

Optimization of highly kinase selective bis-anilino pyrimidine PAK1 inhibitors

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Supplementary Material

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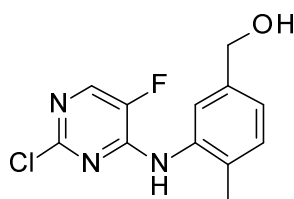
- Procedures for synthesis and characterization of compounds
- Correlation of pPAK1 cell assay with PAK1 enzyme
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Procedures for synthesis and characterization of compounds

General

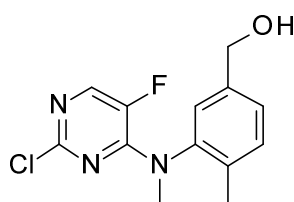
All solvents and chemicals used were reagent grade. Anhydrous solvents tetrahydrofuran (THF), methanol (MeOH), dichloromethane (DCM), dimethoxyethane (DME) were purchased from Aldrich. Flash column chromatography was carried out using prepacked silica cartridges (from 4 g to 300 g) from Biotage or Crawford and eluted using an Isco Companion system. Preparative HPLC was carried out on a Waters XBridge Prep C18 OBD column, 5 μ silica, 19 mm diameter, 100 mm length, gradient 5 to 95% MeCN/1% NH₃ in H₂O. Purity and characterization of compounds were established by a combination of liquid chromatography–mass spectroscopy (LC-MS) and NMR analytical techniques. ¹H NMR spectra were recorded on a Bruker Avance or Avance II spectrometer at a proton frequency of 300, 400 or 500 MHz and were determined in CDCl₃, DMSO-*d*₆ or MeOD. Chemical shifts are reported in ppm relative to tetramethylsilane (TMS) (0.00 ppm) or solvent signal. Splitting patterns are indicated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad peak. Merck precoated thin layer chromatography (TLC) plates (silica gel 60 F₂₅₄, 0.25 mm) were used for TLC analysis.

(3-((2-Chloro-5-fluoropyrimidin-4-yl)amino)-4-methylphenyl)methanol (**22**, R=H)



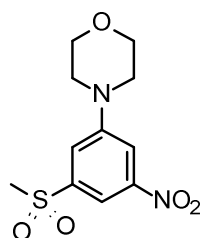
A solution of (3-amino-4-methylphenyl)methanol (CAS: 81863-45-8) (9.9 g, 72.2 mmol), 2,4-dichloro-5-fluoropyrimidine (13.2 g, 79.4 mmol) and DIPEA (32.1 mL, 180.4 mmol) in *n*-BuOH (10 mL) was stirred at 100 °C for 1 d. The reaction mixture was evaporated and diluted with water to afford a brown solid which was filtered, slurried in DCM (100 mL) for 20 min and filtered to afford (3-((2-chloro-5-fluoropyrimidin-4-yl)amino)-4-methylphenyl)methanol (**22**, R=H) (12.9 g, 67%) as a white solid. ¹H NMR (400 MHz, CDCl₃) 1.73 (t, *J*=5.7 Hz, 1H), 2.30 (s, 3H), 4.71 (d, *J*=5.1 Hz, 2H), 6.75 (s, 1H), 7.17 (dd, *J*=1.5, 7.8 Hz, 1H), 7.25 (d, *J*=6.2 Hz, 1H), 7.81 (s, 1H), 8.06 (d, *J*=2.8 Hz, 1H); MS (ESI) [M + H]⁺ *m/z* 268.

(3-((2-Chloro-5-fluoropyrimidin-4-yl)(methyl)amino)-4-methylphenyl)methanol



To a stirred suspension of (3-((2-chloro-5-fluoropyrimidin-4-yl)amino)-4-methylphenyl)methanol (3.05 g, 11.4 mmol) and Cs₂CO₃ (4.1 g, 12.5 mmol) in DMF (25 mL) was added iodomethane (0.70 mL, 11.39 mmol) and the mixture was stirred at rt for 2 h. The reaction mixture was diluted with water (75 mL), and the suspension was stirred at rt for 15 min, then the precipitate was collected by filtration, washed with water (2 x 75 mL) and dried under vacuum to afford (3-((2-chloro-5-fluoropyrimidin-4-yl)(methyl)amino)-4-methylphenyl)methanol (2.74 g, 85%) as a cream solid. ¹H NMR (400 MHz, DMSO) 2.14 (s, 3H), 3.33 (s, 3H), 4.47 (d, *J*=5.7 Hz, 2H), 5.23 (t, *J*=5.7 Hz, 1H), 7.2-7.32 (m, 3H), 8.12 (d, *J*=5.4 Hz, 1H); MS (ESI) [M + H]⁺ *m/z* 282.

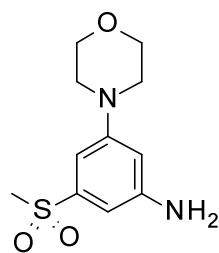
4-(3-(Methylsulfonyl)-5-nitrophenyl)morpholine



Morpholine (16.8 g, 193 mmol) was added to a solution of 1-fluoro-3-(methylsulfonyl)-5-nitrobenzene (CAS: 7087-27-6) (10.6 g, 48.3 mmol) in DMSO (150 mL) and the mixture was stirred and heated to 110 °C for 30 min. After cooling the reaction was diluted with water (800 mL) and CH₂Cl₂ (800 mL). The layers were separated, and the aqueous phase was extracted with CH₂Cl₂ (3 x 500 mL). The

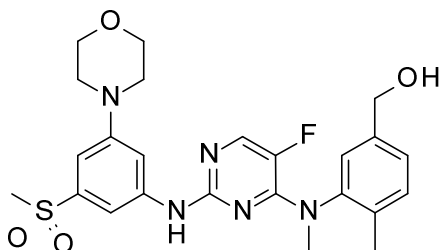
combined organic phases were washed with saturated aqueous NaHCO₃ (3 x 800 mL) and saturated brine (800 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (EtOAc/Hexane=1:2) to afford the 4-(3-(methylsulfonyl)-5-nitrophenyl)morpholine (13.6 g, 98%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) 3.10 (s, 3H), 3.33-3.38 (m, 4H), 3.87-3.92 (m, 4H), 7.66 (dd, *J*=1.4, 2.5 Hz, 1H), 7.88-7.91 (m, 1H), 8.12-8.15 (m, 1H); MS (ESI) [*M* + *H*]⁺ *m/z* 285.

3-(Methylsulfonyl)-5-morpholinoaniline



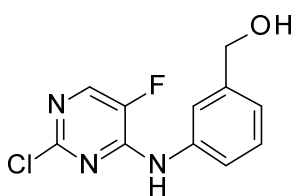
Raney Ni (wet, 5 g) was added to a solution of 4-(3-(methylsulfonyl)-5-nitrophenyl)morpholine (13.6 g, 47 mmol) in MeOH (200 mL). The reaction mixture was pressurized to 10 atm H₂ and shaken for 10 h. The reaction mixture was filtered through a pad of celite[®] and the solids were washed with MeOH (100 mL). The filtrate was concentrated and the residue was further triturated with EtOH/Hexane (1/5, 50 mL), filtered and dried to afford 3-(methylsulfonyl)-5-morpholinoaniline (10.5 g, 86%) as an off-white solid. ¹H NMR (400 MHz, CDCl₃) 3.01 (s, 3H), 3.15-3.21 (m, 4H), 3.82-3.86 (m, 4H), 3.90 (s, 2H), 6.36 (t, *J*=2.1 Hz, 1H), 6.70 (t, *J*=1.7 Hz, 1H), 6.8-6.83 (m, 1H); MS (ESI) [*M* + *H*]⁺ *m/z* 257.

(3-((5-Fluoro-2-(3-(methylsulfonyl)-5-morpholinophenylamino)pyrimidin-4-yl)(methylamino)-4-methylphenyl)methanol (3)



A mixture of (3-((2-chloro-5-fluoropyrimidin-4-yl)(methylamino)-4-methylphenyl)methanol (188 mg, 0.67 mmol), 3-(methylsulfonyl)-5-morpholinoaniline (183 mg, 0.71 mmol), and TsOH.H₂O (252 mg, 1.32 mmol) in *n*-BuOH (4 mL) was heated to 100 °C for 17 h. The reaction was allowed to cool and partitioned between EtOAc (50 mL) and saturated NaHCO₃ (50 mL). The aqueous layer was further extracted with EtOAc (50 mL) and the organic extracts were combined, dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (0 to 20% EtOAc in hexane) to give a viscous, pale yellow oil which was lyophilized from a MeCN/H₂O solution to afford (3-((5-fluoro-2-(3-(methylsulfonyl)-5-morpholinophenylamino)pyrimidin-4-yl)(methylamino)-4-methylphenyl)methanol (3) (115 mg, 34%) as a pale yellow solid. ¹H NMR (400 MHz, DMSO) 2.15 (s, 3H), 3.14 (s, 3H), 3.15-3.20 (m, 4H), 3.37 (s, 3H), 3.72-3.78 (m, 4H), 4.46 (d, *J*=5.0 Hz, 2H), 5.17 (t, *J*=5.0 Hz, 1H), 6.95-6.98 (m, 1H), 7.16-7.20 (m, 2H), 7.23-7.27 (m, 1H), 7.47-7.50 (m, 1H), 7.89-7.92 (m, 1H), 8.07-8.10 (m, 1H), 9.48 (s, 1H); MS (ESI) [*M* + *H*]⁺ *m/z* 502.

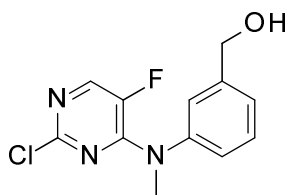
[3-[(2-Chloro-5-fluoropyrimidin-4-yl)amino]phenyl]methanol



A mixture of (3-aminophenyl)methanol (123 mg, 1.00 mmol), 2,4-dichloro-5-fluoropyrimidine (166 mg, 0.99 mmol) and DIPEA (0.35 mL, 1.96 mmol) in IPA (2 mL) was stirred at 80 °C for 2 h. The resulting mixture was concentrated, the residue was partitioned between EtOAc (10 mL) and water (10 mL). The aqueous layer was further extracted with EtOAc (3 x 10 mL), the organic layers combined, dried over MgSO₄, filtered and evaporated. The residue was purified by silica gel chromatography (10 to 50% EtOAc in petroleum ether) to afford [3-

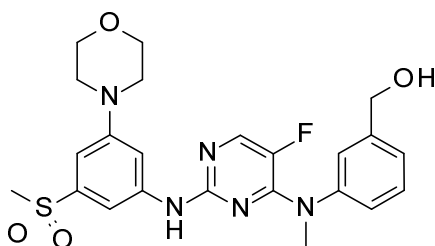
[(2-chloro-5-fluoropyrimidin-4-yl)amino]phenyl]methanol (98 mg, 39%) as an off-white solid. ¹H NMR (300 MHz, DMSO) 4.51 (d, *J*=5.6 Hz, 2 H), 5.22 (t, *J*=5.7 Hz, 1 H), 7.10 (d, *J*=7.7 Hz, 1 H), 7.33 (t, *J*=7.8 Hz, 1 H), 7.54-7.63 (m, 2 H), 8.31 (d, *J*=3.6 Hz, 1 H), 9.95 (s, 1 H); MS (ESI) [*M* + *H*]⁺ *m/z* 254.

