Combination of an EGFR T790M Inhibitor and an EGFR Inhibitor for the Treatment of Non-Small Cell Lung Cancer

Field of the Invention

This invention relates to a method of treating non-small cell lung cancer by administering a combination of an EGFR T790M inhibitor in combination with a low-dose amount of a panHER inhibitor. This invention also relates to a method of treating non-small cell lung cancer by administering a combination of an irreversible EGFR T790M inhibitor in combination with an EGFR inhibitor.

Background

Non-small cell lung cancer is the leading cause of cancer death worldwide, with an estimated 1.4 million new cases diagnosed each year. In lung adenocarcinoma, which is the most common form of non-small cell lung cancer, patients harboring mutations in the epidermal growth factor receptor (EGFR) constitute between 10-30 % of the overall population. It is this segment of patients for whom EGFR inhibitors such as erlotinib or gefitinib can be most effective (Paez *et al*. Science 2004; Lynch *et al*. NEJM 2004; Pao *et al*, PNAS 2004). The most common activating mutations associated with good response to these inhibitors are deletions within exon 19 (e.g. E746-A750) and point mutations in the activation loop (exon 21, in particular, L858R). Additional somatic mutations identified to date but to a lesser extent include point mutations: G719S, G719C, G719A, L861 and small insertions in Exon 20 (Shigematsu *et al* JNCI 2005; Fukuoka *et al*. JCO 2003; Kris *et al* JAMA 2003 and Shepherd *et al* NEJM 2004).

While these agents can be effective treatments for the EGFR mutant sub-population, the majority of patients who initially respond develop resistance. The primary mechanism of resistance, observed in approximately 50 % of patients, is due to a second mutation (T790M) which occurs at the gatekeeper threonine residue (Kosaka *et al* CCR 2006; Balak *et al* CCR 2006 and Engelman *et al* Science 2007).

Improved therapies for the treatment of non-small cell lung cancer comprise a large unmet medical need and the identification of novel combination regimens are required to improve treatment outcome.

Summary of the Invention

Each of the embodiments described below can be combined with any other embodiment described herein not inconsistent with the embodiment with which it is combined. Furthermore, each of the embodiments described herein envisions within its scope pharmaceutically acceptable salts of the compounds described herein. Accordingly, the phrase "or a pharmaceutically acceptable salt thereof" is implicit in the description of all compounds described herein.

Some embodiments described herein relate to a method of treating non-small cell lung cancer comprising administering to a patient in need thereof an effective amount of an irreversible EGFR T790M inhibitor in combination with an effective amount of an EGFR inhibitor.

In further embodiments of the method of the present invention, the irreversible EGFR T790M inhibitor is 1-{(3*R*,4*R*)-3-[({5-chloro-2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)methyl]-4-methoxypyrrolidin-1-yl}prop-2-en-1-one, or a pharmaceutically acceptable salt thereof.

In some embodiments of the method of the present invention, the irreversible EGFR T790M inhibitor is *N*-methyl-*N*-[*trans*-3-({2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-5-(pyridin-2-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)cyclobutyl]prop-2-enamide, or a pharmaceutically acceptable salt thereof.

In certain embodiments of the method of the present invention, the irreversible EGFR T790M inhibitor is *N*-[*trans*-3-({5-chloro-2-[(1,3-dimethyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}amino)cyclobutyl]-*N*-methylprop-2-enamide, or a pharmaceutically acceptable salt thereof.

In further embodiments of the method of the present invention, the irreversible EGFR T790M inhibitor is 1-{(3*R*,4*R*)-3-[({5-chloro-2-[(3-methoxy-1-methyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)methyl]-4-methoxypyrrolidin-1-yl}prop-2-en-1-one, or a pharmaceutically acceptable salt thereof.

In embodiments of the method of the present invention, the EGFR inhibitor is selected from the group consisting of gefitinib, erlotinib, icotinib, vandetanib, lapatinib, neratinib, afatinib, pelitinib, dacomitinib, canertinib, cetuximab and panitumumab, or a pharmaceutically acceptable salt thereof.

In further embodiments of the method of the present invention, the EGFR inhibitor is selected from the group consisting of gefitinib, erlotinib, icotinib, vandetanib,

lapatinib, neratinib, afatinib, pelitinib, dacomitinib, and canertinib, or a pharmaceutically acceptable salt thereof.

In some embodiments of the method of the present invention, the EGFR inhibitor is selected from the group consisting of gefitinib, erlotinib, afatinib, and dacomitinib, or a pharmaceutically acceptable salt thereof.

In certain embodiments of the method of the present invention, the EGFR inhibitor is gefitinib, or a pharmaceutically acceptable salt thereof.

In some embodiments of the method of the present invention, the EGFR inhibitor is erlotinib, or a pharmaceutically acceptable salt thereof.

In embodiments of the method of the present invention, the EGFR inhibitor is afatinib, or a pharmaceutically acceptable salt thereof.

In some embodiments of the method of the present invention, the EGFR inhibitor is dacomitinib, or a pharmaceutically acceptable salt thereof.

In further embodiments of the method of the present invention, the EGFR inhibitor is a reversible EGFR inhibitor.

In further embodiments of the method of the present invention, the reversible EGFR inhibitor is selected from the group consisting of gefitinib, erlotinib, icotinib, vandetanib, and lapatinib, or a pharmaceutically acceptable salt thereof.

In embodiments of the method of the present invention, the reversible EGFR inhibitor is gefitinib, or a pharmaceutically acceptable salt thereof.

In further embodiments of the method of the present invention, the reversible EGFR inhibitor is erlotinib, or a pharmaceutically acceptable salt thereof.

In additional embodiments of the method of the present invention, the EGFR inhibitor is an irreversible EGFR inhibitor.

In embodiments of the method of the present invention, the irreversible EGFR inhibitor is selected from the group consisting of neratinib, afatinib, pelitinib, dacomitinib, and canertinib, or a pharmaceutically acceptable salt thereof.

In further embodiments of the method of the present invention, the irreversible EGFR inhibitor is afatinib, or a pharmaceutically acceptable salt thereof.

In some embodiments of the method of the present invention, the irreversible EGFR inhibitor is dacomitinib, or a pharmaceutically acceptable salt thereof.

Some embodiments of the present invention relate to a method of treating non-small cell lung cancer comprising administering to a patient in need thereof an effective

amount of an EGFR T790M inhibitor in combination with a panHER inhibitor, wherein the panHER inhibitor is administered according to a non-standard clinical dosing regimen.

In further embodiments of the method of the present invention, the non-standard clinical dosing regimen is a non-standard clinical dose or a non-standard dosing schedule.

In some embodiments of the method of the present invention, the non-standard clinical dosing regimen is a low-dose amount of the panHER inhibitor.

In embodiments of the method of the present invention, the non-standard clinical dosing regimen is an intermittent dosing regimen.

In further embodiments of the method of the present invention, the EGFR T790M inhibitor is selected from the group consisting of Go6976, PKC412, AP26113, HM61713, WZ4002, CO-1686 and TAS-2913, or a pharmaceutically acceptable salt thereof.

In further embodiments of the method of the present invention, the EGFR T790M inhibitor is 1-{(3*R*,4*R*)-3-[({5-chloro-2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)methyl]-4-methoxypyrrolidin-1-yl}prop-2-en-1-one, or a pharmaceutically acceptable salt thereof.

In embodiments of the method of the present invention, the EGFR T790M inhibitor is *N*-methyl-*N*-[*trans*-3-({2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-5-(pyridin-2-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)cyclobutyl]prop-2-enamide, or a pharmaceutically acceptable salt thereof.

In additional embodiments of the method of the present invention, the EGFR T790M inhibitor is *N*-[*trans*-3-({5-chloro-2-[(1,3-dimethyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}amino)cyclobutyl]-*N*-methylprop-2-enamide, or a pharmaceutically acceptable salt thereof.

In further embodiments of the method of the present invention, the EGFR T790M inhibitor is 1-{(3*R*,4*R*)-3-[({5-chloro-2-[(3-methoxy-1-methyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)methyl]-4-methoxypyrrolidin-1-yl}prop-2-en-1-one, or a pharmaceutically acceptable salt thereof.

In some embodiments of the method of the present invention, the panHER inhibitor is selected from the group consisting of lapatinib, neratinib, afatinib, pelitinib, dacomitinib, and canertinib, or a pharmaceutically acceptable salt thereof.

In certain embodiments of the method of the present invention, the panHER inhibitor is selected from the group consisting of lapatinib, neratinib, afatinib, pelitinib, dacomitinib, and canertinib, or a pharmaceutically acceptable salt thereof.

In further embodiments of the method of the present invention, the panHER inhibitor is afatinib, or a pharmaceutically acceptable salt thereof.

In embodiments of the method of the present invention, the panHER inhibitor is dacomitinib, or a pharmaceutically acceptable salt thereof.

In further embodiments of the method of the present invention, the panHER inhibitor is an irreversible EGFR inhibitor.

In certain embodiments of the method of the present invention, the irreversible panHER inhibitor is selected from the group consisting of neratinib, afatinib, pelitinib, dacomitinib, and canertinib, or a pharmaceutically acceptable salt thereof.

In embodiments of the method of the present invention, the irreversible panHER inhibitor is afatinib, or a pharmaceutically acceptable salt thereof.

In some embodiments of the method of the present invention, the irreversible panHER inhibitor is dacomitinib, or a pharmaceutically acceptable salt thereof.

Certain embodiments of the present invention relate to a method of treating non-small cell lung cancer comprising administering to a patient in need thereof a synergistic amount of an EGFR T790M inhibitor in combination with an EGFR inhibitor.

In some embodiments of the method of the present invention, the EGFR T790M inhibitor is selected from the group consisting of Go6976, PKC412, AP26113, HM61713, WZ4002, CO-1686 and TAS-2913, or a pharmaceutically acceptable salt thereof.

In further embodiments of the method of the present invention, the EGFR T790M inhibitor is 1-{(3*R*,4*R*)-3-[({5-chloro-2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)methyl]-4-methoxypyrrolidin-1-yl}prop-2-en-1-one, or a pharmaceutically acceptable salt thereof.

In embodiments of the method of the present invention, the EGFR T790M inhibitor is *N*-methyl-*N*-[*trans*-3-({2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-5-(pyridin-2-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)cyclobutyl]prop-2-enamide, or a pharmaceutically acceptable salt thereof.

In certain embodiments of the method of the present invention, the EGFR T790M inhibitor is *N*-[*trans*-3-({5-chloro-2-[(1,3-dimethyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-

d]pyrimidin-4-yl}amino)cyclobutyl]-*N*-methylprop-2-enamide, or a pharmaceutically acceptable salt thereof.

In some embodiments of the method of the present invention, the EGFR T790M inhibitor is 1-{(3*R*,4*R*)-3-[({5-chloro-2-[(3-methoxy-1-methyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)methyl]-4-methoxypyrrolidin-1-yl}prop-2-en-1-one, or a pharmaceutically acceptable salt thereof.

In further embodiments of the method of the present invention, the EGFR inhibitor is selected from the group consisting of gefitinib, erlotinib, icotinib, vandetanib, lapatinib, neratinib, afatinib, pelitinib, dacomitinib, canertinib, cetuximab and panitumumab, or a pharmaceutically acceptable salt thereof.

In some embodiments of the method of the present invention, the EGFR inhibitor is selected from the group consisting of gefitinib, erlotinib, icotinib, vandetanib, lapatinib, neratinib, afatinib, pelitinib, dacomitinib, and canertinib, or a pharmaceutically acceptable salt thereof.

In certain embodiments of the method of the present invention, the EGFR inhibitor is selected from the group consisting of gefitinib, erlotinib, afatinib, and dacomitinib, or a pharmaceutically acceptable salt thereof.

In some embodiments of the method of the present invention, the EGFR inhibitor is gefitinib, or a pharmaceutically acceptable salt thereof.

In embodiments of the method of the present invention, the EGFR inhibitor is erlotinib, or a pharmaceutically acceptable salt thereof.

In some embodiments of the method of the present invention, the EGFR inhibitor is a panHER inhibitor.

In further embodiments of the method of the present invention, the panHER inhibitor is selected from the group consisting of lapatinib, neratinib, afatinib, pelitinib, dacomitinib, and canertinib, or a pharmaceutically acceptable salt thereof.

In embodiments of the method of the present invention, the panHER inhibitor is selected from the group consisting of lapatinib, neratinib, afatinib, pelitinib, dacomitinib, and canertinib, or a pharmaceutically acceptable salt thereof.

In some embodiments of the method of the present invention, the panHER inhibitor is afatinib, or a pharmaceutically acceptable salt thereof.

In additional embodiments of the method of the present invention, the panHER inhibitor is dacomitinib, or a pharmaceutically acceptable salt thereof.

In additional embodiments of the method of the present invention, the panHER inhibitor is an irreversible EGFR inhibitor.

In some embodiments of the method of the present invention, the irreversible panHER inhibitor is selected from the group consisting of neratinib, afatinib, pelitinib, dacomitinib, and canertinib, or a pharmaceutically acceptable salt thereof.

In embodiments of the method of the present invention, the irreversible panHER inhibitor is afatinib, or a pharmaceutically acceptable salt thereof.

In certain embodiments of the method of the present invention, the irreversible panHER inhibitor is dacomitinib, or a pharmaceutically acceptable salt thereof.

Some embodiments of the present invention relate to a synergistic combination of (a) an EGFR T790M inhibitor; and (b) an EGFR inhibitor, wherein component (a) and component (b) are synergistic.

In some embodiments of the combination of the present invention, the EGFR T790M inhibitor is selected from the group consisting of Go6976, PKC412, AP26113, HM61713, WZ4002, CO-1686 and TAS-2913, or a pharmaceutically acceptable salt thereof.

In further embodiments of the combination of the present invention, the EGFR T790M inhibitor is 1-{(3*R*,4*R*)-3-[({5-chloro-2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)methyl]-4-methoxypyrrolidin-1-yl}prop-2-en-1-one, or a pharmaceutically acceptable salt thereof.

In embodiments of the combination of the present invention, the EGFR T790M inhibitor is *N*-methyl-*N*-[*trans*-3-({2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-5-(pyridin-2-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)cyclobutyl]prop-2-enamide, or a pharmaceutically acceptable salt thereof.

In additional embodiments of the combination of the present invention, the EGFR T790M inhibitor is *N*-[*trans*-3-({5-chloro-2-[(1,3-dimethyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}amino)cyclobutyl]-*N*-methylprop-2-enamide, or a pharmaceutically acceptable salt thereof.

In some embodiments of the combination of the present invention, the EGFR T790M inhibitor is 1-{(3*R*,4*R*)-3-[({5-chloro-2-[(3-methoxy-1-methyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)methyl]-4-methoxypyrrolidin-1-yl}prop-2-en-1-one, or a pharmaceutically acceptable salt thereof.

In some embodiments of the combination of the present invention, the EGFR inhibitor is selected from the group consisting of gefitinib, erlotinib, icotinib, vandetanib, lapatinib, neratinib, afatinib, pelitinib, dacomitinib, canertinib, cetuximab and panitumumab, or a pharmaceutically acceptable salt thereof.

In embodiments of the combination of the present invention, the EGFR inhibitor is selected from the group consisting of gefitinib, erlotinib, icotinib, vandetanib, lapatinib, neratinib, afatinib, pelitinib, dacomitinib, and canertinib, or a pharmaceutically acceptable salt thereof.

In embodiments of the combination of the present invention, the EGFR inhibitor is selected from the group consisting of gefitinib, erlotinib, afatinib, and dacomitinib, or a pharmaceutically acceptable salt thereof.

In certain embodiments of the combination of the present invention, the EGFR inhibitor is gefitinib, or a pharmaceutically acceptable salt thereof.

In further embodiments of the combination of the present invention, the EGFR inhibitor is erlotinib, or a pharmaceutically acceptable salt thereof.

In some embodiments of the combination of the present invention, the EGFR inhibitor is a panHER inhibitor.

In further embodiments of the combination of the present invention, the panHER inhibitor is selected from the group consisting of lapatinib, neratinib, afatinib, pelitinib, dacomitinib, and canertinib, or a pharmaceutically acceptable salt thereof.

In additional embodiments of the combination of the present invention, the panHER inhibitor is selected from the group consisting of lapatinib, neratinib, afatinib, pelitinib, dacomitinib, and canertinib, or a pharmaceutically acceptable salt thereof.

In embodiments of the combination of the present invention, the panHER inhibitor is afatinib, or a pharmaceutically acceptable salt thereof.

In some embodiments of the combination of the present invention, the panHER inhibitor is dacomitinib, or a pharmaceutically acceptable salt thereof.

In embodiments of the combination of the present invention, the panHER inhibitor is an irreversible EGFR inhibitor.

In additional embodiments of the combination of the present invention, the irreversible panHER inhibitor is selected from the group consisting of neratinib, afatinib, pelitinib, dacomitinib, and canertinib, or a pharmaceutically acceptable salt thereof.

In some embodiments of the combination of the present invention, the irreversible panHER inhibitor is afatinib, or a pharmaceutically acceptable salt thereof.

In embodiments of the combination of the present invention, the irreversible panHER inhibitor is dacomitinib, or a pharmaceutically acceptable salt thereof.

Brief Description of the Drawings

Figure 1 shows that Sanger sequencing identified a C>T EGFR T790M mutation in RPC9 clone 3 and clone 6, with the percentages shown representing castPCR quantified EGFR T790M alleles with respect to the total EGFR alleles.

Figure 2 shows dose response curves in cell viability assays for PC9 and RPC9 clones 3 and 6 that were treated with various concentration of dacomitinib (Figure 2A) or erlotinib (Figure 2B).

Figure 3 shows dose response curves in an RPC9 clone 6 cell viability assay. Figure 3A shows dose response curves of Compound A ("compd A") and dacomitinib ("daco") alone and in combination. Figure 3B shows dose response curves of Compound A and erlotinib ("erlo") alone and in combination. Figure 3C shows the percent of inhibition at selected concentrations of Compound A as a single agent and in combination with dacomitinib and erlotinib.

Figure 4 shows dose response curves in an RPC9 clone 6 cell viability assay. Figure 4A shows dose response curves of Compound B ("compd B") and dacomitinib ("daco") alone and in combination. Figure 4B shows dose response curves of Compound B and erlotinib ("erlo") alone and in combination. Figure 4C shows the percent of inhibition at selected concentrations of Compound B as a single agent and in combination with dacomitinib and erlotinib.

Figure 5 shows dose response curves in an RPC9 clone 6 cell viability assay. Figure 5A shows dose response curves of Compound A ("compd A") alone and in combination with dacomitinib ("daco"). Figure 5B shows dose response curves of Compound A alone and in combination with gefitinib ("gefi"). Figure 5C shows dose response curves of Compound A alone and in combination with afatinib ("afat"). Figure 5D shows the percent of inhibition at selected concentrations of Compound A as a single agent and in combination with dacomitinib, gefitinib and afatinib.

Figure 6 shows dose response curves in an RPC9 clone 6 cell viability assay. Figure 6A shows dose response curves of Compound B ("compd B") alone and in

combination with dacomitinib ("daco"). Figure 6B shows dose response curves of Compound B alone and in combination with gefitinib ("gefi"). Figure 6C shows dose response curves of Compound B alone and in combination with afatinib ("afat"). Figure 4D shows the percent of inhibition at selected concentrations of Compound B as a single agent and in combination with dacomitinib, gefitinib and afatinib.

Figure 7 shows a Western immunoblot of the phosphorylation levels of EGFR, AKT, and ERK in RPC9 clone 6 cells. GAPDH was included as a protein loading control. Figure 7A shows the RPC9 clone 6 cells treated with DMSO, dacomitinib, Compound A or a combination of dacomitinib + Compound A ("Compd A"). Figure 7B shows the RPC9 clone 6 cells treated with DMSO, erlotinib, Compound A or a combination of erlotinib + Compound A ("Compd A").

Figure 8 shows the densitometry results on the bands of the Western immunoblot (Figure 7A) of the RPC9 clone 6 cells treated with DMSO, dacomitinib, Compound A or a combination of dacomitinib ("Daco") + Compound A ("Compd A"). Inhibition of pEGFR Y1068 (Figure 8A), pAKT S473 (Figure 8B), and pERK T202/Y204 (Figure 8C) was determined by comparison to the DMSO control.

Figure 9 shows the densitometry results on the bands of the Western immunoblot (Figure 7B) of the RPC9 clone 6 cells treated with DMSO, erlotinib, Compound A or a combination of erlotinib ("Erlo") + Compound A ("Compd A"). Inhibition of pEGFR Y1068 (Figure 9A), pAKT S473 (Figure 9B), and pERK T202/Y204 (Figure 9C) was determined by comparison to the DMSO control.

Figure 10 shows a Western immunoblot of the phosphorylation levels of EGFR, AKT, and ERK in RPC9 clone 6 cells. GAPDH was included as a protein loading control. Figure 10A shows the RPC9 clone 6 cells treated with DMSO, dacomitinib, Compound B or a combination of dacomitinib + Compound B ("Compd B"). Figure 10B shows the RPC9 clone 6 cells treated with DMSO, erlotinib, Compound B or a combination of erlotinib + Compound B ("Compd B").

Figure 11 shows the densitometry results on the bands of the Western immunoblot (Figure 10A) of the RPC9 clone 6 cells treated with DMSO, dacomitinib, Compound B or a combination of dacomitinib ("Daco") + Compound B ("Compd B"). Inhibition of pEGFR Y1068 (Figure 11A), pAKT S473 (Figure 11B), and pERK T202/Y204 (Figure 11C) was determined by comparison to the DMSO control.

Figure 12 shows the densitometry results on the bands of the Western immunoblot (Figure 10B) of the RPC9 clone 6 cells treated with DMSO, erlotinib, Compound B or a combination of erlotinib ("Erlo") + Compound B ("Compd B"). Inhibition of pEGFR Y1068 (Figure 12A), pAKT S473 (Figure 12B), and pERK T202/Y204 (Figure 12C) was determined by comparison to the DMSO control.

Figure 13 graphs the results of the xenograft model with RPC9 clone 6 tumor bearing SCID mice, which were randomized, daily and orally treated with vehicle, dacomitinib, Compound A, or dacomitinib ("Daco") + Compound A ("Compd A"). Figure 13A graphs the tumor volumes, which were measured 3 times per week and graphed with mean and standard error of the mean. Figure 13B graphs the body weight of each group, which was recorded daily and percentage changes were graphed with mean and standard error of the mean.

Figure 14 graphs the results of the xenograft model with RPC9 clone 6 tumor bearing SCID mice, which were randomized, daily and orally treated with vehicle, dacomitinib, Compound B, or dacomitinib ("Daco") + Compound B (Compd B"). Figure 14A graphs the tumor volumes, which were measured 3 times per week and graphed with mean and standard error of the mean. Figure 14B graphs the body weight of each group, which was recorded daily and percentage changes were graphed with mean and standard error of the mean.

Figure 15 graphs the results of the xenograft model with RPC9 clone 6 tumor bearing SCID mice, which were randomized, daily and orally treated with vehicle, dacomitinib, Compound B, or dacomitinib ("Daco") + Compound B ("Compd B"). Figure 15A graphs the tumor volumes of single agent treatment groups. Figure 15B graphs the tumor volumes of combination treatment groups.

Figure 16 graphs the results of the xenograft model with RPC9 clone 6 tumor bearing SCID mice, which were randomized, daily and orally treated with vehicle, erlotinib, Compound A, or erlotinib ("Erlo") + Compound A (Compd A"). Figure 16A graphs the tumor volumes, which were measured 3 times per week and graphed with mean and standard error of the mean. Figure 16B graphs the body weight of each group, which was recorded daily and percentage changes were graphed with mean and standard error of the mean.

Detailed Description of the Invention

The members of the human epidermal growth factor receptor/epidermal growth factor receptor (HER/EGFR) family of receptors include EGFR/HER-1, HER2/neu/erbB-2, HER3/erbB-3 and HER4/erbB-4.

EGFR inhibitors effectively inhibit the common activating mutations (L858R and delE746-A750) of EGFR. The common activating mutations are also referred to as single mutants or single mutant forms. Examples of EGFR inhibitors include gefitinib, erlotinib, icotinib, vandetanib, lapatinib, neratinib, afatinib, pelitinib, dacomitinib and canertinib. Monoclonal antibody inhibitors of EGFR, such as cetuximab and panitumumab, are also EGFR inhibitors, as defined in the present invention.

