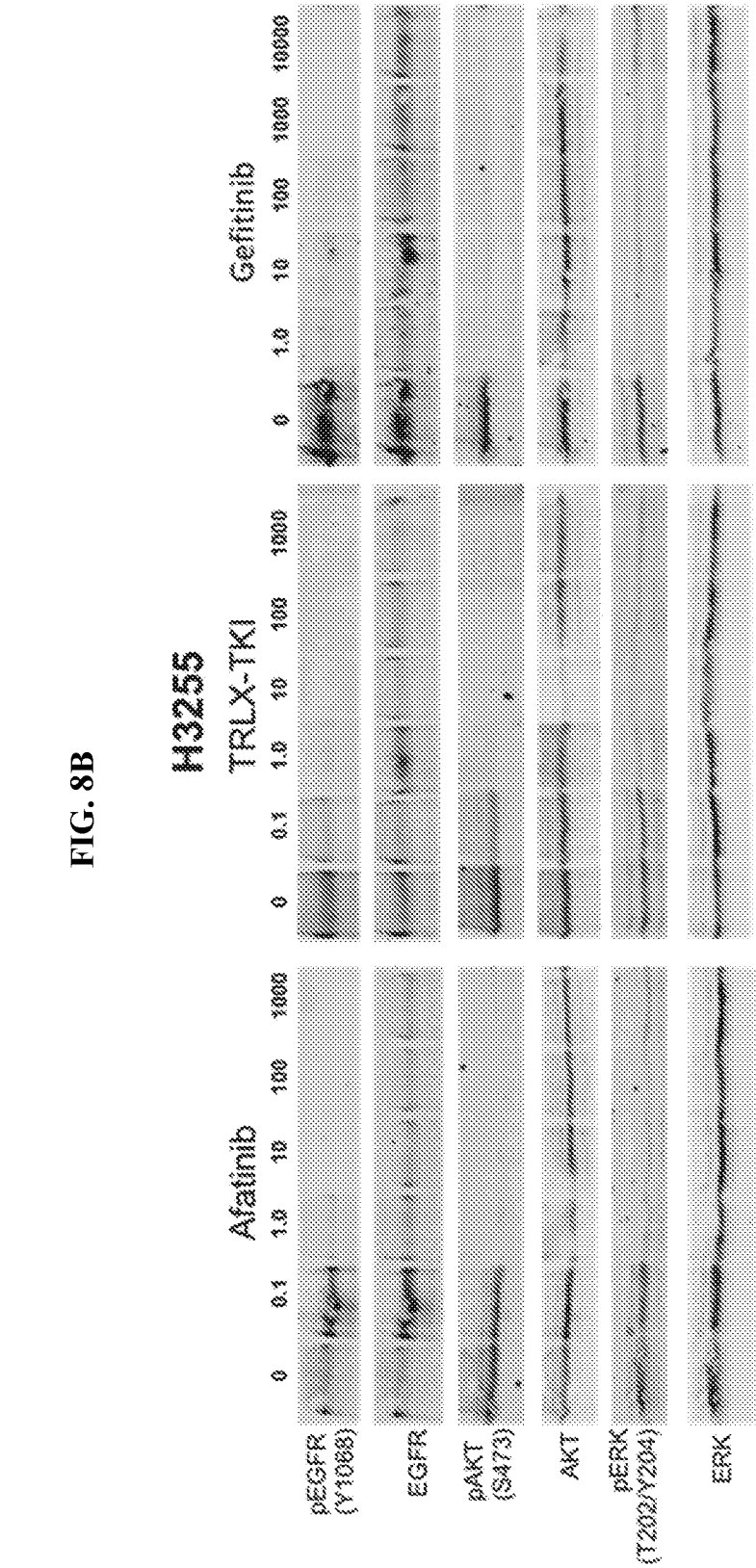

H3255
Afatinib
TRLX-TKI
Gefitinib
0
0.1
1.0
10
100
1000
0
0.1
1.0
10
100
1000
0
1.0
10
100
1000
10000
pEGFR (Y1068)
EGFR
pAKT (S473)
AKT
pERK (T202/Y204)
ERK

FIG. 8C

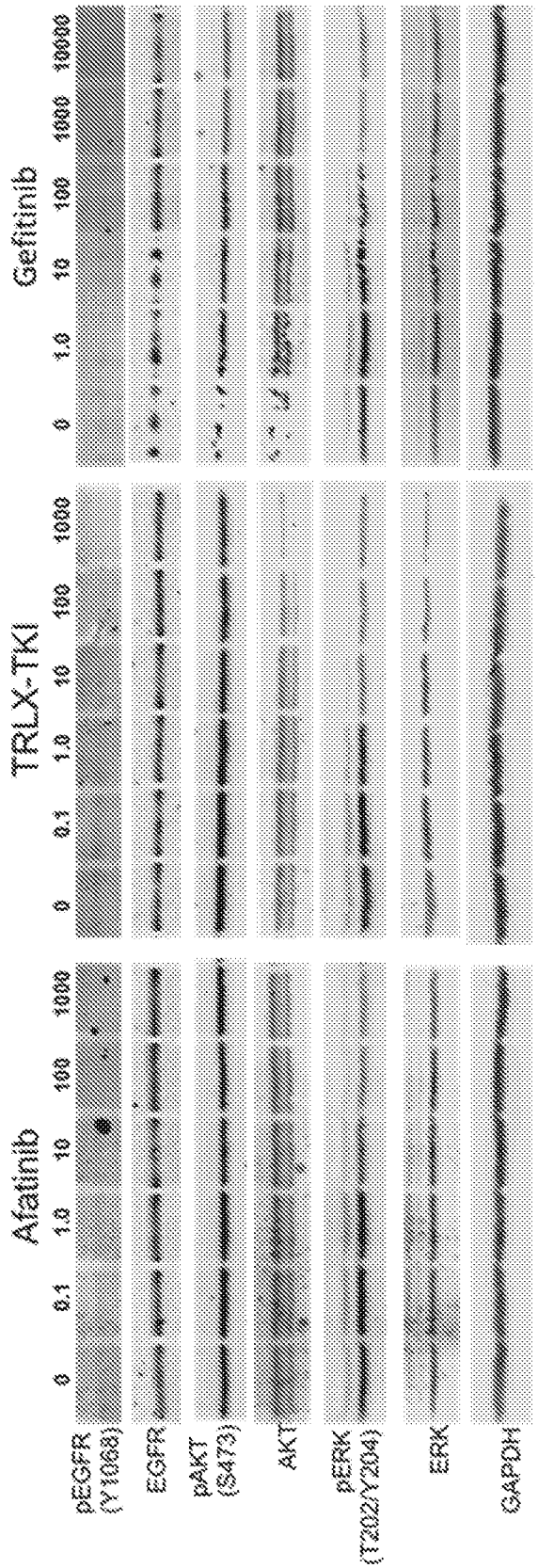

A549
Afatinib
TRLX-TKI
Gefitinib
0
0.1
1.0
10
100
1000
10000
pEGFR (Y1068)
EGFR
pAKT (S473)
AKT
pERK (T202/Y204)
ERK
GAPDH