[3-[(2-Chloro-5-fluoropyrimidin-4-yl)(methyl)amino]phenyl]methanol



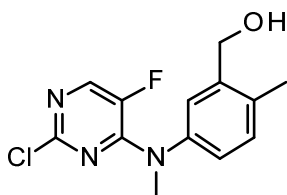
A mixture of [3-[(2-chloro-5-fluoropyrimidin-4-yl)amino]phenyl]methanol (3.1 g, 12.22 mmol), iodomethane (0.76 mL, 12.2 mmol) and Cs₂CO₃ (5.97 g, 18.27 mmol) in DMF (7 mL) was stirred at 60 °C for 4 h. The reaction mixture was diluted with water (90 mL) and extracted into EtOAc (4 x 20 mL). The organic extracts were combined, dried over Na₂SO₄, filtered and evaporated. The residue was purified by silica gel chromatography (10 to 20% EtOAc in petroleum ether) to afford [3-[(2-chloro-5-fluoropyrimidin-4-yl)(methyl)amino]phenyl]methanol (2.3 g, 70%) as an off-white solid. ¹H NMR (300 MHz, CDCl₃) δ 3.51 (s, 3 H), 4.74 (s, 2 H), 7.12 (d, 1 H), 7.24-7.42 (m, 3 H), 7.85 (d, 1 H); MS (ESI) [*M* + *H*]⁺ *m/z* 268.

[3-[(5-Fluoro-2-[[3-(methanesulfonyl)-5-(4-morpholinyl)phenyl]amino}-4-pyrimidinyl)(methyl)amino]phenyl]methanol (4)



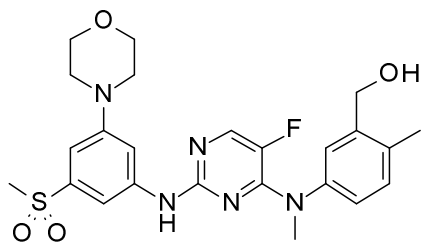
A solution of [3-[(2-chloro-5-fluoropyrimidin-4-yl)(methyl)amino]phenyl]methanol (150 mg, 0.56 mmol) in IPA (6 mL), 3-methanesulfonyl-5-(morpholin-4-yl)aniline (159 mg, 0.62 mmol), concentrated HCl (1 drop) was stirred overnight at 95 °C for 17 h. The resulting mixture was concentrated and the residue was recrystallised from EtOH (15 mL) to afford [3-[(5-fluoro-2-[[3-methanesulfonyl-5-(morpholin-4-yl)phenyl]amino]pyrimidin-4-yl)(methyl)amino]phenyl]methanol (**4**) (73.2 mg, 27%) as a white solid. ¹H NMR (300 MHz, CD₃OD) δ 3.15 (s, 3 H), 3.25-3.33 (m, 4 H), 3.37 (s, 3 H), 3.85-3.88 (m, 4 H), 4.66 (s, 2 H), 7.27-7.31 (m, 3 H), 7.41-7.49 (m, 3 H), 7.92-7.94 (m, 2 H) NH and OH not observed; MS (ESI) [*M* + *H*]⁺ *m/z* 488.

(5-((2-Chloro-5-fluoropyrimidin-4-yl)(methyl)amino)-2-methylphenyl)methanol



(2-Methyl-5-(methylamino)phenyl)methanol (CAS: 1404300-71-5) (56 mg, 0.37 mmol), DIPEA (0.13 mL, 0.74 mmol) and 2,4-dichloro-5-fluoropyrimidine (62 mg, 0.37 mmol) in IPA (1.5 mL) was heated at 80 °C for 17 h. The reaction mixture was concentrated, the residue was partitioned between EtOAc (10 mL) and water (10 mL). The aqueous layer was further extracted with EtOAc (2 x 10 mL), the organic layers combined, dried over MgSO₄, filtered and evaporated. The residue was purified by silica gel chromatography (10 to 100% EtOAc in hexane) to afford (5-((2-chloro-5-fluoropyrimidin-4-yl)(methyl)amino)-2-methylphenyl)methanol (0.08 g, 77%) as a solid. MS (ESI) [*M* + *H*]⁺ *m/z* 282.

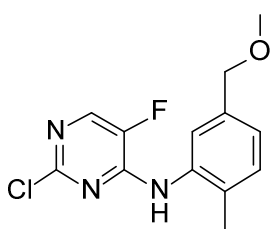
(5-((5-Fluoro-2-(3-(methanesulfonyl)-5-morpholinophenylamino)pyrimidin-4-yl)(methyl)amino)-2-methylphenyl)methanol (5)



A solution of (5-((2-chloro-5-fluoropyrimidin-4-yl)(methyl)amino)-2-methylphenyl)methanol (80 mg, 0.28 mmol) and 3-(methylsulfonyl)-5-morpholinoaniline (80 mg, 0.31 mmol) and concentrated HCl (1 drop) in IPA (2 mL) was heated at 80 °C overnight. The reaction mixture was concentrated and the residue was purified by silica gel chromatography (20 to 40% MeOH in DCM) to afford (5-((5-fluoro-2-(3-(methylsulfonyl)-5-morpholinophenylamino)pyrimidin-4-yl)(methyl)amino)-2-methylphenyl)methanol (**5**) (71 mg, 41%).

¹H NMR (300 MHz, DMSO) 2.23 (s, 3 H), 3.15 (s, 3 H), 3.17-3.18 (m, 4 H), 3.46 (s, 3 H), 3.72-3.80 (m, 4 H), 4.49 (s, 2 H), 6.99 (s, 1 H), 7.10 (d, *J*=2.07 Hz, 1 H), 7.12-7.18 (m, 1 H), 7.25-7.31 (m, 1 H), 7.50 (s, 1 H), 7.99 (d, *J*=5.65 Hz, 1 H), 8.05 (s, 1 H), 9.55 (s, 1 H) OH not observed; MS (ESI) [*M* + *H*]⁺ *m/z* 502.

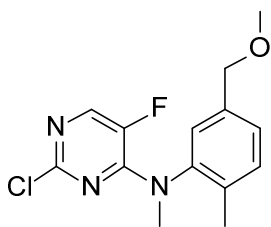
2-Chloro-5-fluoro-N-[5-(methoxymethyl)-2-chloro-5-fluoro-N-[5-(methoxymethyl)-2-methylphenyl]pyrimidin-4-amine



5-(Methoxymethyl)-2-methylaniline (CAS: 64549-36-6) (582 mg, 3.85 mmol), DIPEA (1.1 mL, 6.66 mmol), 2,4-dichloro-5-fluoropyrimidine (639 mg, 3.83 mmol) in IPA (10 mL) was stirred at 80 °C for 16 h. The mixture was concentrated, diluted with water (30 mL) and extracted with EtOAc (3 x 10 mL). The organic extracts were combined and dried over MgSO₄, filtered and evaporated to afford 2-chloro-5-fluoro-N-[5-(methoxymethyl)-2-chloro-5-fluoro-N-[5-(methoxymethyl)-2-methylphenyl]pyrimidin-4-amine (1.0 g, 93%)

as a yellow solid. MS (ESI) [*M* + *H*]⁺ *m/z* 282.

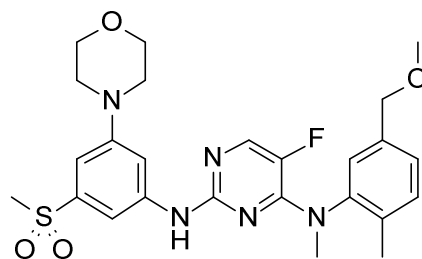
2-Chloro-5-fluoro-N-[5-(methoxymethyl)-2-methylphenyl]-N-methylpyrimidin-4-amine



A mixture of 2-chloro-5-fluoro-N-[5-(methoxymethyl)-2-methylphenyl]pyrimidin-4-amine (500 mg, 1.77 mmol), Cs₂CO₃ (1.15 g, 3.54 mmol), iodomethane (251 mg, 1.77 mmol) in DMF (10 mL) was stirred for at rt for 12 h. The solution was diluted with water (20 mL) and extracted with EtOAc (3 x 20 mL). The organic extracts were combined and dried over MgSO₄, filtered and evaporated to afford 2-chloro-5-fluoro-N-[5-(methoxymethyl)-2-methylphenyl]-N-methylpyrimidin-4-amine (200 mg, 38%)

as a white solid. MS (ESI) [*M* + *H*]⁺ *m/z* 296.

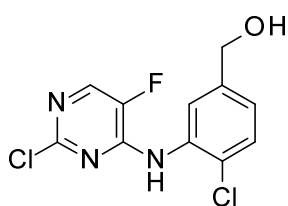
5-Fluoro-N-4-(5-(methoxymethyl)-2-methylphenyl)-N4-methyl-N2-(3-(methylsulfonyl)-5-morpholinophenyl)pyrimidine-2,4-diamine (**6**)



A mixture of 2-chloro-5-fluoro-N-[5-(methoxymethyl)-2-methylphenyl]-N-methylpyrimidin-4-amine (150 mg, 0.51 mmol), 3-methanesulfonyl-5-(morpholin-3-yl)aniline (130 mg, 0.51 mmol), concentrated HCl (1 drop) in IPA (5 mL) was stirred at 80 °C for 12 h. The resulting mixture was concentrated and the crude product was purified by preparative HPLC (1#-Waters 2767-5): Column, SunFire Prep C18, 5μm, 19*100mm; 15-95% H₂O to MeCN to afford fluoro-2-N-[3-methanesulfonyl-5-(morpholin-3-yl)phenyl]-

4-N-[5-(methoxymethyl)-2-methylphenyl]-4-N-methylpyrimidine-2,4-diamine (**6**) (93.7 mg, 36%) as a white solid. ¹H NMR (400MHz, DMSO) 2.17 (s, 3H), 3.15-3.19 (m, 7H), 3.26 (s, 3H), 3.38 (s, 3H), 3.76 (m, 4H), 4.37 (s, 2H), 6.98 (s, 1H), 7.20 (d, 2H), 7.29 (d, 1H), 7.50 (s, 1H), 7.93 (d, 1H), 8.10 (s, 1H), 9.51 (s, 1H); MS (ESI) [M + H]⁺ *m/z* 516.

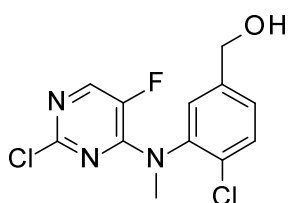
(4-Chloro-3-((2-chloro-5-fluoropyrimidin-4-yl)amino)phenyl)methanol



A mixture of (3-amino-4-chlorophenyl)methanol (CAS: 104317-94-4) (475 mg, 3.01 mmol), 2,4-dichloro-5-fluoropyrimidine (528 mg, 3.16 mmol) and DIPEA (700 μ L, 4.02 mmol) in IPA (12 mL) was heated to 85 °C for 72 h. The reaction mixture was concentrated and the residue was purified by silica gel chromatography (40 to 60% EtOAc in hexane) to afford a ~2:1 mixture of the desired product to the unreacted SM (501 mg) as a pale yellow. This crude material was used directly without further purification. MS (ESI) [M + H]⁺

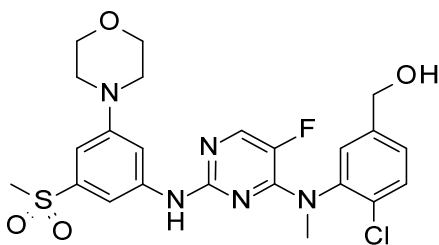
m/z 288, 290.