Inhibitors of EGFR may be reversible or irreversible inhibitors. Reversible inhibitors of the tyrosine kinase domain of the EFGR molecule attach to and periodically detach from the receptor. Gefitinib, erlotinib, icotinib, vandetanib and lapatinib are examples of reversible EGFR inhibitors. Irreversible inhibitors of the tyrosine kinase domain of the EFGR molecule bind to EGFR irreversibly. Neratinib, afatinib, pelitinib, dacomitinib and canertinib are examples of irreversible EGFR inhibitors.

EGFR inhibitors are inhibitors of at least one member of the HER family. Gefitinib, erlotinib, icotinib and vandetanib are selective EGFR/HER-1 tyrosine kinase inhibitors (TKI). Cetuximab and panitumumab are monoclonal antibodies specific to EGFR/HER-1.

A pan-HER inhibitor is an agent that block multiple members of the HER family. Lapatinib, neratinib, afatinib, pelitinib, dacomitinib and canertinib are examples of pan-HER inhibitors. Lapatinib, neratinib, afatinib and pelitinib inhibit the EGFR and HER2 members of the HER family. Dacomitinib and canertinib inhibit the EGFR, HER2, and HER4 members of the HER family.

EGFR T790M inhibitors effectively inhibit the common activating mutations (L858R and delE746-A750) and the gatekeeper mutation (T790M). The EGFR T790M inhibitors of the present invention preferentially inhibit the double mutant forms of EGFR (L858R/T790M and delE746-A750/T790M) over the single mutants (L858R and delE746-A750). Examples of EGFR T790M inhibitors include Go6976, PKC412, AP26113, HM61713, WZ4002, CO-1686 and TAS-2913.

Inhibitors of EGFR T790M may be reversible or irreversible inhibitors. Go6976, PKC412 and AP26113 are examples of reversible EGFR T790M inhibitors. HM61713, WZ4002, CO-1686 and TAS-2913 are examples irreversible EGFR T790M inhibitors.

EGFR T790M inhibitors of the present invention also include 1-{(3*R*,4*R*)-3-[({5-chloro-2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)methyl]-4-methoxypyrrolidin-1-yl}prop-2-en-1-one ("Compound A"), *N*-methyl-*N*-[*trans*-3-({2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-5-(pyridin-2-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)cyclobutyl]prop-2-enamide ("Compound B"), *N*-[*trans*-3-({5-chloro-2-[(1,3-dimethyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}amino)cyclobutyl]-*N*-methylprop-2-enamide ("Compound C"); and 1-{(3*R*,4*R*)-3-[({5-chloro-2-[(3-methoxy-1-methyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)methyl]-4-methoxypyrrolidin-1-yl}prop-2-en-1-one ("Compound D"), or a pharmaceutically acceptable salt thereof. Compound A, Compound B, Compound C and Compound D are examples of irreversible EGFR T790M inhibitors.

The following abbreviations may be used herein: Ac (acetyl); APCI (atomic pressure chemical ionization); Boc (*tert*-butoxycarbonyl); Boc_2O (di-*tert*-butyl dicarbonate); BrettPhos Palladacycle (chloro[2-(dicyclohexylphosphino)-3,6-dimethoxy-2′,4′, 6′-triisopropyl-1,1′-biphenyl][2-(2-aminoethyl)phenyl]palladium(II)); DCC (1,3-dicyclohexylcarbodiimide); DCM (dichloromethane); Deoxo-Fluor® (bis(2-methoxyethyl)aminosulfur trifluoride); DIAD (diisopropyl azodicarboxylate); DIEA (diisopropylethylamine); DIPEA (*N,N*-diisopropylethylamine); DMAP (4-dimethylaminopyridine); DMEM (Dulbecco's modified Eagle's medium); DMF (dimethylformamide); DMSO (dimethylsulphoxide); DPPA (diphenyl phosphorazidate); EGTA ([Ethylenebis(oxyethylenenitrilo)]tetraacetic acid); eq (equivalent); Et (ethyl); EtOH (ethanol); EtOAc (ethyl acetate); Et_2O (diethyl ether); FBS (fetal bovine serum); HATU (2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate); HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); HMDS (bis(trimethylsilyl)amine, which is also known as hexamethyldisilazane or hexamethyldisiloxane); HOAc (acetic acid); HPLC (high-performance liquid chromatography); iPr (isopropyl); iPrMgCl (isopropylmagnesium chloride); iPrOH (isopropyl alcohol); KHMDS (potassium bis(trimethylsilyl)amide); LAH (lithium aluminum hydride); LCMS (liquid chromatography-mass spectrometry); LiHMDS (lithium bis(trimethylsilyl)amide); Me (methyl); MeOH (methanol); MeCN (acetonitrile); MTBE (methyl *tert*-butyl ether); N (normal); N/A (not available); NaHMDS (sodium bis(trimethylsilyl)amide); N/D (not determined); NIS (*N*-iodosuccinimide); NMM (*N*-methylmorpholine); NMR (nuclear magnetic resonance); $Pd_2(dba)_3$

(tris(dibenzylideneacetone)dipalladium(0)); PG (protecting group); Ph (phenyl); $PhI(OAc)_2$ (iodobenzene diacetate); PMSF (phenylmethylsulfonyl fluoride); psi (pounds per square inch); Rf (retention factor); RPMI (Roswell Park Memorial Institute); rt (room temperature); sat. (saturated); SCX (strong cation exchange); SEM (2-(trimethylsilyl)ethoxymethyl); SEM-Cl (2-(trimethylsilyl)ethoxymethyl chloride); SFC (supercritical fluid chromatography); TBAF (tetrabutylammonium fluoride); TBDPS (*tert*-butyldiphenylsilyl); TBS (*tert*-butyldimethylsilyl); t-BuXPhos Palladacycle (chloro[2-(di-*tert*-butylphosphino)-2′,4′,6′-triisopropyl-1,1′-biphenyl][2-(2-aminoethyl)phenyl)]palladium(II); TFA (trifluoroacetate); THF (tetrahydrofuran); TLC (thin layer chromatography); toluene (methylbenzene); tosyl (p-toluenesulfonyl); and Xantphos (4,5-bis(diphenylphosphino)-9,9-dimethylxanthene).

Some embodiments relate to the pharmaceutically acceptable salts of the compounds described herein. Pharmaceutically acceptable salts of the compounds described herein include the acid addition and base addition salts thereof.

Some embodiments also relate to the pharmaceutically acceptable acid addition salts of the compounds described herein. Suitable acid addition salts are formed from acids which form non-toxic salts. Non-limiting examples of suitable acid addition salts, i.e., salts containing pharmacologically acceptable anions, include, but are not limited to, the acetate, acid citrate, adipate, aspartate, benzoate, besylate, bicarbonate/carbonate, bisulphate/sulphate, bitartrate,borate, camsylate, citrate, cyclamate, edisylate, esylate, ethanesulfonate, formate, fumarate, gluceptate, gluconate, glucuronate, hexafluorophosphate, hibenzate, hydrochloride/chloride, hydrobromide/bromide, hydroiodide/iodide, isethionate, lactate, malate, maleate, malonate, mesylate, methanesulfonate, methylsulphate, naphthylate, 2-napsylate, nicotinate, nitrate, orotate, oxalate, palmitate, pamoate, phosphate/hydrogen phosphate/dihydrogen phosphate, pyroglutamate, saccharate, stearate, succinate, tannate, tartrate, p-toluenesulfonate, tosylate, trifluoroacetate and xinofoate salts.

Additional embodiments relate to base addition salts of the compounds described herein. Suitable base addition salts are formed from bases which form non-toxic salts. Non-limiting examples of suitable base salts include the aluminium, arginine, benzathine, calcium, choline, diethylamine, diolamine, glycine, lysine, magnesium, meglumine, olamine, potassium, sodium, tromethamine and zinc salts.

The compounds described herein that are basic in nature are capable of forming a wide variety of salts with various inorganic and organic acids. The acids that may be used to prepare pharmaceutically acceptable acid addition salts of such basic compounds described herein are those that form non-toxic acid addition salts, e.g., salts containing pharmacologically acceptable anions, such as the hydrochloride, hydrobromide, hydroiodide, nitrate, sulfate, bisulfate, phosphate, acid phosphate, isonicotinate, acetate, lactate, salicylate, citrate, acid citrate, tartrate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucuronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate and pamoate [i.e., 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)] salts. The compounds described herein that include a basic moiety, such as an amino group, may form pharmaceutically acceptable salts with various amino acids, in addition to the acids mentioned above.

The chemical bases that may be used as reagents to prepare pharmaceutically acceptable base salts of those compounds of the compounds described herein that are acidic in nature are those that form non-toxic base salts with such compounds. Such non-toxic base salts include, but are not limited to those derived from such pharmacologically acceptable cations such as alkali metal cations (e.g., potassium and sodium) and alkaline earth metal cations (e.g., calcium and magnesium), ammonium or water-soluble amine addition salts such as N-methylglucamine-(meglumine), and the lower alkanolammonium and other base salts of pharmaceutically acceptable organic amines.

The compounds of the embodiments described herein include all stereoisomers (e.g., *cis* and *trans* isomers) and all optical isomers of compounds described herein (e.g., *R* and *S* enantiomers), as well as racemic, diastereomeric and other mixtures of such isomers. While all stereoisomers are encompassed within the scope of our claims, one skilled in the art will recognize that particular stereoisomers may be preferred.

In some embodiments, the compounds described herein can exist in several tautomeric forms, including the enol and imine form, and the keto and enamine form and geometric isomers and mixtures thereof. All such tautomeric forms are included within the scope of the present embodiments. Tautomers exist as mixtures of a tautomeric set in solution. In solid form, usually one tautomer predominates. Even though one

tautomer may be described, the present embodiments includes all tautomers of the present compounds.

The present embodiments also include atropisomers of the compounds described herein. Atropisomers refer to compounds that can be separated into rotationally restricted isomers.

Hemisalts of acids and bases may also be formed, for example, hemisulphate and hemicalcium salts.

For a review on suitable salts, see Handbook of Pharmaceutical Salts: Properties, Selection, and Use by Stahl and Wermuth (Wiley-VCH, 2002). Methods for making pharmaceutically acceptable salts of compounds described herein are known to one of skill in the art.

The term “solvate” is used herein to describe a molecular complex comprising a compound described herein and one or more pharmaceutically acceptable solvent molecules, for example, ethanol.

The compounds described herein may also exist in unsolvated and solvated forms. Accordingly, some embodiments relate to the hydrates and solvates of the compounds described herein.

Compounds described herein containing one or more asymmetric carbon atoms can exist as two or more stereoisomers. Where a compound described herein contains an alkenyl or alkenylene group, geometric cis/trans (or Z/E) isomers are possible. Where structural isomers are interconvertible via a low energy barrier, tautomeric isomerism (‘tautomerism’) can occur. This can take the form of proton tautomerism in compounds described herein containing, for example, an imino, keto, or oxime group, or so-called valence tautomerism in compounds which contain an aromatic moiety. A single compound may exhibit more than one type of isomerism.

Included within the scope of the present embodiments are all stereoisomers, geometric isomers and tautomeric forms of the compounds described herein, including compounds exhibiting more than one type of isomerism, and mixtures of one or more thereof. Also included are acid addition or base salts wherein the counterion is optically active, for example, d-lactate or l-lysine, or racemic, for example, dl-tartrate or dl-arginine.

Cis/trans isomers may be separated by conventional techniques well known to those skilled in the art, for example, chromatography and fractional crystallisation.

Conventional techniques for the preparation/isolation of individual enantiomers include chiral synthesis from a suitable optically pure precursor or resolution of the racemate (or the racemate of a salt or derivative) using, for example, chiral high pressure liquid chromatography (HPLC).

Alternatively, the racemate (or a racemic precursor) may be reacted with a suitable optically active compound, for example, an alcohol, or, in the case where a compound described herein contains an acidic or basic moiety, a base or acid such as 1-phenylethylamine or tartaric acid. The resulting diastereomeric mixture may be separated by chromatography and/or fractional crystallization and one or both of the diastereoisomers converted to the corresponding pure enantiomer(s) by means well known to a skilled person.

The term "treating", as used herein, unless otherwise indicated, means reversing, alleviating, inhibiting the progress of, or preventing the disorder or condition to which such term applies, or one or more symptoms of such disorder or condition. The term "treatment", as used herein, unless otherwise indicated, refers to the act of treating as "treating" is defined immediately above.

A patient to be treated according to this invention includes any warm-blooded animal, such as, but not limited to human, monkey or other lower-order primate, horse, dog, rabbit, guinea pig, or mouse. For example, the patient is human. Those skilled in the medical art are readily able to identify individual patients who are afflicted with non-small cell lung cancer and who are in need of treatment.

The term "additive" means that the result of the combination of the two compounds or targeted agents is the sum of each agent individually. The terms "synergy" or "synergistic" are used to mean that the result of the combination of the two agents is more than the sum of each agent together. A "synergistic amount" is an amount of the combination of the two agents that result in a synergistic effect.

Determining a synergistic interaction between one or two components, the optimum range for the effect and absolute dose ranges of each component for the effect may be definitively measured by administration of the components over different w/w ratio ranges and doses to patients in need of treatment. For humans, the complexity and cost of carrying out clinical studies on patients renders impractical the use of this form of testing as a primary model for synergy. However, the observation of synergy in *in vitro* models or *in vivo* models can be predictive of the effect in humans and other species and *in vitro*

models or *in vivo* models exist, as described herein, to measure a synergistic effect and the results of such studies can also be used to predict effective dose and plasma concentration ratio ranges and the absolute doses and plasma concertrations required in humans and other species by the application of pharmacokinetic/pharmacodynamic methods.

In an embodiment, the method of the invention is related to a method of treating non-small cell lung cancer comprising administering to a patient in need thereof an effective amount of an EGFR T790M inhibitor in combination with a panHER inhibitor, where the panHER inhibitor is administered according to a non-standard clinical dosing regimen, in amounts sufficient to achieve synergistic effects. In this embodiment, the method of the invention is related to a synergistic combination of targeted therapeutic agents, specifically an EGFR T790M inhibitor and a panHER inhibitor.

In an embodiment, the method of the invention is related to a method of treating non-small cell lung cancer comprising administering to a patient in need thereof an effective amount of an EGFR T790M inhibitor in combination with a low-dose amount of a panHER inhibitor, in amounts sufficient to achieve synergistic effects. In this embodiment, the method of the invention is related to a synergistic combination of targeted therapeutic agents, specifically an EGFR T790M inhibitor and a panHER inhibitor.

In another embodiment, the method of the invention is related to a method of treating non-small cell lung cancer comprising administering to a patient in need thereof an effective amount of an irreversible EGFR T790M inhibitor in combination with an effective amount of an EGFR inhibitor, in amounts sufficient to achieve synergistic effects. In this embodiment, the method of the invention is related to a synergistic combination of targeted therapeutic agents, specifically an irreversible EGFR T790M inhibitor and an EGFR inhibitor.

As used herein, an “effective” amount refers to an amount of a substance, agent, compound, or composition that is sufficient to prevent or inhibit the growth of tumor cells or the progression of cancer metastasis in the combination of the present invention. Therapeutic or pharmacological effectiveness of the doses and administration regimens may also be characterized as the ability to induce, enhance, maintain or prolong remission in patients experiencing specific tumors.

A "non-standard clinical dosing regimen," as used herein, refers to a regimen for administering a substance, agent, compound, or composition, which effectively inhibits the single mutant forms (L858R and delE746-A750) of EGFR, but which is different than the amount or dose typically used in a clinical setting. A "non-standard clinical dosing regimen," includes a "non-standard clinical dose" or a "non-standard dosing schedule".

A "low-dose amount", as used herein, refers to an amount or dose of a substance, agent, compound, or composition, which effectively inhibits the single mutant forms (L858R and delE746-A750) of EGFR, but which is an amount or dose lower than the amount or dose typically used in a clinical setting.

Those skilled in the art will be able to determine, according to known methods, the appropriate amount or dosage of each compound, as used in the combination of the present invention, to administer to a patient, taking into account factors such as age, weight, general health, the compound administered, the route of administration, the nature and advancement of the non-small cell lung cancer requiring treatment, and the presence of other medications.

The practice of the method of this invention may be accomplished through various administration regimens. The compounds of the combination of the present invention can be administered intermittently, concurrently or sequentially. Repetition of the administration regimens may be conducted as necessary to achieve the desired reduction or diminution of cancer cells. In an embodiment, the compounds of the combination of the present invention can be administered in an intermittent dosing regimen.

Administration of the compounds of the combination of the present invention can be effected by any method that enables delivery of the compounds to the site of action. These methods include oral routes, intraduodenal routes, parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion), topical, and rectal administration.

The compounds of the method or combination of the present invention may be formulated prior to administration. The formulation will preferably be adapted to the particular mode of administration. These compounds may be formulated with pharmaceutically acceptable carriers as known in the art and administered in a wide variety of dosage forms as known in the art. In making the pharmaceutical compositions of the present invention, the active ingredient will usually be mixed with a pharmaceutically acceptable carrier, or diluted by a carrier or enclosed within a carrier.

Such carriers include, but are not limited to, solid diluents or fillers, excipients, sterile aqueous media and various non-toxic organic solvents. Dosage unit forms or pharmaceutical compositions include tablets, capsules, such as gelatin capsules, pills, powders, granules, aqueous and nonaqueous oral solutions and suspensions, lozenges, troches, hard candies, sprays, creams, salves, suppositories, jellies, gels, pastes, lotions, ointments, injectable solutions, elixirs, syrups, and parenteral solutions packaged in containers adapted for subdivision into individual doses.

Parenteral formulations include pharmaceutically acceptable aqueous or nonaqueous solutions, dispersion, suspensions, emulsions, and sterile powders for the preparation thereof. Examples of carriers include water, ethanol, polyols (propylene glycol, polyethylene glycol), vegetable oils, and injectable organic esters such as ethyl oleate. Fluidity can be maintained by the use of a coating such as lecithin, a surfactant, or maintaining appropriate particle size. Exemplary parenteral administration forms include solutions or suspensions of the compounds of the invention in sterile aqueous solutions, for example, aqueous propylene glycol or dextrose solutions. Such dosage forms can be suitably buffered, if desired.

Additionally, lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc are often useful for tableting purposes. Solid compositions of a similar type may also be employed in soft and hard filled gelatin capsules. Preferred materials, therefor, include lactose or milk sugar and high molecular weight polyethylene glycols. When aqueous suspensions or elixirs are desired for oral administration the active compound therein may be combined with various sweetening or flavoring agents, coloring matters or dyes and, if desired, emulsifying agents or suspending agents, together with diluents such as water, ethanol, propylene glycol, glycerin, or combinations thereof.

Methods of preparing various pharmaceutical compositions with a specific amount of active compound are known, or will be apparent, to those skilled in this art. For examples, see Remington's Pharmaceutical Sciences, Mack Publishing Company, Easter, Pa., 15th Edition (1975).

The invention also relates to a kit comprising the therapeutic agents of the combination of the present invention and written instructions for administration of the therapeutic agents. In one embodiment, the written instructions elaborate and qualify

the modes of administration of the therapeutic agents, for example, for simultaneous or sequential administration of the therapeutic agents of the present invention.

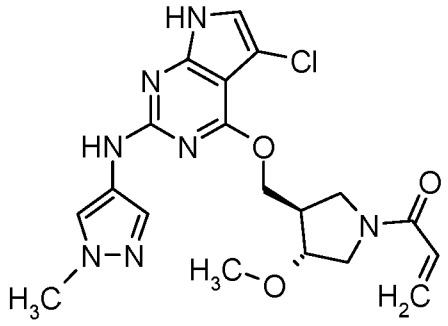
The examples and preparations provided below further illustrate and exemplify the compounds described herein and methods of preparing such compounds. The scope of the embodiments described herein is not limited in any way by the following examples and preparations. In the following examples, molecules with a single chiral center, unless otherwise noted, exist as a racemic mixture. Those molecules with two or more chiral centers, unless otherwise noted, exist as a racemic mixture of diastereomers. Single enantiomers/diastereomers may be obtained by methods known to those skilled in the art.

In the examples shown, salt forms were occasionally isolated as a consequence of the mobile phase additives during HPLC based chromatographic purification. In these cases, salts such as formate, trifluorooacetate and acetate were isolated and tested without further processing. It will be recognized that one of ordinary skill in the art will be able to realize the free base form by standard methodology (such as using ion exchange columns, or performing simple basic extractions using a mild aqueous base).

In general, the compounds described herein may be prepared by processes known in the chemical arts, particularly in light of the description contained herein. Certain processes for the manufacture of the compounds described herein are provided as further features of the embodiments and are illustrated in the reaction schemes provided below and in the experimental section.

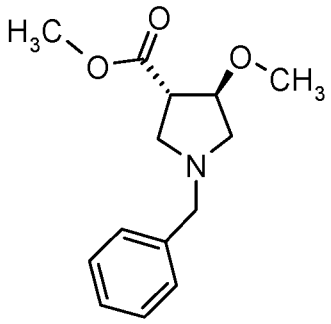
Examples

Example 1: Preparation of 1-{(3*R*,4*R*)-3-[5-chloro-2-(1-methyl-1*H*-pyrazol-4-ylamino)-7*H*-pyrrolo[2,3-d]pyrimidin-4-yloxymethyl]-4-methoxy-pyrrolidin-1-yl}propenone trifluoroacetate (also known as "1-{(3*R*,4*R*)-3-[({5-chloro-2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)methyl]-4-methoxypyrrolidin-1-yl}prop-2-en-1-one trifluoroacetate" and "1-((3R,4R)-3-(((5-chloro-2-((1-methyl-1H-pyrazol-4-yl)amino)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)oxy)methyl)-4-methoxypyrrolidin-1-yl)prop-2-en-1-one trifluoroacetate") (trifluoroacetate salt of "Compound A")



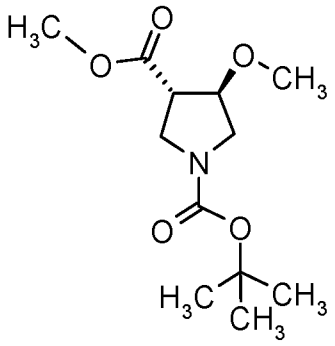
·TFA

Step 1: Preparation of (3*S*,4*R*)-1-benzyl-4-methoxy-pyrrolidine-3-carboxylic acid methyl ester



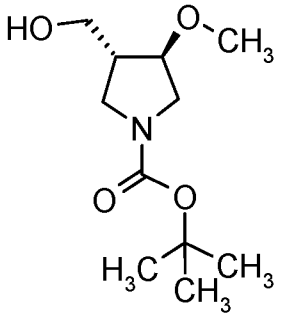
To a solution of (*E*)-3-methoxy-acrylic acid methyl ester (50 g, 430.6 mmol) in 2-Me-THF (600 mL) and TFA (6.7 mL) at 0 °C was added *N*-(methoxymethyl)-*N*-(trimethylsilylmethyl)-benzylamine (204 g, 2 eq) dropwise. After addition, reaction was allowed to warm to rt and stirred for 2 hrs. Reaction was transferred to a separatory funnel and washed with sat. $NaHCO_3$, sat. NaCl, then dried over Na_2SO_4 and the solvent removed to leave the crude racemic product as a yellow oil which was purified on SiO_2 (10 % – 35 % EtOAc/heptane) to give the racemic *trans* product as a yellow oil (82.7 g). Enantiomer separation by chiral-SFC (Chiralpak AD-H 4.6 x 250 mm column 4 % MeOH w/0.1 % diethylamine, 140 bar, 3.0 mL/min) gave the desired single isomer product which was verified by comparison with a known standard (34 g, 31.7 % yield). Specific rotation $[\alpha]_D^{27}$ = +23.8° (C=1.3, MeOH). ^{1}H NMR (400 MHz, DMSO-*d*6) δ ppm 2.55 - 2.63 (m, 2 H) 2.69 (dd, *J*=9.95, 6.42 Hz, 1 H) 2.82 - 2.88 (m, 1 H) 2.90 - 2.96 (m, 1 H) 3.23 (s, 3 H) 3.51 - 3.63 (m, 2 H) 3.66 (s, 3 H) 4.07 - 4.12 (m, 1 H) 7.22 - 7.39 (m, 5 H). *m/z* (APCI+) for ($C_{14}H_{19}NO_3$) 250.0 $(M+H)^+$.

