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Supporting Information

Discovery of a Highly Potent and Broadly Effective Epidermal Growth Factor Receptor and HER2 Exon 20 Insertion Mutant Inhibitor

*Jaebong Jang, Jieun Son, Eunyoung Park, Takayuki Kosaka, Jamie A. Saxon, Dries J. H. De Clercq, Hwan Geun Choi, Junko Tanizaki, Michael J. Eck, Pasi A. Jänne, and Nathanael S. Gray**

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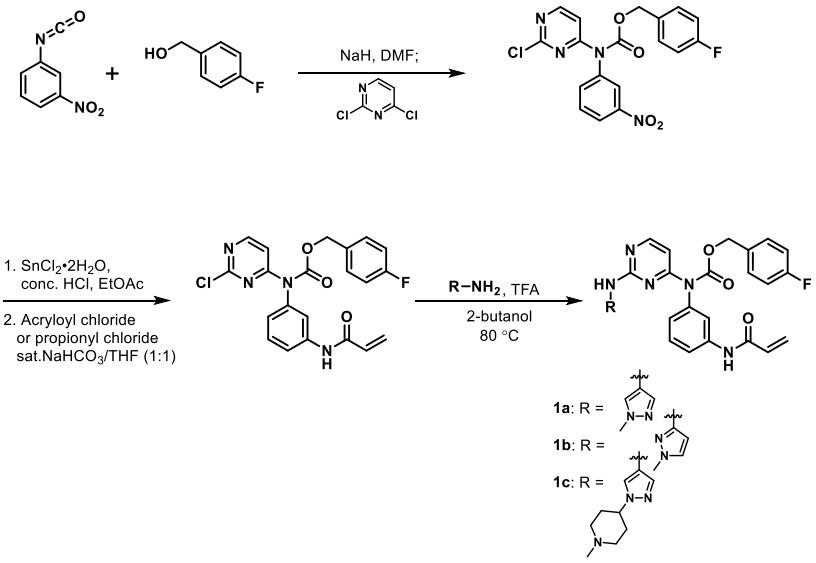
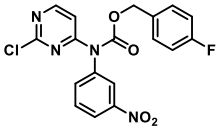
Chemical Synthesis

General Methods. Starting materials, reagents and solvents were purchased from commercial suppliers and were used without further purification unless otherwise noted. All reactions were monitored using a Waters Acquity UPLC/MS system (Waters PDA eλ Detector, QDa Detector, Sample manager - FL, Binary Solvent Manager) using Acquity UPLC® BEH C18 column (2.1 x 50 mm, 1.7 µm particle size): solvent gradient = 85 % A at 0 min, 1 % A at 1.7 min; solvent A = 0.1 % formic acid in Water; solvent B = 0.1 % formic acid in Acetonitrile; flow rate: 0.6 mL/min. Reaction products were purified by flash column chromatography using CombiFlash®Rf with Teledyne Isco Redi*Sep*® normal-phase silica flash columns (4 g, 12 g, 24 g, 40 g or 80 g) and Waters HPLC system using SunFire™ Prep C18 column (19 x 100 mm, 5 µm particle size): solvent gradient = 80 % A at 0 min, 10 % A at 25 min; solvent A = 0.035 % TFA in Water; solvent B = 0.035 % TFA in MeOH; flow rate : 25 mL/min. ^{1}H NMR spectra were recorded on 500 MHz Bruker Avance III spectrometers and ^{13}C NMR spectra were recorded on 125 MHz Bruker Avance III spectrometer. Chemical shifts are reported in parts per million (ppm, δ) downfield from tetramethylsilane (TMS). Coupling constants (*J*) are reported in Hz. Spin multiplicities are described as br (broad), s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet).

Scheme S1. Synthesis of compounds **1a**-**c** and **2a**.

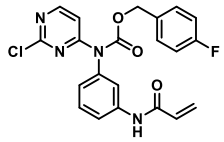


4-fluorobenzyl (2-chloropyrimidin-4-yl)(3-nitrophenyl)carbamate.



To a solution of 4-fluorobenzyl alcohol (1.50 g, 11.9 mmol) in *N*,*N*-dimethylformamide (30 ml) was added portionwise sodium hydride (60 % in mineral oil, 1.2 g, 29.7 mmol) at 0 °C. After stirring for 30 min at room temperature, 3-nitrophenyl isocyanate (2.1 g, 12.5 mmol) was added to the reaction mixture at 0 °C and the resulting mixture was stirred for additional 10 min at room temperature. Then, 2,4-dichloropyrimidine (1.9 g, 12.5 mmol) was added to the reaction mixture at 0 °C then, the resulting mixture was slowly warmed to room temperature. After completed, the reaction mixture was cooled to 0 °C, then diluted with cold EtOAc. The resulting mixture was quenched with ice-cold water and immediately extracted with EtOAc. Then, the organic layer was washed with water five times and brine once, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography to provide 4-fluorobenzyl (2-chloropyrimidin-4-yl)(3-nitrophenyl)carbamate (3.1 g, 65 %) as an off-white sold. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.69 (d, J = 5.8 Hz, 1H), 8.38 (t, J = 2.0 Hz, 1H), 8.28 - 8.24 (m, 1H), 8.14 (d, J = 5.8 Hz, 1H), 7.89 - 7.85 (m, 1H), 7.76 (t, J = 8.2 Hz, 1H), 7.32 - 7.27 (m, 2H), 7.27 - 7.11 (m, 2H), 5.22 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.9, 161.9, 160.9, 160.5, 158.4, 152.4, 148.2, 139.7, 136.0, 131.4, 130.5, 130.0, 129.9, 124.2, 123.1, 115.3, 115.1, 111.5, 67.6; LC/MS (ESI) calcd. m/z 403.06 $[M+H]^+$, found 402.98; Retention time: 1.43 min.