(4-Chloro-3-((2-chloro-5-fluoropyrimidin-4-yl)(methyl)amino)phenyl)methanol



A mixture of (4-chloro-3-((2-chloro-5-fluoropyrimidin-4-yl)amino)phenyl)methanol (501 mg, maximum of 1.74 mmol, ~66% purity;) Cs₂CO₃ (1.12 g, 3.44 mmol) and iodomethane (175 μ L, 2.81 mmol) and in DMF (6 mL) was allowed to stir at rt for 30 min. The reaction mixture was partitioned between EtOAc (50 mL) and water (50 mL). The aqueous layer was further extracted with EtOAc (50 mL), the organic layers combined, washed with water (50 mL) dried over MgSO₄, filtered and evaporated. The crude material (329 mg) was used directly without further purification. MS (ESI) [M + H]⁺ *m/z* 302, 304.

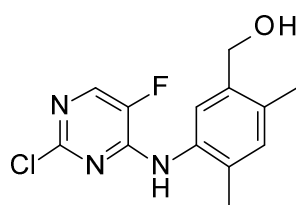
(4-Chloro-3-((5-fluoro-2-((3-(methylsulfonyl)-5-morpholinophenyl)amino)pyrimidin-4-yl)(methyl)amino)phenyl)methanol (**7**)



A mixture of (4-chloro-3-((2-chloro-5-fluoropyrimidin-4-yl)(methyl)amino)phenyl)methanol (329 mg, maximum of 1.09 mmol, ~60% purity), 3-(methylsulfonyl)-5-morpholinoaniline (152 mg, 0.59 mmol) and TsOH.H₂O (200 mg, 1.05 mmol) in *n*-BuOH (4 mL) was heated to 100 °C for 17 h. The reaction mixture was partitioned between EtOAc (50 mL) and saturated NaHCO₃ (50 mL). The aqueous layer was further extracted with EtOAc (20 mL), the organic layers combined, dried over MgSO₄, filtered and

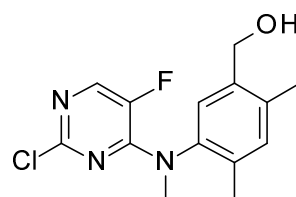
evaporated. The residue was purified by silica gel chromatography (20 to 80% EtOAc in hexane) give a viscous pale yellow oil. This was lyophilized from a MeCN/H₂O solution to afford (4-chloro-3-((5-fluoro-2-((3-(methylsulfonyl)-5-morpholinophenyl)amino)pyrimidin-4-yl)(methyl)amino)phenyl)methanol (**7**) as a pale yellow solid (140 mg, 45%). ¹H NMR (400 MHz, DMSO) 3.14 (s, 3H), 3.15-3.21 (m, 4H), 3.41 (s, 3H), 3.71-3.79 (m, 4H), 4.50 (d, *J*=5.0 Hz, 2H), 5.34 (t, *J*=5.0 Hz, 1H), 6.96-7.00 (m, 1H), 7.29-7.35 (m, 1H), 7.43-7.49 (m, 2H), 7.49-7.55 (m, 1H), 7.95-8.01 (m, 1H), 8.07 (s, 1H), 9.54 (s, 1H); MS (ESI) [M + H]⁺ *m/z* 522, 524.

(5-(2-Chloro-5-fluoropyrimidin-4-ylamino)-2,4-dimethylphenyl)methanol (22, R=Me)



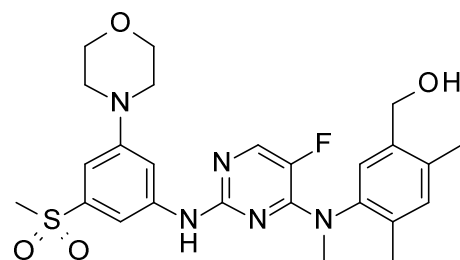
A mixture of (5-amino-2,4-dimethylphenyl)methanol (CAS: 1334148-06-9) (573 mg, 3.79 mmol), 2,4-dichloro-5-fluoropyrimidine (696 mg, 4.17 mmol) and DIPEA (1.7 mL, 9.47 mmol) in *n*-BuOH (10 mL) and stirred at 100 °C for 17 h. The reaction mixture was evaporated, diluted with water to afford a brown solid, this was filtered and then added to DCM (100 mL), the mixture was stirred for 20 min and filtered to afford as light yellow solid. This was dissolved in DCM (50 mL) and diethyl ether and heptane were added and the solvent was slowly removed until only the heptane was remaining, the resultant solid was filtered to afford (5-((2-chloro-5-fluoropyrimidin-4-yl)amino)-2,4-dimethylphenyl)methanol (820 mg, 77%) as a white solid. ¹H NMR (400 MHz, CDCl₃) 1.57 (t, *J*=5.8 Hz, 1H), 2.25 (s, 3H), 2.32 (s, 3H), 4.69 (d, *J*=5.5 Hz, 2H), 6.67 (s, 1H), 7.07 (s, 1H), 7.68 (s, 1H), 8.03 (d, *J*=2.8 Hz, 1H); MS (ESI) [*M* + *H*]⁺ *m/z* 282.

(5-((2-Chloro-5-fluoropyrimidin-4-yl)(methyl)amino)-2,4-dimethylphenyl)methanol (23)



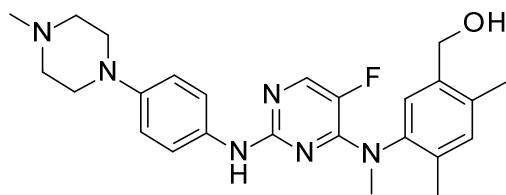
A mixture of (5-((2-chloro-5-fluoropyrimidin-4-yl)amino)-2,4-dimethylphenyl)methanol (175 mg, 0.62 mmol), Cs₂CO₃ (429 mg, 1.32 mmol), iodomethane (60 μL, 0.96 mmol) in DMF (4 mL) was stirred at rt for 1 h. The reaction was diluted with water (50 mL) and extracted with EtOAc (2 x 50 mL). The organic extracts were combined washed with water (50 mL), dried over MgSO₄, filtered and evaporated to afford (5-((2-chloro-5-fluoropyrimidin-4-yl)(methyl)amino)-2,4-dimethylphenyl)methanol (**23**) (184 mg, 100%) as a yellow gum which was used directly in the next step without purification. ¹H NMR (400 MHz, CDCl₃) 1.61 (t, *J*=5.6 Hz, 1H), 2.15 (s, 3H), 2.32 (s, 3H), 3.40 (s, 3H), 4.67 (d, *J*=5.6 Hz, 2H), 7.07 (s, 1H), 7.16 (s, 1H), 7.79 (d, *J*=5.1 Hz, 1H); MS (ESI) [*M* + *H*]⁺ *m/z* 296.

(5-((5-Fluoro-2-((3-(methylsulfonyl)-5-morpholinophenyl)amino)pyrimidin-4-yl)(methyl)amino)-2,4-dimethylphenyl)methanol (8)



A mixture of (5-((2-chloro-5-fluoropyrimidin-4-yl)(methyl)amino)-2,4-dimethylphenyl)methanol (0.62 mmol), 3-(methylsulfonyl)-5-morpholinoaniline (170 mg, 0.66 mmol), TsOH.H₂O (144 mg, 0.76 mmol) in *n*-BuOH (4 mL) was heated to 100 °C for 17 h. The reaction mixture was partitioned between EtOAc (50 mL) and saturated NaHCO₃ (50 mL). The aqueous layer was further extracted with EtOAc (20 mL), the organic layers combined, dried over MgSO₄, filtered and evaporated. The residue was purified by silica gel chromatography (20 to 80% EtOAc in hexane) to give a viscous, yellow oil. The material was further purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5μ silica, 19 mm diameter, 100 mm length, 25-65% H₂O/0.2% NH₃ to MeCN) to afford (5-((5-fluoro-2-((3-(methylsulfonyl)-5-morpholinophenyl)amino)pyrimidin-4-yl)(methyl)amino)-2,4-dimethylphenyl)methanol (**8**) as a colorless solid (152 mg, 48%). ¹H NMR (400 MHz, DMSO) 2.11 (s, 3H), 2.20 (s, 3H), 3.14 (s, 3H), 3.14-3.18 (m, 4H), 3.35 (s, 3H), 3.72-3.77 (m, 4H), 4.43 (d, *J*=6.0 Hz, 2H), 5.08 (t, *J*=6.0 Hz, 1H), 6.95-6.99 (m, 1H), 7.04-7.06 (m, 1H), 7.16-7.19 (m, 1H), 7.47-7.50 (m, 1H), 7.87-7.91 (m, 1H), 8.08-8.11 (m, 1H), 9.47 (s, 1 H); MS (ESI) [*M* + *H*]⁺ *m/z* 516.

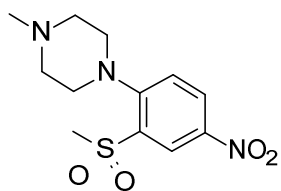
(5-((5-Fluoro-2-((4-(4-methylpiperazin-1-yl)phenyl)amino)pyrimidin-4-yl)(methylamino)-2,4-dimethylphenyl)methanol (9)



A mixture of 4-(4-methylpiperazin-1-yl)aniline (CAS: 16153-81-4) (46 mg, 0.24 mmol) and (5-((2-chloro-5-fluoropyrimidin-4-yl)(methylamino)-2,4-dimethylphenyl)methanol (60 mg, 0.2 mmol) and concentrated HCl (0.5 mL) in IPA (2 mL) was heated to 100 °C for 16 h. The reaction mixture was concentrated and the

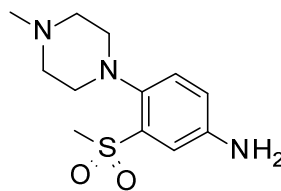
residue purified by reverse phase HPLC to afford (5-((5-fluoro-2-((4-(4-methylpiperazin-1-yl)phenyl)amino)pyrimidin-4-yl)(methylamino)-2,4-dimethylphenyl)methanol (**9**) (31 mg, 34%) as a tan solid. ¹H NMR (400 MHz, DMSO) 2.11 (s, 3H), 2.22 (s, 3H), 2.23 (s, 3H), 2.42-2.47 (m, 4H), 2.95-3.08 (m, 4H), 3.33 (s, 3H), 4.46 (d, *J*=4.5 Hz, 2H), 5.08 (t, *J*=5.2 Hz, 1H), 6.80 (d, *J*=8.7 Hz, 2H), 7.07 (s, 1H), 7.18 (s, 1H), 7.49 (d, *J*=7.5 Hz, 2H), 7.82 (d, *J*=5.9 Hz, 1H), 8.90 (s, 1H); MS (ESI) [*M* + *H*]⁺ *m/z* 451.