Step 2: Preparation of (3*S*,4*R*)-4-methoxy-pyrrolidine-1,3-dicarboxylic acid 1-*tert*-butyl ester 3-methyl ester



A solution of (3*S*,4*R*)-1-benzyl-4-methoxy-pyrrolidine-3-carboxylic acid methyl ester (35 g, 140.4 mmol) in ethanol (500 mL) was purged with nitrogen and then $Pd(OH)_2$ (2 g, 0.1 eq) was added and the mixture stirred overnight under an atmosphere of hydrogen gas at approximately 15 psi (*via* hydrogen balloon). The reaction was then filtered through Celite and di-*tert*-butyldicarbonate (30.9 g, 1 eq) was added to the resulting filtrate slowly with stirring. After one hr the reaction was concentrated and the crude material was purified through a short silica column eluting with 10 % EtOAc/heptane for 2 volumes then 1:1 EtOAc/heptane until the product was completely eluted. Product fractions were combined and concentrated to give the title compound as a clear oil, (35.81 g, 98 % yield). ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 1.39 (s, 9 H) 3.17 (br. s., 1 H) 3.23 - 3.28 (m, 4 H) 3.35 - 3.53 (m, 3 H) 3.65 (s, 3 H) 4.06 (d, J=4.78 Hz, 1 H). *m/z* (APCI+) for product minus Boc ($C_7H_{13}NO_3$) 160.1 $(M+H)^+$. Specific Rotation: [a]D= -12.5 degrees (C=0.87, MeOH).

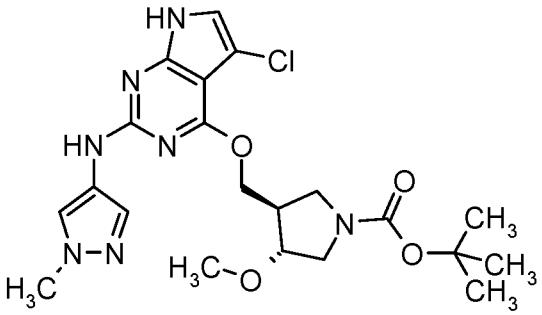
Step 3: Preparation of (3*R*,4*R*)-3-hydroxymethyl-4-methoxy-pyrrolidine-1-carboxylic acid *tert*-butyl ester



Lithium borohydride (12.7 g, 4 eq) was added portionwise to a solution of (3*S*,4*R*)-4-methoxy-pyrrolidine-1,3-dicarboxylic acid 1-*tert*-butyl ester 3-methyl ester (35.81 g, 138.1 mmol) in THF (600 mL), then the reaction was heated to 60 °C for 4 hrs. The reaction was quenched with water at 0 °C and extracted with EtOAc. The organic layer was washed with sat. NaCl and dried over Na_2SO_4. The solvent was removed and

the residue was purified through a plug of SiO_2 (3:1 EtOAc/heptane) to yield the title compound as a clear oil (29.35 g, 92 % yield). ^{1}H NMR (400 MHz, chloroform-d) δ ppm 1.46 (s, 9 H) 2.37 - 2.47 (m, 1 H) 3.19 (dd, J=11.08, 5.29 Hz, 1 H) 3.33 (d, J=4.03 Hz, 4 H) 3.50 - 3.66 (m, 4H) 3.77 - 3.83 (m, 1 H). *m/z* (APCI+) for product minus Boc ($C_6H_{13}NO_2$) 132.2 $(M+H)^+$. Specific Rotation: [a]D= +9.3 degrees (C=0.86, MeOH).

Step 4: Preparation of (3*R*,4*R*)-3-[5-chloro-2-(1-methyl-1*H*-pyrazol-4-ylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yloxymethyl]-4-methoxy-pyrrolidine-1-carboxylic acid *tert*-butyl ester



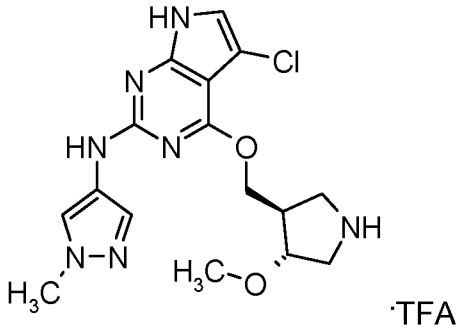
Method A: (using microwave heating)

To a solution 2,4,5-trichloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (904 mg, 4.1 mmol) and (3*R*,4*R*)-3-hydroxymethyl-4-methoxy-pyrrolidine-1-carboxylic acid *tert*-butyl ester (940 mg, 4.1 mmol) in 1,4-dioxane (15 mL) in a microwave vial was added potassium *tert*-pentoxide (25 % w/w in toluene, 1.6 mL, 3.5 mmol). The resulting solution was stirred at ambient temperature for 15 min. LCMS showed a quantitative formation of (3*R*,4*R*)-3-(2,5-dichloro-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yloxymethyl)-4-methoxy-pyrrolidine-1-carboxylic acid *tert*-butyl ester. To this resulting reaction solution was added 1-methyl-1*H*-pyrazol-4-ylamine (474 mg, 4.9 mmol) and t-BuXPhos palladacycle (110 mg, 0.04 mol eq). The reaction mixture was stirred and heated to 100 °C using microwave at normal absorption level for 45 min. The reaction mixture was filtered through Celite and the filtrate was evaporated to give a dark color residue. The crude material was purified *via* flash chromatography eluting with a gradient of 0 % – 100 % EtOAc in heptanes to give the title compound (1.78 g, 76 % yield). ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 11.50 (br. s., 1 H) 9.06 (s, 1 H) 7.85 (s, 1 H) 7.52 (s, 1 H) 7.05 (d, J=2.27 Hz, 1 H) 4.30 - 4.53 (m, 2 H) 3.86 - 3.96 (m, 1 H) 3.80 (s, 3 H) 3.55 - 3.68 (m, 1 H) 3.43 - 3.53 (m, 1 H) 3.24 - 3.31 (m, 3 H) 2.71 (br. s., 1 H) 1.39 (br. s., 9 H). *m/z* (APCI+) for product minus Boc; $C_{16}H_{20}ClN_7O_2$ 378.1 $(M+H)^+$ with Cl isotope pattern.

Method B: using thermal heating

To a solution 2,4,5-trichloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (9.28 g, 41.7 mmol) and (3*R*,4*R*)-3-hydroxymethyl-4-methoxy-pyrrolidine-1-carboxylic acid *tert*-butyl ester (9.65 g, 41.7 mmol) in 1,4-dioxane (100 mL) in a round bottom flask was added potassium *tert*-pentoxide (25 % w/w in toluene, 80 mL, 167 mmol). The resulting reaction solution was stirred at ambient temperature for 30 min. LCMS showed a quantitative formation of (3*R*,4*R*)-3-(2,5-dichloro-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yloxymethyl)-4-methoxy-pyrrolidine-1-carboxylic acid *tert*-butyl ester. To the resulting reaction solution was added 1-methyl-1*H*-pyrazol-4-ylamine (4.86 g, 50.1 mmol) and t-BuXPhos palladacycle (1.1 g, 1.67 mmol, 0.04 mol eq). The reaction mixture was stirred and heated to 90 °C in an oil bath for 1 hr. The reaction mixture was then filtered through Celite and the filtrate was evaporated to remove the volatiles to give a dark gum that was then dissolved in ethyl acetate (300 mL) and filtered through a silica gel plug. The filtrate was evaporated and the residue was purified *via* flash chromatography eluting with a gradient of 0 % – 100 % EtOAc in heptanes to give the title compound (12.4 g, 62 % yield). ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 11.51 (br. s., 1 H) 9.07 (s, 1 H) 7.86 (s, 1 H) 7.52 (s, 1 H) 7.06 (d, J=2.20 Hz, 1 H) 4.31 - 4.54 (m, 2 H) 3.92 (br. s., 1 H) 3.80 (s, 3 H) 3.55 - 3.68 (m, 1 H) 3.44 - 3.55 (m, 1 H) 3.30 (d, J=18.34 Hz, 3 H) 2.72 (br. s., 1 H) 1.39 (br. s., 9 H). *m/z* (APCI+) for $C_{21}H_{28}ClN_7O_4$ 378.2 $(M+H)^+$ with Cl isotope pattern.

Step 5: Preparation of [5-chloro-4-((3*R*,4*R*)-4-methoxy-pyrrolidin-3-ylmethoxy)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-(1-methyl-1*H*-pyrazol-4-yl)-amine trifluoroacetate

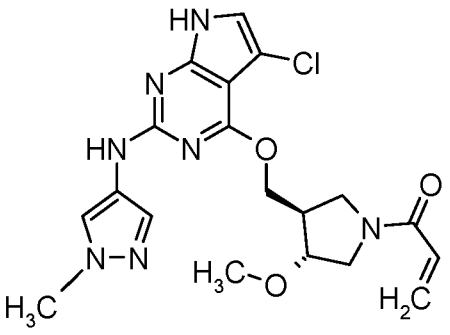


·TFA

To a solution of (3*R*,4*R*)-3-[5-chloro-2-(1-methyl-1*H*-pyrazol-4-ylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yloxymethyl]-4-methoxy-pyrrolidine-1-carboxylic acid *tert*-butyl ester (12.40 g, 26 mmol) in DCM (60 mL) at 0 °C was added TFA (10.1 mL, 208 mmol) and the resulting solution was stirred at ambient temperature for 2.5 hrs. The volatiles were removed and to the residue was added ethyl ether (150 mL). The resulting suspension was stirred for 2 hrs then filtered to afford a light pink solid. This was washed with ethyl ether (30 mL) and dried to give the title compound (15.69 g, quant) as

a TFA salt. ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 11.56 (br. s., 1 H) 9.09 (s, 3 H) 7.85 (s, 1 H) 7.54 (s, 1 H) 7.09 (d, J=2.32 Hz, 1 H) 4.48 (d, J=6.48 Hz, 2 H) 4.11 (br. s., 1 H) 3.81 (s, 3 H) 3.46 - 3.60 (m, 1 H) 3.35 - 3.45 (m, 2 H) 3.32 (s, 3 H) 3.15 (dq, J=12.01, 6.02 Hz, 1 H) 2.88 (m, J=6.42, 6.42 Hz, 1 H). *m/z* (APCI+) for parent molecule $C_{16}H_{20}ClN_7O_2$ 378.2 $(M+H)^+$ with Cl isotope pattern.

Step 6: Preparation of 1-{(3*R*,4*R*)-3-[5-chloro-2-(1-methyl-1*H*-pyrazol-4-ylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yloxymethyl]-4-methoxy-pyrrolidin-1-yl}propenone trifluoroacetate

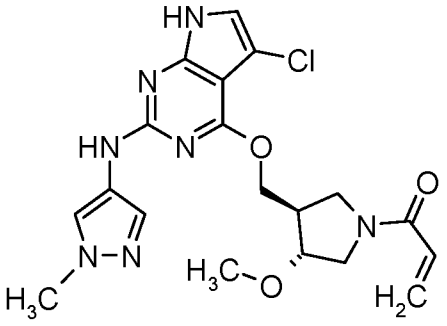


·TFA

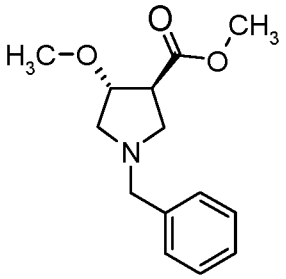
A mixture of [5-chloro-4-((3*R*,4*R*)-4-methoxy-pyrrolidin-3-ylmethoxy)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-(1-methyl-1*H*-pyrazol-4-yl)-amine (15.0 g (2 TFA salt)), 24.7 mmol), ethyl acetate (200 mL) and saturated aqueous $NaHCO_3$ (100 mL) was stirred at 0 °C for 10 min. Acryloyl chloride (2.3 mL, 29 mmol, 1.1 mol eq) was added dropwise and the resulting mixture was stirred at ambient temperature for 30 min. Ethyl acetate (150 mL) was added and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (150 mL) and the combined organic layers were dried over Na_2SO_4 and evaporated to give a solid that was purified by SFC (ZymorSPHER HAP 5µ 21.2 x 150 mm column eluting with 35 % EtOH in CO_2 at 120 bar, flow 64 mL/min) to give the title compound as an off white solid (8.3 g, 78 % yield). ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 11.51 (s, 1 H) 9.07 (s, 1 H) 7.86 (s, 1 H) 7.52 (s, 1 H) 7.05 (s, 1 H) 6.59 (ddd, J=16.75, 10.27, 1.34 Hz, 1 H) 6.14 (dd, J=16.75, 2.32 Hz, 1 H) 5.68 (dt, J=10.27, 2.32 Hz, 1 H) 4.44 (d, J=6.24 Hz, 2 H) 3.82 - 4.09 (m, 2 H) 3.80 (s, 3 H) 3.57 - 3.76 (m, 2 H) 3.47 - 3.54 (m, 1 H) 3.31 (d, J=4.65 Hz, 3 H) 2.67 - 2.92 (m, 1 H). *m/z* (APCI+) for parent molecule $C_{19}H_{22}ClN_7O_3$ 431.9 $(M+H)^+$ with Cl isotope pattern.

Alternate Example 1: Preparation of 1-{(3*R*,4*R*)-3-[({5-chloro-2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)methyl]-4-

methoxypyrrolidin-1-yl}prop-2-en-1-one (also known as, "1-((3R,4R)-3-(((5-chloro-2-((1-methyl-1H-pyrazol-4-yl)amino)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)oxy)methyl)-4-methoxypyrrolidin-1-yl)prop-2-en-1-one") ("Compound A")



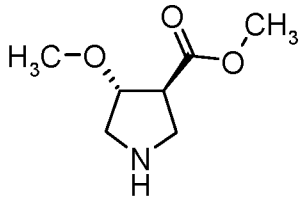
Step 1: Preparation of methyl (3,4-*trans*)-1-benzyl-4-methoxypyrrolidine-3-carboxylate



Under a nitrogen atmosphere with magnetic stirring, methyl *trans*-3-methoxyacrylate (500 mL, 540 g, 4.65 mol) and benzyl methoxymethyltrimethylsilylamine (595 mL, 552.1 g, 2.3 mol) were mixed. To this mixture was added TFA (2.7 mL, 4.14 g, 36.3 mmol) which resulted in an exotherm to approximately 95 °C in 30 seconds. The resulting mixture was then heated at reflux for 1 hr (note: at the beginning the reflux temperature was approximately at 104 °C and after 1 hr it had dropped to approximately at 90 °C). Three batches of this scale plus another batch using 325 mL benzyl methoxymethyltrimethylsilylamine compound were performed. Two of these batches were combined and poured into 2 N HCl (5 L). The mixture was extracted with EtOAc (3 L and 2 L). With cooling on ice, the aqueous layer was brought to pH 9 by adding 50 % NaOH (aq). The aqueous layer was extracted with EtOAc (2.5 L, 1.5 L and 1 L). The combined organic layers were washed with brine (3 L) and dried over Na_2SO_4. The same workup was done for the remaining batches. All organic layers were filtered and the filtrate was concentrated *in vacuo* to give crude title compound (racemic-trans, 1640 g). After purification in batches by bulb-to-bulb

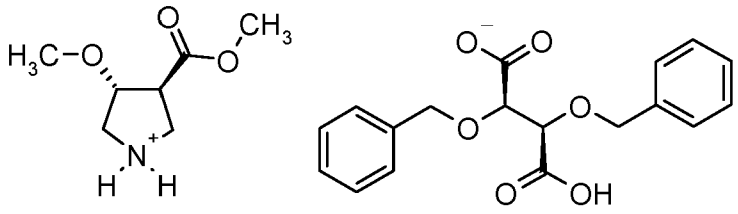
distillation (0.1 mbar, 100 °C – 145 °C) the title compound was isolated as a yellow oil (69 % overall yield).

Step 2: Preparation of methyl (3,4-*trans*)-4-methoxypyrrolidine-3-carboxylate



Methyl (3,4-*trans*)-1-benzyl-4-methoxypyrrolidine-3-carboxylate (463.3 g, 1858 mmol) was dissolved in iPrOH (2 L). To this solution was added 20 % $Pd(OH)_2/C$ (50 g, 37 % moist, Aldrich) and the mixture was stirred vigorously. A pressure of 11.8 bar H_2 was applied and refilling was done several times until ^{1}H-NMR showed complete conversion. The mixture was filtered through Celite and the Celite was rinsed with iPrOH. The filtrate was concentrated *in vacuo* to give the title compound as a dark yellow liquid (266 g, 90 % yield). Used as is in next step.

Step 3: Preparation of (3*R*,4*S*)-3-methoxy-4-(methoxycarbonyl)pyrrolidinium (2*R*,3*R*)-2,3-*bis*(benzyloxy)-3-carboxypropanoate



To a warm solution of O,O-dibenzoyl-L-tartaric acid (1 kg, 2.79 mol) in ethanol (5 L) was added a solution of methyl (3,4-*trans*)-4-methoxypyrrolidine-3-carboxylate (480 g, 2.84 mole) in ethanol (1 L). The clear solution was seeded and allowed to crystallize overnight. The resulting solid was isolated and washed with ethanol. This enriched material was recrystallized 5 times from ethanol (2 times 5 L, 4 L, 3.5 L and 3 L) to afford 216 g (15 % yield) of the salt with an enantiomeric excess of 98 % (Rt 12.18 min using condition below).

Chiral purity determination:

Sample preparation: 5 mg salt was mixed with DCM (1.5 mL) and 2 N NaOH (0.2 mL). The DCM layer was dried and analysed by gas chromatography.

Column: Agilent Cyclosil B; 30m x 250 cm x 0.25 cm

Temp: 90 °C (0 min) to 5 °C/min to 180 °C (4 min). Total run time 22 min.

Inj temp.: 250 °C

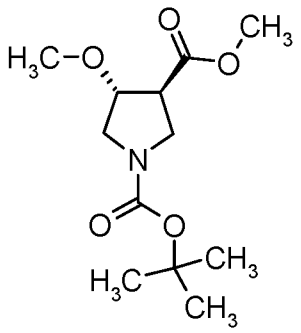
Detector: 250 °C; FID

Inj vol.: 1.0 µL

Split ratio: 25:1

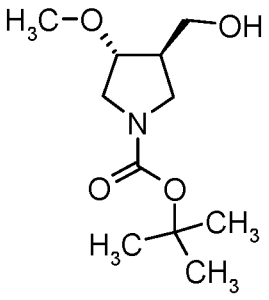
Column flow: 2.2 mL/min (H2)

Step 4: Preparation of 1-*tert*-butyl 3-methyl (3*S*,4*R*)-4-methoxypyrrolidine-1,3-dicarboxylate



A mixture of (3*R*,4*S*)-3-methoxy-4-(methoxycarbonyl)pyrrolidinium (2*R*,3*R*)-2,3-bis(benzyloxy)-3-carboxypropanoate (216 g, 420 mmol) in DCM and saturated $NaHCO_3$ was stirred mechanically, and Boc_2O (119 g, 546 mmol) was added portionwise. The mixture was stirred overnight at rt and the layers were separated. The aqueous phase was extracted with DCM and the combined organic layers washed with brine, dried and concentrated. This afforded 138 g of the crude title product mixed with 33 % Boc_2O (85 % yield). Used directly in next step.

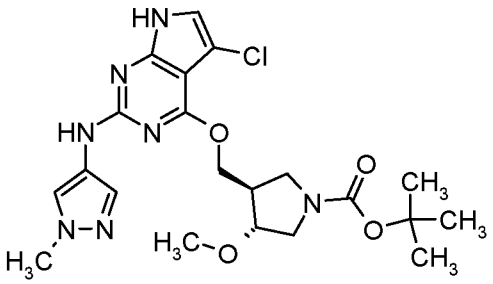
Step 5: Preparation of *tert*-butyl (3*R*,4*R*)-3-(hydroxymethyl)-4-methoxypyrrolidine-1-carboxylate



1-*Tert*-butyl 3-methyl (3*S*,4*R*)-4-methoxypyrrolidine-1,3-dicarboxylate (138 g, containing Boc_2O, approx. 2:1, ~ 0.35 mol) was dissolved in 2 L THF. The solution was mechanically stirred and cooled to -78 °C. A solution of lithium aluminium hydride (2.4 M in THF, 200 mL, 0.5 mol) was added dropwise over 30 min, keeping the temperature below -70 °C. The temperature was then allowed to rise to -30 °C. A saturated solution

of sodium potassium tartrate tetrahydrate (aqueous, 100 mL) was added slowly to quench the reaction. The solid material was filtered off over a bed of Na_2SO_4. The filtrate was concentrated *in vacuo* to give the title compound (66 g, 0.28 mol, ~ 80 % yield) as an almost colorless syrup. ^{1}H-NMR (300 MHz, CDCl3): ppm 3.80 (q, J= 5.1 Hz, 1H), 3.62 (d, J=6.2 Hz, 2H), 3.56 (m, 1H), 3.52 (d, J=7.4 Hz, 1H), 3.40-3.30 (m, 1H), 3.36 (s, 3H), 2.42 (m, 1H), 1.46 (s, 9H). *m/z* (GCMS) for $C_{11}H_{21}NO_4$ 231.2 $(M)^+$. *m/z* (APCI+) for $C_{11}H_{21}NO_4$ 132.0 $(M+H)^+$. Specific rotation: [a]D = +11.6 degrees (c 0.77, MeOH). Chiral purity determination method: Chiralpak AD-H 21.2 x 250 mm 5u column eluted with mobile phase of 12 % MeOH: 88 % CO_2 at 35 ºC and held to 120 bar. Flow rate of 62 mL/min. Rt at 3.36 min.

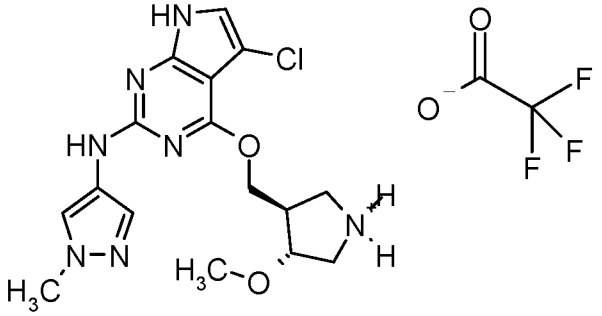
Step 6: Preparation of *tert*-butyl (3*R*,4*R*)-3-[({5-chloro-2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)methyl]-4-methoxypyrrolidine-1-carboxylate



To a solution 2,4,5-trichloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (9.28 g, 41.7 mmol) and *tert*-butyl (3*R*,4*R*)-3-(hydroxymethyl)-4-methoxypyrrolidine-1-carboxylate 9.65 g, 41.7 mmol) in 1,4-dioxane (100 mL) in a round bottom flask was added potassium tert-pentoxide (25 % w/w in toluene, 80 mL, 167 mmol). The resulting reaction solution was stirred at ambient temperature for 30 min. LCMS showed a quantitative formation of intermediate *tert*-butyl (3*R*,4*R*)-3-{[(2,5-dichloro-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy]methyl}-4-methoxypyrrolidine-1-carboxylate. To the above reaction solution was added 1-methyl-1*H*-pyrazol-4-ylamine (4.86 g, 50.1 mmol), t-BuXPhos palladacycle (1.1 g, 1.67 mmol, 0.04 mol eq) and the reaction mixture was stirred and heated to 90 °C in an oil bath for 1 hr. LCMS indicated the reaction was complete. The reaction mixture was filtered through Celite, and the Celite washed with ethyl acetate (200 mL). The combined filtrates were evaporated to remove the volatiles to give a dark color residue. This residue was dissolved in ethyl acetate (300 mL) and filtered through a silica gel plug. The filtrate was evaporated and the residue was purified *via* flash chromatography

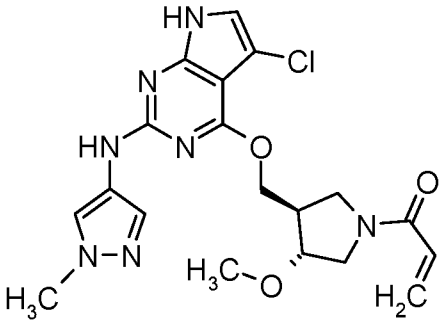
eluting with a gradient of 0 – 100 % EtOAc in heptanes to give the title compound (12.4 g, 62 % yield). ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 11.51 (br. s., 1 H) 9.07 (s, 1 H) 7.86 (s, 1 H) 7.52 (s, 1 H) 7.06 (d, J=2.20 Hz, 1 H) 4.31 - 4.54 (m, 2 H) 3.92 (br. s., 1 H) 3.80 (s, 3 H) 3.55 - 3.68 (m, 1 H) 3.44 - 3.55 (m, 1 H) 3.30 (d, J=18.34 Hz, 3 H) 2.72 (br. s., 1 H) 1.39 (br. s., 9 H). *m/z* (APCI+) for $C_{21}H_{28}ClN_7O_4$ 378.2 $(M+H)^+$ with Cl isotope pattern. Optical rotation: [a]d= -8.3 degrees (c=0.24, MeOH).

