

Pseudo Cyclization through Intramolecular Hydrogen Bond Enables Discovery of Pyridine Substituted Pyrimidines as New Mer Kinase Inhibitors

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Supporting Information

Synthesis of Analogues

Experimental

Microwave reaction was carried out using a CEM Discover-S reactor with a vertically-focused IR external temperature sensor and an Explorer 72 autosampler. The dynamic mode was used to set up the desired temperature and hold time with the following fixed parameters: PreStirring, 1 min; Pressure, 200 psi; Power, 200 W; PowerMax, off; Stirring, high. Flash chromatography was carried out with pre-packed silica gel disposable columns. Preparative HPLC was performed with the UV detection at 220 or 254 nm. Samples were injected onto a 75 x 30 mm, 5 μ M, C18(2) column at room temperature. The flow rate was 30 mL/min. Various linear gradients were used with A being H₂O + 0.5% TFA and B being MeOH. Analytical thin-layer chromatography (TLC) was performed with silica gel 60 F₂₅₄, 0.25 mm pre-coated TLC plates. TLC plates were visualized using UV₂₅₄ and phosphomolybdic acid with charring. All ¹H NMR spectra were obtained with a 400 MHz spectrometer using CDCl₃ (7.26 ppm), DMSO-*d*₆ (2.50 ppm), or CD₃OD (2.05 ppm) as an internal reference. Signals are reported as m (multiplet), s (singlet), d (doublet), t (triplet), q (quartet), and bs (broad singlet); and coupling constants are reported in Hertz (Hz). ¹³C NMR spectra were obtained with a 100 MHz spectrometer using CDCl₃ (77.2 ppm), DMSO-*d*₆ (39.5 ppm), or CD₃OD (49.0 ppm) as the internal standard. LC-MS was performed using an analytical instrument with the UV detector set to 220 nm, 254 nm,

and 280 nm, and a single quadrupole mass spectrometer using electrospray ionization (ESI) source. Samples were injected (2 μ L) onto a 4.6 x 50 mm, 1.8 μ M, C18 column at room temperature. A linear gradient from 10% to 100% B (MeOH + 0.1% Acetic Acid) in 5.0 min was followed by pumping 100% B for another 2 or 4 minutes with A being H₂O + 0.1% acetic acid. The flow rate was 1.0 mL/min. High-resolution (positive ion) mass spectra (HRMS) were acquired using a LCMS-TOF mass spectrometer.

General synthesis of lithium trihydroxy borate salt of 2-pyridines¹ A solution of 2-bromopyridine (14.3 g, 150 mmol) and triisopropylborate (150 mL, 150 mmol) in a mixture of toluene (280 mL) and THF (70 mL) was added a 2.5 M solution of *n*-butyl lithium in hexane (60 mL, 150 mmol) slowly (over 60 min) at -78°C. The reaction mixture (brown) was stirred for 135 min, then the cooling bath was removed. The reaction mixture was stirred at room temperature (turned into a greenish-brown mixture) until the temperature reached 0°C (1.5 h) and then quenched with 10 mL of *i*-propanol (left overnight). The solvent was removed under reduced pressure and the residue was treated with 90% acetone in water until white solid was precipitated. The solid was filtered and washed with 90% acetone in water to afford the desired compound as white fine powder (16.6 g, 75%).

***N*4-(*trans*-4-Aminocyclohexyl)methyl-*N*2-butyl-5-(pyridin-2-yl)pyrimidine-2,4-diamine (2)**

A solution of 2,4-dichloro-5-bromopyrimidine (456 mg, 2 mmol) and *tert*-butyl (4-(aminomethyl)cyclohexyl)carbamate (480 mg, 2.1 mmol) was heated at 80°C under microwave irradiation for 20 min, then BuNH₂ (1.0 mL) was added. The resulting reaction mixture was heated under microwave irradiation at 100°C for 30 min, then quenched with brine and extracted with CH₂Cl₂ (3x). The organic layer was dried (MgSO₄) and concentrated. A solution of this residue in a mixture of DMF (8 mL) and H₂O (2 mL) was added lithium 2-pyridinyl