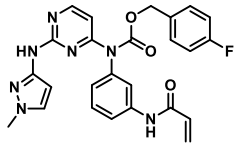
4-fluorobenzyl (3-acrylamidophenyl)(2-chloropyrimidin-4-yl)carbamate.



To a solution of 4-fluorobenzyl (2-chloropyrimidin-4-yl)(3-nitrophenyl)carbamate (3.0 g, 7.45 mmol) in EtOAc (30 ml) were added $SnCl_2 \cdot 2H_2O$ (5.0 g) and conc. HCl (1 ml). After stirring at 40 °C for 3 hrs, the mixture was diluted with EtOAc and neutralized using ammonium hydroxide. The resulting suspension was filtered through a pad of celite and the filtrate was washed with sat. $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure to obtain a crude 4-fluorobenzyl (3-aminophenyl)(2-chloropyrimidin-4-yl)carbamate which was used in the next step without further purification.

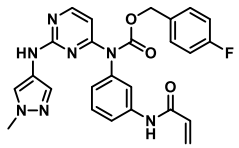
The crude residue was dissolved in 1:1 mixture of THF (15 ml) and sat. $NaHCO_3$ (15 ml). Then, acryloyl chloride (0.78 ml) was added dropwise to the mixture at 0 °C. After stirring for 20 min, the reaction mixture was diluted with DCM and washed with water and then, the aqueous layer was extracted twice with DCM. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (DCM : EtOAc = 100 : 0 to 50 : 50) to give 4-fluorobenzyl (3-acrylamidophenyl)(2-chloropyrimidin-4-yl)carbamate (2.3 g, 73 %) as an off-white solid.
^{1}H NMR 500 MHz (DMSO-d_6) δ 10.29 (s, 1H), 8.66 (d, J = 5.8 Hz, 1H), 8.00 (d, J = 5.8 Hz, 1H), 7.68 (t, J = 2.0 Hz, 1H), 7.63 - 7.59 (m, 1H), 7.41 (t, J = 7.9 Hz, 1H), 7.34 - 7.28 (m, 2H), 7.17 - 7.11 (m, 2H), 7.07 - 7.01 (m, 1H), 6.43 (dd, J = 17.1, 10.1, Hz, 1H), 6.26 (dd, J = 17.0, 1.8 Hz, 1H), 5.77 (t, J = 10.1, 1.9 Hz, 1H), 5.22 (s, 2H); ^{13}C NMR 125 MHz (DMSO-d_6) δ 163.3, 162.8, 162.3, 160.8, 160.5, 158.7, 153.0, 139.8, 138.9, 131.7, 131.7, 131.6, 129.8, 129.7, 129.5, 127.2, 123.8, 119.3, 119.0, 115.3, 115.1, 111.9, 67.3; LC/MS (ESI) calcd. m/z 427.10 $[M+H]^+$, found 426.84; Retention time: 1.24 min.

4-fluorobenzyl (3-acrylamidophenyl)(2-((1-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-4-yl)carbamate. (1b)



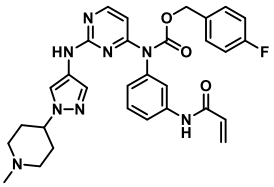
To a solution of 4-fluorobenzyl (3-acrylamidophenyl)(2-chloropyrimidin-4-yl)carbamate (1.0 g, 2.35 mmol) in 2-butanol (8 ml) were added 1-methyl-1*H*-pyrazol-3-amine (342 mg, 3.52 mmol) and trifluoroacetic acid (2 ml). After stirring at 80 °C for 4 hrs, the reaction mixture was cooled to room temperature and diluted with DMSO. The mixture was purified by preparative HPLC to obtain 4-fluorobenzyl (3-acrylamidophenyl)(2-((1-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-4-yl)carbamate (859 mg, 75 %) as an off-white sold.
^{1}H NMR 500 MHz (DMSO-d_6) δ 10.23 (s, 1H), 9.62 (s, 1H), 8.33 (d, J = 5.5 Hz, 1H), 7.70 - 7.66 (m, 1H), 7.59 - 7.57 (m, 1H), 7.40 (t, J = 8.0 Hz, 1H), 7.33 - 7.26 (m, 3H), 7.17 - 7.10 (m, 3H), 7.05 - 7.02 (m, 1H), 6.40 (dd, J = 17.1, 10.1 Hz, 1H), 6.26 (dd, J = 17.0, 2.0 Hz, 1H), 5.75 (dd, J = 10.1, 1.8 Hz, 1H), 5.42 (br s, 1H), 5.19 (s, 2H), 3.61 (s, 3H); ^{13}C NMR 125 MHz (DMSO-d_6) δ 163.3, 162.7, 161.0, 160.8, 159.1, 158.2, 153.1, 140.3, 139.9, 132.1, 131.6, 129.7, 129.6, 128.6, 127.3, 124.3, 123.0, 119.8, 118.9, 118.5, 115.3, 115.1, 101.0, 66.8, 38.4; LC/MS (ESI) calcd. m/z 488.18 $[M+H]^+$, found 487.88; Retention time: 1.04 min.