Step 7: Preparation of (3*R*,4*R*)-3-[({5-chloro-2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)methyl]-4-methoxypyrrolidinium trifluoroacetate



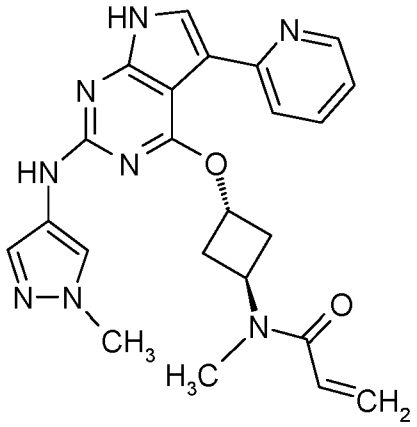
To a solution of *tert*-butyl (3*R*,4*R*)-3-[({5-chloro-2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)methyl]-4-methoxypyrrolidine-1-carboxylate (12.40 g, 26 mmol) in DCM (60 mL) in a water bath was added TFA (10.1 mL, 208 mmol) and the resulting solution was stirred at ambient temperature for 2.5 hrs. The volatiles were removed to give a residue to which was added ethyl ether (150 mL). The resulting suspension was stirred for 2 hrs and the light pink solid was collected by filtration, washed with ethyl ether (30 mL) and dried to give the title product (15.69 g, 100 % yield) ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 11.56 (br. s., 1 H) 9.09 (s, 3 H) 7.85 (s, 1 H) 7.54 (s, 1 H) 7.09 (d, J=2.32 Hz, 1 H) 4.48 (d, J=6.48 Hz, 2 H) 4.11 (br. s., 1 H) 3.81 (s, 3 H) 3.46 - 3.60 (m, 1 H) 3.35 - 3.45 (m, 2 H) 3.32 (s, 3 H) 3.15 (dq, J=12.01, 6.02 Hz, 1 H) 2.88 (m, J=6.42, 6.42 Hz, 1 H). *m/z* (APCI+) for parent molecule $C_{16}H_{20}ClN_7O_2$ 378.2 $(M+H)^+$ with Cl isotope pattern. Optical rotation: [a]d= -4.1 degrees (c=0.24, MeOH).

Step 8: Preparation of 1-{(3*R*,4*R*)-3-[5-chloro-2-(1-methyl-1*H*-pyrazol-4-ylamino)-7*H*-pyrrolo[2,3-d]pyrimidin-4-yloxymethyl]-4-methoxy-pyrrolidin-1-yl}prop2-en-1-one

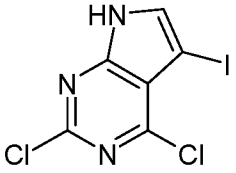


A mixture of (3*R*,4*R*)-3-[({5-chloro-2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)methyl]-4-methoxypyrrolidinium trifluoroacetate (15.0 g 24.7 mmol), ethyl acetate (200 mL) and saturated aqueous $NaHCO_3$ (100 mL) was stirred at 0 °C for 10 min. Acryloyl chloride (2.3 mL, 29 mmol, 1.1 mol eq) was added dropwise and the resulting mixture was stirred at ambient temperature for 30 min. Ethyl acetate (150 mL) was added and the organic layer was separated; the aqueous layer was extracted with ethyl acetate (150 mL) and the combined organic layers were dried over Na_2SO_4 and evaporated to give a solid, which was purified *via* flash chromatography eluting with a gradient of 0 – 50 % ethanol in ethyl acetate to give a white solid. This solid was then recrystallized from ethanol (10 mL of ethanol for 1 g of crude) with light heating using heat gun and seeded with crystal seeds. Upon cooling the white crystals were collected by filtration and washed with ethanol (3 mL of ethanol for 1 g of crude) to give the title compound (7.47 g, 70 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 11.50 (br. s., 1 H) 9.06 (s, 1 H) 7.85 (s, 1 H) 7.51 (s, 1 H) 7.04 (d, J=2.32 Hz, 1 H) 6.58 (ddd, J=16.78, 10.30, 1.16 Hz, 1 H) 6.13 (dd, J=16.81, 2.38 Hz, 1 H) 5.67 (dt, J=10.33, 2.23 Hz, 1 H) 4.43 (d, J=6.24 Hz, 2 H) 3.95 - 4.05 (m, 1 H) 3.68-3.85 (m, 4 H) 3.56 - 3.66 (m, 2 H) 3.44 - 3.53 (m, 1 H) 3.30 (d, J=4.65 Hz, 3 H) 2.68 - 2.90 (m, 1 H). *m/z* (APCI+) for parent molecule $C_{19}H_{22}ClN_7O_3$ 432.1 $(M+H)^+$ with Cl isotope pattern. Chiral purity determination: Whelk-O1 (R,R) 4.6 x 250 mm column 30 % EtOH at 140 bar, 3mL/min. Rt= ~8.8 min, Peak 1, >99 % ee. Optical rotation: [a]D22 = -3.1 degrees (c 0.14, EtOH). Elemental analysis: Theoretical: C, 52.84; H, 5.13; Cl, 8.21; N, 22.70;. Found: C, 52.45; H, 5.38; Cl, 7.91; N, 22.02.

Example 2: Preparation of *N*-methyl-*N*-[*trans*-3-({2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-5-(pyridin-2-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)cyclobutyl]prop-2-enamide ("Compound B")

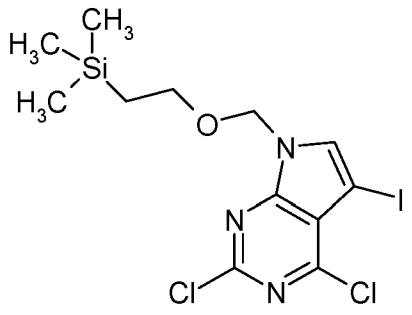


Step 1: Preparation of 2,4-dichloro-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidine



To a solution of 2,4-dichloro-7*H*-pyrrolo[2,3-*d*]pyrimidine, (50.0 g, 266 mmol, 1.00 equiv) in DMF (266 mL, 1.0 M) was added *N*-iodosuccinimide (62.8 g, 279 mmol, 1.05 equiv) at a rate such that the internal temperature was maintained below 50 °C. The reaction mixture was stirred vigorously and cooled in an ambient temperature bath for 1.5 hrs. The reaction mixture was diluted with ice water (1.5 L), and the resulting precipitate was isolated by filtration. The precipitate was washed with ice water (2 x 500 mL) and dried *in vacuo* at 45 °C for 36 hrs to give the title compound (81.5 g, 98 % yield) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 13.09 (br. s., 1 H), 7.95 (s, 1 H). *m/z* (APCI+) for $C_6H_2Cl_2IN_3$ 313.9 $(M+H)^+$.

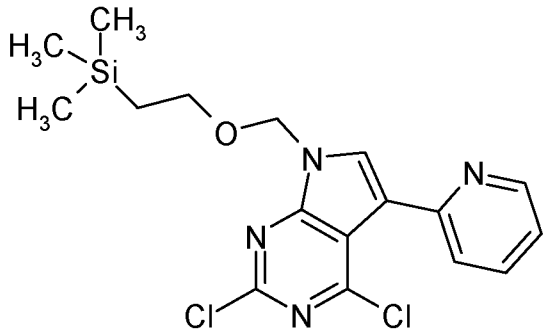
Step 2: Preparation of 2,4-dichloro-5-iodo-7-{[2-(trimethylsilyl)ethoxy]methyl}-7*H*-pyrrolo[2,3-*d*]pyrimidine



To a cooled (0 °C) solution of 2,4-dichloro-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidine (81.4 g, 259 mmol, 1.00 equiv) and diisopropylethylamine (105 mL, 596 mmol, 2.30 equiv) in THF (600 mL) was added 2-(trimethylsilyl)ethoxymethyl chloride (59.5 mL, 337

mmol, 1.30 equiv) in a dropwise manner over 5 min. The reaction mixture was allowed to stir at 0 °C for 3 hrs. At that time the reaction mixture was filtered, and the filtrate was concentrated *in vacuo*. The resulting thick oil was diluted with EtOAc (400 mL), washed sequentially with sat. aqueous NH_4Cl (2 x 200 mL) and brine (2 x 200 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The resulting material was dissolved in a minimum volume of DCM (50 mL) and diluted with heptane (200 mL). This solution was concentrated to a total volume of 150 mL which facilitated the trituration of the title compound. This mixture was filtered to give the title compound (84.1 g) and a filtrate that was concentrated and further purified *via* flash chromatography eluting with a gradient of 0 – 15 % EtOAc in heptane to provide an additional portion of the title compound (26.5 g). These two portions were combined to give the desired product (110.6 g) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.50 (s, 1 H), 5.57 (s, 2 H), 3.61 (t, J=8.0 Hz, 2 H), 0.95 (t, J=8.0 Hz, 2 H), 0.01 (s, 9 H). *m/z* (APCI+) for $C_{12}H_{16}Cl_2IN_3OSi$ 444.0 $(M+H)^+$.

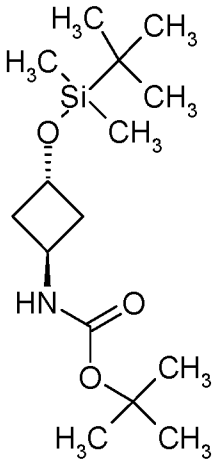
Step 3: Preparation of 2,4-dichloro-5-(pyridin-2-yl)-7-{[2-(trimethylsilyl)ethoxy]methyl}-7*H*-pyrrolo[2,3-*d*]pyrimidine



To a cooled (-78 °C) solution of 2,4-dichloro-5-iodo-7-{[2-(trimethylsilyl)ethoxy]methyl}-7*H*-pyrrolo[2,3-*d*]pyrimidine (30.0 g, 68.0 mmol, 1.00 equiv) in THF (350 mL) was added a solution of *i*-PrMgCl (47.3 mL, 94.6 mmol, 1.40 equiv, 1.00 M THF) in a dropwise manner over 4 min. The reaction mixture was stirred at -78 °C for 2 h and then treated with a freshly prepared solution of $ZnBr_2$ (24.7 g, 110 mmol, 1.62 equiv, dried at 130 °C) in THF (100 mL) in a dropwise manner over 15 min. The mixture was stirred at -78 °C for an additional 1 hr then warmed to ambient temperature and stirred for an additional 0.5 hrs. At this time the reaction mixture was treated with $Pd(PPh_3)_4$ (3.94 g, 3.38 mmol, 0.05 equiv) and 2-iodopyridine (10.8 mL, 101 mol, 1.50 equiv) and heated to 65 °C for 10 hrs. Upon cooling to ambient temperature, the reaction mixture was concentrated to a volume of ~200 mL, diluted with water (600

mL), sat. aqueous sodium potassium tartrate (100 mL) and EtOAc (400 mL). The layers were separated and the aqueous layer was extracted with EtOAc (4 x 300 mL). The combined organics were washed with brine (300 mL), dried (Na_2SO_4), and concentrated *in vacuo*. The resulting oil was purified *via* flash chromatography eluting with a gradient of 0 – 30 % EtOAc in heptane to provide the title compound (23.5 g, 87 % yield) as an oil that converted on standing to a light tan solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.76 - 8.63 (m, 1 H), 7.83 - 7.77 (m, 1 H), 7.74 (s, 1 H), 7.67 (d, J=7.8 Hz, 1 H), 7.35 - 7.29 (m, 1 H), 5.68 (s, 2 H), 3.61 (dd, J=7.6, 8.9 Hz, 2 H), 1.03 - 0.92 (m, 2 H), -0.01 (s, 9 H). *m/z* (APCI+) for $C_{17}H_{20}Cl_2N_4OSi$ 395.1 $(M+H)^+$.

Step 4: Preparation of *tert*-butyl (*trans*-3-{[*tert*-butyl(dimethyl)silyl]oxy}cyclobutyl)-carbamate

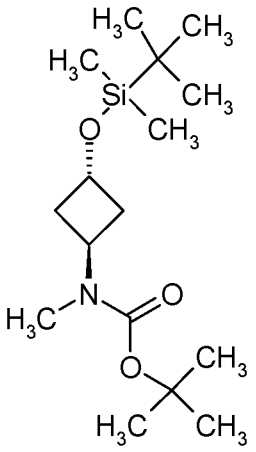


To a cooled (0 °C) solution of *tert*-butyl (*cis*-3-hydroxycyclobutyl)carbamate (62.0 g, 330 mmol, 1.00 equiv) and 4-nitrobenzoic acid (60.8 g, 360 mmol, 1.10 equiv) in THF (1.0 L) was sequentially treated with PPh_3 (130 g, 490 mol, 1.48 equiv) and diethyl azodicarboxylate (86.3 g, 490 mmol, 1.48 equiv). After the addition the reaction mixture was refluxed for 4 days, cooled to ambient temperature, and concentrated *in vacuo*. The residue was crystallized from *i*-PrOH to give a white solid (63 g).

To a solution of the above obtained 4-nitro benzoate ester (63 g) in MeOH (1.0 L) and H_2O (200 mL) was added K_2CO_3 (51.6 g, 370 mmol). The resulting mixture was refluxed for 2 hrs, cooled to ambient temperature, and filtered. The filtrate was concentrated *in vacuo*, and partitioned between EtOAc and aqueous 10 % Na_2CO_3. The resulting organic layer was washed with brine and concentrated to afford a white solid (31.0 g).

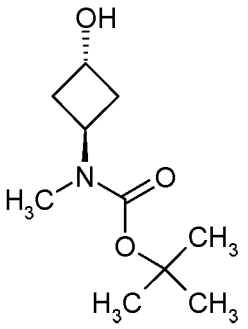
To a solution of the above obtained alcohol (75.0 g, 400 mmol, 1.00 equiv) in pyridine (1.0 L) was added TBSCl (91.0 g, 600 mmol, 1.50 equiv). The reaction mixture was stirred at ambient temperature for 2 hrs and then concentrated *in vacuo*. The resulting residue was purified *via* flash chromatography eluting with a gradient of 0 – 10 % EtOAc in petroleum ether to provide the title compound (111 g, 92 % yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 7.13 (d, 1 H) 4.38 - 4.47 (m, 1 H) 3.90 (br. s., 1 H) 2.00 - 2.16 (m, 4 H) 1.36 (s, 9 H) 0.81 - 0.89 (m, 9 H) -0.01 - 0.01 (m, 6 H). *m/z* (APCI+) for $C_{10}H_{23}NOSi$ 202.1 $(M\text{-}Boc+H)^+$.

Step 5: Preparation of *tert*-butyl (*trans*-3-{[*tert*-butyl(dimethyl)silyl]oxy}cyclobutyl)-methylcarbamate



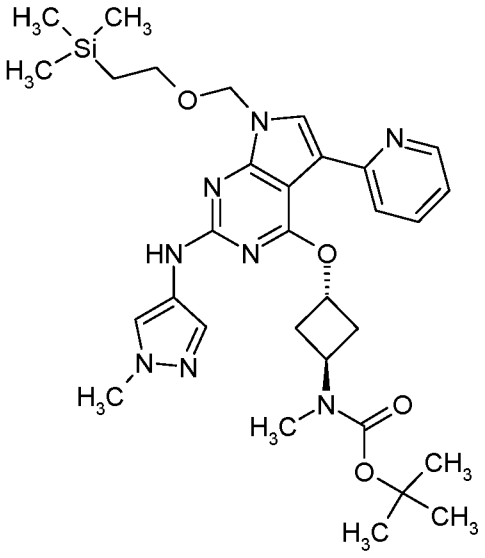
To a solution of *tert*-butyl (*trans*-3-{[*tert*-butyl(dimethyl)silyl]oxy}cyclobutyl)carbamate (111 g, 370 mmol, 1.00 equiv) in THF (1.0 L) was added NaH (60 % dispersion in oil, 22.2 g, 550 mmol, 1.50 equiv) in portions. After the addition, the reaction mixture was stirred for an additional 0.5 hrs, cooled (0 °C), and treated with methyl iodide (38.2 mL, 615 mmol, 1.66 equiv) in a dropwise manner. After an additional 5 hrs at ambient temperature, the reaction mixture concentrated *in vacuo*, and the resulting residue was purified *via* flash chromatography eluting with 10 % EtOAc in petroleum ether to provide the title compound (97.5 g, 84 % yield) as an oil. ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 4.64 (br. s., 1 H) 4.29 - 4.37 (m, 1 H) 2.73 (s, 3 H) 2.31 - 2.43 (m, 2 H) 1.97 - 2.08 (m, 2 H) 1.37 (s, 9 H) 0.86 (s, 9 H) -0.01 - 0.05 (m, 6 H). *m/z* (APCI+) for $C_{11}H_{25}NOSi$ 216.2 $(M\text{-}Boc+H)^+$.

Step 6: Preparation of *tert*-butyl (*trans*-3-hydroxycyclobutyl)methylcarbamate



To a solution of *tert*-butyl (*trans*-3-{[*tert*-butyl(dimethyl)silyl]oxy}cyclobutyl)-methylcarbamate (195 g, 600 mmol, 1.00 equiv) in THF (1.0 L) was added TBAF (930 mL, 930 mmol, 1.55 equiv, 1 M in THF). The reaction mixture was stirred at ambient temperature for 3 hrs and then concentrated *in vacuo*. The resulting residue was partitioned between EtOAc (1.0 L) and sat. aqueous NH_4Cl (500 mL). The resulting organic layer was concentrated *in vacuo*, and the resulting residue was purified *via* flash chromatography eluting with 10 % EtOAc in petroleum ether to provide the title compound (88 g, 76 % yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.78 (s, 1 H), 4.41-4.38 (m, 1 H), 2.82 (s, 3 H), 2.41-2.38 (m, 2 H), 2.23-2.20 (m, 2 H), 1.47(s, 9 H). *m/z* (ESI+) for $C_{10}H_{18}NO_3$ 146.1 $(M-{}^tBu+H)^+$.

Step 7: Preparation of *tert*-butyl methyl{*trans*-3-[(2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-5-(pyridin-2-yl)-7-{[2-(trimethylsilyl)ethoxy]methyl}-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy]cyclobutyl}carbamate

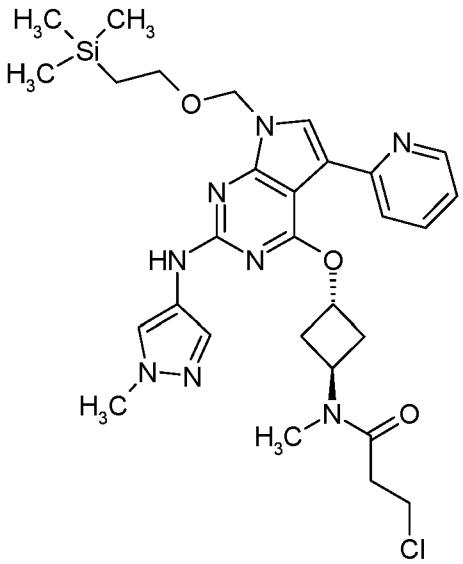


To a cooled solution of *tert*-butyl (*trans*-3-hydroxycyclobutyl)methylcarbamate (12.9 g, 64.0 mmol, 1.15 equiv) in THF (100 mL) was added potassium bis(trimethylsilyl)amide (12.4 g, 62.3 mmol, 1.12 equiv) in three portions. After the

addition, the alkoxide solution was allowed to warm to ambient temperature over 0.5 hr. A separate flask was charged with 2,4-dichloro-5-(pyridin-2-yl)-7-{[2-(trimethylsilyl)ethoxy]methyl}-7*H*-pyrrolo[2,3-*d*]pyrimidine (22.0 g, 55.6 mmol, 1.00 equiv) and THF (300 mL) and cooled (0 °C). This solution was treated with the alkoxide solution *via* cannula over 5 min, and then stirred for an additional 0.5 hr at 0 °C. The reaction mixture was then diluted with brine (100 mL), water (200 mL), and EtOAc (600 mL). The layers were separated and the aqueous layer was extracted with EtOAc (4 x 200 mL). The combined organic layer was then washed with brine (200 mL), dried (Na_2SO_4), and concentrated *in vacuo*. The resulting viscous oil (31.0 g) was used without further purification in the next step.

To a solution of the above obtained oil (31.0 g) in 1,4-dioxane (300 mL) was added 1-methyl-1*H*-pyrazol-4-amine (7.57 g, 77.9 mmol, 1.40 equiv), Pd_2dba_3 (2.68 g, 2.78 mmol, 0.05 equiv), 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (3.25 g, 5.56 mmol, 0.10 equiv), and Cs_2CO_3 (45.8 g, 139 mmol, 2.5 equiv). The reaction mixture was then sparged with a stream of nitrogen gas for 20 min and heated at 105 °C for 10 hrs with vigorous stirring. Upon cooling to ambient temperature, the reaction mixture was diluted with EtOAc (500 mL), filtered through Celite, and concentrated *in vacuo*. The resulting residue was purified *via* flash chromatography eluting with a gradient of 0 – 80 % EtOAc in heptane to provide the title compound (25.6 g, 74 % yield) as an orange foam. ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 9.17 (s, 1 H), 8.56 (d, J=4.3 Hz, 1 H), 8.14 (d, J=7.9 Hz, 1 H), 7.93 (br. s., 1 H), 7.87 - 7.79 (m, 1 H), 7.69 (s, 1 H), 7.55 (s, 1 H), 7.23 (dd, J=5.0, 7.2 Hz, 1 H), 5.57 (br. s., 2 H), 5.47 (br. s., 1 H), 4.84 - 4.66 (m, 1 H), 3.82 (s, 3 H), 3.63 - 3.53 (m, 2 H), 2.84 (s, 3 H), 2.75 - 2.62 (m, 2 H), 2.47 - 2.34 (m, 2 H), 1.38 (s, 9 H), 0.86 (t, J=8.0 Hz, 2 H), -0.11 (s, 9 H). *m/z* (APCI+) for $C_{31}H_{44}N_8O_4Si$ 621.3 $(M+H)^+$.

Step 8: Preparation of 3-chloro-*N*-methyl-*N*-{*trans*-3-[(2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-5-(pyridin-2-yl)-7-{[2-(trimethylsilyl)ethoxy]methyl}-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy]cyclobutyl}propanamide

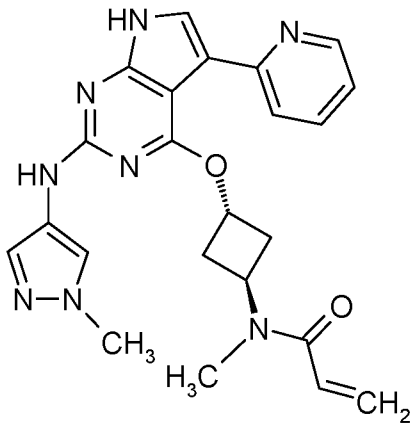


To a cooled (0 °C) solution of *tert*-butyl methyl{*trans*-3-[(2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-5-(pyridin-2-yl)-7-{[2-(trimethylsilyl)ethoxy]methyl}-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy]cyclobutyl}carbamate (25.0 g, 40.3 mmol, 1.00 equiv) in MeCN (600 mL) was added TFA (70.0 mL, 914 mmol, 22.7 equiv) in a dropwise manner. The reaction mixture was stirred at 0 °C and then allowed to warm to ambient temperature overnight. The reaction mixture was then cooled (0 °C), adjusted to pH = 8 with aqueous NaOH, and the phases separated. The aqueous phase was extracted with EtOAc (3 x 300 mL), and the combined organic phases were washed with brine (2 x 200 mL), dried (Na_2SO_4), and concentrated *in vacuo*. The resulting residue was partially purified by *via* flash chromatography eluting with a gradient of 2 – 7 % MeOH in DCM to provide 4-{[*trans*-3-(methylamino)cyclobutyl]oxy}-*N*-(1-methyl-1*H*-pyrazol-4-yl)-5-pyridin-2-yl-7-{[2-(trimethylsilyl)ethoxy]methyl}-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine as a yellow gum, which was used directly in the next step.