trihydroxyborate (882 mg, 6 mmol), copper bromide (58 mg, 0.4 mmol), potassium carbonate (6 mmol) and PdCl₂(dppf) (164 mg, 0.2 mmol). The reaction mixtures were heated at 120°C for 30 min. The mixture was then diluted with EtOAc (15 mL) and the insoluble material was removed by a filtration through a short pad of celite. The filtrate was washed with water (2x), dried (MgSO₄), and concentrated. The residue was purified by prep-HPLC to afford Boc-protected compound **2** which was treated with a 3.0 N HCl solution in MeOH. The Solvent was removed to afford the title compound **2** as a light yellow solid (116 mg, 25% over 4 steps) (The last Boc-deprotection step is not needed for compounds without Boc protecting group). ¹H NMR (400 MHz, CD₃OD) δ 8.67 (d, *J* = 5.0 Hz, 1H), 8.27 (s, 1H), 8.05 (dd, *J* = 11.1, 4.5 Hz, 1H), 7.94 (d, *J* = 8.1 Hz, 1H), 7.52 (dd, *J* = 7.2, 5.5 Hz, 1H), 3.59–3.46 (m, 4H), 3.07 (ddd, *J* = 11.9, 8.2, 4.0 Hz, 1H), 2.08 (d, *J* = 9.4 Hz, 2H), 1.96 (d, *J* = 12.2 Hz, 2H), 1.77 (d, *J* = 3.1 Hz, 1H), 1.66 (dt, *J* = 14.9, 7.4 Hz, 2H), 1.52–1.33 (m, 4H), 1.22 (dd, *J* = 24.8, 11.1 Hz, 2H), 0.99 (t, *J* = 7.4 Hz, 3H). LC-MS (ESI⁺): t_R = 3.756 min, MS *m/z* 355.0 [M+1]⁺, > 98% purity.

***trans*-4-((2-(Butylamino)-5-(pyridin-2-yl)pyrimidin-4-yl)amino)cyclohexanol (3)** The title compound **3** (24.6 mg, 36% over 3 steps) was prepared according to general procedure A from 2-chloropyridine (22.7 mg, 0.20 mmol) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ 10.67 (s, 1H), 8.50 (d, *J* = 4.7 Hz, 1H), 8.12 (s, 1H), 7.79–7.70 (m, 1H), 7.56 (d, *J* = 8.3 Hz, 1H), 7.24–7.14 (m, 1H), 4.13–4.04 (m, 1H), 3.79–3.71 (m, 1H), 3.48–3.38 (m, 2H), 2.26–2.13 (m, 2H), 2.11–2.01 (m, 2H), 1.69–1.60 (m, 2H), 1.54–1.34 (m, 6H), 0.95 (t, *J* = 7.4 Hz, 3H); ¹³C NMR (101 MHz, cdcl₃) δ 164.4, 159.9, 147.2, 137.5, 131.6, 118.9, 115.1, 104.4, 77.3, 77.0, 76.7, 69.5, 49.6, 41.2, 33.6, 31.4, 29.8, 20.1, 13.8. LC-MS (ESI⁺): MS *m/z* 342.25 [M+H]⁺, >95% purity.

***trans*-4-((5-Bromo-2-(butylamino)pyrimidin-4-yl)amino)cyclohexanol (IIa)** A solution of 5-bromo-2,4-dichloropyrimidine (34.2 g, 150 mmol) and 4-aminocyclohexanol (17.3 g, 150 mmol)