1-Methyl-4-(2-(methylsulfonyl)-4-nitrophenyl)piperazine



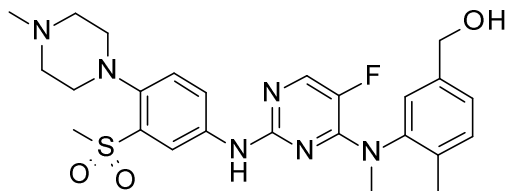
A mixture of 1-methylpiperazine (0.53 mL, 4.79 mmol), 1-fluoro-2-(methylsulfonyl)-4-nitrobenzene (500 mg, 2.3 mmol) were suspended in DMSO (1.8 mL) and sealed into a microwave tube and heated to 100 °C for 10 min in the microwave reactor and cooled to rt. The mixture was diluted with EtOAc (50 mL) and washed with saturated aqueous NaHCO₃ (50 mL) and saturated brine (50 mL). The organic layer was dried over Na₂SO₄, filtered and evaporated to afford 1-methyl-4-(2-(methylsulfonyl)-4-nitrophenyl)piperazine (490 mg, 72%) as a yellow solid. ¹H NMR (300 MHz, DMSO) δ 2.26 (s, 3H), 2.49-2.62 (m, 4H), 3.14-3.38 (m, 4H), 3.45 (s, 3H), 7.75 (d, *J*=8.7 Hz, 1H), 8.50 (dd, *J*=2.8, 8.8 Hz, 1H), 8.64 (d, *J*=2.6 Hz, 1H); MS (ESI) [*M* + *H*]⁺ *m/z* 300.

4-(4-Methylpiperazin-1-yl)-3-(methylsulfonyl)aniline



A degassed mixture of 1-methyl-4-(2-(methylsulfonyl)-4-nitrophenyl)piperazine (490 mg, 1.64 mmol) and 10% palladium on carbon (44 mg) in MeOH (10 mL) was stirred at rt under a balloon of hydrogen for 2 d. The mixture was filtered and the filtrate was concentrated under reduced pressure to afford 4-(4-methylpiperazin-1-yl)-3-(methylsulfonyl)aniline (400 mg, 92%) as a light pink solid. ¹H NMR (300 MHz, DMSO) 2.012.70 (br. m, 4H, obsc.), 2.22 (s, 3H), 2.83 (t, *J*=4.5 Hz, 4H), 3.33 (s, 3H), 5.46 (s, 2H), 6.81 (dd, *J*=2.8, 8.5 Hz, 1H), 7.11 (d, *J*=2.6 Hz, 1H), 7.25 (d, *J*=8.7 Hz, 1H); MS (ESI) [*M* + *H*]⁺ *m/z* 270.

(3-((5-Fluoro-2-((4-(4-methylpiperazin-1-yl)-3-(methylsulfonyl)phenyl)amino)pyrimidin-4-yl)(methylamino)-4-methylphenyl)methanol (10)



A mixture of (3-((2-chloro-5-fluoropyrimidin-4-yl)(methylamino)-4-methylphenyl)methanol (200 mg, 0.71 mmol), 4-(4-methylpiperazin-1-yl)-3-(methylsulfonyl)aniline (200 mg, 0.74 mmol), and TsOH.H₂O (150 mg, 0.78 mmol) in IPA (4 mL) was heated to 80 °C for 18 h. The reaction was

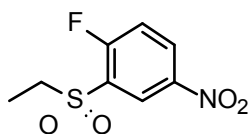
allowed to cool and partitioned between DCM (50 mL) and saturated NaHCO₃ (50 mL). The aqueous layer was further extracted with DCM (50 mL) and the organic extracts were combined, dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (5 to 20% MeOH in DCM). The resulting residue (343 mg) was repurified by flash silica chromatography (20 to 40% MeOH in DCM) to afford (3-((5-fluoro-2-((4-(4-methylpiperazin-1-yl)-3-(methylsulfonyl)phenyl)amino)pyrimidin-4-yl)(methyl)amino)-4-methylphenyl)methanol (**10**) (240 mg, 64%) as a white foam. ¹H NMR (300 MHz, DMSO) 2.12-2.19 (m, 3H), 2.28 (s, 3H), 2.36-2.66 (m, 4H), 2.90-2.97 (m, 4H), 3.39 (s, 6H), 4.47 (d, *J*=5.6 Hz, 2H), 5.18 (t, *J*=5.8 Hz, 1H), 7.03-7.33 (m, 3H), 7.46 (d, *J*=9.0 Hz, 1H), 7.84 (dd, *J*=2.1, 8.8 Hz, 1H), 7.91 (d, *J*=5.6 Hz, 1H), 8.55 (br. s., 1H), 9.59 (s, 1H); MS (ESI) [*M* + *H*]⁺ *m/z* 515.

2-Fluoro-5-nitrobenzene-1-sulfonyl chloride



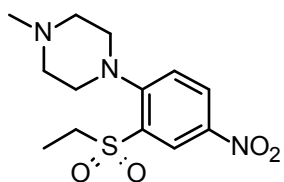
A mixture of chlorosulfonic acid (36 mL, 546 mmol) and 1-fluoro-4-nitrobenzene (18 g, 128 mmol) was stirred at 100 °C for 3 d. The reaction mixture was allowed to cool, cautiously poured into ice water (500 mL) (*CAUTION: exothermic*), extracted with EtOAc (3 x 300 mL), the organic extracts were combined and washed with saturated NaHCO₃ (2 x 150 mL), and saturated brine (300 mL). The organic layer was dried over MgSO₄, filtered and evaporated to afford crude product which was purified by silica gel chromatography (0 to 20% EtOAc in heptane) to afford 2-fluoro-5-nitrobenzene-1-sulfonyl chloride (18.5 g, 60%) as a brown oil. ¹H NMR (400 MHz, CDCl₃) 7.53-7.59 (m, 1H), 8.64 (ddd, *J*=2.8, 4.0, 9.1 Hz, 1H), 8.89 (dd, *J*=2.8, 5.8 Hz, 1H); GCMS (EI) calcd for C₆H₃ClFNO₄S 238.9455 [*M*]⁺ *m/z*, found 238.9456.

2-(Ethylsulfonyl)-1-fluoro-4-nitrobenzene (19)



Sodium sulfite (4.44 g, 35.2 mmol) and NaHCO₃ (2.96 g, 35.2 mmol) were added to a stirred mixture of 2-fluoro-5-nitrobenzene-1-sulfonyl chloride (4.22 g, 17.61 mmol) in EtOH (20 mL) and H₂O (40 mL) (*efferevescence occurred after addition of NaHCO₃*) and the resulting solution was stirred at 50 °C for 1 h. The reaction mixture was evaporated and the residue was suspended in DMF (100 mL), iodoethane (7.1 mL, 88.1 mmol) was added and the resulting mixture was stirred at rt for 2 h. The reaction mixture was diluted with EtOAc (150 mL), and washed with water (100 mL) and saturated brine (2 x 100 mL). The organic layer was dried over MgSO₄, filtered and evaporated to afford crude product which was purified by silica gel chromatography (0 to 30% EtOAc in heptane) to afford 2-(ethylsulfonyl)-1-fluoro-4-nitrobenzene (**19**) (1.71 g, 41%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) 1.36 (td, *J*=0.5, 7.5 Hz, 3H), 3.34-3.41 (m, 2H), 7.42-7.48 (m, 1H), 8.54 (ddd, *J*=2.9, 4.1, 9.0 Hz, 1H), 8.86 (dd, *J*=2.9, 5.8 Hz, 1H); HRMS (EI-TOF) calcd for C₈H₈FNO₄S 233.0158 [*M*]⁺ *m/z*, found 233.0147.

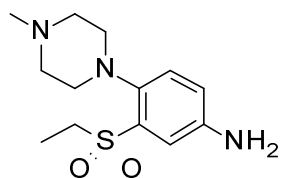
1-(2-(Ethylsulfonyl)-4-nitrophenyl)-4-methylpiperazine



1-Methylpiperazine (220 μL, 1.98 mmol) was added to a solution of 2-(ethylsulfonyl)-1-fluoro-4-nitrobenzene (192 mg, 0.82 mmol) in DMF (4 mL) and the resulting solution was heated to 80 °C for 2 h. The reaction was partitioned between EtOAc (50 mL) and water (50 mL). The aqueous layer was further extracted with EtOAc (50 mL), the organic extracts were

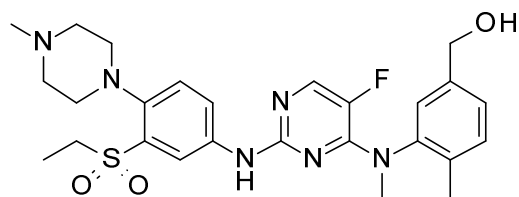
combined, dried over MgSO_4 , filtered and evaporated to afford 1-(2-(ethylsulfonyl)-4-nitrophenyl)-4-methylpiperazine as an orange solid (259 mg, 42%) that was used without further purification. MS (ESI) $[\text{M} + \text{H}]^+ m/z$ 314.

3-(Ethylsulfonyl)-4-(4-methylpiperazin-1-yl)aniline



A suspension of 1-(2-(ethylsulfonyl)-4-nitrophenyl)-4-methylpiperazine (259 mg, 0.83 mmol) and 10% Pd/C (101 mg) was in MeOH (6 mL) was stirred at rt under a balloon atmosphere of hydrogen for 17 h. The mixture was filtered through a plug of celite[®] and the filtrate was evaporated to afford 3-(ethylsulfonyl)-4-(4-methylpiperazin-1-yl)aniline (209 mg, 89%) as a colorless solid that was used without further purification. MS (ESI) $[\text{M} + \text{H}]^+ m/z$ 284.

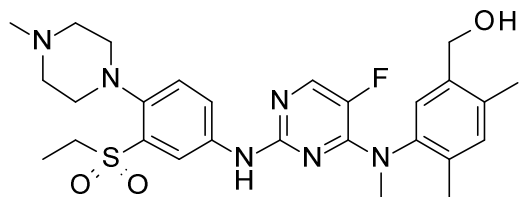
(3-((2-((3-(Ethylsulfonyl)-4-(4-methylpiperazin-1-yl)phenyl)amino)-5-fluoropyrimidin-4-yl)(methyl)amino)-4-methylphenyl)methanol (11)



A mixture of 3-(ethylsulfonyl)-4-(4-methylpiperazin-1-yl)aniline (133 mg, 0.47 mmol), (3-((2-chloro-5-fluoropyrimidin-4-yl)(methyl)amino)-4-methylphenyl)methanol (142 mg, 0.50 mmol), and TsOH.H₂O (127 mg, 0.67 mmol) in IPA (4 mL) was heated to 85 °C for 17 h. The reaction was allowed to cool and

partitioned between EtOAc (50 mL) and saturated NaHCO₃ (50 mL). The aqueous layer was further extracted with EtOAc (50 mL) and the organic extracts were combined, dried over MgSO_4 , filtered and concentrated under reduced pressure. The residue was purified by reverse phase HPLC to give (3-((2-((3-(ethylsulfonyl)-4-(4-methylpiperazin-1-yl)phenyl)amino)-5-fluoropyrimidin-4-yl)(methyl)amino)-4-methylphenyl)methanol (**11**) (176 mg, 66%) as a white solid. ¹H NMR (400 MHz, DMSO) 1.04 (t, $J=7.5$ Hz, 3H), 2.14 (s, 3H), 2.21 (s, 3H), 2.45-2.52 (m, 4H), 2.84-2.95 (m, 4H), 3.38 (s, 3H), 3.61 (q, $J=7.5$ Hz, 2H), 4.46 (d, $J=5.0$ Hz, 2H), 5.17 (t, $J=5.0$ Hz, 1H), 7.16-7.21 (m, 2H), 7.23-7.27 (m, 1H), 7.40-7.47 (m, 1H), 7.78-7.86 (m, 1H), 7.88-7.92 (m, 1H), 8.51 (br s, 1H), 9.58 (s, 1H); MS (ESI) $[\text{M} + \text{H}]^+ m/z$ 529.