To a solution of 4-{[*trans*-3-(methylamino)cyclobutyl]oxy}-*N*-(1-methyl-1*H*-pyrazol-4-yl)-5-pyridin-2-yl-7-{[2-(trimethylsilyl)ethoxy]methyl}-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine and DIPEA (16.0 mL, 91.9 mmol, 1.61 equiv) in DCM (500 mL) was added 3-chloropropionyl chloride (8.25 mL, 86.4 mmol, 1.51 equiv) in a dropwise manner and then stirred at ambient temperature for 1 hr. The reaction mixture was then washed with H_2O (2 x 100 mL) and brine (100 mL), dried (Na_2SO_4), and concentrated *in vacuo*. The resulting gum was triturated with MTBE (250 mL), and the solid was filtered and dried *in vacuo* to give the title compound (24.0 g, 68.9 % yield) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 9.10 - 9.25 (m, 1 H) 8.51 - 8.61 (m, 1 H) 8.10 - 8.25 (m, 1 H) 7.92 - 8.05 (m, 1 H) 7.82 - 7.91 (m, 1 H) 7.67 - 7.76 (m, 1 H) 7.47 - 7.60 (m, 1 H) 7.17 -

7.30 (m, 1 H) 5.55 - 5.64 (m, 2 H) 5.40 - 5.54 (m, 1 H) 4.63 - 5.31 (m, 1 H) 3.83 (s, 3 H) 3.74 - 3.81 (m, 2 H) 3.53 - 3.66 (m, 2 H) 2.92 - 3.08 (m, 3 H) 2.81 - 2.89 (m, 2 H) 2.58 - 2.79 (m, 2 H) 2.29 - 2.46 (m, 2 H) 0.75 - 0.93 (m, 2 H) -0.10 (s, 9 H). *m/z* (APCI+) for $C_{29}H_{39}ClN_8O_3Si$ 611.2 $(M+H)^+$.

Step 9: Preparation of *N*-methyl-*N*-[*trans*-3-({2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-5-(pyridin-2-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)cyclobutyl]prop-2-enamide



To a solution of 3-chloro-*N*-methyl-*N*-{*trans*-3-[(2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-5-(pyridin-2-yl)-7-{[2-(trimethylsilyl)ethoxy]methyl}-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy]cyclobutyl}propanamide (24.0 g, 39.2 mmol, 1.00 equiv) in DCM/iPrOH (9:1, 250 mL) was added an HCl solution (250 mL, 4 M in 1,4-dioxane). The reaction mixture was stirred at ambient temperature overnight, concentrated *in vacuo* to afford 3-chloro-*N*-[*trans*-3-({7-(hydroxymethyl)-2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-5-pyridin-2-yl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)cyclobutyl]-*N*-methylpropanamide that was used in the next step without purification.

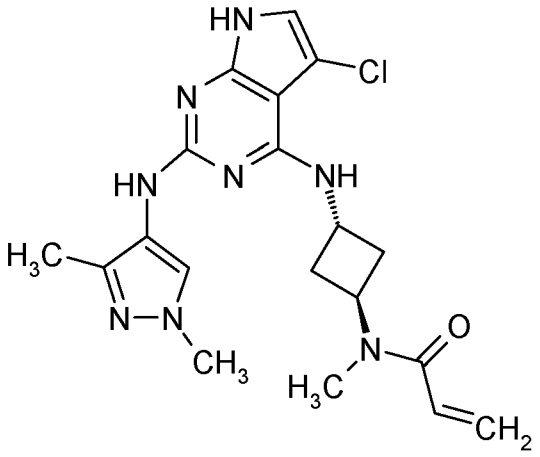
The intermediate from the previous step was dissolved in 1,4-dioxane (80 mL) and concentrated aqueous NH_4OH (50 mL). The reaction mixture was stirred at ambient temperature for 3 hrs and then concentrated *in vacuo*. The residue was triturated with MTBE (100 mL), and the solid filtered and dried *in vacuo* to give 3-chloro-*N*-methyl-*N*-[*trans*-3-({2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-5-pyridin-2-yl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)cyclobutyl]propanamide as a yellow solid that was used in the next step without further purification.

To a solution of 3-chloro-*N*-methyl-*N*-[*trans*-3-({2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-5-pyridin-2-yl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)cyclobutyl]propanamide in EtOH (400 mL) was added K_2CO_3 (21.4 g, 155 mmol, 3.95 equiv) and the reaction mixture was stirred at ambient temperature overnight. The reaction mixture was filtered

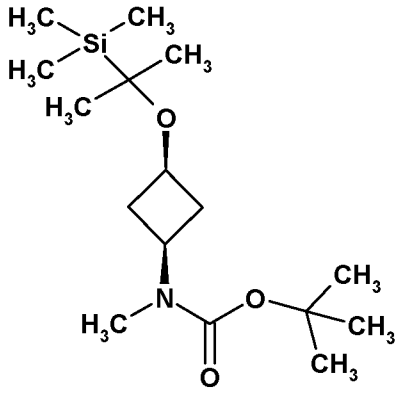
to remove inorganic salts, concentrated *in vacuo*, and dissolved in EtOAc (100 mL). MTBE (200 mL) was added to precipitate the crude product, which was collected by filtration. The filtrate was concentrated and purified *via* flash chromatography eluting with a gradient of 2 – 7 % MeOH in DCM to provide an additional portion of the crude product. The combined crude material was purified *via* reverse phase chromatography using a YMC-Actus Triact C18 (150 mm x 30 mm x 5 μm) column eluting with a gradient of 5% MeCN in H_2O (0.225 % HCOOH) to 25 % MeCN in H_2O (0.225 % HCOOH) to give the formate salt of the title compound (7.14 g, 37 % over 3 steps) as a yellow solid.

To a solution of the formate salt of the title compound (5.14 g) in H_2O (200 mL) was added sat. aqueous $NaHCO_3$ (100 mL) and EtOAc (200 mL) sequentially. The layers were separated, and the aqueous layer was extracted with EtOAc (8 x 100 mL). The combined organics were dried (Na_2SO_4) and concentrated *in vacuo* to give the title compound as an amorphous solid. A portion of this amorphous solid (~3 g) was dissolved in a minimum volume of EtOH/EtOAc (~1:1, ~120 mL), concentrated *in vacuo* to a volume of ~10 mL, and diluted with EtOAc (40 mL). This solution was seeded with ~5 mg of crystalline product and allowed to stir at ambient temperature overnight. The crystallization flask was cooled (0 °C) for 1 hr to promote further crystallization. The product was collected by filtration and dried *in vacuo* to give the title compound (2.63 g) as a white crystalline material containing EtOAc (0.038 equiv) and EtOH (0.03 equiv). mp = 204.9 °C. ^{1}H NMR (400 MHz, DMSO-d6, 30 °C) δ ppm 11.65 (s, 1 H), 8.93 (s, 1 H), 8.54 (d, J=3.9 Hz, 1 H), 8.14 (d, J=8.1 Hz, 1 H), 7.86 (s, 1 H), 7.82 (t, J=7.3 Hz, 1 H), 7.53 (s, 1 H), 7.52 (s, 1 H), 7.20 (ddd, J=1.0, 4.9, 7.4 Hz, 1 H), 6.71 (br. s., 1 H), 6.08 (br. s., 1 H), 5.66 (br. s., 1 H), 5.53 (br. s., 1 H), 5.31 - 4.79 (m, 1 H), 3.82 (s, 3 H), 3.15 - 2.93 (m, 3 H), 2.76 (br. s., 2 H), 2.46 (br. s., 2 H). ^{1}H NMR (400 MHz, DMSO-d6, 80 °C) δ ppm 11.41 (br. s., 1 H), 8.59 (s, 1 H), 8.55 - 8.52 (m, 1 H), 8.13 (d, J=8.1 Hz, 1 H), 7.84 (s, 1 H), 7.80 (dt, J=1.9, 7.7 Hz, 1 H), 7.55 (s, 1 H), 7.50 (s, 1 H), 7.18 (ddd, J=1.0, 4.8, 7.4 Hz, 1 H), 6.66 (dd, J=10.6, 16.8 Hz, 1 H), 6.05 (dd, J=2.3, 16.8 Hz, 1 H), 5.63 (dd, J=2.3, 10.5 Hz, 1 H), 5.59 - 5.53 (m, 1 H), 5.00 (t, J=8.0 Hz, 1 H), 3.82 (s, 3 H), 3.04 (s, 3 H), 2.82 - 2.71 (m, 2 H), 2.55 - 2.45 (m, 2 H). *m/z* (APCI+) for $C_{23}H_{24}N_8O_2$ 445.2 $(M+H)^+$. Elemental analysis: found C, 61.96; H, 5.50; N, 24.93. $C_{23}H_{24}N_8O_2$ + 0.038 EtOAc + 0.030 equiv EtOH requires C, 62.06; H, 5.49; N, 24.95.

Example 3: *N*-[*trans*-3-({5-chloro-2-[(1,3-dimethyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}amino)cyclobutyl]-*N*-methylprop-2-enamide ("Compound C")



Step 1: Preparation of *tert*-butyl methyl{*cis*-3-[1-methyl-1-(trimethylsilyl)ethoxy]cyclobutyl}carbamate

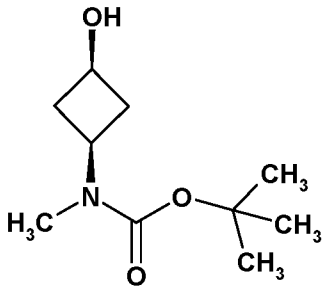


To a solution of *tert*-butyl (*cis*-3-hydroxycyclobutyl)carbamate (10.5 g, 56 mmol, 1.00 equiv) in pyridine (150 mL) was added TBSCl (12.7 g, 84 mmol, 1.50 equiv). After addition, the mixture was stirred at ambient temperature for 3 hrs. TLC (petroleum ether / EtOAc = 3/1) showed the starting material was completely consumed. The reaction mixture was concentrated, and the residue was extracted with EtOAc (3 x 100 mL). The combined organic layers were concentrated to give crude *tert*-butyl {*cis*-3-[1-methyl-1-(trimethylsilyl)ethoxy]cyclobutyl}carbamate as an oil, which was used for the next step without further purification.

Crude *tert*-butyl {*cis*-3-[1-methyl-1-(trimethylsilyl)ethoxy]cyclobutyl}carbamate was diluted in THF (300 mL) and treated with NaH (60 %, 3.36 g, 84 mmol, 1.50 equiv) in portions and stirred at rt for 30 min. The mixture was cooled to 0 °C and treated with methyl iodide (23.9 g, 168 mmol, 3.0 equiv) in a dropwise manner. After addition, the

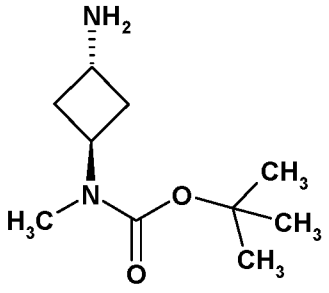
reaction mixture was stirred at ambient temperature for 5 hrs. TLC (petroleum ether / EtOAc = 10/1) indicated that the starting material had been consumed completely. The reaction mixture was concentrated and purified *via* silica gel chromatography (petroleum ether / EtOAc = 10/1) to give the title compound (18 g, 100 %) as an oil.

Step 2: Preparation of *tert*-butyl (*cis*-3-hydroxycyclobutyl)methylcarbamate



To a solution of *tert*-butyl methyl{*cis*-3-[1-methyl-1-(trimethylsilyl)ethoxy]cyclobutyl}carbamate (18 g, 56 mmol, 1.0 equiv) in THF (200 mL) was added TBAF (22.0 g, 84 mmol, 1.50 equiv) in portions. After addition, the mixture was stirred at ambient temperature for 3 hrs. TLC (petroleum ether: EtOAc = 2:1) showed the starting material was completely consumed. The reaction mixture was concentrated, and the residue was purified by column chromatography (petroleum ether: EtOAc = 2:1) to afford the title compound (8.9 g, 79 % yield) as a white solid.

Step 3: Preparation of *tert*-butyl (*trans*-3-aminocyclobutyl)methylcarbamate



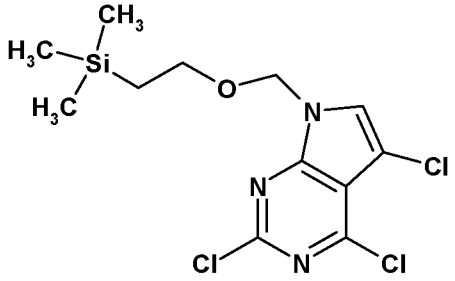
To a vigorously stirred cooled (-30 °C) solution of *tert*-butyl (*cis*-3-hydroxycyclobutyl)methylcarbamate (18.0 g, 0.089 mol, 1.0 equiv) and triethylamine (37 mL, 0.267 mol, 3.0 equiv) in DCM (300 mL) was added MsCl (14.1 g, 0.123 mol, 1.38 equiv) in a dropwise manner over 30 min. The reaction mixture was then allowed to warm to ambient temperature and stirred for 1 hr. The reaction mixture was diluted with water (100 mL) and DCM (200 mL). The organic phase was separated, washed with water (2 x 100 mL), sat. aqueous NH_4Cl (3 x 100 mL) and brine (100 mL), dried over anhydrous Na_2SO_4, and concentrated to give crude *cis*-3-[(*tert*-

butoxycarbonyl)(methyl)amino]cyclobutyl methanesulfonate as a yellow solid, which was used in the next step without further purification.

The above obtained crude *cis*-3-[(*tert*-butoxycarbonyl)(methyl)amino]cyclobutyl methanesulfonate was dissolved in DMF (250 mL) and treated with NaN_3 (28.77 g, 0.44 mol, 5 equiv). The resulting mixture was then heated to 70 °C and stirred overnight. After cooling, water (1500 mL) and EtOAc (300 mL) were added to the reaction mixture. The phases were separated and the aqueous layer was extracted with EtOAc (3 x 300 mL). The combined organic phases were washed with sat. aqueous $NaHCO_3$ (2 x 100 mL), water (2 x 200 mL) and brine (100 mL), dried over anhydrous Na_2SO_4, and evaporated to give a crude *tert*-butyl (*trans*-3-azidocyclobutyl)methylcarbamate, which was used directly in the next step.

To a mixture of the above crude *tert*-butyl (*trans*-3-azidocyclobutyl)methylcarbamate and Pd/C (2.5 g) in MeOH (100 mL) under a hydrogen atmosphere was added saturated NH_3 in MeOH (200 mL) *via* syringe. The resulting mixture was stirred at ambient temperature for 36 hrs. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. This crude material was purified by column chromatography with EtOAc/petroleum ether from 1/10 to 1/1 to afford the title compound (13.6 g, 76.4 % yield over 3 steps) as yellow liquid.

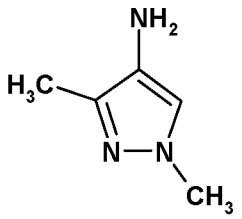
Step 4: Preparation of 2,4,5-trichloro-7-{[2-(trimethylsilyl)ethoxy]methyl}-7*H*-pyrrolo[2,3-*d*]pyrimidine



To a solution of 2,4-dichloro-7-{[2-(trimethylsilyl)ethoxy]methyl}-7*H*-pyrrolo[2,3-*d*]pyrimidine (100.0 g, 316 mmol, 1.0 equiv), as prepared in Example 2, step 1, in DMF (1800 mL) was added *N*-chlorosuccinimide (44.5 g, 332 mmol, 1.05 equiv) at ambient temperature. The resulting mixture was then stirred at 80 °C for 3 hrs. The reaction mixture was then cooled to ambient temperature and poured into ice water (3 L). The white precipitate formed was collected and dried *in vacuo* to give the title compound (99.7 g, 90 % yield) as a gray solid. ^1H NMR (400 MHz, $CDCl_3$) δ ppm = 7.35 (s, 1H),

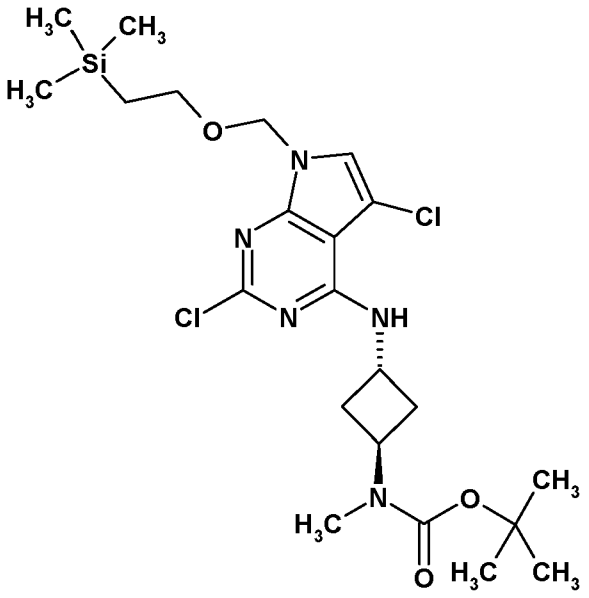
5.58 (s, 2H), 3.60 - 3.49 (m, 2H), 1.00 - 0.89 (m, 2H), -0.02 (s, 9H). *m/z* (APCI+) for $C_{12}H_{16}Cl_3N_3OSi$ 352.0 $(M+H)^+$.

Step 5: Preparation of 1,3-dimethyl-1*H*-pyrazol-4-amine



A solution of 1,3-dimethyl-1*H*-pyrazol-4-amine hydrochloride (800 mg) in MeOH (15 mL) was treated with hydroxide resin (Bio Rad AG 1-X2 resin, catolog #143-1255) until pH ~ 8 was obtained. The mixture was stirred for 15 min. The resin was filtered off and washed with several portions of MeOH. The filtrate was concentrated under reduced pressure to give the title compound (615.3 mg, 94 % yield). This material was used without further purification. ^{1}H NMR (400 MHz, DMSO-d6) δ ppm = 6.89 (s, 1H) 3.57 (s, 3H) 3.54 (br. s., 2H) 1.96 (s, 3H). *m/z* (APCI+) for $C_5H_9N_3$ 112.1 $(M+H)^+$.

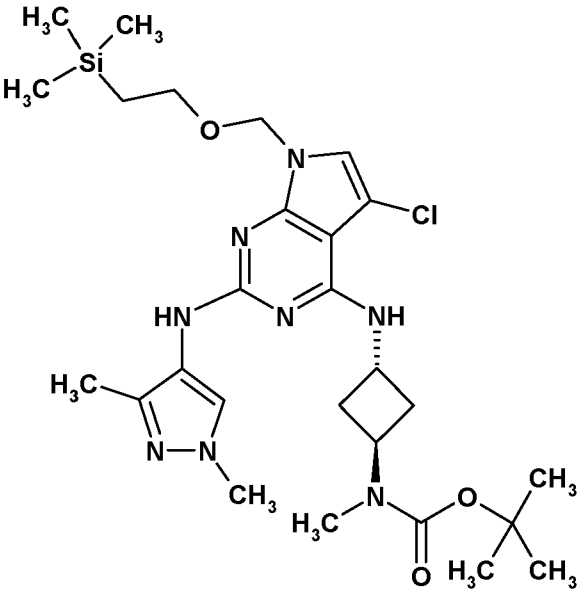
Step 6: Preparation of *tert*-butyl {*trans*-3-[(2,5-dichloro-7-{[2-(trimethylsilyl)ethoxy]methyl}-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino]cyclobutyl}methylcarbamate



A mixture of *tert*-butyl (*trans*-3-aminocyclobutyl)methylcarbamate (1020 mg, 5.1mmol, 1.2 equiv), 2,4,5-trichloro-7-{[2-(trimethylsilyl)ethoxy]methyl}-7*H*-pyrrolo[2,3-*d*]pyrimidine (1500 mg, 4.253 mmol, 1.0 equiv) and DIPEA (2.12 mL, 12.8 mmol, 3.0 equiv) in MeCN (21.0 mL, 0.2M) was heated at 80 °C for 5.5 hrs. The reaction mixture was diluted with water and extracted with EtOAc. The organic layer was dried over

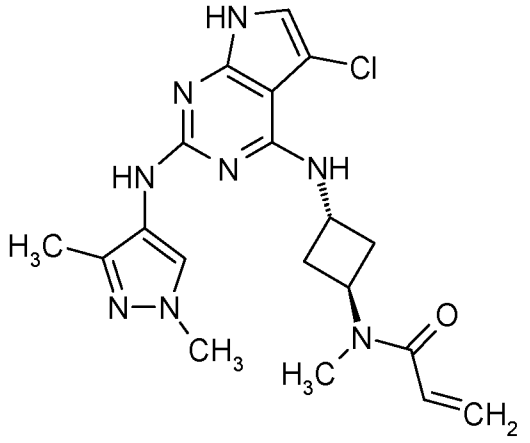
Na_2SO_4 and concentrated. The residue was purified *via* flash chromatography (10 to 30 % EtOAc in heptane) to give the tile compound (2.22 g, 100 % yield) as a clear gum. ^{1}H NMR (400 MHz, DMSO-d6) δ ppm = 7.54 (s, 1H) 7.05 (d, *J*=5.99 Hz, 1H) 5.40 (s, 2H) 4.74 (br. s., 1H) 4.41 - 4.54 (m, 1H) 3.45 - 3.54 (m, 2H) 2.83 (s, 3H) 2.52 - 2.63 (m, 2H) 2.28 - 2.42 (m, 2H) 1.40 (s, 9H) 0.78 - 0.89 (m, 2H) -0.08 (s, 9H). *m/z* (APCI+) for $C_{22}H_{35}Cl_2N_5O_3Si$ 516.2 $(M+H)^+$.

Step 7: Preparation of *tert*-butyl {*trans*-3-[(5-chloro-2-[(1,3-dimethyl-1*H*-pyrazol-4-yl)amino]-7-{[2-(trimethylsilyl)ethoxy]methyl}-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino]cyclobutyl}methylcarbamate



A flask containing 1,3-dimethyl-1*H*-pyrazol-4-amine (567 mg, 5.10 mmol, 1.2 equiv) and *tert*-butyl {*trans*-3-[(2,5-dichloro-7-{[2-(trimethylsilyl)ethoxy]methyl}-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino]cyclobutyl}methylcarbamate (2197 mg, 4.253 mmol, 1.00 equiv) was charged with $Pd_2(dba)_3$ (393 mg, 0.425 mmol, 0.1 equiv), Xantphos (259 mg, 0.425 mmol, 0.1 equiv) and Cs_2CO_3 (4160 mg, 12.8 mmol, 3.0 equiv). 1,4-Dioxane (42 mL, 0.1 M) was added and the mixture was heated to 105 °C for 18 hrs. The reaction was cooled to rt and filtered through a pad of Celite. The filtrate was concentrated, and the residue was purified *via* flash chromatography (40 – 60 % EtOAc in heptane) to give the title compound (1950 mg, 78 % yield) as a foam. ^{1}H NMR (400 MHz, DMSO-d6) δ ppm = 8.03 (s, 1H) 7.87 (s, 1H) 7.10 (s, 1H) 6.33 (d, J=6.24 Hz, 1H) 5.35 (s, 2H) 4.67 (br. s., 1H) 4.55 (br. s., 1H) 3.68 - 3.75 (m, 3H) 3.45 - 3.53 (m, 2H) 2.85 (s, 3H) 2.52 - 2.60 (m, 2H) 2.29 - 2.38 (m, 2H) 2.12 (s, 3H) 1.40 (s, 9H) 0.78 - 0.88 (m, 2H) -0.10 (s, 9H). *m/z* (APCI+) for $C_{27}H_{43}ClN_8O_3Si$ 591.3 $(M+H)^+$.