FIG. 9A

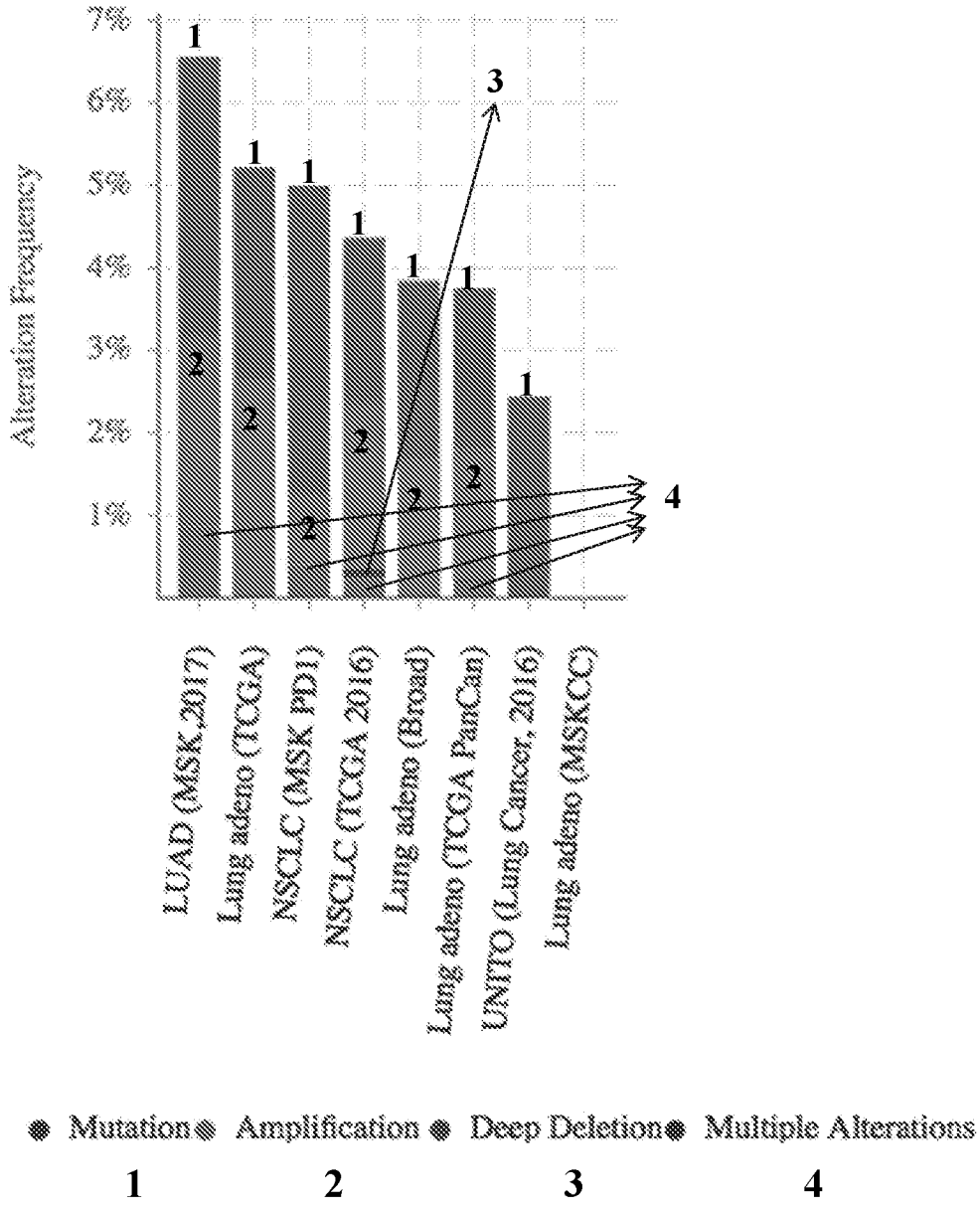

Alteration Frequency
7%
6%
5%
4%
3%
2%
1%
LUAD (MSK,2017)
Lung adeno (TCGA)
NSCLC (MSK PD1)
NSCLC (TCGA 2016)
Lung adeno (Broad)
Lung adeno (TCGA PanCan)
UNITO (Lung Cancer, 2016)
Lung adeno (MSKCC)
Mutation
Amplification
Deep Deletion
Multiple Alterations
1
2
3
4

FIG. 9B

Study of origin	ERBB2 Genetic Alteration	Study of origin	ERBB2 Genetic Alteration	Study of origin	ERBB2 Genetic Alteration	Study of origin	ERBB2 Genetic Alteration
n	f	n	f	p	f	p	a
l	f	n	f	p	f	p	a
p	f, a	n	f	p	f	l	a
n	f, a	n	f	m	f	p	a
n	f, a	n	f	l	f	m	a
o	f, a	o	f	p	f	p	a
n	f, a	n	f	p	f	m	a
p	f, a	n	f	l	f	l	a
n	f, b	n	f	p	g	p	a
n	f, b	p	f	j	a	p	c
n	f, d	p	f	j	a	n	c
p	f, d	p	f	p	a	n	c
n	f, c	p	f	n	a	n	c
p	f	p	f	n	a	o	c
j	f	p	f	n	a	n	c
j	f	m	f	n	a	n	c
p	f	l	f	n	a	n	c
j	f	p	f	n	a	n	c
p	f	m	f	n	a	o	c
p	f	l	f	n	a	m	c
n	f	p	f	o	a	l	c
n	f	m	f	n	a	p	c
n	f	l	f	n	a	i	d
n	f	p	f	n	a	j	d
n	f	p	f	n	a	p	d
n	f	p	f	n	a	n	d
n	f	l	f	n	a	o	d
n	f	p	f	n	a	m	d
o	f	m	f	o	a		
n	f	l	f	o	a		
n	f	p	f	l	a		
n	f	m	f	p	a		
n	f	l	f	m	a		
n	f	p	f	l	a		