[5-[(2-[[3-(Ethanesulfonyl)-4-(4-methylpiperazin-1-yl)phenyl]amino]-5-fluoropyrimidin-4-yl)(methyl)amino]-2,4-dimethylphenyl]methanol (12)

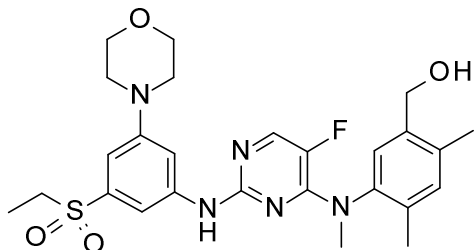


A mixture of 3-(ethanesulfonyl)-4-(4-methylpiperazin-1-yl)aniline (100 mg, 0.35 mmol) and [5-[(2-chloro-5-fluoropyrimidin-4-yl)(methyl)amino]-2,4-dimethylphenyl]methanol (105 mg, 0.36 mmol) in IPA (5 mL) and HCl (0.04 mL, 1.32 mmol) was stirred at 100 °C for 17 h. The mixture was concentrated under vacuum and

the residue was purified by preparative HPLC (XBridge Prep C18 OBD Column, 5μm, 19 x 150mm,; mobile phase, water with 0.03% NH₃/H₂O and MeCN (15.0% MeCN up to 62.0% in 8 min, hold 95.0% in 1 min, down to 15.0% in 2 min) to afford [5-[(2-[[3-(ethanesulfonyl)-4-(4-methylpiperazin-1-yl)phenyl]amino]-5-fluoropyrimidin-4-yl)(methyl)amino]-2,4-dimethylphenyl]methanol (**12**) (94.7 mg, 49%) as a white solid. ¹H NMR (300 MHz, CD₃OD) 1.17 (t, $J=7.5$ Hz, 3H), 2.13 (s, 3H), 2.34 (s, 3H), 2.37 (s, 3H), 2.55-2.66 (brs, 4H), 3.04 (s, 4H), 3.35 (3H, s), 3.61 (q, $J=7.5$ Hz, 2H), 4.60 (s, 2H), 7.10 (s,

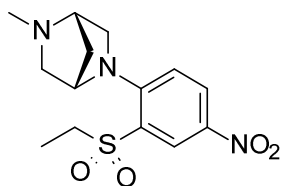
1H), 7.20 (s, 1H), 7.37-7.40 (m, 1H), 7.69-7.76 (m, 2H), 8.58 (s, 1H), NH and OH not observed; MS (ESI) $[M + H]^+$ m/z 543.

(5-((2-(3-(Ethylsulfonyl)-5-morpholinophenylamino)-5-fluoropyrimidin-4-yl)(methyl)amino)-2,4-dimethylphenyl)methanol (13)



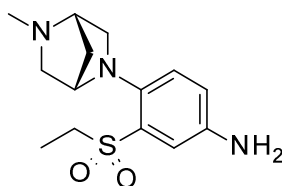
A mixture of (5-((2-chloro-5-fluoropyrimidin-4-yl)(methyl)amino)-2,4-dimethylphenyl)methanol (100 mg, 0.33 mmol), 3-(ethylsulfonyl)-5-morpholinobenzenamine (87 mg, 0.33 mmol), concentrated HCl (1 drop) in IPA (5 mL) was stirred at 80 °C for 12 h. The mixture was concentrated under vacuum and the residue was purified by preparative HPLC (SunFire Prep C18, 5μm, 19 x 100mm; mobile phase, H₂O and MeCN (15% MeCN up to 55% in 8 min, up to 100% in 0.1 min, hold 100% in 0.9 min, down to 15% in 0.1 min, hold 15% in 1.4 min) to afford (5-((2-(3-(ethylsulfonyl)-5-morpholinophenylamino)-5-fluoropyrimidin-4-yl)(methyl)amino)-2,4-dimethylphenyl)methanol (**13**) (18.7 mg, 9.5%) as a white solid. ¹H NMR (400 MHz, DMSO) 1.26 (t, $J=8.0$ Hz, 3H), 2.19 (s, 3H), 2.34 (s, 3H), 3.18-3.26 (m, 6H), 3.46 (s, 3H), 3.85-3.88 (m, 4H), 4.61 (s, 2H), 7.03-7.04 (m, 1H), 7.11 (s, 1H), 7.21 (s, 1H), 7.39-7.40 (m, 1H), 7.77 (d, 1H), 8.18 (s, 1H), NH and OH not observed; MS (ESI) $[M + H]^+$ m/z 530.

(1S,4S)-2-(2-(Ethylsulfonyl)-4-nitrophenyl)-5-methyl-2,5-diazabicyclo[2.2.1]heptane



A solution of 2-(ethylsulfonyl)-1-fluoro-4-nitrobenzene (1.0 g, 4.29 mmol), (1S,4S)-2-methyl-2,5-diazabicyclo[2.2.1]heptane dihydrobromide (CAS 125224-62-6) (1.2 g, 4.29 mmol) and K₂CO₃ (2.4 g, 17.1 mmol) in DMSO (10 mL) was stirred at 55 °C for 90 min. The reaction mixture was allowed to cool, diluted with EtOAc (125 mL), and washed with water (100 mL) and saturated brine (125 mL). The organic layer was dried over MgSO₄, filtered and evaporated and the residue was purified silica gel chromatography (0 to 10% MeOH/NH₃ in DCM) to afford (1S,4S)-2-(2-(ethylsulfonyl)-4-nitrophenyl)-5-methyl-2,5-diazabicyclo[2.2.1]heptane (0.584 g, 42%) as a yellow gum. ¹H NMR (400 MHz, CDCl₃) 1.31 (t, $J=7.4$ Hz, 3H), 1.89 (d, $J=10$ Hz, 1H), 2.01-2.08 (m, 1H), 2.42 (s, 3H), 2.86-2.98 (m, 2H), 3.28-3.43 (m, 2H), 3.50 (s, 1H), 3.65 (dd, $J=1.4$, 10.2 Hz, 1H), 3.83 (dd, $J=2.5$, 10.2 Hz, 1H), 4.54 (s, 1H), 6.93 (d, $J=9.4$ Hz, 1H), 8.20 (dd, $J=2.8$, 9.3 Hz, 1H), 8.84 (d, $J=2.7$ Hz, 1H); MS (ESI) $[M + H]^+$ m/z 326.

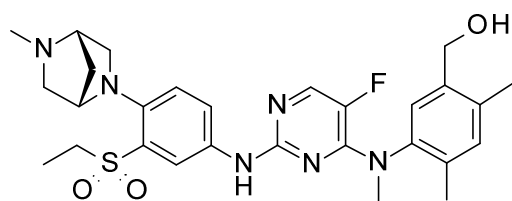
3-(Ethylsulfonyl)-4-((1S,4S)-5-methyl-2,5-diazabicyclo[2.2.1]heptan-2-yl)aniline (20)



10% Pd/C (57.3 mg, 0.05 mmol) was added to a stirred solution of (1S,4S)-2-(2-(ethylsulfonyl)-4-nitrophenyl)-5-methyl-2,5-diazabicyclo[2.2.1]heptane (584 mg, 1.79 mmol) in EtOH (15 mL) and EtOAc (10 mL). The reaction flask was evacuated and purged with hydrogen 3 times, and the suspension was then stirred under a balloon atmosphere of hydrogen at rt for 18 h. The reaction mixture was filtered through celite[®] to afford 3-(ethylsulfonyl)-4-((1S,4S)-5-methyl-2,5-diazabicyclo[2.2.1]heptan-2-yl)aniline (**20**) (495 mg, 93%) as a yellow gum. ¹H NMR (400 MHz, DMSO) 1.04 (t, $J=7.4$ Hz, 3H), 1.76 (s, 2H), 2.35 (s, 3H), 2.74 (d, $J=9.6$ Hz, 1H), 2.98 (dd, $J=2.2$,

9.4 Hz, 1H), 3.34 (s, 2H), 3.4-3.5 (m, 2H), 3.53-3.59 (m, 1H), 3.61 (s, 1H), 5.36 (s, 2H), 6.81 (dd, $J=2.8, 8.6$ Hz, 1H), 7.12 (d, $J=2.8$ Hz, 1H), 7.20 (d, $J=8.6$ Hz, 1H); MS (ESI) $[M + H]^+$ m/z 296.

(5-((2-((3-(Ethylsulfonyl)-4-((1*S*,4*S*)-5-methyl-2,5-diazabicyclo[2.2.1]heptan-2-yl)phenyl)amino)-5-fluoropyrimidin-4-yl)(methyl)amino)-2,4-dimethylphenyl)methanol (14)

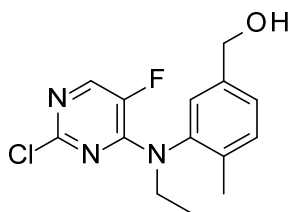


A mixture of 3-(ethylsulfonyl)-4-((1*S*,4*S*)-5-methyl-2,5-diazabicyclo[2.2.1]heptan-2-yl)aniline (250 mg, 0.85 mmol), (5-((2-chloro-5-fluoropyrimidin-4-yl)(methyl)amino)-2,4-dimethylphenyl)methanol (250 mg, 0.85 mmol) and TsOH.H₂O (322 mg, 1.69 mmol) in IPA (10 mL) was stirred at 90 °C for 24 h. The reaction mixture was diluted with 2M

NaOH (25 mL), extracted with EtOAc (2 x 50 mL), the organic layer was dried over MgSO₄, filtered and evaporated to afford a yellow foam. The residue was purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5μ silica, 50 mm diameter, 100 mm length, 5-95% H₂O/1% NH₃ to MeCN) followed by preparative HPLC (Waters SunFire column, 5μ silica, 50 mm diameter, 100 mm length, 5-95% H₂O/0.1% formic acid to MeCN). An additional preparative HPLC (Waters XBridge Prep C18 OBD column, 5μ silica, 50 mm diameter, 100 mm length, 5-95% H₂O/1% NH₃ to MeCN) afforded

(5-((2-((3-(ethylsulfonyl)-4-((1*S*,4*S*)-5-methyl-2,5-diazabicyclo[2.2.1]heptan-2-yl)phenyl)amino)-5-fluoropyrimidin-4-yl)(methyl)amino)-2,4-dimethylphenyl)methanol (**14**) (140 mg, 30%) as a white solid. ¹H NMR (400 MHz, DMSO) 1.05 (t, $J=7.4$ Hz, 3H), 1.80 (s, 2H), 2.12 (s, 3H), 2.24 (s, 3H), 2.36 (s, 3H), 2.54-2.57 (m, 1H), 2.73 (d, $J=9.4$ Hz, 1H), 3.04-3.1 (m, 1H), 3.35 (s, 1H), 3.38 (s, 3H), 3.42-3.53 (m, 2H), 3.61 (dd, $J=7.4, 13.8$ Hz, 1H), 3.79 (s, 1H), 4.46 (d, $J=5.2$ Hz, 2H), 5.06 (t, $J=5.3$ Hz, 1H), 7.07 (s, 1H), 7.19 (s, 1H), 7.33 (d, $J=7.5$ Hz, 1H), 7.79 (d, $J=9.2$ Hz, 1H), 7.89 (d, $J=5.8$ Hz, 1H), 8.47 (s, 1H), 9.47 (s, 1H); ¹³C NMR (101 MHz, DMSO, 30°C) 7.12, 16.64, 17.63, 33.90, 39.10, 41.45, 47.03, 56.34, 59.57, 60.43, 63.36, 65.25, 118.84, 123.62, 124.75, 125.23, 131.88, 132.42 ($J=3.0$), 134.00, 134.48, 137.28, 139.02, 141.12 ($J=247.3$ Hz), 141.58, 142.45 ($J=23.5$ Hz), 142.48, 151.28 ($J=5.1$ Hz), 155.12 ($J=2.6$ Hz); HRMS (ESI-TOF) calcd for C₂₈H₃₆FN₆O₃S 555.2548 $[M + H]^+$ m/z , found 555.2546.