in *i*-PrOH (300 mL) was added DIPEA (39.2 mL, 225 mmol). The reaction mixture was stirred at room temperature for 12 h, and then the solvent was evaporated under reduced pressure. The residue was dissolved in EtOAc and washed with brine. The organic layer was dried (Na₂SO₄) and concentrated to yield 4-((5-bromo-2-chloropyrimidin-4-yl)amino)cyclohexanol (46.0 g, 150 mmol). A solution of 4-((5-bromo-2-chloropyrimidin-4-yl)amino)cyclohexanol (46.0 g, 150 mmol) in toluene (150 mL) was added *n*-butylamine (150 mL, 1.5 mol). The reaction mixture was heated at 80°C for 12 h, then the solvent was evaporated under reduced pressure. The residue was dissolved in EtOAc and washed with brine. The organic layer was dried (Na₂SO₄) and concentrated to yield the title compound **IIa** (47.5 g, 92%). ¹H NMR (400 MHz, CDCl₃) δ 7.82 (s, 1H), 5.01–4.80 (m, 2H), 3.97–3.82 (m, 1H), 3.65 (tt, *J* = 10.4, 4.2 Hz, 1H), 3.31 (dt, *J* = 7.1, 6.1 Hz, 2H), 2.21 (s, 1H), 2.16–2.06 (m, 2H), 2.06–1.95 (m, 2H), 1.59–1.49 (m, 2H), 1.49–1.34 (m, 4H), 1.34–1.20 (m, 2H), 0.92 (t, *J* = 7.3 Hz, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 161.14 (s), 157.59 (s), 155.72 (s), 69.70 (s), 48.85 (s), 41.39 (s), 33.94 (s), 31.84 (s), 30.62 (s), 20.12 (s), 13.86 (s). LC-MS (ESI+): MS *m/z* 343.0 [M+1]⁺, >98% purity.

***trans*-4-((2-(Butylamino)-5-(pyridin-3-yl)pyrimidin-4-yl)amino)cyclohexanol (4)** (General Procedure B) A mixture of lithium 3-pyridinyl trihydroborate (74 mg, 0.5 mmol, 2.5 eq.), copper bromide (6.0 mg, 0.040 mmol, 0.2 eq.), potassium carbonate (56 mg, 0.40 mmol, 2.0 eq.) and **IIa** (69 mg, 0.20 mmol, 1.0 eq) in DMF/H₂O (2.0 mL/0.50 mL) was added PdCl₂(dppf) (16.3 mg, 0.020 mmol, 0.1 eq.). The reaction mixture was heated at 120°C in the open air for 30 min, and then cooled to room temperature. The solid was removed by filtration through a short pad of celite (washed with DMF). The solvent was removed under reduced pressure and the residue was purified by an ISCO silica gel column to afford the title compound **4** as a light yellow solid (52 mg, 62%). ¹H NMR (400 MHz, CD₃OD) δ 8.96 (s, 1H), 8.91 (d, *J* = 5.6 Hz, 1H), 8.59 (d, *J* = 8.1

Hz, 1H), 8.14 (dd, $J = 8.0, 5.8$ Hz, 1H), 7.74 (s, 1H), 4.13 (s, 1H), 3.74–3.55 (m, 1H), 3.50 (s, 2H), 1.98 (d, $J = 6.5$ Hz, 4H), 1.67 (dt, $J = 14.9, 7.4$ Hz, 2H), 1.40 (ddd, $J = 23.6, 14.3, 5.7$ Hz, 6H), 0.99 (t, $J = 7.4$ Hz, 3H). LC-MS (ESI⁺): $t_R = 3.704$ min, MS m/z 342.0 $[M+1]^+$, >98% purity.

***trans*-4-((2-(butylamino)-5-(4-fluorophenyl)pyrimidin-4-yl)amino)cyclohexan-1-ol (5)** The title compound **5** (81.4 mg, 88%) was prepared according to general procedure A from (4-fluorophenyl)boronic acid (55.0 mg, 0.39 mmol) as a white solid. ¹H NMR (400 MHz, CD₃OD) δ 7.45 (s, 1H), 7.41–7.37 (m, 2H), 7.26–7.20 (m, 2H), 4.17–4.07 (m, 1H), 3.55–3.41 (m, 3H), 2.01–1.92 (m, 4H), 1.71–1.62 (m, 2H), 1.49–1.33 (m, 6H), 1.00 (t, $J = 7.4$ Hz, 3H). LC-MS: >97% purity.

***trans*-4-((2-(Butylamino)pyrimidin-4-yl)amino)cyclohexanol (6)** The title compound **6** (25.7 mg, 49%) is a side product of the synthesis of compound **16**. ¹H NMR (400 MHz, CD₃OD) δ 7.46 (d, $J = 7.3$ Hz, 1H), 5.97 (d, $J = 7.2$ Hz, 1H), 3.97–3.87 (m, 1H), 3.60–3.51 (m, 1H), 3.48–3.31 (m, 2H), 2.07–1.92 (m, 4H), 1.65–1.55 (m, 2H), 1.44–1.31 (m, 6H), 0.96 (t, $J = 7.4$ Hz, 3H). LC-MS: >97% purity.