FIG. 9C

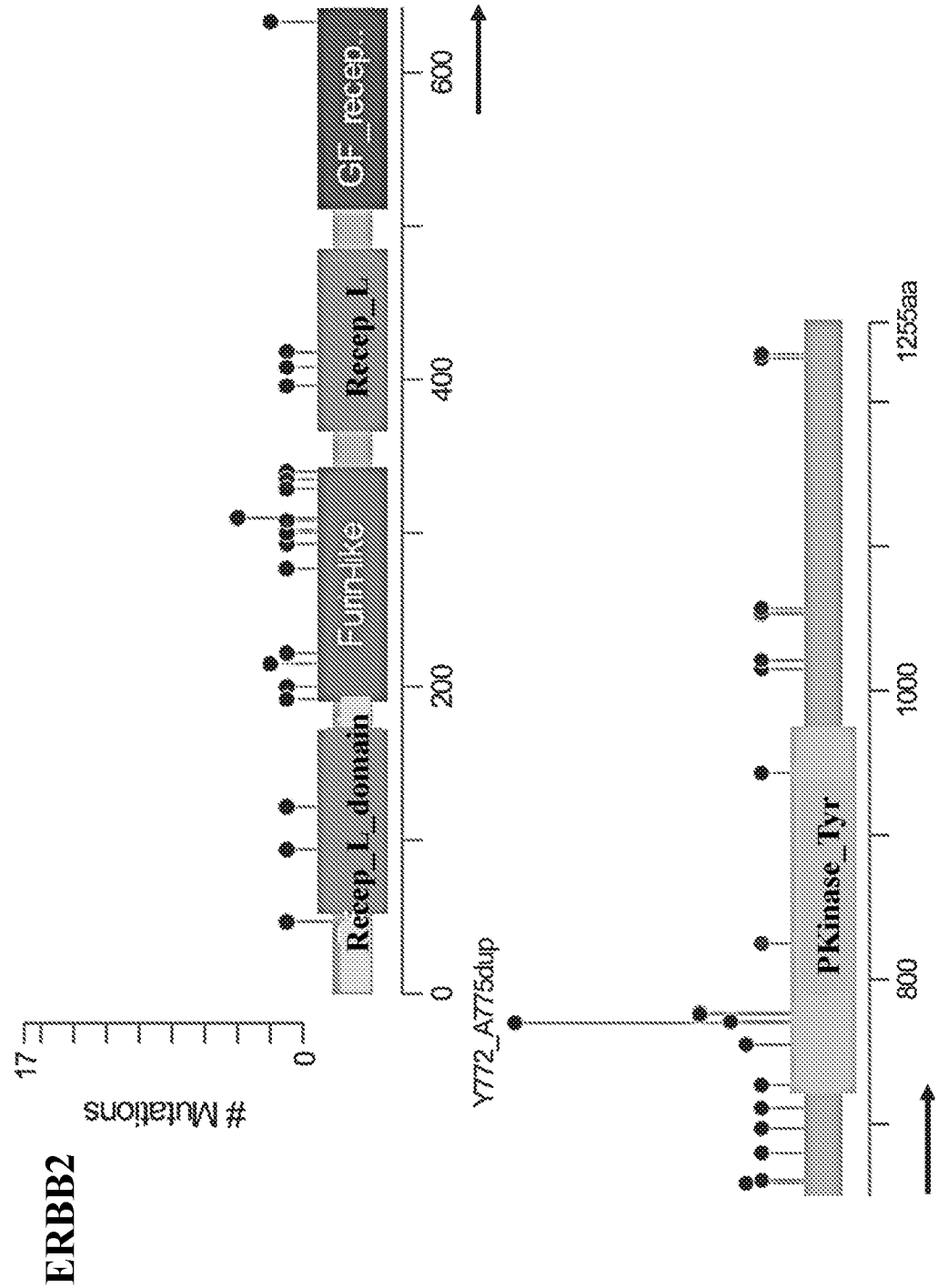

ERBB2
Mutations
17
0
Recep_L_domain
Furin-like
Recep_L
GF_recep_
0
200
400
600
Y772_A775dup
PKinase_Tyr
800
1000
1255aa

FIG. 10A

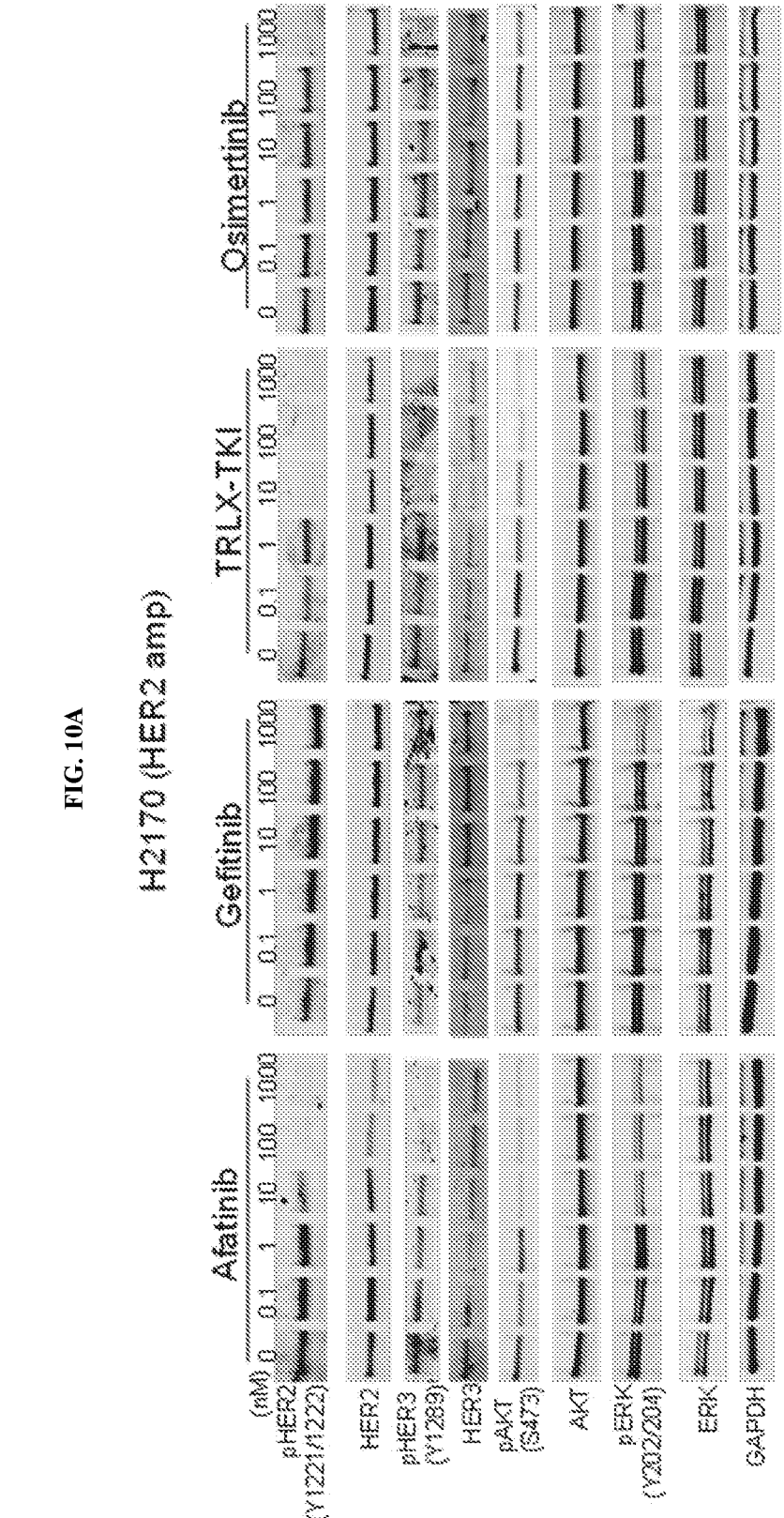

H2170 (HER2 amp)
Afatinib
Gefitinib
TRLX-TKI
Osimertinib
(nM) 0 0.1 1 10 100 1000
pHER2 (Y1221/1222)
HER2
pHER3 (Y1289)
HER3
pAKT (S473)
AKT
pERK (Y202/204)
ERK
GAPDH