4-fluorobenzyl (3-acrylamidophenyl)(2-((1-methyl-1*H*-pyrazol-4-yl)amino)pyrimidin-4-yl)carbamate. (1a)



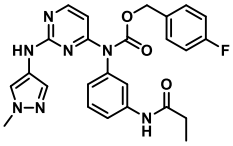
^{1}H NMR 500 MHz (DMSO-d_6) δ 10.33 (br s, 1H), 9.42 (br s, 1H), 8.32 (d, J = 5.2 Hz, 1H), 7.83 - 7.73 (m, 1H), 7.62 (br s, J = 7.0 Hz, 1H), 7.56 - 7.46 (m, 1H), 7.39 (br s, 1H), 7.29 (dd, J = 7.9, 5.8 Hz, 2H), 7.18 - 7.11 (m, 2H), 7.08 (br d, 1H), 7.02 (br s, 1H), 6.47 (br s, 1H), 6.41 (dd, J = 16.8, 10.1 Hz, 1H), 6.26 (dd, J = 17.0, 1.8 Hz, 1H), 5.77 (dd, J = 10.1, 1.8 Hz, 1H), 5.19 (s, 2H), 3.50 (s, 3H); ^{13}C NMR 125 MHz (DMSO-d_6) δ 163.3, 162.7, 161.0, 160.8, 159.1, 158.2, 153.1, 140.3, 139.9, 132.1, 131.6, 129.7, 129.6, 128.6, 127.3, 124.3, 123.0, 119.8, 118.9, 118.5, 115.3, 115.1, 101.0, 66.8, 38.4; LC/MS (ESI) calcd. m/z 488.18 $[M+H]^+$, found 487.88; Retention time: 1.03 min.

4-fluorobenzyl (3-acrylamidophenyl)(2-((1-(1-methylpiperidin-4-yl)-1*H*-pyrazol-4-yl)amino)pyrimidin-4-yl)carbamate. (1c)



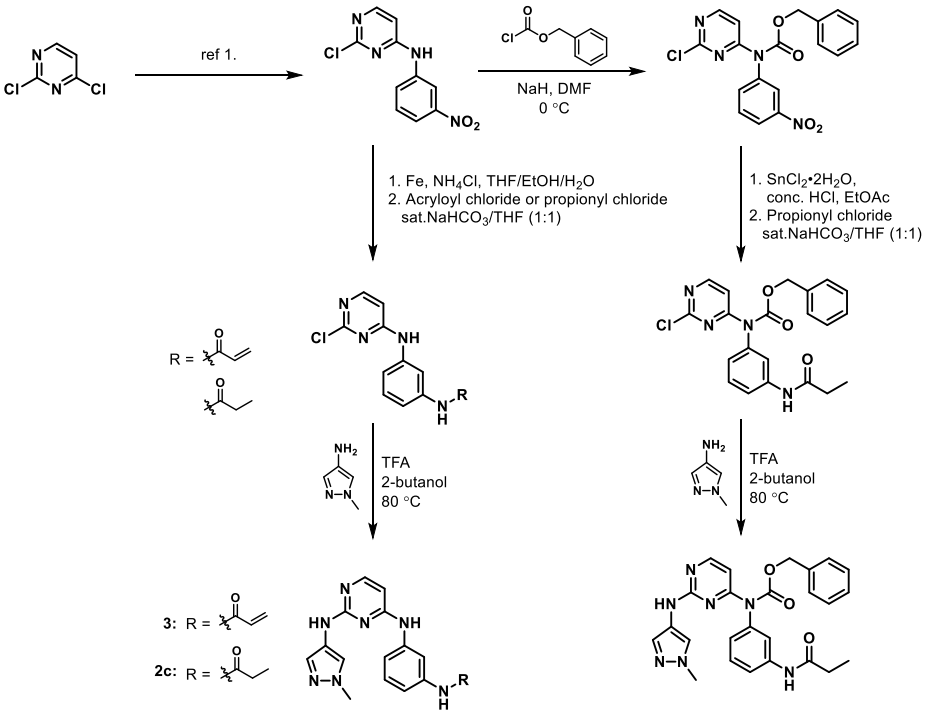
^{1}H NMR 500 MHz (DMSO-d_6) δ 10.35 (br s, 1H), 9.44 (br s, 1H), 8.33 (d, J = 5.5 Hz, 1H), 7.89 - 7.79 (m, 1H), 7.1 (s, 1H), 7.54 - 7.45 (m, 1H), 7.38 - 7.32 (m, 1H), 7.29 (dd, J = 8.1, 6.0 Hz, 2H), 7.17 - 7.03 (m, 4H), 6.60 (br s, 1H), 6.42 (dd, J = 17.1, 10.1 Hz, 1H), 6.26 (dd, J = 16.9, 2.0 Hz, 1H), 5.78 (dd, J = 10.2, 2.0 Hz, 1H), 5.19 (s, 2H), 3.60 (br s, 1H), 2.96 - 2.83 (m, 2H), 2.29 (s, 3H), 2.26 - 2.09 (m, 2H), 1.91 - 1.76 (m, 2H), 1.70 - 1.55 (m, 2H); ^{13}C NMR 125 MHz (DMSO-d_6) δ 163.3, 162.7, 161.1, 160.8, 159.1, 158.3, 153.2, 140.4, 139.9, 132.1, 131.6, 129.7, 129.6, 129.6, 128.7, 127.3, 124.1, 122.4, 119.7, 118.5, 117.2, 115.3, 115.1, 101.3, 66.8, 57.4, 53.9, 45.3, 31.5; LC/MS (ESI) calcd. m/z 571.26 $[M+H]^+$, found 570.87; Retention time: 0.86 min.