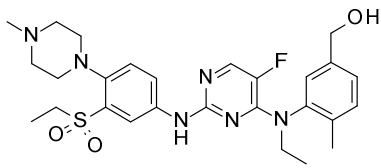
(3-((2-Chloro-5-fluoropyrimidin-4-yl)(ethyl)amino)-4-methylphenyl)methanol (3-((2-chloro-5-fluoropyrimidin-4-yl)(ethyl)amino)-4-methylphenyl)methanol



A mixture of (3-((2-chloro-5-fluoropyrimidin-4-yl)amino)-4-methylphenyl)methanol (1.0 g, 3.74 mmol), Cs₂CO₃ (1.3 g, 4.11 mmol) and ethylbromide (0.28 mL, 3.74 mmol) in DMF (20 mL) and stirred at rt for 1 h. The reaction mixture was diluted with water (50 mL) and extracted with ether (2 x 50 mL). The organic layer was dried over MgSO₄ concentrated and the resulting residue was purified by silica gel chromatography (80 to 100% ether in heptane) to afford (3-((2-chloro-5-fluoropyrimidin-4-yl)(ethyl)amino)-4-methylphenyl)methanol (900 mg, 81%) as a colorless gum.

¹H NMR (400 MHz, CDCl₃) 1.25 (t, $J=7.1$ Hz, 3H), 1.77 (t, $J=5.9$ Hz, 1H), 2.18 (s, 3H), 3.58-3.77 (m, 1H), 4.05-4.21 (m, 1H), 4.69 (d, $J=5.7$ Hz, 2H), 7.14 (s, 1H), 7.27 (d, $J=1.1$ Hz, 2H), 7.79 (d, $J=5.1$ Hz, 1H); MS (ESI) $[M + H]^+$ m/z 296.

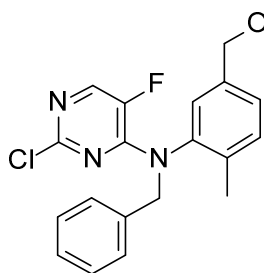
(3-(Ethyl(2-((3-(ethylsulfonyl)-4-(4-methylpiperazin-1-yl)phenyl)amino)-5-fluoropyrimidin-4-yl)amino)-4-methylphenyl)methanol (15)



A mixture of 3-(ethylsulfonyl)-4-(4-methylpiperazin-1-yl)aniline (162 mg, 0.57 mmol) and (3-((2-chloro-5-fluoropyrimidin-4-yl)(ethyl)amino)-4-methylphenyl)methanol (169 mg, 0.57 mmol), 6 N HCl in IPA (9.52 μ L, 0.06 mmol) and TsOH.H₂O (217 mg, 1.14 mmol) in IPA (10 mL) was stirred at 80 °C for 36 h. 2M NaOH (25 mL) was

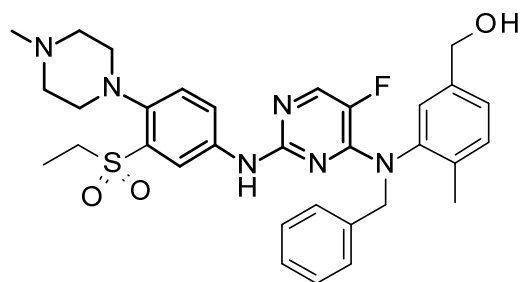
added and the mixture extracted with EtOAc (2 x 50 mL), the organic layer was dried over MgSO₄, filtered and evaporated to afford yellow foam. This was purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5 μ silica, 50 mm diameter, 100 mm length, 5-95% H₂O/1% NH₃ to MeCN) to afford (3-(ethyl(2-((3-(ethylsulfonyl)-4-(4-methylpiperazin-1-yl)phenyl)amino)-5-fluoropyrimidin-4-yl)amino)-4-methylphenyl)methanol (**15**) (117 mg, 38%) as a white solid. ¹H NMR (500 MHz, DMSO) 1.07 (t, *J*=7.4 Hz, 3H), 1.20 (t, *J*=7.0 Hz, 3H), 2.14 (s, 3H), 2.24 (s, 3H), 2.42-2.5 (m, 4H), 2.92 (t, *J*=4.4 Hz, 4H), 3.63 (q, *J*=7.4 Hz, 2H), 3.71 (brs, 1H), 4.10 (brs, 1H), 4.49 (d, *J*=5.4 Hz, 2H), 5.18 (t, *J*=5.7 Hz, 1H), 7.18 (s, 1H), 7.22 (d, *J*=7.7 Hz, 1H), 7.28 (d, *J*=7.8 Hz, 1H), 7.45 (s, 1H), 7.83 (s, 1H), 7.91 (d, *J*=5.9 Hz, 1H), 8.44 (s, 1H), 9.54 (s, 1H); MS (ESI) [*M* + *H*]⁺ *m/z* 543.

(3-(Benzyl(2-chloro-5-fluoropyrimidin-4-yl)amino)-4-methylphenyl)methanol

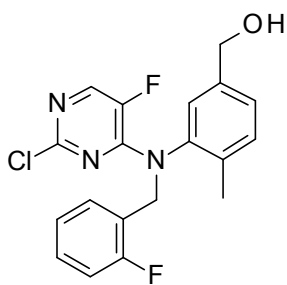


A mixture of (3-((2-chloro-5-fluoropyrimidin-4-yl)amino)-4-methylphenyl)methanol (1.0 g, 3.74 mmol), Cs₂CO₃ (1.3 g, 4.11 mmol) and benzyl bromide (0.45 mL, 3.74 mmol) in DMF (10 mL) was stirred at rt for 1 h. The reaction mixture was diluted with EtOAc (75 mL), and washed with saturated brine (3 x 75 mL). The organic layer was dried over MgSO₄, filtered and evaporated to afford crude product which was purified by silica gel chromatography (20 to 80% EtOAc in heptane) to afford (3-(benzyl(2-chloro-5-fluoropyrimidin-4-yl)amino)-4-methylphenyl)methanol (1.2 g, 90%) as a pale yellow oil. ¹H NMR (400 MHz, CDCl₃) 1.62 (t, *J*=5.9 Hz, 1H), 1.94 (s, 3H), 4.58 (d, *J*=5.8 Hz, 2H), 4.85 (d, *J*=14.1 Hz, 1H), 5.29 (d, *J*=14.1 Hz, 1H), 6.91-6.95 (m, 1H), 7.16-7.31 (m, 7H), 7.81 (d, *J*=5.1 Hz, 1H); MS (ESI) [*M* + *H*]⁺ *m/z* 358.

(3-(Benzyl(2-((3-(ethylsulfonyl)-4-(4-methylpiperazin-1-yl)phenyl)amino)-5-fluoropyrimidin-4-yl)amino)-4-methylphenyl)methanol (**16**)



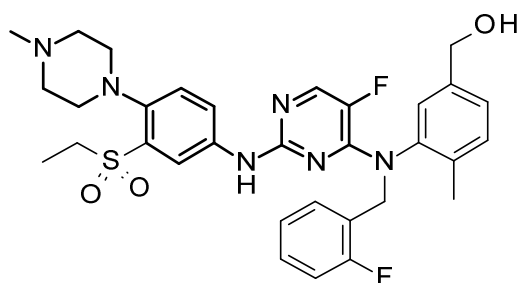
A mixture of 3-(ethylsulfonyl)-4-(4-methylpiperazin-1-yl)aniline (79 mg, 0.28 mmol), (3-(benzyl(2-chloro-5-fluoropyrimidin-4-yl)amino)-4-methylphenyl)methanol (100 mg, 0.28 mmol) and TsOH.H₂O (4.81 mg, 0.03 mmol) in *n*-BuOH (6 mL) was heated to 100 °C for 17 h. Additional TsOH.H₂O (100 mg, 0.28 mmol) was added and the mixture was stirred at 90 °C for a further 17 h. The reaction mixture was evaporated to afford crude product which was purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5 μ silica, 50 mm diameter, 100 mm length, 5-95% H₂O/1% NH₃ to MeCN) to afford (3-(benzyl(2-((3-(ethylsulfonyl)-4-(4-methylpiperazin-1-yl)phenyl)amino)-5-fluoropyrimidin-4-yl)amino)-4-methylphenyl)methanol (**16**) (6.6 mg, 3.9%) as a beige solid. ¹H NMR (500 MHz, DMSO) 1.05 (t, *J*=7.4 Hz, 3H), 1.96 (s, 3H), 2.28 (s, 3H), 2.46-2.54 (m, 4H), 2.94 (t, *J*=4.6 Hz, 4H), 3.63 (q, *J*=7.4 Hz, 2H), 4.47 (s, 2H), 5.19 (s, 3H), 7.17 (s, 1H), 7.23 (s, 2H), 7.27-7.34 (m, 5H), 7.39 (d, *J*=8.8 Hz, 1H), 7.91 (d, *J*=8.6 Hz, 1H), 8.02 (d, *J*=5.8 Hz, 1H), 8.34 (s, 1H), 9.62 (s, 1H); MS (ESI) [*M* + *H*]⁺ *m/z* 604.



(3-((2-Chloro-5-fluoropyrimidin-4-yl)(2-fluorobenzyl)amino)-4-methylphenyl)methanol A mixture of (3-((2-chloro-5-fluoropyrimidin-4-yl)amino)-4-methylphenyl)methanol (500 mg, 1.87 mmol), Cs_2CO_3 (669 mg, 2.05 mmol) and 1-(bromomethyl)-2-fluorobenzene (0.23 mL, 1.87 mmol) in DMF (5 mL) was stirred at rt 2 h. The reaction mixture was diluted with water (50 mL), extracted with EtOAc (2 x 50 mL), the organic layer was separated, dried over MgSO_4 , filtered and evaporated to afford crude product which was purified by silica gel chromatography (10 to 100% EtOAc in heptane) to afford

(3-((2-chloro-5-fluoropyrimidin-4-yl)(2-fluorobenzyl)amino)-4-methylphenyl)methanol (592 mg, 84%) as a colourless gum. ^1H NMR (400 MHz, CDCl_3) 0.84-0.92 (m, 1H), 1.51 (s, 3H), 4.57 (d, $J=6.0$ Hz, 2H), 4.94 (d, $J=14.4$ Hz, 1H), 5.39 (d, $J=13.7$ Hz, 1H), 6.89-6.98 (m, 2H), 7.09 (td, $J=1.1$, 7.5 Hz, 1H), 7.17-7.28 (m, 3H), 7.51 (td, $J=1.7$, 7.5 Hz, 1H), 7.84 (d, $J=5.0$ Hz, 1H); MS (ESI) $[\text{M} + \text{H}]^+ m/z$ 376.