FIG. 10B

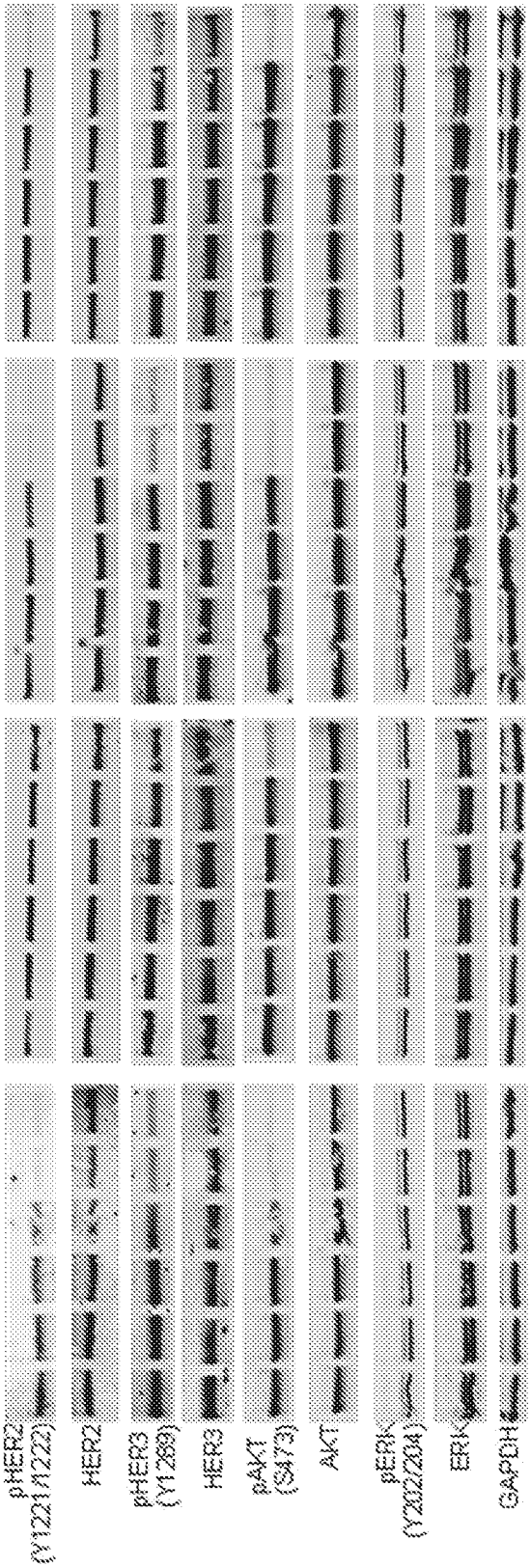

Calu-3 (HER2 amp)
pHER2 (Y1221/1222)
HER2
pHER3 (Y1289)
HER3
pAKT (S473)
AKT
pERK (Y202/204)
ERK
GAPDH