4-fluorobenzyl (2-((1-methyl-1*H*-pyrazol-4-yl)amino)pyrimidin-4-yl)(3-propionamidophenyl)carbamate. (2a)

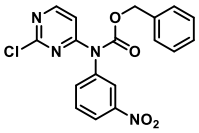


4-fluorobenzyl (2-((1-methyl-1*H*-pyrazol-4-yl)amino)pyrimidin-4-yl)(3-propionamidophenyl)carbamate was synthesized using analogous procedures which were used to make **1a**.
^{1}H NMR 500 MHz (DMSO-d_6) δ 10.04 (br s, 1H), 9.41 (br s, 1H), 8.32 (d, J = 5.5 Hz, 1H), 7.74 - 7.65 (m, 1H), 7.60 (br s, 1H), 7.51 - 7.43 (m, 1H), 7.38 (br s, 1H), 7.32 - 7.25 (m, 2H), 7.18 - 7.11 (m, 2H), 7.03 (br s, 1H), 6.45 (br s, 1H), 5.19 (s, 2H), 3.51 (br s, 3H), 2.31 (q, J = 7.4 Hz, 2H), 1.06 (t, J = 7.5 Hz, 1H); ^{13}C NMR 125 MHz (DMSO-d_6) δ 172.3, 162.7, 161.0, 160.8, 159.1, 158.2, 153.2, 140.3, 140.2, 132.1, 132.1, 129.6, 129.6, 128.6, 123.6, 123.0, 119.4, 119.0, 118.1, 115.3, 115.1, 101.0, 66.7, 38.4, 29.5, 9.5; LC/MS (ESI)) calcd. m/z 490.20 $[M+H]^+$, found 489.89; Retention time: 1.06 min.

Scheme S2. Synthesis of compounds **2b**-**c** and **3**.

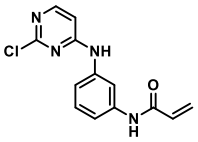


Benzyl (2-chloropyrimidin-4-yl)(3-nitrophenyl)carbamate.



To a solution of 2-chloro-*N*-(3-nitrophenyl)pyrimidin-4-amine[1] (1.3 g, 5.19 mmol) in *N*,*N*-dimethylformamide (30 ml) was added portionwise sodium hydride (60 % dispersion in mineral oil, 312 mg, 7.79 mmol) at 0 °C. After stirring for 30 min at room temperature, benzyl chloroformate (0.81 ml, 5.71 mmol) was added to the reaction mixture at 0 °C and the resulting mixture was stirred for additional 1 hr at room temperature. After completed, the reaction mixture was cooled to 0 °C, then diluted with cold EtOAc. The resulting mixture was quenched with ice-cold water and immediately extracted with EtOAc. Then, the organic layer was washed with water five times and brine once, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography to obtain benzyl (2-chloropyrimidin-4-yl)(3-nitrophenyl)carbamate (1.2 g, 62 %) as an off-white sold. ^{1}H NMR 500 MHz (DMSO-d_6) δ 8.69 (d, J = 5.8 Hz, 1H), 8.40 (t, J = 2.1 Hz, 1H), 8.29 - 8.24 (m, 1H), 8.16 (d, J = 5.8 Hz, 1H), 7.90 - 7.86 (m, 1H), 7.77 (t, J = 8.2 Hz, 1H), 7.34 - 7.26 (m, 3H), 7.25 - 7.19 (m, 2H), 5.24 (s, 2H); ^{13}C NMR 125 MHz (DMSO-d_6) δ 162.4, 161.0, 158.9, 152.9, 148.7, 140.3, 136.5, 135.7, 131.0, 128.9, 128.6, 127.9, 124.7, 123.6, 112.0, 68.8; LC/MS (ESI) calcd. m/z 385.07 $[M+H]^+$, found 384.79; Retention time: 1.41 min.

***N*-(3-((2-chloropyrimidin-4-yl)amino)phenyl)acrylamide.**

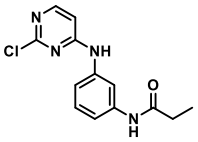


To a solution of 2-chloro-*N*-(3-nitrophenyl)pyrimidin-4-amine (500 mg, 1.99 mmol) in THF/EtOH (5 ml/ 2 ml) were added sat NH_4Cl (2 ml) and iron powder (222 mg, 3.98 mmol). After stirring at 50 °C for 3 hrs, the mixture was filtered through celite and the filtrate was diluted with EtOAc. The resulting mixture was washed with sat.$NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to obtain crude N^1-(2-chloropyrimidin-4-yl)benzene-1,3-diamine which was used in the next step without further purification.

The crude residue was dissolved in 1:1 mixture of THF (5 ml) and sat. $NaHCO_3$ (5 ml). Then, acryloyl chloride (0.24 ml, 2.99 mmol) was added dropwise to the mixture at 0 °C. After stirring for 20 min, the reaction mixture was diluted with DCM and washed with water and then, the aqueous layer was extracted twice with DCM. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (DCM : MeOH = 100 : 0 to 80 : 20) to give *N*-(3-((2-chloropyrimidin-4-yl)amino)phenyl)acrylamide (295 mg, 54 %, two steps) as an off-white solid.