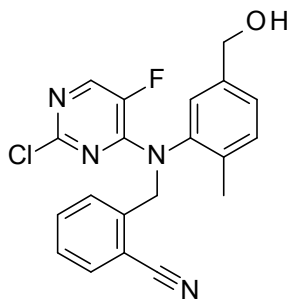
(3-((2-((3-(Ethylsulfonyl)-4-(4-methylpiperazin-1-yl)phenyl)amino)-5-fluoropyrimidin-4-yl)(2-fluorobenzyl)amino)-4-methylphenyl)methanol (17)



A mixture of (3-((2-chloro-5-fluoropyrimidin-4-yl)(2-fluorobenzyl)amino)-4-methylphenyl)methanol (147 mg, 0.39 mmol), 3-(ethylsulfonyl)-4-(4-methylpiperazin-1-yl)aniline (111 mg, 0.39 mmol) and $\text{TsOH} \cdot \text{H}_2\text{O}$ (81 mg, 0.47 mmol) in IPA (4 mL) was heated at 80 °C for 48 h. After cooling to rt the mixture was diluted with MeOH (5 mL) and was purified by ion exchange chromatography, using an SCX column (100% 0.35M NH_3/MeOH) followed by silica gel chromatography (0 to 10% [9:1 MeOH/ NH_3] in

DCM) to afford a yellow residue. This was dissolved in 1:1 DCM/ether and product was precipitated out by addition of heptane. The solid was collected by filtration and dried to afford (3-((2-((3-(ethylsulfonyl)-4-(4-methylpiperazin-1-yl)phenyl)amino)-5-fluoropyrimidin-4-yl)(2-fluorobenzyl)amino)-4-methylphenyl)methanol (17) (125 mg, 51%) as a yellow solid. ^1H NMR (400 MHz, DMSO) 0.99 (t, $J=7.4$ Hz, 3H), 1.96 (s, 3H), 2.23 (s, 3H), 2.39-2.47 (m, 4H), 2.89 (t, $J=4.5$ Hz, 4H), 3.57 (q, $J=7.4$ Hz, 2H), 4.39 (d, $J=5.6$ Hz, 2H), 5.10 (t, $J=5.7$ Hz, 1H), 5.15 (s, 2H), 7.05-7.15 (m, 3H), 7.17 (s, 2H), 7.26-7.32 (m, 1H), 7.33 (d, $J=8.6$ Hz, 1H), 7.45 (t, $J=7.0$ Hz, 1H), 7.84 (dd, $J=2.5$, 8.7 Hz, 1H), 7.98 (d, $J=5.8$ Hz, 1H), 8.30 (d, $J=2.5$ Hz, 1H), 9.58 (s, 1H); MS (ESI) $[\text{M} + \text{H}]^+ m/z$ 623.

2-(((2-Chloro-5-fluoropyrimidin-4-yl)(5-(hydroxymethyl)-2-methylphenyl)amino)methyl)benzonitrile (24)

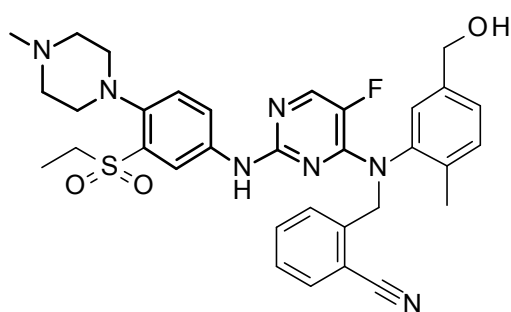


A mixture of (3-((2-chloro-5-fluoropyrimidin-4-yl)amino)-4-methylphenyl)methanol (1.0 g, 3.74 mmol), Cs_2CO_3 (1.3 g, 4.11 mmol) and 2-cyanobenzyl bromide (0.73 g, 3.74 mmol) in DMF (20 mL) was stirred at rt for 1 h. The reaction mixture was diluted with water (50 mL) and extracted with ether (2 x 50 mL). The organic layer was dried over MgSO_4 concentrated and the resulting residue was purified by silica gel chromatography (80 to 100% ether in heptane) to afford 2-(((2-chloro-5-fluoropyrimidin-4-yl)(5-(hydroxymethyl)-2-methylphenyl)amino)methyl)benzonitrile (24) (1.1 g, 79%) as a white solid. ^1H NMR (400 MHz, CDCl_3) 1.85 (t, $J=6.0$ Hz, 1H),

1.92 (s, 3H), 4.59 (d, $J=5.7$ Hz, 2H), 5.29 (d, $J=14.8$ Hz, 1H), 5.44 (d, $J=14.7$ Hz, 1H), 7.07 (d, $J=1.4$

Hz, 1H), 7.18 (d, $J=7.8$ Hz, 1H), 7.25 (d, $J=8.1$ Hz, 1H), 7.38 (td, $J=1.2, 7.7$ Hz, 1H), 7.52 (dd, $J=1.1, 7.8$ Hz, 1H), 7.60 (td, $J=1.4, 7.7$ Hz, 1H), 7.85-7.88 (m, 1H), 7.88 (d, $J=4.9$ Hz, 1H); MS (ESI) $[M + H]^+$ m/z 383.

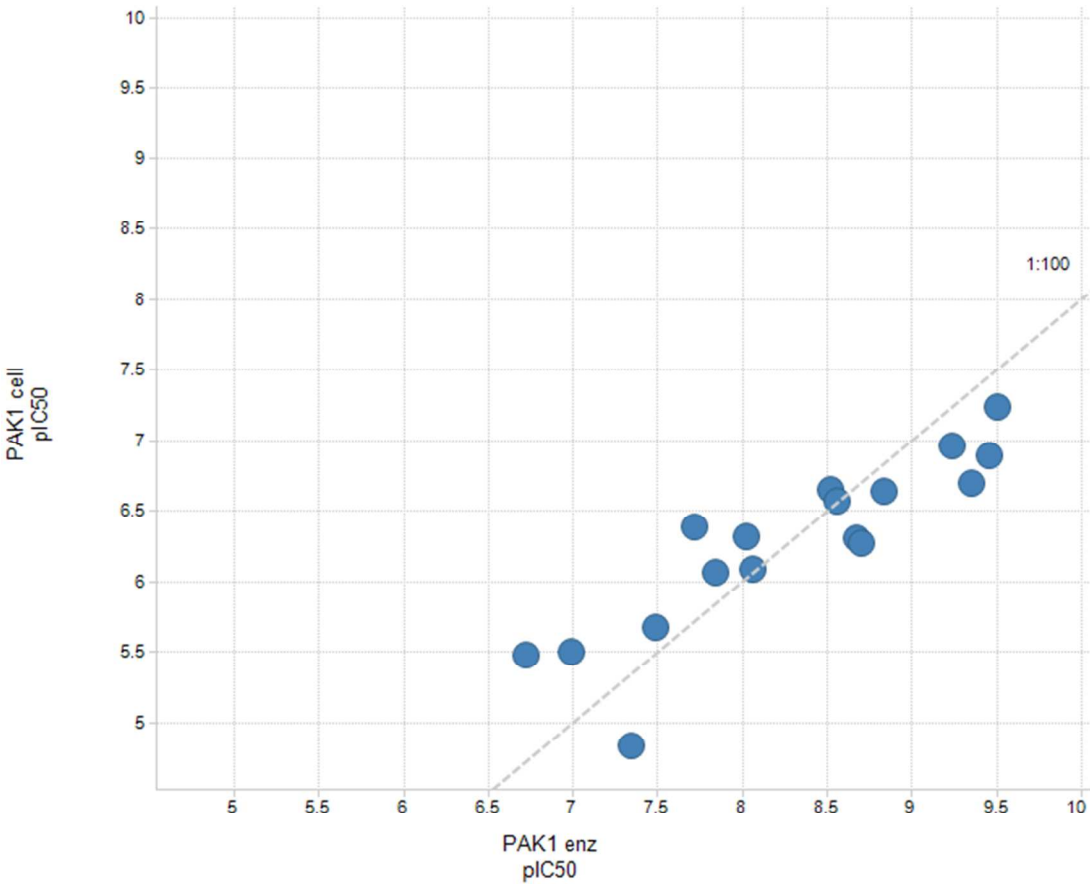
2-(((2-((3-(Ethylsulfonyl)-4-(4-methylpiperazin-1-yl)phenyl)amino)-5-fluoropyrimidin-4-yl)(5-(hydroxymethyl)-2-methylphenyl)amino)methyl)benzonitrile (18)



A solution of 3-(ethylsulfonyl)-4-(4-methylpiperazin-1-yl)aniline (111 mg, 0.39 mmol) and 2-(((2-chloro-5-fluoropyrimidin-4-yl)(5-(hydroxymethyl)-2-methylphenyl)amino)methyl)benzonitrile (150 mg, 0.39 mmol), 6 N HCl in IPA (6.53 μ l, 0.04 mmol) in IPA (10 mL) and was stirred at 80 °C for 18 h. TsOH.H₂O (149 mg, 0.78 mmol) was added and the reaction was again stirred at 80 °C for a further 18 h. 2M NaOH (25 mL) was added and the mixture extracted with EtOAc (2 x 50 mL), the organic layer

was dried over MgSO₄, filtered and evaporated to afford yellow foam. This was purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5 μ silica, 50 mm diameter, 100 mm length, 5-95% H₂O/1% NH₃ to MeCN) followed by silica gel chromatography (70 to 100% EtOAc in heptane) to afford a yellow foam, which was triturated with ether to give a solid which was collected by filtration and dried under vacuum to give 2-(((2-((3-(ethylsulfonyl)-4-(4-methylpiperazin-1-yl)phenyl)amino)-5-fluoropyrimidin-4-yl)(5-(hydroxymethyl)-2-methylphenyl)amino)methyl)benzonitrile (**18**) (116 mg, 47%) as a beige solid. ¹H NMR (500 MHz, DMSO) 0.98 (t, $J=7.4$ Hz, 3H), 2.04 (s, 3H), 2.23 (s, 3H), 2.37-2.51 (m, 4H), 2.89 (t, $J=4.5$ Hz, 4H), 3.31 (s, 1H), 3.56 (q, $J=7.4$ Hz, 2H), 4.37 (d, $J=5.0$ Hz, 2H), 5.10 (t, $J=5.6$ Hz, 1H), 5.53 (s, 1H), 7.03 (s, 1H), 7.15-7.24 (m, 2H), 7.34 (d, $J=8.8$ Hz, 1H), 7.46 (td, $J=1.4, 7.6$ Hz, 1H), 7.61-7.7 (m, 2H), 7.78 (dd, $J=1.1, 7.8$ Hz, 2H), 8.02 (d, $J=5.7$ Hz, 1H), 8.30 (d, $J=2.4$ Hz, 1H), 9.63 (s, 1H); ¹³C NMR (101 MHz, DMSO) 7.02, 16.94, 45.66, 47.87, 51.82, 52.90, 55.01, 61.99, 111.34, 117.08, 118.96, 123.85, 124.37, 125.69, 125.73, 128.16, 129.43, 130.38, 132.73, 133.19, 133.44 ($J=3.6$), 134.47, 138.36, 140.59, 140.95 ($J=248.2$), 141.21, 141.28 ($J=2.8$), 143.60 ($J=23.5$), 144.74, 150.90 ($J=5.0$), 154.99 ($J=2.6$); HRMS (ESI-TOF) calcd for C₃₃H₃₇FN₇O₃S 630.2657 $[M + H]^+$ m/z , found 630.2659.