FIG. 10C

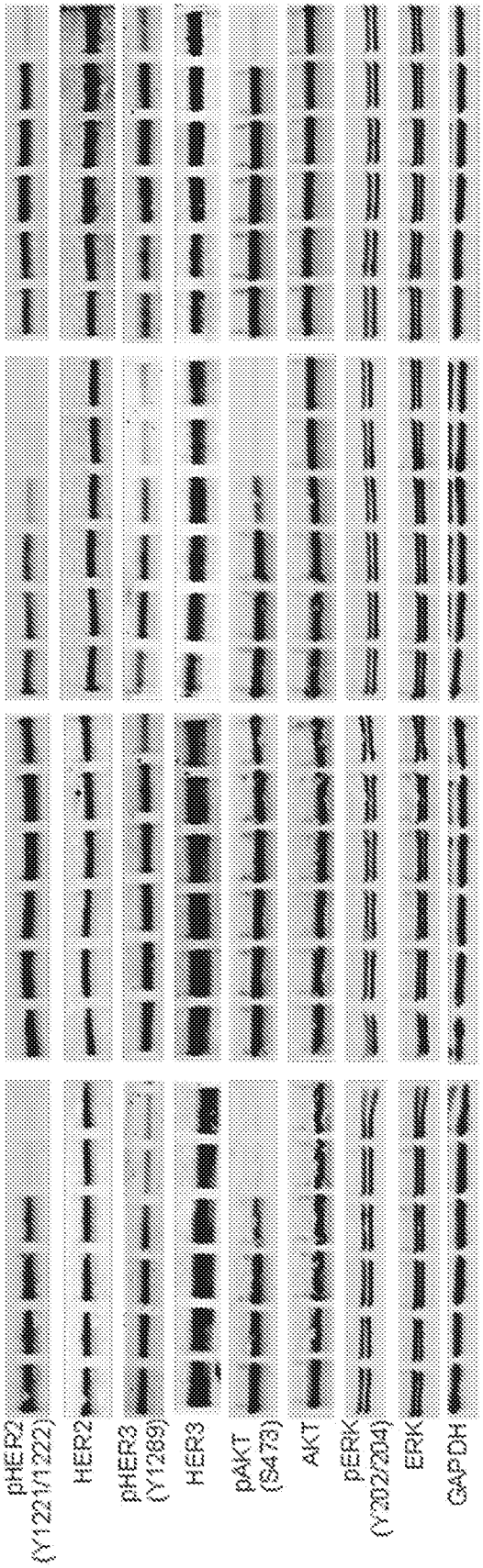

H1781 (p.G776Ins V_G/C)
pHER2 (Y1221/1222)
HER2
pHER3 (Y1289)
HER3
pAKT (S473)
AKT
pERK (Y202/204)
ERK
GAPDH

FIG. 11

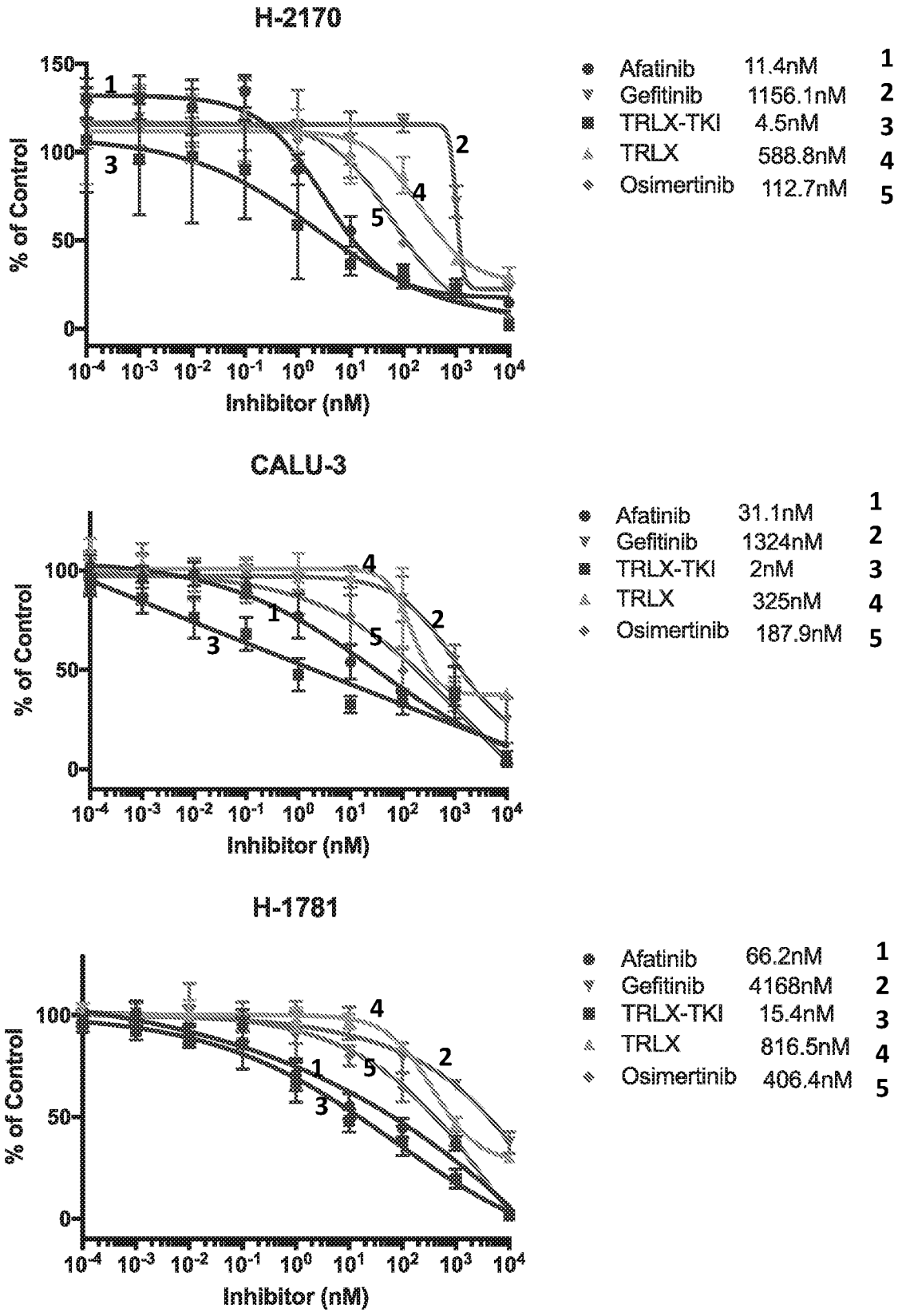

H-2170
% of Control
Inhibitor (nM)
Afatinib 11.4nM 1
Gefitinib 1156.1nM 2
TRLX-TKI 4.5nM 3
TRLX 588.8nM 4
Osimertinib 112.7nM 5
CALU-3
% of Control
Inhibitor (nM)
Afatinib 31.1nM 1
Gefitinib 1324nM 2
TRLX-TKI 2nM 3
TRLX 325nM 4
Osimertinib 187.9nM 5
H-1781
% of Control
Inhibitor (nM)
Afatinib 66.2nM 1
Gefitinib 4168nM 2
TRLX-TKI 15.4nM 3
TRLX 816.5nM 4
Osimertinib 406.4nM 5