^{1}H NMR 500 MHz (DMSO-d_6) δ 10.46 (s, 1H), 10.30 (s, 1H), 8.15 (d, J = 5.8 Hz, 1H), 7.92 (s, 1H), 7.45 (d, J = 6.4 Hz, 1H), 7.40 (d, J = 8.2 Hz, 1H), 7.29 (d, J = 8.1 Hz, 1H), 6.87 (d, J = 5.8 Hz, 1H), 6.55 (dd, J = 16.9, 10.2 Hz, 1H), 6.25 (dd, J = 16.9, 1.7 Hz, 1H), 5.74 (dd, J = 10.1, 1.8 Hz, 1H); ^{13}C NMR 125 MHz (DMSO-d_6) δ 163.2, 161.6, 159.3, 157.1, 139.6, 138.9, 132.0, 129.1, 126.7, 115.7, 114.8, 111.5, 105.8; LC/MS (ESI) calcd. m/z 275.07 $[M+H]^+$, found 274.87; Retention time: 0.79 min.

***N*-(3-((2-chloropyrimidin-4-yl)amino)phenyl)propionamide.**

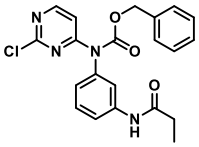


N^1-(2-chloropyrimidin-4-yl)benzene-1,3-diamine (80 mg, 0.363 mmol) was dissolved in 1:1 mixture of THF (2 ml) and sat. $NaHCO_3$ (2 ml). Then, propionyl chloride (0.05 ml, 0.543 mmol) was added dropwise to the mixture at 0 °C. After stirring for 20 min, the reaction mixture was diluted with DCM and washed with water and then, the aqueous layer was extracted twice with DCM. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (DCM : MeOH = 100 : 0 to 80 : 20) to give *N*-(3-((2-chloropyrimidin-4-yl)amino)phenyl)propionamide (91 mg, 91 %) as an off-white solid.

^{1}H NMR 500 MHz (DMSO-d_6) δ 10.01 (s, 1H), 9.91 (s, 1H), 8.15 (d, J = 5.8 Hz, 1H), 7.82 (s, 1H), 7.38 (d, J = 6.7 Hz, 1H), 7.30 - 7.22 (m, 2H), 6.76 (d, J = 5.8 Hz, 1H), 2.33 (q, J = 7.4 Hz, 2H), 1.08 (t, J = 7.6 Hz, 1H); ^{13}C NMR 125 MHz (DMSO-d_6) δ 172.1, 161.5,

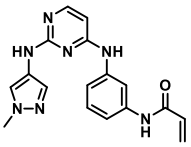
159.4, 157.2, 139.9, 138.8, 129.1, 115.3, 114.5, 111.3, 105.6, 29.5, 9.7; LC/MS (ESI) calcd. m/z 277.09 $[M+H]^+$, found 276.93; Retention time: 0.79 min.

Benzyl (2-chloropyrimidin-4-yl)(3-propionamidophenyl)carbamate.



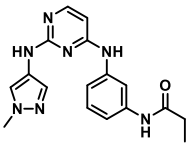
^{1}H NMR 500 MHz (DMSO-d_6) δ 10.00 (s, 1H), 8.65 (d, J = 5.8 Hz, 1H), 8.00 (d, J = 5.8 Hz, 1H), 7.63 (s, 1H), 7.55 (d, J = 8.2 Hz, 1H), 7.38 (t, J = 8.1 Hz, 1H), 7.35 - 7.27 (m, 3H), 7.25 - 7.21 (m, 2H), 7.03 - 6.97 (m, 1H), 5.23 (s, 2H) 2.32 (q, J = 7.4 Hz, 1H), 1.07 (t, J = 7.5 Hz, 1H); ^{13}C NMR 125 MHz (DMSO-d_6) δ 172.2, 162.3, 160.4, 158.7, 153.0, 140.2, 138.8, 135.5, 129.4, 128.4, 128.0, 127.2, 123.2, 118.9, 118.5, 111.8, 68.0, 29.5, 9.6; LC/MS (ESI) calcd. m/z 411.12 $[M+H]^+$, found 410.86; Retention time: 1.24 min.

***N*-(3-((2-((1-methyl-1H-pyrazol-4-yl)amino)pyrimidin-4-yl)amino)phenyl)acrylamide. (3)**



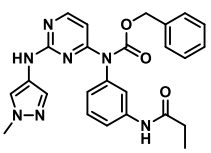
To a solution of *N*-(3-((2-chloropyrimidin-4-yl)amino)phenyl)acrylamide (60 mg, 0.218 mmol) in 2-butanol (0.7 ml) were added 1-methyl-1*H*-pyrazol-4-amine (44 mg, 0.328 mmol) and trifluoroacetic acid (0.3 ml). After stirring at 80 °C for 4 hrs, the reaction mixture was cooled to room temperature and diluted with DMSO. The mixture was purified by preparative HPLC to give *N*-(3-((2-((1-methyl-1*H*-pyrazol-4-yl)amino)pyrimidin-4-yl)amino)phenyl)acrylamide (58 mg, 80 %) as an off-white sold.
^{1}H NMR 500 MHz (CD_3OD) δ 7.97 (br s, 1H), 7.88 (d, J = 5.8 Hz, 1H), 7.82 (s, 1H), 7.46 (s, 1H), 7.38 - 7.26 (m, 3H), 6.45 (dd, J = 17.1, 10.1 Hz, 1H), 6.36 (dd, J = 16.8, 1.8 Hz, 1H), 6.15 (d, J = 5.8 Hz, 1H), 5.77 (dd, J = 10.1, 1.8 Hz, 1H), 3.77 (s, 3H); ^{13}C NMR 125 MHz (CD_3OD) δ 164.8, 161.7, 159.4, 155.2, 140.1, 138.8, 131.1, 130.7, 128.8, 126.6, 123.3, 122.4, 117.5, 114.9, 113.3, 96.9, 37.6; LC/MS (ESI) calcd. m/z 336.16 $[M+H]^+$, found 335.84; Retention time: 0.47 min.