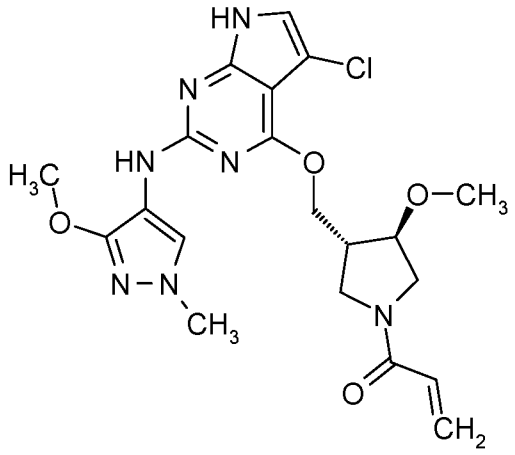
Step 8: Preparation of *N*-[*trans*-3-({5-chloro-2-[(1,3-dimethyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}amino)cyclobutyl]-*N*-methylprop-2-enamide



To a cooled (0 °C) solution of *tert*-butyl {*trans*-3-[(5-chloro-2-[(1,3-dimethyl-1*H*-pyrazol-4-yl)amino]-7-{[2-(trimethylsilyl)ethoxy]methyl}-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino]cyclobutyl}methylcarbamate (1950 mg, 3.30 mmol, 1.0 equiv) in DCM (42 mL) was added TFA (31 mL). The reaction mixture was allowed to come to rt and was stirred for an additional 16 hrs. The reaction mixture was then diluted with toluene (30 mL) and concentrated to dryness. The resulting crude residue was dissolved in 1,4-dioxane (20 mL) and concentrated aqueous NH_4OH (20 mL) and stirred at ambient temperature for 3 hrs. The reaction mixture was then evaporated to dryness. The resulting crude solid was partitioned between EtOAc (140 mL) and sat. aqueous Na_2CO_3 (140 mL) and treated with acryloyl chloride (0.420 mL, 5.19 mmol, 1.58 equiv) with vigorous stirring for 1 hr. At this time the layers were separated and the aqueous layer was extracted with EtOAc (50 mL). The combined organic layers were dried (Na_2SO_4), diluted with toluene (30 mL), and evaporated to dryness. The resulting solid was purified by SFC using a ZymorSpher HAP 150 x 21.2 mm column with 20 – 40 % EtOH @ 4 %/min, 140 bar, 55 mL/min to afford the title compound (950 mg, 69 % yield) as a grey powder. ^{1}H NMR (400 MHz, DMSO-d6, 26 °C) δ ppm = 11.16 (br. s., 1H), 7.80 (br. s., 1H), 7.77 (s, 1H), 6.88 (d, *J*=2.4 Hz, 1H), 6.74 (dd, *J*=10.5, 16.7 Hz, 1H), 6.32 (br. s., 1H), 6.07 (d, *J*=15.9 Hz, 1H), 5.66 (d, *J*=10.4 Hz, 1H), 5.30 - 4.75 (m, 1H), 4.59 (br. s., 1H), 3.71 (s, 3H), 3.15 - 2.88 (m, 3H), 2.62 (br. s., 2H), 2.40 (br. s., 2H), 2.08 (s, 3H). ^{1}H NMR (400 MHz, DMSO-d6, 80 °C) δ ppm = 10.93 (br. s., 1H), 7.73 (s, 1H), 7.41 (br. s., 1H), 6.82 (d, *J*=2.2 Hz, 1H), 6.68 (dd, *J*=10.5, 16.9 Hz, 1H), 6.16 (d, *J*=5.9 Hz, 1H), 6.05 (dd, *J*=2.4, 16.8 Hz, 1H), 5.63 (dd, *J*=2.4, 10.5 Hz, 1H), 4.94 (t, *J*=8.0 Hz, 1H), 4.60 (dd, *J*=4.0, 8.7

Hz, 1H), 3.72 (s, 3H), 3.04 (s, 3H), 2.72 - 2.57 (m, 2H), 2.41 (ddd, *J*=4.5, 8.9, 13.6 Hz, 2H), 2.10 (s, 3H). *m/z* (APCI+) for $C_{19}H_{23}ClN_8O_3$ 415.1 $(M+H)^+$.

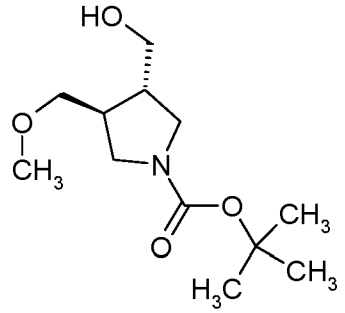
Example 4: Preparation of 1-{(3*R*,4*R*)-3-[({5-chloro-2-[(3-methoxy-1-methyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)methyl]-4-methoxypyrrolidin-1-yl}prop-2-en-1-one ("Compound D")



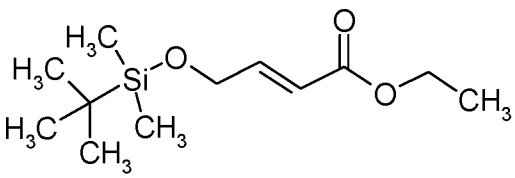
Prepared in a manner analogous to Example 1, substituting 1-methyl-1*H*-pyrazol-4-amine with 3-methoxy-1-methyl-1*H*-pyrazol-4-amine and other non-critical substitutions.

Experimental Procedures for Key Intermediates

Preparation 1. Preparation of *tert*-butyl-3-(hydroxymethyl)-4-(methoxymethyl)pyrrolidine-1-carboxylate

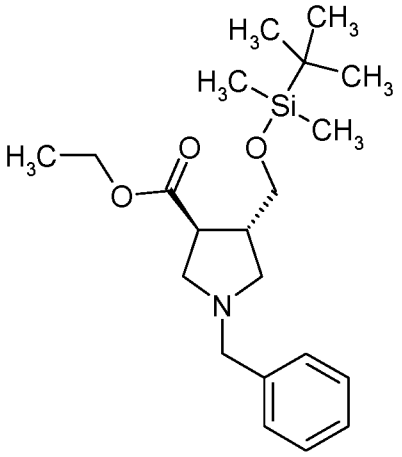


Step 1: Preparation of ethyl (2*E*)-4-{[*tert*-butyl(dimethyl)silyl]oxy}but-2-enoate



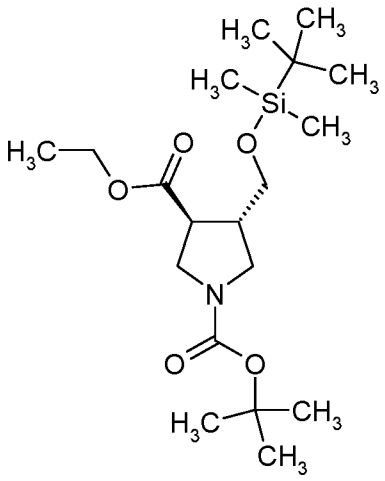
DIEA (2.75 mL, 16.6 mmol) and LiCl (5.54 g, 129 mmol) were added to a solution of *tert*-butyldimethylsilyloxy acetaldehyde (3.22 g, 18.5 mmol) and diethylmethylphosphonoacetate (4.66 g, 22.2 mmol) in CH_3CN (40 mL) and the mixture was stirred at rt for 24 hrs. The mixture was quenched with water (50 mL) and extracted with EtOAc (50 mL). The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified *via* flash chromatography eluting with 25 % EtOAc/heptane to give the title compound as a colorless oil (3.27 g, 72 % yield). ^{1}H NMR (400 MHz, chloroform-d) δ ppm 6.91 (dt, J=15.42, 3.49 Hz, 1 H) 6.01 (dt, J=15.61, 2.27 Hz, 1 H) 4.25 (dd, J=3.27, 2.27 Hz, 2 H) 4.12 (q, J=7.22 Hz, 2 H) 1.21 (t, J=7.18 Hz, 3 H) 0.84 (s, 9 H) 0.00 (s, 6 H).

Step 2: Preparation of *trans*-ethyl-1-benzyl-4-({[tertbutyl(dimethyl)silyl]oxy}methyl) pyrrolidine-3-carboxylate



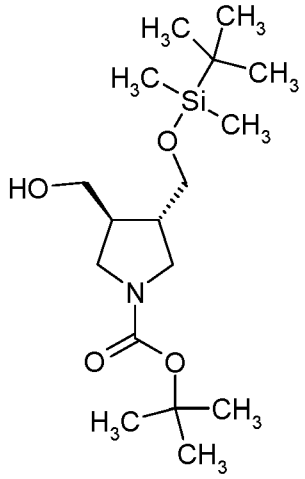
To a solution of ethyl (2*E*)-4-{[*tert*-butyl(dimethyl)silyl]oxy}but-2-enoate (3.27 g, 13.4 mmol) and *N*-benzyl-1-methoxy-*N*-((trimethylsilyl)methyl)methanamine (4.14 g, 17.5 mmol) in CH_2Cl_2 (30 mL) was added TFA (0.280 mL, 3.64 mmol) at 0 °C. The reaction was stirred at rt overnight. The mixture was quenched with water (50 mL) and extracted with EtOAc (two x 50 mL). The combined organic layers were dried over $MgSO_4$ and concentrated. The residue was purified *via* flash chromatography eluting with 20 % EtOAc/heptane to give the title compound as a pale yellow oil (2.61 g, 53 % yield). ^{1}H NMR (400 MHz, chloroform-d) δ ppm 7.08 - 7.41 (m, 5H), 4.10 (q, J = 7.13 Hz, 2H), 3.42 - 3.73 (m, 4H), 2.37 - 2.90 (m, 6H), 1.22 (t, J = 7.05 Hz, 3H), 0.84 (s, 9H), 0.00 (d, J = 1.26 Hz, 6H).

Step 3: Preparation of *trans*-1-*tert*-butyl 3-ethyl-4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl) pyrrolidine-1,3-dicarboxylate



To a solution of *trans*-ethyl-1-benzyl-4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl) pyrrolidine-3-carboxylate (*trans* mixture) (3.25 g, 8.61 mmol) in EtOH (40 mL) was added $Pd(OH)_2$ (300 mg) and Boc_2O (1.90 g, 8.61 mmol). The mixture was stirred under H_2 (50 psi, 50 °C) overnight. The mixture was filtered through Celite and the filtrate was concentrated. The residue was purified *via* flash chromatography eluting with 5 % –10 % EtOAc/heptane to give the title compound as a colorless oil (3.08 g, 92 % yield). ^{1}H NMR (400 MHz, chloroform-d) δ ppm 4.13 - 4.25 (m, 2 H) 3.65 (m, 5 H) 3.14 - 3.29 (m, 1 H) 2.84 - 3.00 (m, 1 H) 2.47 - 2.70 (m, 1 H) 1.46 (s, 9 H) 1.27 (td, J=7.11, 2.64 Hz, 3 H) 0.85 - 0.92 (m, 9 H) 0.05 (s, 6 H).

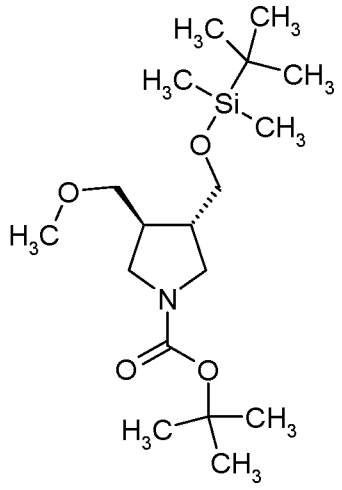
Step 4: Preparation of *trans-tert*-butyl-3-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-4-(hydroxymethyl)pyrrolidine-1-carboxylate



$LiBH_4$ (911 mg, 39.7 mmol) was added to a solution of *trans*-1-*tert*-butyl 3-ethyl-4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)pyrrolidine-1,3-dicarboxylate (3.08 g, 7.95 mmol) in THF (25 mL). The mixture was heated to reflux for 3 hrs. The reaction mixture was cooled to rt, then quenched with water (15 mL) and stirred at rt for 1 hr. The mixture

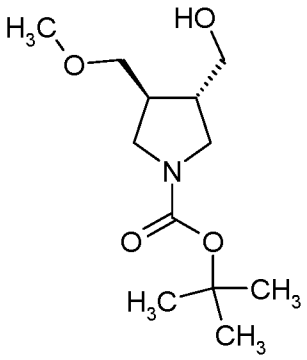
was diluted with water (60 mL) and extracted with ethyl acetate (two x 80 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to give a colorless oil. The crude product was purified *via* flash chromatography eluting with 30 % EtOAc/heptane to give the title compound as a colorless oil (2.34 g, 86 % yield). ^{1}H NMR (400 MHz, chloroform-d) δ ppm 3.73 (m, 1H), 3.61 (m, 2H), 3.52 (m, 2H), 3.45 (m, 1H), 2.90 - 3.09 (m, 2H), 2.04 - 2.32 (m, 2H), 1.46 (s, 9H), 0.92 (s, 9H), 0.10 (d, J = 1.01 Hz, 6H).

Step 5: Preparation of *trans-tert*-butyl-3-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-4-(methoxymethyl)pyrrolidine-1-carboxylate



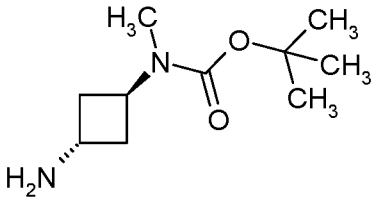
Tetrabutylammonium iodide (0.110 g, 0.28 mmol), 50 % aqueous NaOH (20 mL) and dimethyl sulfate (0.325 mL, 3.41 mmol) were added to a solution of *trans-tert*-butyl-3-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-4-(hydroxymethyl)pyrrolidine-1-carboxylate (0.982 g, 2.84 mmol) in CH_2Cl_2 (20 mL). The reaction was stirred at rt overnight. TLC showed some starting material remaining so additional dimethyl sulfate (0.150 mL) was added to the reaction mixture and stirred at rt for 3 hrs. Aqueous NH_3OH (30 mL) was added to the reaction mixture and stirred at rt for 1 hr. The mixture was diluted with water (20 mL) and extracted with CH_2Cl_2 (two x 30 mL). The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified *via* flash chromatography eluting with 10 % EtOAc/heptane to give the title compound as a colorless oil (451 mg, 44 % yield). ^{1}H NMR (400 MHz, chloroform-d) δ ppm 3.60 - 3.70 (m, 1H), 3.55 (br. s., 2H), 3.37 - 3.48 (m, 1H), 3.34 (m, 4H), 3.05 - 3.23 (m, 2H), 2.22 - 2.40 (m, 1H), 2.07 - 2.21 (m, 1H), 1.43 - 1.49 (m, 9H), 0.89 (s, 9H), 0.05 (s, 6H).

Step 6: Preparation of *trans-tert*-butyl-3-(hydroxymethyl)-4-(methoxymethyl)pyrrolidine-1-carboxylate

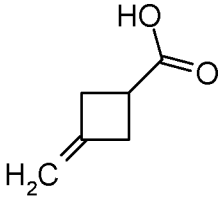


TBAF (1.0 M in THF, 2.45 mL, 2.45 mmol) was added to a solution of *trans-tert*-butyl-3-({[tert-butyl(dimethyl)silyl]oxy}methyl)-4-(methoxymethyl)pyrrolidine-1-carboxylate (290 mg, 0.81 mmol) in THF (5 mL). The mixture was stirred at rt for 1 hr. The mixture was quenched with water and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated. The crude product was used without purification in subsequent steps.

Preparation 2. Preparation of *tert*-butyl (*trans*-3-aminocyclobutyl)methylcarbamate

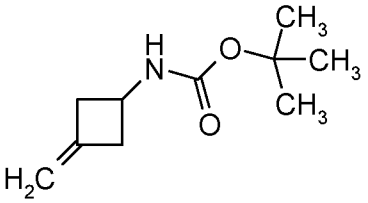


Step 1: Preparation of 3-methylidenecyclobutanecarboxylic acid



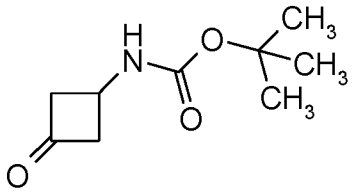
To a solution of 3-methylidenecyclobutanecarbonitrile (110 g, 1.18 mol) in ethanol (500 mL) and water (500 mL) was added potassium hydroxide (264 g, 4.7 mol) and the resulting mixture was refluxed overnight. The ethanol was removed under reduced pressure, and then the solution was cooled to below 10 °C and acidified with concentrated HCl to pH 1. The mixture was extracted with EtOAc (two x 500 mL) and the combined organic extracts were dried over anhydrous sodium sulfate and concentrated under vacuum to afford compound the title compound (132 g, 100 % yield) as yellow oil.

Step 2: Preparation of *tert*-butyl (3-methylidenecyclobutyl)carbamate



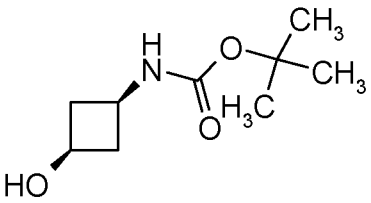
To a solution of 3-methylidenecyclobutanecarboxylic acid (132 g, 1.17 mol) and Et_3N (178 g, 1.76 mol) in *tert*-butyl alcohol (1 L) was added dropwise DPPA (574 g, 1.41 mol) and the resulting mixture was refluxed overnight. The mixture was then quenched with water (100 mL). After removal of the *tert*-butyl alcohol, the residue was treated with sat. NH_4Cl (500 mL), and the resulting solid precipitate was collected, washed with sat. NH_4Cl and sat. $NaHCO_3$ to give the title compound (165 g, 77 % yield) as a white solid.

Step 3: Preparation of *tert*-butyl (3-oxocyclobutyl)carbamate



To a solution of *tert*-butyl (3-methylidenecyclobutyl)carbamate (165 g, 0.91 mol) in CH_2Cl_2 (1000 mL) and MeOH (1000 mL) was bubbled O_3 at -78 °C until the solution turned blue. TLC (petroleum ether: EtOAc = 10:1) showed that the starting material was consumed completely. Nitrogen gas was then bubbled through the reaction to remove excess O_3, and then the mixture was quenched with Me_2S (200 mL) and stirred for an hour. The solution was concentrated to give a residue, which was washed with sat. $NaHCO_3$ and water to yield the title compound (118 g, 70 % yield) as a white solid.

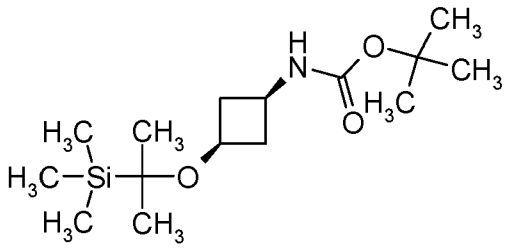
Step 4: Preparation of *tert*-butyl (*cis*-3-hydroxycyclobutyl)carbamate



To a solution of *tert*-butyl (3-oxocyclobutyl)carbamate (100 g, 54 mmol) in THF (2000 mL) at -72 °C was added dropwise a solution of lithium trisec-butylhidridoborate (648 mL, 1 M) in THF over 1.5 hrs. The resulting solution was allowed to warm up to rt and stirred for another 1 hr. TLC (petroleum ether: EtOAc = 2:1) showed that the starting material was consumed completely. The reaction was quenched with NH_4Cl aqueous. Water (1000 mL) and EtOAc (2000 mL) were added to the mixture. The

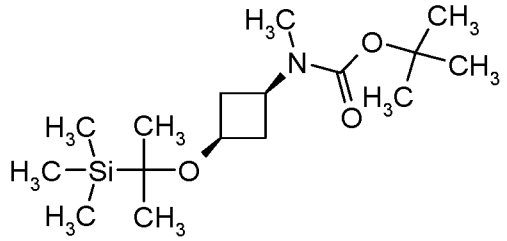
organic layer was separated, dried over $MgSO_4$ and concentrated to give crude material, which was purified by column chromatography with petroleum ether: EtOAc from 10:1 to 1:2 to afford the title compound (62 g, 61 % yield) as a white solid.

Step 5: Preparation of *tert*-butyl {*cis*-3-[1-methyl-1-(trimethylsilyl)ethoxy]cyclobutyl}carbamate



To a solution of *tert*-butyl (*cis*-3-hydroxycyclobutyl)carbamate (62 g, 0.33 mol) in pyridine (1 L) was added TBSCl (159 g, 1.056 mol). After addition, the mixture was stirred at ambient temperature overnight. TLC (petroleum ether: EtOAc = 2:1) showed the starting material was consumed completely. The reaction was then concentrated and diluted with EtOAc (1 L), and the organic layer was separated and washed with water (three x 300 mL) and brine (200 mL), dried over $MgSO_4$, filtered and concentrated to dryness to give crude title compound (108 g), which was used for the next step directly without further purification.

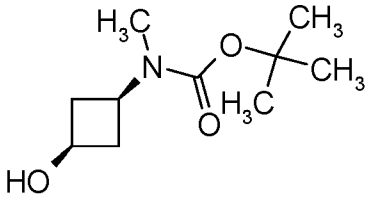
Step 6: Preparation of *tert*-butyl methyl{*cis*-3-[1-methyl-1-(trimethylsilyl)ethoxy]cyclobutyl}carbamate



To a solution of crude *tert*-butyl {*cis*-3-[1-methyl-1-(trimethylsilyl)ethoxy]cyclobutyl}carbamate (108 g) in THF (1 L) was added NaH (60 % in oil, 39.6 g, 0.99 mol) in portions and the resulting mixture was stirred at rt for 30 min. The mixture was then cooled to 0 ^oC and iodomethane (140.58 g, 0.99 mol) was added dropwise. After addition, the mixture was stirred from 0 ^oC to rt overnight. The mixture was quenched with sat. NH_4Cl, and water was added (200 mL), and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, then evaporated

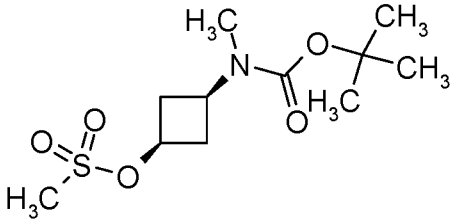
to give crude product which was purified *via* silica gel chromatography to give the title compound (68.9 g, 87 % yield) as an oil.

Step 7: Preparation of *tert*-butyl (*cis*-3-hydroxycyclobutyl)methylcarbamate



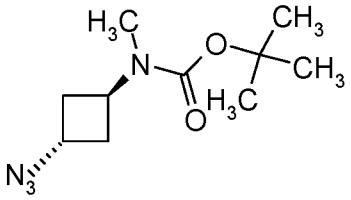
To a solution of *tert*-butyl methyl{*cis*-3-[1-methyl-1-(trimethylsilyl)ethoxy]cyclobutyl}carbamate (68.9 g, 0.217 mol) in pyridine (800 mL) was added TBAF (62 g, 0.24 mol) in portions. After addition, the mixture was stirred at rt for 2 hrs. The mixture was evaporated to dryness, and the residue was dissolved in 1000 mL of ethyl acetate and washed with conc. NH_4Cl (three x 200 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated to give the crude product, which was purified by column chromatography with EtOAc/petroleum ether from 1/20 to 1/5 to afford the title compound (26.3 g, 60 % yield) as a white solid.

Step 8: Preparation of *cis*-3-[(*tert*-butoxycarbonyl)(methyl)amino]cyclobutyl methanesulfonate



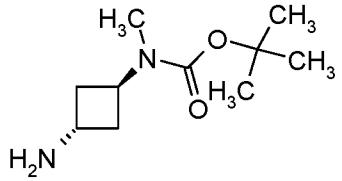
Triethylamine (4.14 mL, 29.79 mmol) was added into the solution of *tert*-butyl (*cis*-3-hydroxycyclobutyl)methylcarbamate (2.0 g, 9.93 mmol) in CH_2Cl_2 (30 mL) and the resulting mixture was cooled to -30 ^oC upon vigorous stirring. Mesyl chloride (1.36 g, 11.91 mmol) was added dropwise over a ten minute period. The mixture was then allowed to warm to rt and stirred for an hour until TLC analysis ($MeOH/CH_2Cl_2$ = 1/15) showed the reaction was complete. The reaction mixture was then washed with water (two x 10 mL), aq. NH_4Cl (10 mL), brine (10 mL), dried over anhydrous Na_2SO_4 and concentrated to give the title compound (2.5 g, 91 % yield) as yellow solid, which was used for next step directly.