FIG. 12

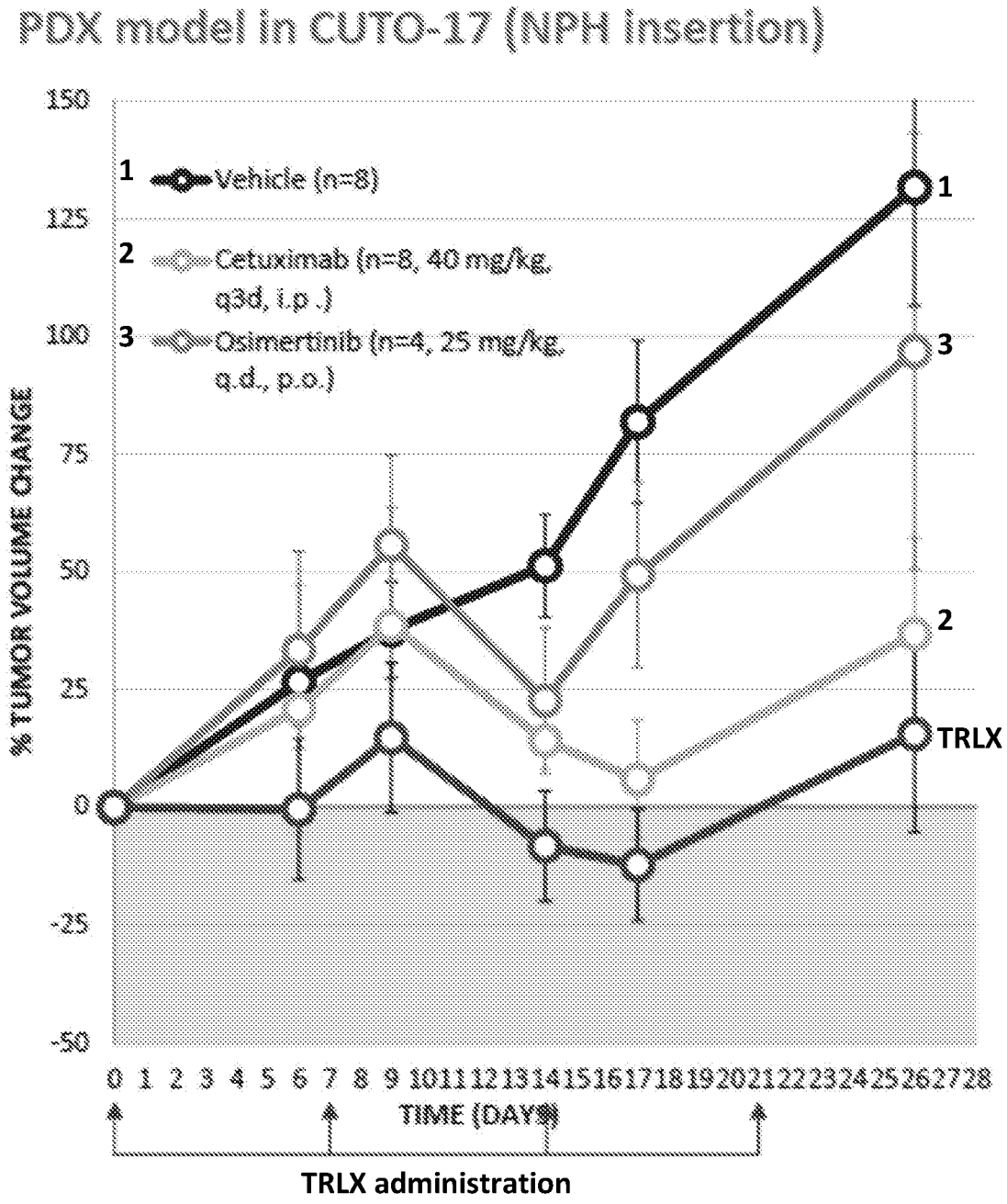

PDX model in CUTO-17 (NPH insertion)
% TUMOR VOLUME CHANGE
150
125
100
75
50
25
0
-25
-50
1 Vehicle (n=8)
2 Cetuximab (n=8, 40 mg/kg, q3d, i.p .)
3 Osimertinib (n=4, 25 mg/kg, q.d., p.o.)
1
2
3
TRLX
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
TIME (DAYS)
TRLX administration

FIG. 13

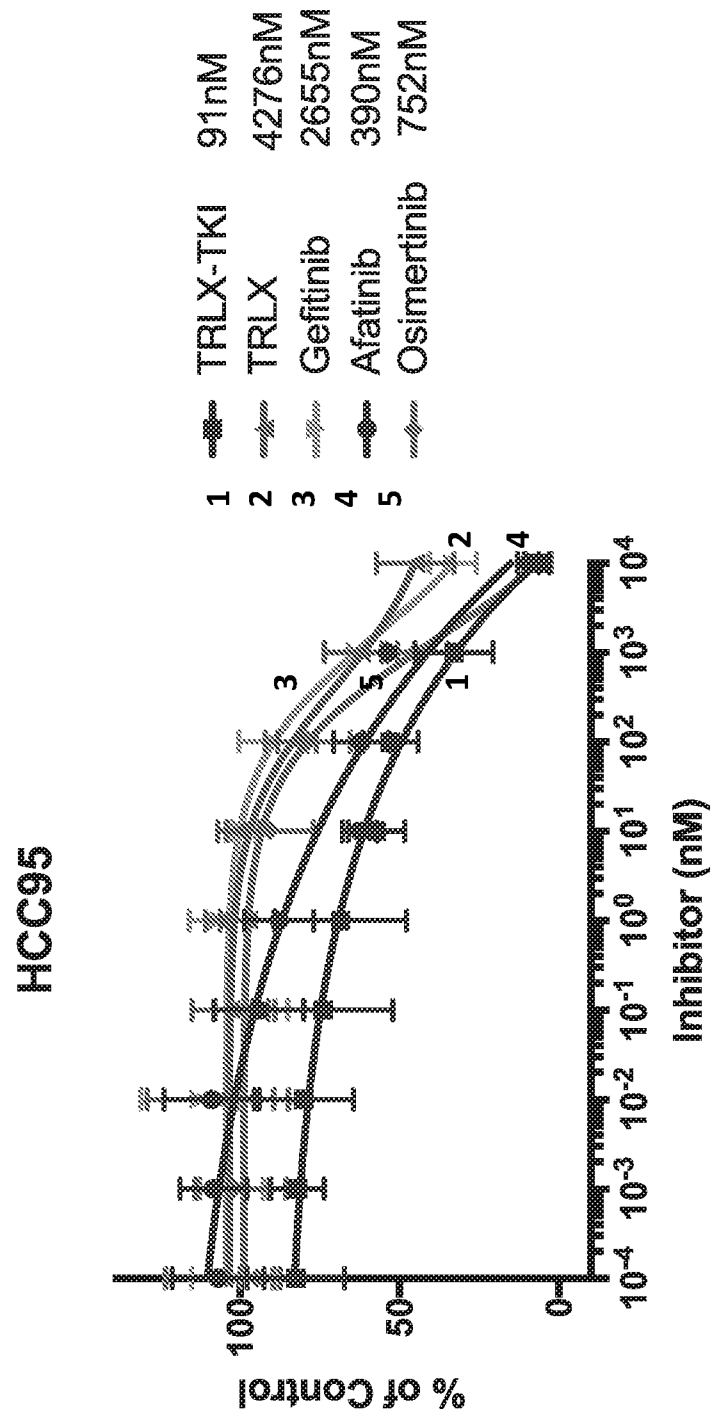

HCC95
% of Control
100
50
0
Inhibitor (nM)
10-4
10-3
10-2
10-1
100
101
102
103
104
1 TRLX-TKI 91nM
2 TRLX 4276nM
3 Gefitinib 2655nM
4 Afatinib 390nM
5 Osimertinib 752nM

FIG. 14

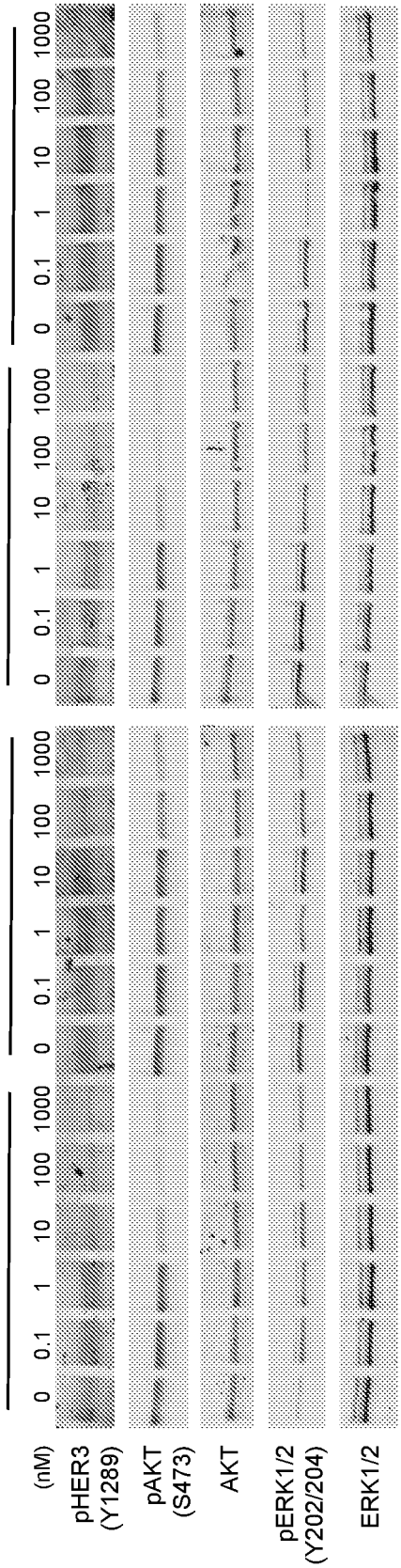

Afatinib
Gefitinib
TRLX-TKI
Osimertinib
(nM)
0 0.1 1 10 100 1000
pHER3 (Y1289)
pAKT (S473)
AKT
pERK1/2 (Y202/204)
ERK1/2