Correlation of pPAK1 cell pIC50 with PAK1 enzyme pIC50



Kinase selectivity data for compound **18** (Source: Millipore)

Table 1. %inhibition data for compound **18** tested at 100 nM concentration

Kinase Name	Mean Inhibition (%)
ALK(h)	20
ALK4(h)	17
AMPKa2(h)	15
ARK5(h)	-2
Abl(h)	23
Arg(h)	67
Aurora-B(h)	-14
Aurora-C(h)	-3
Axl(h)	11
BRK(h)	25
BTK(h)	-4
Blk(h)	47
Bmx(h)	27
CDK1/cyclinB(h)	20
CDK2/cyclinA(h)	9
CDK6/cyclinD3(h)	-1
CDK7/cyclinH/MAT1(h)	-3
CDK9/cyclinT1(h)	10
CHK1(h)	4
CK1g1(h)	3
CK2a2(h)	4
CaMKI(h)	46
CaMKIIb(h)	4
DDR2(h)	29
DMPK(h)	-2
DRAK1(h)	-4
DYRK2(h)	-7
EGFR(T790M,L858R)(h)	63
EGFR(h)	3
EphA5(h)	29
EphB1(h)	15
EphB4(h)	14
ErbB4(h)	5
FAK(h)	-1
FGFR1(V561M)(h)	-3
FGFR1(h)	-3
FGFR2(h)	10
FGFR3(h)	24
FGFR4(h)	8
Fer(h)	2

Kinase Name	Mean Inhibition (%)
Lyn(h)	91
MAPKAP-K2(h)	2
MARK1(h)	-7
MEK1(h)	-6
MINK(h)	38
MKK7b(h)	-27
MLCK(h)	-1
MLK1(h)	12
MSK1(h)	28
MST1(h)	2
Met(h)	-4
Mnk2(h)	-4
NEK2(h)	-15
PAK1(h)	100
PAK2(h)	95
PAK4(h)	-5
PAK5(h)	19
PAR-1Ba(h)	7
PDGFRb(h)	44
PDK1(h)	-17
PI3 Kinase (p110a/p85a)(h)	-1
PI3 Kinase (p110b/p85a)(h)	3
PI3 Kinase (p110d/p85a)(h)	-2
PI3 Kinase (p120g)(h)	1
PI3KC2g(h)	9
PIP4K2a(h)	1
PIP5K1a(h)	3
PIP5K1g(h)	1
PKA(h)	42
PKBa(h)	-2
PKBb(h)	5
PKCa(h)	41
PKCe(h)	76
PKCq(h)	91
PKG1a(h)	9
PRAK(h)	9
Pim-2(h)	25
Plk1(h)	15
ROCK-I(h)	-3
ROCK-II(h)	5

Fes(h)	45
Flt1(h)	-11
Flt3(h)	1
Fms(h)	-7
Fyn(h)	87
GSK3a(h)	11
GSK3b(h)	33
IGF-1R(h)	-1
IGF-1R(h) activated	17
IKKb(h)	2
IR(h)	24
IR(h) activated	2
IRAK4(h)	17
IRR(h)	38
JAK2(h)	10
JAK3(h)	18
JNK1a1(h)	8
KDR(h)	-2
LKB1(h)	22
LOK(h)	17
Lck(h)	82
Lck(h) activated	83

Ret(V804L)(h)	12
Ret(V804M)(h)	9
Ret(h)	5
Ron(h)	13
Ros(h)	-22
Rse(h)	-1
Rsk1(h)	9
SAPK2a(h)	4
SGK(h)	19
SRPK1(h)	4
Src(1-530)(h)	86
TAK1(h)	4
TBK1(h)	-10
TGFBR1(h)	3
TrkA(h)	13
ULK2(h)	-2
Yes(h)	98
c-Kit(h)	21
c-RAF(h)	-5
cSRC(h)	74
eEF-2K(h)	12
mTOR(h)	2
p70S6K(h)	4

Effect of 18 in *in vitro* radioligand binding, enzyme and functional assays

Target	Binding K_i or Enzyme IC₅₀ (μM)	Functional IC₅₀ or EC₅₀ (μM)	Pharmacological mode of action
Muscarinic Acetylcholine Receptor 5	0.10	nt	nd
Adrenergic Receptor Alpha 1B	0.24	12	Antagonist
5-Hydroxytryptamine Receptor 2B	0.26	3.3	Antagonist
Muscarinic Acetylcholine Receptor 1	0.28	1.5	Antagonist
Muscarinic Acetylcholine Receptor 2	0.39	15	Antagonist
Dopamine Receptor D3	0.51	nt	nd
Calcium channel, voltage-dependent, L type, alpha 1	0.54	nt	nd
5-Hydroxytryptamine Receptor 2C	0.67	10	Antagonist
Dopamine Transporter	0.93	nt	nd
Adrenergic Receptor Alpha 2C	0.95	nt	nd
Adenosine Transporter	1.1	nt	nd
Adrenergic Receptor Alpha 1A	1.4	8.7	Antagonist
Glucocorticoid Receptor	1.5	nt	nd
Opioid Receptor Mu 1	1.8	nt	nd
Ghrelin Receptor	1.8	2.9	Antagonist
Neurokinin Receptor 1	1.9	16	Antagonist
Nicotinic Acetylcholine Receptor Alpha 7	2.1	nt	nd
Dopamine Receptor D1	2.3	nt	nd
Histamine Receptor H2	2.5	nt	nd
Serotonin Transporter	2.8	nt	nd
Phosphodiesterase 4D	3.0	nt	nd
Dopamine Receptor D2	3.1	6.9	Antagonist
GABAB Receptor	3.3	nt	Antagonist
Adenosine Receptor A2a	3.5	nt	nd
Delta 2 opioid receptor	4.0	14	Antagonist
Adrenergic Receptor Alpha 2A	4.3	8.0	Antagonist
5-Hydroxytryptamine Receptor 7	4.4	nt	nd
Adenosine Receptor A1	4.9	nt	nd
Norepinephrine Transporter	5.5	nt	nd
5-Hydroxytryptamine Receptor 4	5.9	nt	nd
Opioid Receptor Kappa 1	6.5	nt	nd
Nicotinic Acetylcholine Receptor Alpha 4	7.0	nt	nd
Endothelin Receptor A	8.3	nt	Antagonist
Sigma1	8.6	nt	nd
Histamine Receptor H1	8.9	9.8	Antagonist
Glycine receptor?	11	nt	nd
Bradykinin Receptor 2	11	9.0	Antagonist

Protein expression, purification, crystallisation and structure determination for 1 and 2 in PAK1

The kinase domain of human PAK1 (residues 249-545 containing three point mutations D389N, T423E and E503D) was expressed with an TEV cleavable N-terminal 6xHis-tag in *E. coli*, and purified according as described previously^{1,2}. Initial protein crystals were grown using the hanging drop method using a low potency (~1 μ M) ATP-competitive tool compound. The reservoir solution contained 14-16% PEG 3350, 0.2 M ammonium sulfate and 100 mM HEPES (pH 7.6). Drops were set up with 2 μ L protein solution and 2 μ L reservoir solution. Trays were incubated at 20 °C, and crystals appeared after 1 week and reached the final size after 2 weeks. Crystals were soaked in reservoir solution containing 5 mM of compound for 24 h. Crystals were transferred to a soaking solution containing 20% glycerol, and were vitrified in liquid nitrogen. X-ray diffraction data was collected at cryogenic temperature using synchrotron radiation at the Diamond Light Source and the European Synchrotron Radiation Source. Diffraction data was processed with XDS³ and scaled using SCALA⁴, as implemented in the autoPROC routines from Global Phasing⁵. The structure was solved by molecular replacement using MOLREP⁶ and the coordinates of Protein Data Bank accession code 4P90. Protein and inhibitor were modeled into the electron density using Coot⁷, and the model was refined using BUSTER⁸. Crystallographic statistics for the human PAK1 in complex with compound **1** are as follows: space group $P2_1$, unit cell dimensions $a=61.01$ Å, $b=80.94$ Å, $c=66.02$ Å, $\alpha=90^\circ$, $\beta=107^\circ$, $\gamma=90^\circ$; resolution range 63-2.58 Å (2.72-2.58 Å); 80,612 total reflections with 19,584 unique reflections; overall redundancy of 4.1 (4.4); overall completeness of 99.6% (99.9%); R_{merge} of 6.5% (86.2%); and mean $I/\sigma(I)$ of 11.7 (1.5). The final refined model has a R_{work} of 18.5% and R_{free} of 22.9%. Crystallographic statistics for the human PAK1 in complex with compound **2** are as follows: space group $P2_1$, unit cell dimensions $a=61.82$ Å, $b=81.82$ Å, $c=65.91$ Å, $\alpha=90^\circ$, $\beta=107^\circ$, $\gamma=90^\circ$; resolution range 82-2.36 Å (2.49-2.36 Å); 106,519 total reflections with 25,829 unique reflections; overall redundancy of 4.1 (4.3); overall completeness of 99.6% (99.6%); R_{merge} of 5.7% (85.0%); and mean $I/\sigma(I)$ of 12.3 (1.4). The final refined model has a R_{work} of 20.7% and R_{free} of 24.3%. Atomic coordinates and structure factors have been deposited in the Protein Data Bank under accession code (5KBQ and 5KBR).

References.

1. [Lei, M.](#); [Robinson, M.A.](#); [Harrison, S.C.](#) *Structure* **2005** *13*, 769.
2. Lei, M.; Lu, W.; Meng, W.; Parrini, M.C.; Eck, M.J.; Mayer, B.J.; Harrison, S.C. *Cell* **2000** *102*, 387.
3. Kabsch, W. *Acta Crystallogr. D Biol. Crystallogr.* **2010** *66*, 125.
4. Bailey, S. *Acta Crystallogr. D Biol. Crystallogr.* **1994** *50*, 760.
5. Vonnrhein, C.; Flensburg, C.; Keller, P.; Sharff, A.; Smart, O.; Paciorek, W.; Womack, T.; Bricogne, G. *Acta Crystallogr. D Biol. Crystallogr.* **2011** *67*, 293.
6. Vagin, A.; Teplyakov, A. *Acta Crystallogr. D Biol. Crystallogr.* **2010** *66*, 22.
7. Emsley, P.; Lohkamp, B.; Scott, W.G.; Cowtan, K. *Acta Crystallogr. D Biol. Crystallogr.* **2010** *66*, 486.
8. Smart, O.S.; Womack, T.O.; Flensburg, C.; Keller, P.; Paciorek, W.; Sharff, A.; Vonnrhein, C.; Bricogne, G. *Acta Crystallogr. D Biol. Crystallogr.* **2012** *68*, 368.