***N*-(3-((2-((1-methyl-1*H*-pyrazol-4-yl)amino)pyrimidin-4-yl)amino)phenyl)propionamide. (2c)**



^{1}H NMR 500 MHz (DMSO-d_6) δ 10.11 (s, 1H), 9.28 (s, 1H), 8.93 (br s, 1H), 7.96 (d, J = 5.5 Hz, 1H), 7.82 (s, 2H), 7.40 (s, 1H), 7.35 - 7.24 (m, 2H), 6.46 (dd, J = 17.1, 10.1 Hz, 1H), 6.26 (dd, J = 17.1, 1.8 Hz, 1H), 6.14 (d, J = 5.8 Hz, 1H), 5.76 (dd, J = 10.1, 1.8 Hz, 1H), 3.72 (s, 3H); ^{13}C NMR 125 MHz (CD3OD) δ 175.4, 163.1, 160.9, 156.7, 141.4, 140.5, 132.0, 130.0, 124.8, 123.6, 118.6, 116.1, 114.6, 98.2, 38.9, 31.1, 10.2; LC/MS (ESI) calcd. m/z 338.17 $[M+H]^+$, found 337.90; Retention time: 0.43 min.

Benzyl (2-((1-methyl-1*H*-pyrazol-4-yl)amino)pyrimidin-4-yl)(3-propionamidophenyl)carbamate. (2b)



^{1}H NMR 500 MHz (DMSO-d_6) δ 10.07 (br s, 1H), 9.49 (br s, 1H), 8.32 (d, J = 5.8 Hz, 1H), 7.74 - 7.66 (m, 1H), 7.63 (br s, 1H), 7.53 - 7.44 (m, 1H), 7.44 - 7.37 (m, 1H), 7.34 - 7.26 (m, 3H), 7.22 (br s, 1H), 7.21 (br s, 1H), 7.08 - 7.01 (m, 2H), 6.46 (br s, 1H), 5.21 (s, 2H), 3.51 (s, 3H), 2.31 (q, J = 7.6 Hz, 2H), 1.06 (q, J = 7.6 Hz, 3H); ^{13}C NMR 125 MHz (DMSO-d_6) δ 172.7, 161.7, 159.5, 159.0, 153.7, 140.9, 140.8, 136.4, 129.9, 129.4, 128.8, 128.4, 127.6, 124.0, 123.5, 119.9, 119.8, 118.7, 102.0, 67.9, 38.9, 30.0, 10.0; LC/MS (ESI) calcd. m/z 472.21 $[M+H]^+$, found 471.96 $[M+H]^+$; Retention time: 1.01 min.

Structure Determination

The Wild type EGFR kinase domain was crystallized as previously described.[2] **1b** was soaked into the apo-crystals in reservoir solution supplemented with 1 mM for 3 hours. Diffraction data were collected on the NE-CAT beamline (ID24-E) at Argonne National Laboratory at 100K, and were processed and merged with XDS.[3] The structure was determined by molecular replacement using the EGFR kinase structure as a search model (PDB 4ZAU). Repeated rounds of manual re-fitting and crystallographic refinement were performed using COOT[4] and PHENIX.[5] The inhibitor was modeled into closely fitting positive Fo-Fc density and included in subsequent refinement cycles. Topology and parameter files for the inhibitor was generated using PHENIX.[5] Crystallographic data and statistics are presented in Table S3.

Biological and Biochemical Materials and Methods

Cell lines

Wild-type EGFR Ba/F3, wild-type HER2 Ba/F3, mutant Ba/F3 (InsSVD, InsASV, InsGY, InsYVMA, InsGSP, L858R, Del, L858R/T790M and Del/T790M) and DFCI127 cells were previously generated and characterized as described.[6] DFCI127 and all Ba/F3 cells were cultured in RPMI media supplemented with 10% fetal bovine serum (FBS), Penicillin and Streptomycin. Wild-type EGFR Ba/F3 cells were maintained in the same media with the addition of EGF (10 ng/ml). All murine mutant Ba/F3 were sequenced to ensure they possess the correct mutations. All cell lines were tested negative for *Mycoplasma* using the Mycoplasma Plus PCR Primer Set (Agilent).

Cell viability assays

Ba/F3 and DFCI127 cells were treated with increasing concentrations of inhibitors for 72 hours and growth or the inhibition of growth was assessed by MTS assay according to previously established methods.[7] All experiments were conducted in six replicates and were repeated multiple times except **1a-c, 2a** and **3** in Ba/F3 cells.

Western blotting

To assess the effect of compounds on EGFR, HER2 and its downstream pathways, Ba/F3 and DFCI127 cells were treated for 6 hours before cells were lysed with RIPA buffer, supplemented with protease and phosphatase inhibitors, followed by protein quantification. For immunoblotting, 20 µg of total cell lysates were separated by 4 - 12% SDS-PAGE and transferred onto polyvinylidene difluoride membranes. Primary antibodies included: phospho-EGFR (Tyr1068), total-EGFR, phospho-HER2 (Tyr1196), total-HER2, phospho-Erk1/2 (Thr202/Tyr204), total-Erk1/2 and Bim purchased from Cell Signaling Technology, and HSP90 antibody purchased from Santa Cruz Biotechnology.

Biochemical assays

All biochemical activities were determined by Invitrogen kinase assays.

Kinome selectivity profiling

Kinome selectivity profiling was performed using the KINOME*scan* method at a compound concentration of 1 µM. Protocols are available from DiscoverX.