FIG. 15

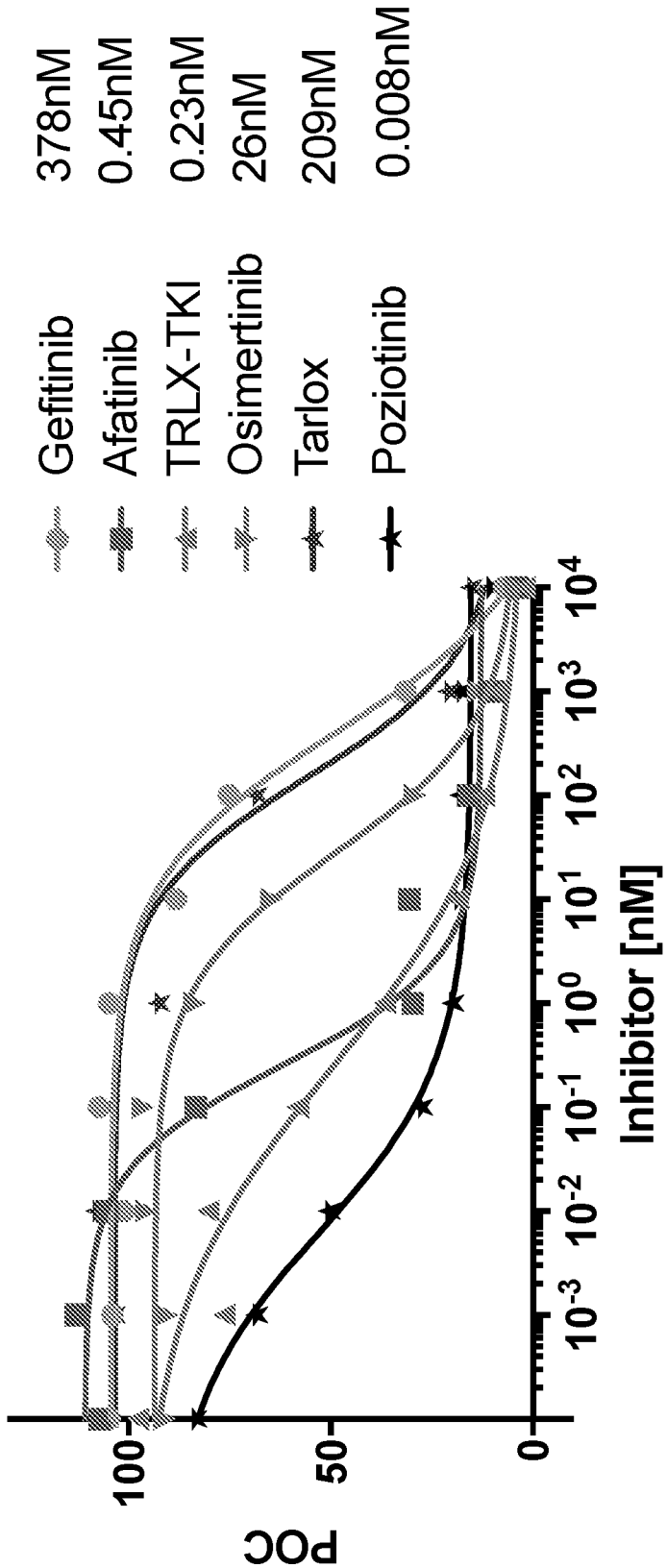

MDA-MB-157
Gefitinib 378nM
Afatinib 0.45nM
TRLX-TKI 0.23nM
Osimertinib 26nM
Tarlox 209nM
Poziotinib 0.008nM
PDC
100
50
0
10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3} 10^{4}
Inhibitor [nM]