Step 9: Preparation of *tert*-butyl (*trans*-3-azidocyclobutyl)methylcarbamate



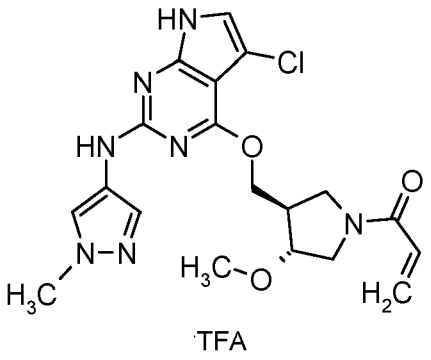
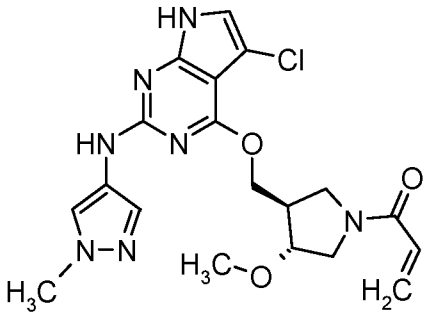
Cis-3-[(*tert*-butoxycarbonyl)(methyl)amino]cyclobutyl methanesulfonate (2.5 g, 8.94 mmol) was dissolved in DMF (25 mL) and NaN_3 (2.84 g, 43.69 mmol) was added. The resulting mixture was then heated to 70 °C and stirred overnight. After cooling, water (150 mL) was added and the mixture was extracted with EtOAc (three x 50 mL). The combined organic phases were washed with water (three x 20 mL) and brine (20 mL), dried over anhydrous Na_2SO_4, then concentrated *in vacuo* to give the title compound (1.8 g, 89 % yield) as a yellow liquid, which was used without further purification.

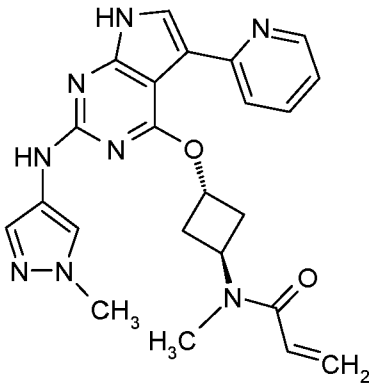
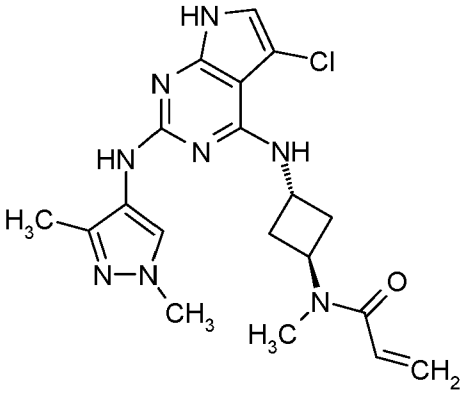
Step 10: Preparation of *tert*-butyl (*trans*-3-aminocyclobutyl)methylcarbamate

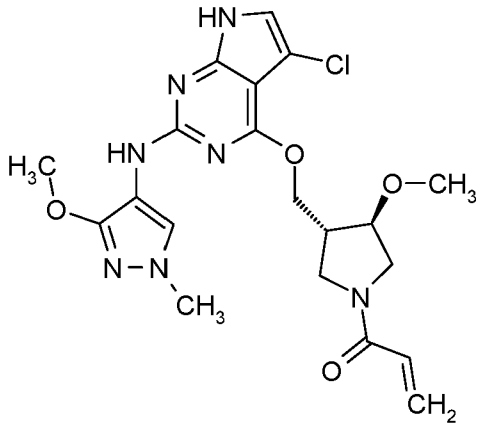


To the mixture of *tert*-butyl (*trans*-3-azidocyclobutyl)methylcarbamate (1.8 g, 7.95 mmol) and Pd/C (200 mg) in MeOH (5 mL) under hydrogen atmosphere (hydrogen balloon) was added NH_3(g)/MeOH (saturated, 50 mL) *via* syringe. The resulting mixture was stirred at rt for three hours until TLC analysis (EtOAc:petroleum ether = 1:2) showed the reaction was complete. Pd/C was filtered off and the resulting solution was concentrated and dried in vacuum to afford crude title compound (1.6 g), which was used for the next steps without further purification.

Table 1

Example No. (Scheme)	Structure and Compound Name	LRMS *m/z*	^{1}H NMR
1 **(Scheme F)** **TFA salt of Compound A**	 TFA 1-{(3*R*,4*R*)-3-[({5-chloro-2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)methyl]-4-methoxypyrrolidin-1-yl}prop-2-en-1-one trifluoroacetate	431.9	^{1}H NMR (400 MHz, DMSO-d6) δ ppm 11.51 (s, 1 H) 9.07 (s, 1 H) 7.86 (s, 1 H) 7.52 (s, 1 H) 7.05 (s, 1 H) 6.59 (ddd, J=16.75, 10.27, 1.34 Hz, 1 H) 6.14 (dd, J=16.75, 2.32 Hz, 1 H) 5.68 (dt, J=10.27, 2.32 Hz, 1 H) 4.44 (d, J=6.24 Hz, 2 H) 3.82 - 4.09 (m, 2 H) 3.80 (s, 3 H) 3.57 - 3.76 (m, 2 H) 3.47 - 3.54 (m, 1 H) 3.31 (d, J=4.65 Hz, 3 H) 2.67 - 2.92 (m, 1 H).
Alternate 1 **Compound A**	 1-{(3*R*,4*R*)-3-[({5-chloro-2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)methyl]-4-methoxypyrrolidin-1-yl}prop-2-en-1-one	432.1	^{1}H NMR (400 MHz, DMSO-d6) δ ppm 11.50 (br. s., 1 H) 9.06 (s, 1 H) 7.85 (s, 1 H) 7.51 (s, 1 H) 7.04 (d, J=2.32 Hz, 1 H) 6.58 (ddd, J=16.78, 10.30, 1.16 Hz, 1 H) 6.13 (dd, J=16.81, 2.38 Hz, 1 H) 5.67 (dt, J=10.33, 2.23 Hz, 1 H) 4.43 (d, J=6.24 Hz, 2 H) 3.95 - 4.05 (m, 1 H) 3.68-3.85 (m, 4 H) 3.56 - 3.66 (m, 2 H) 3.44 - 3.53 (m, 1 H) 3.30 (d, J=4.65 Hz, 3 H) 2.68 - 2.90 (m, 1 H).

Example No. (Scheme)	Structure and Compound Name	LRMS *m/z*	^{1}H NMR
2 **(Scheme I)** **Compound B**	 *N*-methyl-*N*-[*trans*-3-({2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-5-(pyridin-2-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)cyclobutyl]prop-2-enamide	445.1	^{1}H NMR (700 MHz, DMSO) δ ppm 11.68 (br. s., 1 H) 8.96 (s, 1 H) 8.55 (d, J=3.96 Hz, 1 H) 8.15 (d, J=7.70 Hz, 1 H) 7.75 - 7.94 (m, 2 H) 7.54 (d, J=8.36 Hz, 2 H) 7.21 (dd, J=6.93, 5.17 Hz, 1 H) 6.57 - 6.92 (m, 1 H) 5.92 - 6.22 (m, 1 H) 5.61 - 5.82 (m, 1 H) 5.54 (br. s., 1 H) 5.25 (br. s.,1 H) 3.83 (s, 3 H) 2.97 - 3.15 (m, 3 H) 2.76 (br. s., 2 H) 2.34 - 2.49 (m, 2 H)
3 **(Scheme B)** **Compound C**	 *N*-[*trans*-3-({5-chloro-2-[(1,3-dimethyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}amino)cyclobutyl]-*N*-methylprop-2-enamide	415.1	1H NMR (400 MHz, DMSO-d6) δ ppm 11.16 (br. s., 1 H) 7.77 (s, 2 H) 6.88 (s, 1 H) 6.74 (dd, J=16.63, 10.52 Hz, 1 H) 6.30 (d, J=6.11 Hz, 1 H)6.07 (d, J=15.89 Hz, 1 H) 5.66 (d, J=10.03 Hz, 1 H) 4.81 - 5.19 (m, 1 H) 4.59 (br. s., 1 H) 3.71 (s, 3 H) 2.93 - 3.15 (m, 3 H) 2.62 (br. s., 2 H) 2.39(br. s., 2 H) 2.08 (s, 3 H)

Example No. (Scheme)	Structure and Compound Name	LRMS *m/z*	^{1}H NMR
4 **(Scheme F)** **Compound D**	 1-{(3*R*,4*R*)-3-[({5-chloro-2-[(3-methoxy-1-methyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)methyl]-4-methoxypyrrolidin-1-yl}prop-2-en-1-one	462.1	^{1}H NMR (400 MHz, DMSO-d6) δ ppm 11.43 (br. s., 1 H) 7.80 (s, 1 H) 7.56 (d, J=2.27 Hz, 1 H) 6.91 (s, 1 H) 6.49 (dd, J=16.80, 10.23 Hz, 1 H) 6.04 (dd, J=16.67, 2.27 Hz, 1 H) 5.58 (dt, J=10.29, 1.80 Hz, 1 H) 4.21 - 4.39 (m, 2 H) 3.89 - 3.99 (m, 1 H) 3.79 - 3.87 (m, 1 H) 3.64 - 3.74 (m, 3 H) 3.62 (d, J=5.05 Hz, 1 H) 3.55 - 3.60 (m, 3 H) 3.45 - 3.54 (m, 1 H) 3.29 - 3.44 (m, 1 H) 3.20 (d, J=4.55 Hz, 3 H) 2.59 - 2.77 (m, 1 H)

Biological Examples

Example 5: *p*EGFR Y1068 ELISA Assay

In order to profile the effect of EGFR T790M inhibitors in cells with different EGFR mutation status, inhibition of phosphorylation of EGFR at Tyr1068 was determined in cells with wildtype EGFR and EGFR double mutants (L858R+T790M, EGFR delE746-A750 +T790M). Phosphorylation of EGFR at Y1068 was measured by PathScan® Phospho-EGF Receptor (Try1068) Sandwich ELISA kit (# 7240, Cell Signaling Technology®, Danvers, MA). The PathScan® Phospho-EGF Receptor (Tyr1068) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of phospho-EGF Receptor (Tyr1068) protein. The following cell lines were evaluated in this assay: A549 (EGFR wildtype, endogenous), NCI-H1975 (EGFR L858R+T790M, endogenous), NIH3T3/EGFR_wildtype, NIH3T3/EGFR L858R+T790M and PC9-DRH (EGFR delE746-

A750 +T790M). NIH/3T3 parental, A549, and NCI-H1975 cells were purchased from the American Type Culture Collection (Manassas, VA). All cells were cultured according to ATCC recommendations. A549 cells were grown in RPMI media (Invitrogen, Carlsbad) supplemented with 10 % FBS (Sigma, St Louis, MO), and with 1 % Penn/Strep (Invitrogen). NCI-H1975 cells were grown in RPMI (Invitrogen) supplemented with 10% FBS (Sigma), and with 1 % Penn/Strep (Invitrogen). NIH/3T3 cells were grown in DMEM (Invitrogen) supplemented with 10 % newborn calf serum (Invitrogen), and NIH3T3/EGFR mutant cells were grown in complete media with 5 µg/mL puromycin (Invitrogen). PC9-DRH cells were generated and cultured as described in Example 6. Plasmids (pLPCX) with various EGFR constructs were made by GenScript (Piscataway, NJ), and stable pools of NIH/3T3 cells expressing these constructs were made at Pfizer La Jolla. Cells were plated in complete culture media (50 µL/well) on the bottom of clear tissue culture treated microtiter plates (#3595, Corning Inc, Corning, NY) and allowed to adhere overnight at 37 °C, 5 % CO_2. Cells were seeded at the following concentrations: (A549: 40,000/well, NCI-H1975: 40,000/well, NIH3T3: 20,000/well, PC9-DRH: 50,000/well). The following day, compound dilution plates were prepared in 96 well clear V-bottom 0.5 mL polypropylene block plates (#3956, Corning, Inc). All cell lines were not evaluated for each compound. Each compound evaluated was prepared as a DMSO stock solution (10 mM). Compounds were tested in duplicate on each plate, with an 11-point serial dilution curve (1:3 dilution). Compound treatment (50 µL) was added from the compound dilution plate to the cell plate. The highest compound concentration was 1 or 10 µM (final), with a 0.3 % final DMSO (#D-5879, Sigma) concentration. Plates were then incubated for 2 hrs at 37 °C, 5 % CO_2. For NIH3T3/wildtype assay, cells were serum starved for 24 hrs prior to compound treatment; cells were treated in serum-free media as described and then stimulated for 10 min with EGF (100 ng/mL, Calbiochem/EMD Chemicals, Gibbstown, NJ). For A549/wildtype assay, cells were plated in full-serum (10 %) media for 24 hrs prior to compound treatment; cells were treated in full serum media as described and then stimulated for 10 min with EGF (40 ng/mL/starvation media, Invitrogen). Immediately prior to the end of the incubation, ice-cold lysis buffer was prepared (1x Cell Lysis Buffer (#9803, Cell Signaling Technology), 1 mM sodium orthovanadate (Na_3VO_4, #96508, Sigma), 1 mM phenylmethanesulfonyl fluoride (PMSF, 52332, CalBiochem/EMD Chemicals), complete Mini EDTA-free Protease Inhibitor Cocktail Tablet (1 tablet/10 mL, #11836170001, Roche, Indianapolis,

IN), and PhosSTOP Phosphatase Inhibitor Cocktail Tablet (1 tablet/10 mL, #04906837001, Roche) in pure water. At the end of 2 hrs, media was flicked off and cells were washed once with ice-cold 1 mM Na_3VO_4 in PBS (100 μL/well, Invitrogen). The wash was then flicked off and ice-cold lysis buffer was added to the cells (50 μL/well). The plate was shaken for 20-30 min at 4 °C to completely lyse the cells. Sample diluent (50 μL/well) was added to the ELISA plate, and the lysate (50 μL) was diluted into the sample diluent in each well of the ELISA plate. Plates were sealed and incubated overnight at 4 °C with shaking. The next day, wells were washed four times with 1x Wash Buffer; plates were taped on lint-free paper after the final wash prior to adding Add Detection Antibody (green, 100 μL/well) to each well and incubating for 1 hr at 37 °C. After incubation, wells were washed as described. HRP-Linked secondary antibody (red, 100 μL/well) was added to each well and incubated for 30 min at 37 °C. After incubation, the wells were washed as described. TMB Substrate (100 μL/well) was added to each well and the plate incubated for 10 minutes at 37°C or 30 minutes at room temperature maximum. Stop Solution (100 μL/well) was added to each well at the end of the incubation and plates were shaken gently for a few seconds. Absorbance was read at 450 nm within 30 min after addition of Stop Solution on a PerkinElmer EnVision Excite Multilabel Reader Method for Absorbance or on a Molecular Devices SpectraMax^{384} Reader for absorbance. Data were analyzed using a four-parameter fit in Microsoft Excel.

The results of the *p*EGFR Y1068 ELISA assays for the compounds tested are listed in Table 2. The pEGFR ELISA IC_{50} data shown in Table 2 for T790M_L858R is for 3T3 cell lines, unless otherwise indicated.

Table 2

Example Number	*pEGFR*Y1068 ELISA3T3T790M_ L858R IC_{50} (nM)	*pEGFRY1068 PC9-DRH* IC_{50} (nM)	*pEGFRY*1068 ELISAA549 IC_{50} (nM)
1	7	N/D	>4,287
Alternate 1		15	
2	6 (H1975)	N/D	1650
3	27	14	4286
4	12	6	>10,000

Example 6: Generation and Characterization of RPC9 and PC9-DRH Cells

Step 1: Generation of RPC9 cells from PC9 cells

In parental PC9 cells, the EGFR delE746-A750 mutant allele is amplified and no wild-type EGFR allele can be detected. Parental PC9 cells were utilized in the generation of the RPC9 cells. PC9 cells were cultured at 37 °C with 5 % CO_2 in RPMI 1640 medium supplemented with 10 % heat inactivated FBS. To generate EGFR inhibitor resistant cell lines, PC9 cells were initially treated with 0.5 nM dacomitinib. Once cells grew up to 90 % of confluence, they were split and the drug concentration was escalated by two-fold. After six weeks of such treatment, PC9 cells could grow in 2 nM dacomitinib. Single cell clones were generated and ten were selected for futher characterization. Those resistant cells were maintained in growth medium containing 2 μM erlotinib, and were named RPC9 for Resistant PC9.

Step 2: CastPCR analysis

Genomic DNA was extracted from clones of RPC9 cells using Qiagen DNA mini kit following manufacture's recommendations and subjected to castPCR (primer sets: Hs00000106_wt and Hs00000105) following the protocol from the manufacturer (ABI). Data were analyzed via ABI mutation detector software.

Step 3: Cell viability IC_{50} determination

3000 RPC9 cells per well were seeded in 90 μL of growth medium in duplicate wells of a 96 well plate (Corning). 24 hours later, cells were treated with dacomitinib or erlotinib in an 11 point titration of 3-fold dilution in 10 μL growth medium. The highest final concentration was 10 μM. After 72 hours of treatment, cells were analyzed via CTG assay (Promega) following manufacture's instructions.

Step 4: Characterization of RPC9 cells harboring EGFR T790M mutation

In the 10 clones that were generated in Step 1, Sanger sequencing identified the C>T mutation in EGFR exon20, which corresponds to the clinically relevant T790M mutation. Sequences of representative clones, RPC9 clone 3 and clone 6, are shown in Figure 1. Further validation via castPCR showed there were 10.2 % and 11.9 % of EGFR alleles harboring the EGFR T790M mutation in RPC9 clones 3 and 6, respectively (Figure 1). PC9 cells are very sensitive to dacomitinib (Figure 2A). Even the lowest concentration (0.17 nM) of dacomitinib inhibited 96 % of PC9 cell viability (Figure 2A). IC_{50} could not be calculated according to the dose response curve. RPC9 clones 3 and 6 were more resistant with IC_{50}s of 73 and 64 nM (Figure 2A). When

RPC9 clones 3 and 6 were treated with erlotinib, RPC9 clones 3 and 6 showed more than 200 fold increase in IC_{50} compared with the PC9 cells in the cell viability assay (Figure 2B).

Accordingly, RPC9 cells which harbor EGFR T790M mutation and are resistant to EGFR inhibitors such as dacomitinib and erlotinib were generated. The RPC9 cells contain a mixture of both single mutant (EGFR delE746-A750) and double mutant (EGFR delE746-A750 and T790M) EGFR alleles, since the EGFR T790M allele constitutes about 10 % of the total EGFR alleles in RPC9 cells.

Step 5: Generation and Characterization of PC9-DRH cells

In addition to RPC9 cells, PC9-DRH cells (DRH = dacomitinib resistant high T790M) were also generated. The RPC9 cell pool resistant to 2 nM dacomitinib was further challenged with increasing concentrations of dacomitinib from 2 nM to 2 μM in 8 weeks, as described in Step 1. PC9-DRH cells were maintained in growth medium, as described in Step 1, containing 2 μM dacomitinib. PC9-DRH cells were analyzed, as described in Steps 2, 3, and 4. PC9-DRH cells contain 70 % of their EGFR alleles as double mutant EGFR delE746-A750 and T790M. Similar to RPC9 cells, PC9-DRH cells are resistant to dacomitinib (IC_{50} = 1,651 nM), erlotinib (IC_{50} >10,000 nM), and gefitinib (IC_{50} >10,000 nM). When used in the pEGFR Y1068 ELISA assay as described in Example 5, the 2 μM dacomitinib was removed from growth medium and cells were allowed to grow for 36 hours prior to use in the ELISA assay.

Example 7: RPC9 Cell Viability utilizing EGFR T790M inhibitors alone or in combination with dacomitinib or erlotinib

3000 RPC9 cells, as prepared in Example 6, per well were seeded in 90 μL of growth medium into duplicate wells of a 96 well plate (Corning). 24 hours later, cells were treated with one of the EGFR T790M inhibitors, Compound A, Compound B, Compound C or Compound D in an 11 point titration of three-fold dilution with or without either 4 nM dacomitinib or 300 nM erlotinib in 10 μL growth medium. The highest final concentration was 10 μM of Compound A, Compound B, Compound C or Compound D. After 72 hours of treatment, cells were analyzed via CTG assay (Promega) following manufacture's instruction.

The free plasma concentration at steady-state from the standard clinical dosing regimen of dacomitinib and erlotinib are 4 nM and 300 nM, respectively. At those

concentrations, dacomitinib and erlotinib completely inhibited parental PC9 cell viability (Figure 2A and 2B). Neither drug significantly inhibited RPC9 cell viability at the same concentrations (Figure 2A and 2B).

The inhibition of viability in RPC9 clone 6 cells was potentiated by a combination of Compound A with either dacomitinib or erlotinib (Figures 3A and 3B). The viability IC_{50} of Compound A was 17 nM when combined with 4 nM dacomitinib and 15 nM when combined with 300 nM erlotinib (Table 3). The viability IC_{50}s for Compound A in combination decrease over 11 fold compared with that of Compound A treatment alone. Similarly, when RPC9 clone 6 cells were treated with Compound B, dacomitinib and erlotinib also sensitized RPC9 clone 6 to Compound B (Figures 4A and 4B). The IC_{50} of Compound B was 4 nM in combination with dacomitinib and 5 nM in combination with erlotinib (Table 3). The viability IC_{50}s decreased by 9.5 fold and 7.6 fold compared with that of Compound B treatment alone. Importantly, the projected human exposure for Compound A is 190 nM at which concentration Compound A alone inhibited cell viability about 40 %. When combined with dacomitinib or erlotinib, the same concentration of Compound A achieved maximal inhibition (83 %) (Figure 3A and 3B). Similarly for Compound B, the projected human exposure is 90 nM at which concentration, Compound B alone achieved 64 % inhibition. The combinations further potentiated the inhibition up to 84 % (Figure 4A and 4B). Thus, a combination with dacomitinib or erlotinib enhances the viability effect for Compound A and Compound B. In addition to Compound A and Compound B, Compound C and Compound D were synergistic with the clinically relevant concentrations of dacomitinib and erlotinib (Table 3).

Table 3. Viability IC_{50} of EGFR T790M inhibitors alone or in combination with dacomitinib or erlotinib in RPC9 clone 6.

Compound	IC_{50} (nM)		
	single agent	in combination with dacomitinib	in combination with erlotinib
dacomitinib	67		
erlotinib	6433		
Compound A	199	17	15
Compound B	38	4	5
Compound C	234	19	16
Compound D	441	30	21

In conclusion, compounds which specifically target the single mutant form of EGFR, such as dacomitinib and erlotinib, used at their clinically relevant concentrations potentiated compounds which preferentially inhibit the double mutant form of EGFR, such as Compound A, Compound B, Compound C and Compound D, in clinically relevant models that harbor both double mutant and single mutant forms of EGFR.

Example 8: RPC9 Clone 6 Cell Viability utilizing EGFR T790M inhibitors alone or in combination with dacomitinib, gefitinib, or afatinib

Using the method of Example 7, cells were treated with one of the EGFR T790M inhibitors, Compound A or Compound B, with or without either 4 nM dacomitinib, 20 nM gefitinib or 20 nM afatinib.

The free plasma concentration at steady-state from the standard clinical dosing regimen of dacomitinib is 4 nM. The free plasma concentration at steady-state from the standard clinical dosing regimen of gefitinib and afatinib is 20 nM.

The inhibition of viability in RPC9 clone 6 cells was potentiated by a combination of Compound A with either dacomitinib, gefitinib or afatinib (Figures 5A, 5B and 5C). Similarly, when RPC9 clone 6 cells were treated with Compound B, dacomitinib, gefitinib or afatinib also sensitized RPC9 clone 6 to Compound B (Figures 6A, 6B and 6C). Thus, each of Compound A and Compound B were synergistic with the clinically relevant concentrations of dacomitinib, gefitinib and afatinib (Figures 5 and 6). Similar to the discussion in example 7, the viability IC_{50}s of Compound A decreased 19 fold and 14 fold when combined with gefitinib and afatinib, respectively. The IC_{50}s of Compound B decreased 10 fold and 8 fold when combined with gefitinib and afatinib, respectively.

In conclusion, compounds which specifically target the single mutant form of EGFR, such as dacomitinib, gefitinib or afatinib, used at their clinically relevant concentrations potentiated compounds which preferentially inhibit the double mutant form of EGFR, such as Compound A and Compound B, in clinically relevant models that harbor both double mutant and single mutant forms of EGFR.