Supplementary Figures and Tables

Figure S1. A deep hydrophobic pocket in WT EGFR in complex with afatinib (PDB: 4G5J, A) and WT HER2 in complex with TAK-285 (PDB: 3RCD, B).

(A) (B)

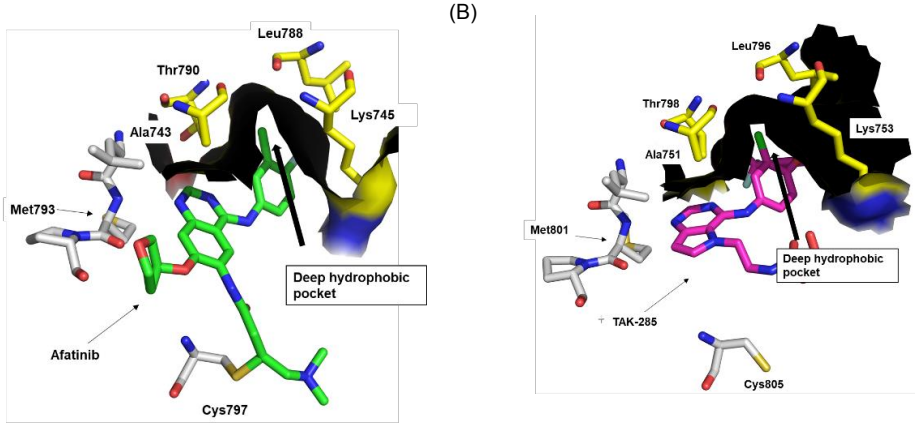


Table S1. Biochemical activities of reversible analogs against wild-type EGFR and EGFR Del746-750.

	Biochemical activities (IC_{50}, nM)	
Compound	WT EGFR[1]	EGFR Del746-750[2]
Afatinib	<0.5	<0.5
Osimertinib	3.1±0.40	0.82±0.03
2c	>5000	2400±260
2b	340±42	340±27
2a	240±38	200±41

[1] Enzymatic inhibitory activity [Invitrogen, Z`-LYTE]; [2] Binding assay [Invitrogen, LanthaScreen binding].

Figure S2. Kinome selectivity of **1a** over 468 kinases at a concentration of 1 μM. The size of circles mapped onto the kinase phylogenetic tree utilizing DiscoverX TREE*spot*™ corresponds to the strength of the binding affinity as indicated in the figure after cut-off data at 30%control. Wild type EGFR, wild type HER2 and EGFR mutants are indicated by blue spots.

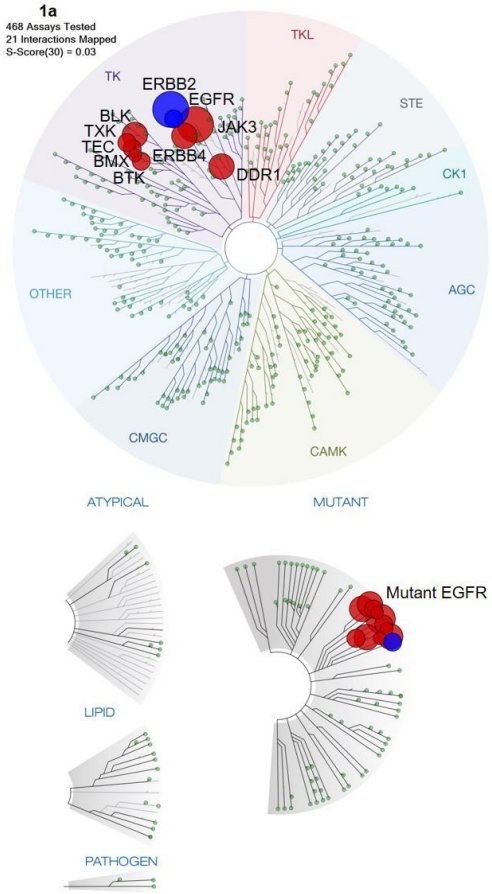


Table S2. Biochemical and antiproliferative activities of **1a**-**c** against common EGFR mutants.

	Biochemical activities (IC_{50}, nM)				Antiproliferative activities (EC_{50}, nM)				
Compound	WT[1]	L858R[2]	Del[2]	L858R/ T790M[2]	WT	L858R	Del	L858R/ T790M	Del/ T790M
Gefitinib	0.6±0.06	<0.5	<0.5	192±6.2	NT[3]	14±0.9	26±0.0	NA[4]	NA[4]
Osimertinib	12.3±2.0	4.41±0.26	0.82±0.03	0.93±0.11	60±15	7.3±1.7	11±1.9	23±5.5	12±3.2
1a[5]	<0.5	<0.5	<0.5	<0.5	7.6±1.9	<1.0	4.3±0.33	2.1±0.23	4.6±0.44
1b[5]	0.8±0.10	<0.5	<0.5	<0.5	21±4.2	<1.0	15±2.3	14±2.2	37±2.7
1c[5]	0.7±0.61	<0.5	<0.5	<0.5	19±2.2	1.1±0.05	13±0.92	9.3±0.85	14±1.3

IC_{50} values were determined at an ATP concentration of [1] 100 μM or [2] Km; [3] Not tested; [4] Not active (EC_{50} value was higher than 10 μM); [5] EC_{50} values were measured from single experiment with six replicates. Errors are reported as ±95% confidence interval.

Figure S3. Cell proliferation assay of DFCI127 cells harboring EGFR InsPNP treated with dose-escalated **1a-c**, **2a** and **3** as well as known EGFR inhibitors.