FIG. 16A

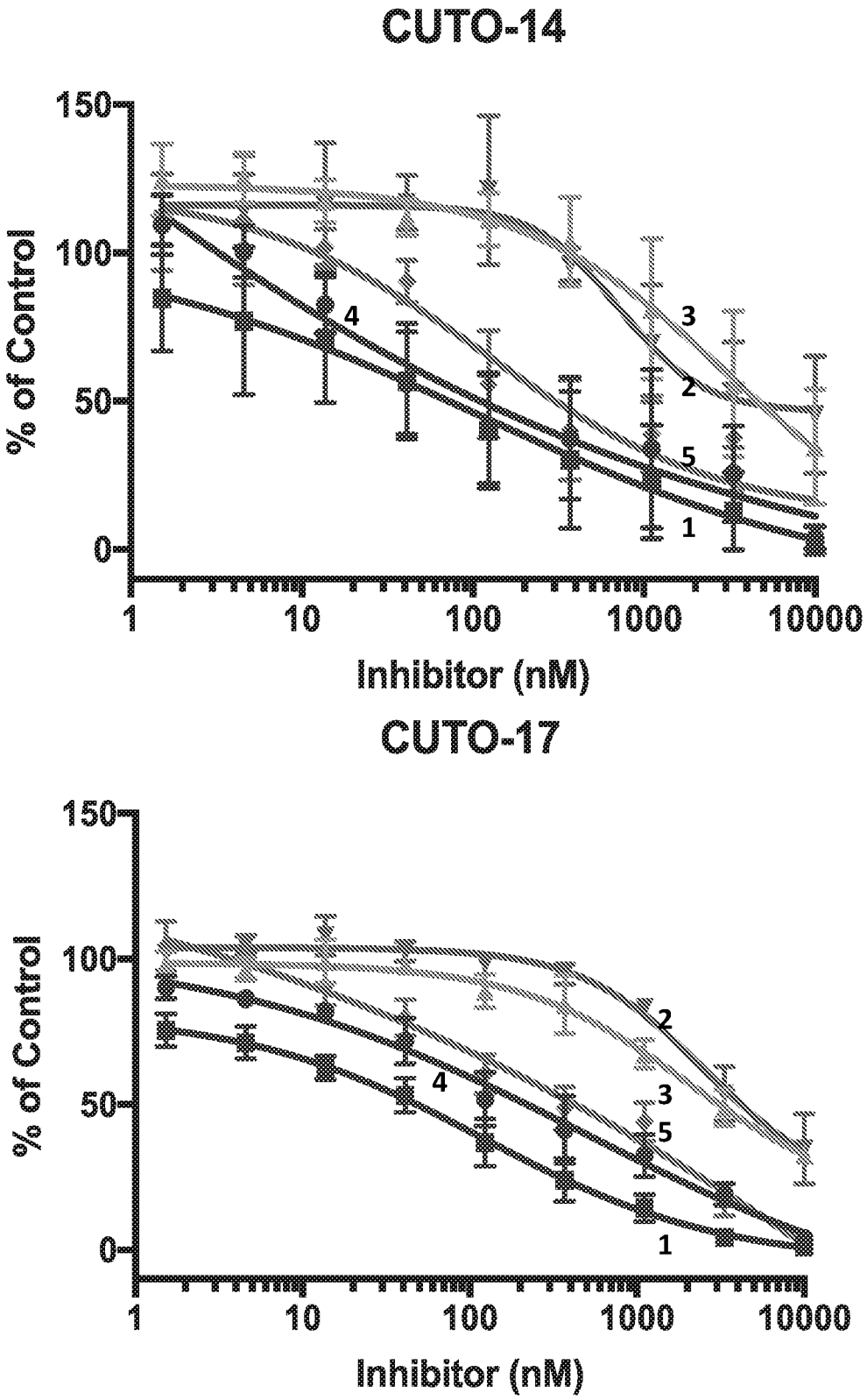
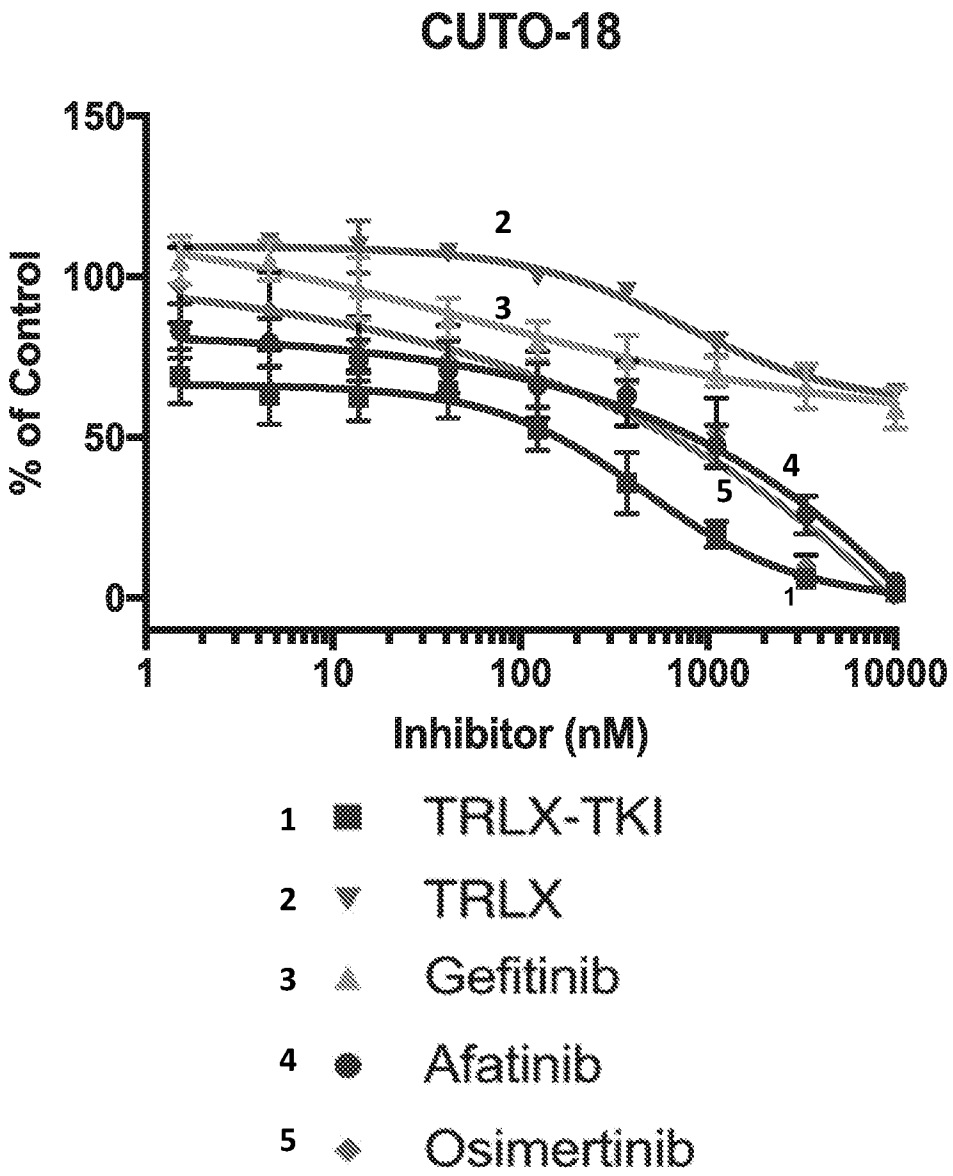

CUTO-14
150
100
50
0
% of Control
1
10
100
1000
10000
Inhibitor (nM)
4
3
2
5
1
CUTO-17
150
100
50
0
% of Control
1
10
100
1000
10000
Inhibitor (nM)
2
4
3
5
1

FIG. 16B


CUTO-18
150
100
50
0
% of Control
1
10
100
1000
10000
Inhibitor (nM)
2
3
4
5
1
1 TRLX-TKI
2 TRLX
3 Gefitinib
4 Afatinib
5 Osimertinib

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/49842

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07K 14/71, A61K 31/519, 31/517 (2018.01)
CPC - C07K 14/71, 14/705; A61K 31/519, 31/517

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ELAMIN et al. Preliminary Results of a Phase II Study of Poziotinib in EGFR Exon 20 Mutant Advanced NSCLC. J Thor Oncol August 2017 Vol 20 No 8 Suppl Page S1536. Especially abstract.	1-8
Y	SHROTRIYA. Spectrum Pharmaceuticals Investor Presentation, January 2017 [online]. [Retrieved 31 October 2018]. Retrieved from the internet: <URL: https://www.sec.gov/Archives/edgar/data/831547/000083154717000002/sppicorporatepresentaf74.htm >. Especially PDF pg 3.	1-8
Y	US 2012/0202832 A1 (SMAILL et al.) 9 August 2012 (09.08.2012). Especially para [0037], [0042], [0170], [0172], [0334], sheet 2 fig 2, sheet 4 fig 4.	1-8

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 October 2018	30 NOV 2018

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/49842

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

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

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

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

3. ☒ Claims Nos.: 9-22
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.