Example 9: EGFR T790M inhibitors in combination with dacomitinib or erlotinib in Allele Mixture Models

Methodology

Cell Culture: RPC9 clone 6 cells were generated and subcloned as described in Example 6. Cells were cultured in RPMI with 10 % FBS and were maintained under selective pressure (2 nM dacomitinib). For experiments, selective pressure was removed, cells plated onto 10 cm dishes and incubated overnight (37 °C, 5 % CO_2) to achieve 70-80 % confluence for treatments.

Treatments: Dacomitinib, erlotinib, Compound A, and Compound B were dissolved in 100 % DMSO. Dacomitinib (4 nM) and erlotinib (300 nM) were used at their free plasma exposure from standard clinical dosing regimen. Compound A and Compound B were used at a range starting below the target modulation IC_{50} value and up to the predicted clinical free plasma exposure for each compound, respectively. Cells were treated with dacomitinib or erlotinib and/or a titration of Compound A or Compound B, or with control (DMSO). Treatment was applied for 6 hours; at the end of the incubation period, cell pellets were collected and frozen until ready for analysis.

Immunoblotting: Cell pellets were treated with lysis buffer (150 mM NaCl, 1.5 mM $MgCl_2$, 50 mM HEPES, 10 % glycerol, 1 mM EGTA, 1 % Triton® X-100, 0.5 % NP-40) supplemented with 1 mM Na_3VO_4, 1 mM PMSF, 1 mM NaF, 1 mM β-glycerophosphate, protease inhibitor cocktail (Roche, Indianapolis, IN), and phosphatase inhibitor cocktail (Roche). Protein concentration of cell lysates was determined using the BCA Protein Assay (Pierce/Thermo Fisher Scientific, Rockford, IL) per the manufacturer's instructions. Protein (10 µg) was resolved by SDS-PAGE and transferred onto nitrocellulose membranes (Bio-Rad Criterion™ System, Hercules, CA). Blots were probed with primary antibodies to detect proteins of interest. EGFR, pEGFR Y1068, AKT, pAKT S473, ERK, and pERK T202/204 antibodies were purchased from Cell Signaling Technology, Inc (Danvers, MA). GAPDH antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). After incubation with secondary antibodies, membranes were visualized by chemiluminescence (Pierce/Thermo Fisher Scientific) and densitometry was performed on the FluorChem Q Imaging System (ProteinSimple, Santa Clara, CA).

Results

Cells treated with both dacomitinib and Compound A exhibited a decrease in pEGFR signaling that is consistent with a greater effect of combination versus single agent therapy at the two lowest doses (77 % inhibition with dacomitinib, 24 % inhibition with 10 nM Compound A versus 91 % with dacomitinib + 10 nM Compound A; 21%

inhibition with 30 nM Compound A versus 99 % with dacomitinib + 30 nM Compound A) (Figure 7A, 8A). These combinations exhibited greater than additive inhibition of pERK signaling at the lowest dose (34 % inhibition with dacomitinib, 27 % inhibition with 10 nM Compound A versus 100 % with dacomitinib + 10 nM Compound A; and an additive effect at the next highest dose of (34 % inhibition with dacomitinib, 64 % inhibition with 30 nM Compound A versus 99 % with dacomitinib + 30 nM Compound A; (Figure 7A, 8C). Higher doses with single agent therapy led to greater inhibition of pEGFR and pERK signaling; additivity calculations were constrained by the maximal inhibition observed in the assay. In contrast, inhibition of pAKT appeared to be additive at the lowest concentration of (37 % inhibition with dacomitinib, 22 % inhibition with 10 nM Compound A versus 64 % with dacomitinib + 10 nM Compound A; but achieved no greater than 60-65 % inhibition even at the higher doses (Figure 7A, 8B).

Cells treated with both erlotinib and Compound A exhibited a decrease in pEGFR signaling greater than the additive effect of single agent therapy at the three lowest concentrations of Compound A (61 % inhibition with erlotinib, 41 % inhibition with 10 nM Compound A versus 86 % inhibition with erlotinib + 10 nM Compound A; 27 % inhibition with 30 nM Compound A versus 91 % inhibition with erlotinib + 30 nM Compound A; 16 % inhibition with 100 nM Compound A versus 95 % inhibition with erlotinib + 100 nM Compound A) (Figure 7B, 9A). These treatments exhibited greater than additive inhibition of pERK signaling at the two lowest doses (31 % inhibition with erlotinib, 38 % inhibition with 10 nM Compound A (versus 100 % with erlotinib + 10 nM Compound A; 54 % inhibition with 30 nM Compound A versus 100 % with erlotinib + 30 nM Compound A; Figure 7B, 9C). Higher doses with single agent therapy led to greater inhibition of pEGFR and pERK signaling; additivity calculations were constrained by the maximal inhibition observed in the assay. In contrast, inhibition of pAKT appeared to be greater than additive at the lowest concentration of Compound A (18 % inhibition with erlotinib, 3 % inhibition with 10 nM Compound A versus 48 % with erlotinib + 10 nM Compound A; Figure 7B, 9B) and additive at the next highest dose (18 % inhibition with erlotinib, 31 % inhibition with 30 nM Compound A versus 49 % with erlotinib + 30 nM Compound A; (Figure 7B, 9B), but achieved no greater than 50 % inhibition even at the higher doses.

Cells treated with both dacomitinib and Compound B exhibited a decrease in pEGFR signaling greater than the additive effect of single agent therapy at the two lowest doses (54 % inhibition with dacomitinib, 46 % inhibition with 3 nM Compound B

versus 81 % with dacomitinib + 3 nM Compound B; 17 % inhibition with 10 nM Compound B versus 90 % with dacomitinib + 10 nM Compound B) and additive at the next highest dose (54 % inhibition with dacomitinib, 33 % inhibition with 30 nM Compound B versus 90 % with dacomitinib + 30 nM Compound B) (Figure 10A, 11A). These same cells exhibited an additive inhibition of pERK signaling at the lowest dose (57 % inhibition with dacomitinib, 55 % inhibition with 3 nM Compound B versus 100 % with dacomitinib + 3 nM Compound B; (Figure 10A, 11C). Higher doses with single agent therapy led to greater inhibition of pEGFR and pERK signaling; additivity calculations were constrained by the maximal inhibition observed in the assay. In contrast, inhibition of pAKT achieved no greater than 72 % inhibition even at the higher doses, and did not achieve partial or greater than additive results (Figure 10A, 11B).

Cells treated with both erlotinib and Compound B exhibited a decrease in pEGFR signaling greater than the additive effect of single agent therapy at all concentrations of Compound B (12 % inhibition with erlotinib, 2 % inhibition with 3 nM Compound B versus 74 % with erlotinib + 3 nM Compound B; 8 % inhibition with 10 nM Compound B versus 66 % inhibition with erlotinib + 10 nM Compound B; 22 % inhibition with 30 nM Compound B versus 82 % with erlotinib + 30 nM Compound B; 60 % inhibition with 100 nM Compound B versus 84 % with erlotinib + 100 nM Compound B) (Figure 10B, 12A). These treatments exhibited greater than additive inhibition of pERK signaling at the lowest dose of Compound B (41 % inhibition with erlotinib, 39 % inhibition with 3 nM Compound B versus 99 % with erlotinib + 3 nM Compound B; Figure 10B, 12C). Higher doses with single agent therapy led to greater inhibition of pEGFR and pERK signaling; additivity calculations were constrained by the maximal inhibition observed in the assay. In contrast, inhibition of pAKT appeared to be greater than additive at the lowest concentration of Compound B (29 % inhibition with erlotinib, 15 % inhibition with 3 nM Compound B versus 54 % with erlotinib + 3 nM Compound B; but achieved no greater than 62 % inhibition even at the higher doses (Figure 10B, 12B).

Example 10: EGFR T790M inhibitors in combination with dacomitinib or erlotinib in the RPC9 clone 6 (dacomitinib and erlotinib resistant) Xenograft Model

Background:

RPC9 clone 6 cells were generated from parental PC9 cells as described in Example 6. Parental PC9 cells contain EGFR delE746-A750 and are sensitive to the

treatments of dacomitinib and erlotinib. RPC9 clone 6 cell line was one of the selected resistant clones generated by dose-escalation treatment with dacomitinib ("daco"). RPC9 clone 6 cells contain approximately 10 % EGFR delE746-A750 and T790M and 90 % EGFR delE746-A750 alleles. Therefore, in the *in vitro* assays, RPC9 clone 6 was resistant to dacomitinib/erlotinib single agent treatments due to the EGFR delE746-A750 and T790M allele as well as resistant to Compound A ("compd A") and Compound B ("compd B") single agent treatments due to the EGFR delE746-A750 allele. Combination of Compound A or Compound B and clinically relevant concentrations of dacomitinib or erlotinib generated a synergistic effect on cell viability via synergistic inhibition of EGFR signal pathway. Therefore, the *in vivo* animal studies were performed to evaluate whether combination of Compound A or Compound B with dacomitinib or erlotinib would generate a synergistic anti-tumor effect in the RPC9 clone 6 xenograft model.

Methods:

Four- to six-week-old SCID beige female mice were obtained from Charles River lab and maintained in pressurized ventilated caging at the Pfizer La Jolla animal facility. All studies were approved by Pfizer Institutional Animal Care and Use Committees. Tumors were established by subcutaneously injecting $5x10^6$ RPC9 clone 6 cells suspended 1:1 (v/v) with reconstituted basement membrane (Matrigel, BD Biosciences). For tumor growth inhibition (TGI) studies, mice with established tumors of ~300 mm^3 were selected and randomized, then treated with EGFR T790M inhibitors as single agent or in combination with dacomitinib or erlotinib using the indicated doses and regimens. Tumor dimensions were measured with vernier calipers and tumor volumes were calculated using the formula of $\pi/6$ x larger diameter x $(\text{smaller diameter})^2$. Tumor growth inhibition percentage (TGI %) was calculated as 100 x $(1-\Delta T/\Delta C)$. Tumor regression percentage was calculated as 100 x (1-ΔT/starting tumor size).

Compound A was formulated in spray dried dispersion suspension in 0.5 % Methocel/20mM Tris Buffer at pH 7.4. Compound B was formulated in in-situ lactate salt solution with 0.5 % Methocel. Dacomitinib was formulated in 0.1 M lactic acid solution at pH 4.5. Erlotinib was formulated in 40 % Captisol®. All drugs were formulated and dosed at the concentration of 10 mL/kg. Tumor bearing mice were orally and daily administrated with indicated treatments; body weight and health observation were recorded daily.

Results:

Study I: Combination of Compound A and dacomitinib

In this study, RPC9 clone 6 tumor-bearing mice were randomized and treated with either single agent of Compound A or dacomitinib, or Compound A in combination with dacomitinib. Dacomitinib at 5 mg/kg gave an average unbound drug concentration of 4 nM in mouse plasma, which matches the average clinical exposure from the clinical dose of 45 mg/kg/day. The body weight change percentages were plotted in Figure 13B, and indicated that all dose groups of this study were well tolerated with body weight loss less than 10 %. Compound A was dosed at 500 mg/kg, 200 mg/kg, and 50 mg/kg as single agent or in combination with dacomitinib at 5 mg/kg as indicated in the Figure 13A. At study day 39, when the tumor sizes of vehicle group reached average 1200 mm^3, single agent treatment of dacomitinib generated a tumor growth stasis and single agent treatments of compound A generated a dose-dependent tumor growth inhibition as illustrated in Figure 13A and Table 4. Combination of all dose ranges of Compound A with dacomitinib generated complete tumor regression as illustrated in Table 4.

To further assess the combination effects on the tumor regression, mice in the single and combination treatment groups of Compound A at 200 mg/kg and 50 mg/kg continued to receive treatments until the study day 61. The tumor growth inhibition and tumor regression were calculated and illustrated in Table 4. Results indicate that (1) combination of compound A with dacomitinib generated complete tumor regression at both dose ranges, (2) tumors in the single agent treatment groups of compound A at both 200 mg/kg and 50 mg/kg progressed in a dose-dependent manner which mimics *in vitro* resistance probably driven by EGFR delE746-A750 allele, and (3) tumors in the single treatment group of dacomitinib also progressed which mimics the *in vitro* resistance probably driven by EGFR delE746-A750 and T790M allele in this RPC9 clone 6 xenograft model.

The treatment period was further extended to assess the *in vivo* resistance by single agent treatment and combination treatment. The single treatment group of dacomitinib continued to progress and was terminated at day 74 when tumor size reached above 1200 mm^3. Similarly, the single treatment group of compound A at 200 mg/kg continued to progress and was terminated at day 95 when tumor size reached above 1400 mm^3. Therefore, tumors in the single treatment groups of either dacomitinib

or compound A continued to progress and demonstrated the *in vivo* resistance similar to the *in vitro* characteristics. Combination of dacomitinib and compound A at 50 mg/kg group was able to achieve 100 % TGI, furthermore, combination of dacomitinib and compound A at 200 mg/kg maintained tumor regression until the end of study at day 120 as shown in Figure 13A and Table 4.

Table 4: Tumor Growth Inhibition and Regression in Study I.

Study day	Day 39		Day 61		Day 120	
	TGI	Regression	TGI	Regression	TGI	Regression
daco_5 mg/kg	96%		45%		NA[b]	
compd A_500 mg/kg		7%		NA*		
compd A_200 mg/kg	91%		65%		NA[c]	
compd A_50 mg/kg	68%		NA[a]			
compd A_500 mg/kg + daco_5 mg/kg		100%		NA*		
compd A_200 mg/kg + daco_5 mg/kg		100%		100%	100%	
compd A_50 mg/kg + daco_5 mg/kg		100%		100%		80%

*: this study group was terminated at day 39, since there were no tumors detectable for combination arm, animals were used for safety end points.

[a]: this study group was terminated at day 53, mean tumor volume was above 1200 mm^3.

[b]: this study group was terminated at day 74, mean tumor volume was above 1200 mm^3.

[c]: this study group was terminated at day 95, mean tumor volume was above 1400 mm^3.

Study II: Combination of Compound B and dacomitinib

In this study, RPC9 clone 6 tumor-bearing mice were randomized and treated with either single agent of Compound B or dacomitinib, or Compound B in combination with dacomitinib. Dacomitinib was dosed at 5 mg/kg and 1.5 mg/kg. Compound B was dosed at 50 mg/kg, 15 mg/kg, and 5 mg/kg as indicated in the Figure 14A. The body weight change percentages were plotted in Figure 14B, and indicated that all dose groups of this study were well tolerated with body weight loss less than 10 %. At study day 36, when the tumor sizes of vehicle group reached average 1000 mm^3, single agent treatments of dacomitinib generated a dose-dependent tumor growth inhibition as illustrated in Figure 15A. Single agent treatments of Compound B at 5 mg/kg and 15 mg/kg were not significantly effective due to the extreme low dosages, while single

agent treatment of Compound B at 50 mg/kg gave a 47 % TGI as shown in Figure 15A. Combination of Compound B and dacomitinib generated dose-dependent tumor regression as shown in Figure 15B. The tumor growth inhibition and regression were calculated and illustrated in Table 5.

Table 5: Tumor Growth Inhibition and Regression in Study II.

Study day	Day 36	
	TGI	Regression
Dacomitinib_5 mg/kg	97%	
Dacomitinib_1.5 mg/kg	80%	
Compound B_50 mg/kg	47%	
Compound B_15 mg/kg	2%	
Compound B_5 mg/kg	18%	
Compound B_50 mg/kg + Dacomitinib_5 mg/kg		92%
Compound B_15 mg/kg + Dacomitinib_5 mg/kg		72%
Compound B_5 mg/kg + Dacomitinib_5 mg/kg		52%
Compound B_50 mg/kg + Dacomitinib_1.5 mg/kg		31%

Study III: Combination of compound A and erlotinib

In this study, RPC9 clone 6 tumor-bearing mice were randomized and treated with either single agent of Compound A or erlotinib, or Compound A in combination with erlotinib. Erlotinib at 25 mg/kg gave an average unbound drug concentration of 300 nM in mouse plasma, which matches the average clinical exposure. The body weight change percentages were plotted in Figure 16B, and indicated that all dose groups of this study were well tolerated with body weight loss less than 10 %. Compound A was dosed at 400 mg/kg, 200 mg/kg, and 50 mg/kg as single agent or in combination with erlotinib at 25 mg/kg as indicated in the Figure 16A. At study day 45, when the tumor sizes of vehicle group reached above 1500 mm^3, single agent treatment of erlotinib generated 51 % tumor growth inhibition and single agent treatments of compound A generated a dose-dependent tumor growth inhibition as illustrated in Figure 16A and Table 6. Combination of all dose ranges of compound A with erlotinib generated tumor regression as illustrated in Table 6.

To further assess the combination effects on the tumor regression, mice in the single and combination treatment groups of Compound A at 200 mg/kg and 50 mg/kg continued to receive treatments until the study day 73. The tumor growth inhibition and tumor regression were calculated and illustrated in Table 6. Similar to the results of Study I in combination with dacomitinib, the results from this study also indicate that combination of Compound A with erlotinib generated tumor regression at both dose ranges, and tumors in the single agent treatment groups of compound A or erlotinib continued to progress indicating the *in vivo* resistance driven by either del or del/T790M allele.

Therefore, in conclusion, combination of compound A with either dacomitinib or erlotinib achieved synergistic antitumor activity to induce tumor regression in the resistant RPC9 clone 6 xenograft model.

Table 6: Tumor Growth Inhibition and Regression in Study III.

Study day	Day 45		Day 73	
	TGI	Regression	TGI	Regression
Erlotinib_25 mg/kg	51%		NA[a]	
Compound A_400 mg/kg		39%		NA*
Compound A_200 mg/kg	94%		76%	
Compound A_50 mg/kg	63%		NA[b]	
Compound A_400 mg/kg + Erlotinib_25 mg/kg		92%		NA*
Compound A_200 mg/kg + Erlotinib_25 mg/kg		80%		75%
Compound A_50 mg/kg + Erlotinib_25 mg/kg		44%		35%

*: these groups were terminated at day 45, since there were no tumors detectable for combination arm, animals were used for safety end points.

[a]: this group was terminated at day 52, mean tumor volume was above 1500 mm^3.

[b]: this group was terminated at day 55, mean tumor volume was above 1500 mm^3.

Conclusions:

RPC9 clone 6 xenograft model which harbors both EGFR delE746-A750 and EGFR delE746-A750/T790M alleles exhibited tumor progression when treated with compound A, compound B, dacomitinib, or erlotinib as single agent treatment. The model showed complete regression when treated with high doses of compound A with clinical relevant dose of dacomitinib or erlotinib as combination therapy, as well as

demonstrated dose-dependent tumor regression when treated with low doses of compound B in combination with dacomitinib. Therefore, current preclinical animal studies have successfully demonstrated that the combination strategy of EGFR T790M selective inhibitors with dacomitinib or erlotinib is a mechanism based and potentially clinically translatable strategy to develop EGFR T790M clinical candidates in NSCLC patients with both primary and acquired EGFR mutations.

What is claimed:

1. A method of treating non-small cell lung cancer comprising administering to a patient in need thereof an effective amount of an irreversible EGFR T790M inhibitor in combination with an effective amount of an EGFR inhibitor.

2. The method of claim 1, wherein the irreversible EGFR T790M inhibitor is 1-{(3*R*,4*R*)-3-[({5-chloro-2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)methyl]-4-methoxypyrrolidin-1-yl}prop-2-en-1-one, or a pharmaceutically acceptable salt thereof.

3. The method of claim 1 or 2, wherein the EGFR inhibitor is selected from the group consisting of gefitinib, erlotinib, icotinib, vandetanib, lapatinib, neratinib, afatinib, pelitinib, dacomitinib, canertinib, cetuximab and panitumumab, or a pharmaceutically acceptable salt thereof.

4. The method of claim 1 or 2, wherein the EGFR inhibitor is selected from the group consisting of gefitinib, erlotinib, afatinib, and dacomitinib, or a pharmaceutically acceptable salt thereof.

5. The method of claim 1 or 2, wherein the EGFR inhibitor is erlotinib, or a pharmaceutically acceptable salt thereof.

6. The method of claim 1 or 2, wherein the EGFR inhibitor is dacomitinib, or a pharmaceutically acceptable salt thereof.

7. A method of treating non-small cell lung cancer comprising administering to a patient in need thereof an effective amount of an EGFR T790M inhibitor in combination with a panHER inhibitor, wherein the panHER inhibitor is administered according to a non-standard clinical dosing regimen.

8. The method of claim 7, wherein the non-standard clinical dosing regimen is a non-standard clinical dose or a non-standard dosing schedule.

9. The method of claim 7, wherein the non-standard clinical dosing regimen is a low-dose amount of the panHER inhibitor.

10. The method of claim 7, wherein the non-standard clinical dosing regimen is an intermittent dosing regimen.

11. The method of any of claims 7-10, wherein the EGFR T790M inhibitor is selected from the group consisting of Go6976, PKC412, AP26113, HM61713, WZ4002, CO-1686 and TAS-2913, or a pharmaceutically acceptable salt thereof.

12. The method of any of claims 7-10, wherein the EGFR T790M inhibitor is 1-{(3*R*,4*R*)-3-[({5-chloro-2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)methyl]-4-methoxypyrrolidin-1-yl}prop-2-en-1-one, or a pharmaceutically acceptable salt thereof.

13. The method of any of claims any of claims 7-10, wherein the panHER inhibitor is selected from the group consisting of lapatinib, neratinib, afatinib, pelitinib, dacomitinib, and canertinib, or a pharmaceutically acceptable salt thereof.

14. The method of any of claims any of claims 7-10, wherein the panHER inhibitor is afatinib, or a pharmaceutically acceptable salt thereof.

15. The method of any of claims any of claims 7-10, wherein the panHER inhibitor is dacomitinib, or a pharmaceutically acceptable salt thereof.

16. A method of treating non-small cell lung cancer comprising administering to a patient in need thereof a synergistic amount of an EGFR T790M inhibitor in combination with an EGFR inhibitor.

17. The method of claim 16, wherein the EGFR T790M inhibitor is selected from the group consisting of Go6976, PKC412, AP26113, HM61713, WZ4002, CO-1686 and TAS-2913, or a pharmaceutically acceptable salt thereof.

18. The method of claim 16, wherein the EGFR T790M inhibitor is 1-{(3*R*,4*R*)-3-[({5-chloro-2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)methyl]-4-methoxypyrrolidin-1-yl}prop-2-en-1-one, or a pharmaceutically acceptable salt thereof.

19. The method of any of claims 16-18, wherein the EGFR inhibitor is selected from the group consisting of gefitinib, erlotinib, icotinib, vandetanib, lapatinib, neratinib, afatinib, pelitinib, dacomitinib, canertinib, cetuximab and panitumumab, or a pharmaceutically acceptable salt thereof.

20. The method of any of claims 16-18, wherein the EGFR inhibitor is selected from the group consisting of gefitinib, erlotinib, afatinib, and dacomitinib, or a pharmaceutically acceptable salt thereof.

21. The method of any of claims 16-18, wherein the EGFR inhibitor is erlotinib, or a pharmaceutically acceptable salt thereof.

22. A synergistic combination of

(a) an EGFR T790M inhibitor; and

(b) an EGFR inhibitor,

wherein component (a) and component (b) are synergistic.

23. The combination of claim 22, wherein the EGFR T790M inhibitor is selected from the group consisting of Go6976, PKC412, AP26113, HM61713, WZ4002, CO-1686 and TAS-2913, or a pharmaceutically acceptable salt thereof.

24. The combination of claim 22, wherein the EGFR T790M inhibitor is 1-{(3*R*,4*R*)-3-[({5-chloro-2-[(1-methyl-1*H*-pyrazol-4-yl)amino]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl}oxy)methyl]-4-methoxypyrrolidin-1-yl}prop-2-en-1-one, or a pharmaceutically acceptable salt thereof.

25. The combination of any of claims 22-24, wherein the EGFR inhibitor is selected from the group consisting of gefitinib, erlotinib, icotinib, vandetanib, lapatinib, neratinib, afatinib, pelitinib, dacomitinib, canertinib, cetuximab and panitumumab, or a pharmaceutically acceptable salt thereof.

26. The combination of any of claims 22-24, wherein the EGFR inhibitor is selected from the group consisting of gefitinib, erlotinib, afatinib, and dacomitinib, or a pharmaceutically acceptable salt thereof.

27. The combination of any of claims 22-24, wherein the EGFR inhibitor is erlotinib, or a pharmaceutically acceptable salt thereof.