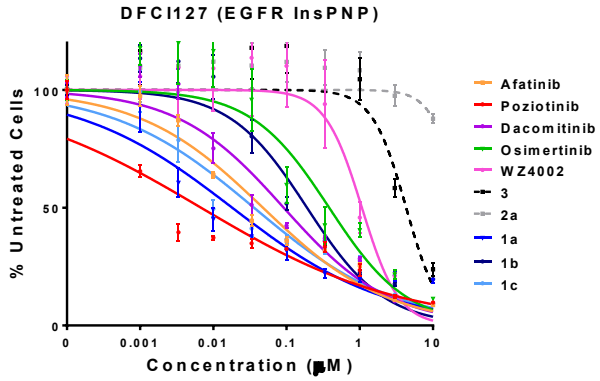


Table S3. Crystallographic data collection and refinement statistics.

PDB ID Code	6D8E
Data collection	
Space group	I23
Cell dimensions	
a, b, c (Å)	146.572 146.572 146.572
α, β, γ (°)	90 90 90
Resolution* (Å)	59.84 - 2.538 (2.628 - 2.538)
R_{merge}*	0.09643 (1.8)
I/σ*	14.38 (0.92)
R_{meas}	*0.105(1.997)*
$CC_{(1/2)}$	0.998(0.313)
Completeness* (%)	99.7 (98.5)
Multiplicity*	4.9 (4.7)
Refinement	
Resolution (Å)	59.84 - 2.538
No. of Reflections	17335 (1629)
R_{work}/ R_{free}	0.2092/0.2421
No. of Atoms	
Protein	2364
Ligand	36
Water	12
B-factors	
Protein	70.5
Ligand	66.8
Water	63
R.m.s deviations	
Bond lengths (Å)	0.009
Bond angles (°)	1.1038
Ramachandran Plot	
Most favored	270 (92%)
Allowed	21 (7.2%)
Outliers	1 (0.34%)

*Numbers in parentheses are for the highest resolution shell.

Figure S4. Electron density map (2Fo-Fc) of compound **1b** and Cys797 (left, contoured at 1.2σ) and Fo-Fc stimulated annealing omit map of inhibitor generated by PHENIX (right, contoured level is 2.5σ).

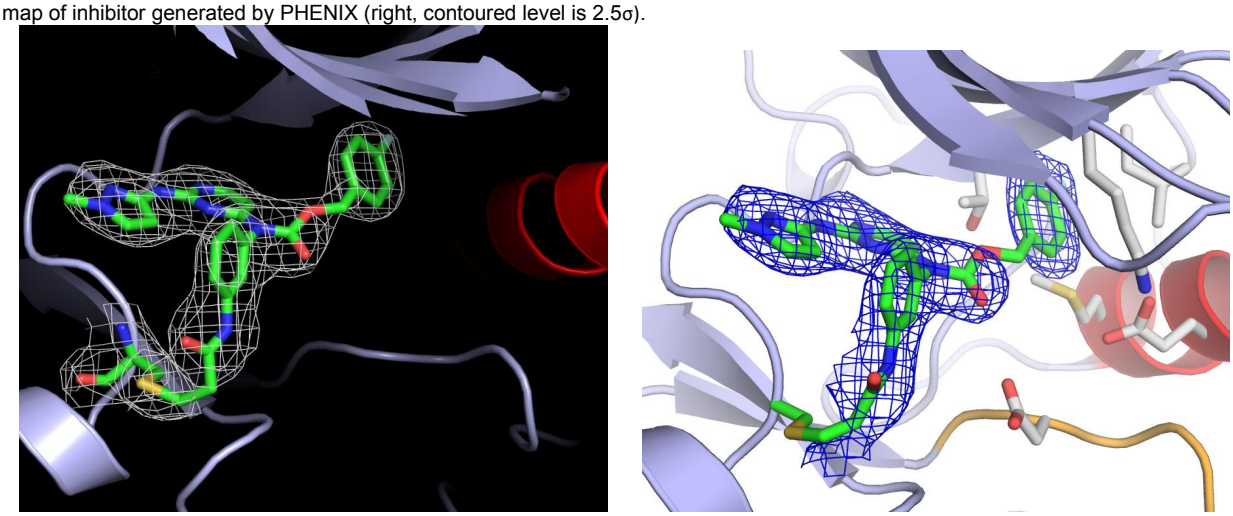


Table S4. In vitro ADME profile of **1a**.

	1a
Thermodynamic solubility at pH 7.4 (PBS)	Not detected in filtrate (Low solubility)
Caco-2 permeability (P_{app}, 10^{-6} cm/s)	0.017 (mean A to B, low permeability)
	0.38 (mean B to A, low permeability)
	22.4 (Efflux ratio)
Mouse liver microsome stability (half-life)	5.9 min

Table S5. Pharmacokinetic properties of **1a.**

ID	route	dose (mg/kg)	$T_{½}$ (hr)	T_{max} (hr)	C_{max} (μM)	AUC_{last} (min·ng/mL)	$Cl_{\_obs}$ (mL/min/Kg)	V_{ss_obs} (L/Kg)	F (%)
1a	IV	3	0.5			33667	90.15	1.65	
	PO	20		0.25	0.7	24580	773.37		11

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Author Contributions

J.J., D.J.H.D.C. and H.G.C. performed the chemical design, synthesis, validation and analyzed data. J.S., T.K., J.A.S. and J.T. performed the cellular experiments. E.P. performed the structure determination for the compound **1b**. M.J.E., P.A.J. and N.S.G. coordinated and supervised the study. J.J. and N.S.G. wrote the manuscript which all authors critically reviewed and revised.