***trans*-4-((2-(Butylamino)-5-(5-fluoropyridin-2-yl)pyrimidin-4-yl)amino)cyclohexanol (7)** The title compound **7** (56 mg, 65%) was prepared according to general procedure B from lithium (5-fluoropyridin-2-yl) trihydroborate (83 mg, 0.5 mmol, 2.5 eq.) as a light yellow solid. ¹H NMR (400 MHz, CD₃OD) δ 8.54 (d, $J = 3.0$ Hz, 1H), 8.26 (s, 1H), 7.93 (dd, $J = 9.0, 4.1$ Hz, 1H), 7.80–7.71 (m, 1H), 4.20–4.05 (m, 1H), 3.67 (td, $J = 10.0, 5.1$ Hz, 1H), 3.48 (s, 2H), 2.16 (d, $J = 8.5$ Hz, 2H), 2.05–1.92 (m, 2H), 1.67 (dt, $J = 12.6, 7.5$ Hz, 2H), 1.59–1.38 (m, 6H), 1.00 (t, $J = 7.4$ Hz, 3H). LC-MS (ESI⁺): $t_R = 4.481$ min, MS m/z 360.0 $[M+1]^+$, >98% purity.

***trans*-4-((2-(Butylamino)-5-(5-(4-methylpiperazin-1-yl)pyridin-2-yl)pyrimidin-4-yl)amino)cyclohexanol (8)** The title compound **8** (66 mg, 60%) was prepared according to general procedure B from lithium (5-(4-methylpiperazin-1-yl)pyridin-2-yl) trihydroborate (123 mg, 0.50 mmol) to as a light yellow solid. ¹H NMR (400 MHz, CD₃OD) δ 8.61 (s, 1H), 8.22 (d, *J* = 7.4 Hz, 1H), 7.98–7.88 (m, 2H), 4.27 (d, *J* = 13.6 Hz, 2H), 4.13 (s, 1H), 3.80–3.64 (m, 3H), 3.62–3.49 (m, 4H), 3.42 (d, *J* = 11.5 Hz, 2H), 2.01 (s, 3H), 1.68 (dt, *J* = 14.6, 7.3 Hz, 2H), 1.59–1.25 (m, 6H), 1.00 (t, *J* = 7.3 Hz, 3H). LC-MS (ESI⁺): *t*_R = 3.546 min, MS *m/z* 440.0 [M+1]⁺, >98% purity.

***trans*-4-((2-(Butylamino)-5-(5-((4-methylpiperazin-1-yl)methyl)pyridin-2-yl)pyrimidin-4-yl)amino)cyclohexanol (9)** (General Procedure C) 4-((5-(5-(1,3-dioxolan-2-yl)pyridin-2-yl)-2-(butylamino)pyrimidin-4-yl)amino)cyclohexanol (83 mg, 0.2 mmol) (prepared according to general procedure B) was dissolved in a 4.0 N HCl solution (10 mL) and heated at 100°C for 30 min. The mixture was quenched at room temperature with a 4.0 N KOH solution (11 mL), extracted with a mixture of CH₂Cl₂ and *i*-propyl alcohol (9:1). The organic layer was dried (MgSO₄) and concentrated. The residue was dissolved in CH₂Cl₂ (10 mL) and was added *N*-methyl piperazine (100 mg, 0.11 mL, 1.0 mmol) and NaBH(OAc)₃ (212 mg, 1.0 mmol). The reaction mixture was stirred at room temperature overnight. The solvent was removed and the residue was dissolved in CH₂Cl₂ and washed with a sat. solution of NaHCO₃ and brine. The organic layer was dried (MgSO₄) and concentrated. The residue was purified by prep-HPLC to afford a TFA salt of the title compound **9**. The TFA salt was dissolved in a 3.0 M solution of HCl in MeOH (0.2 mL) and stirred at room temperature for 12 h. Then the solvent was removed under the reduced pressure to afford a HCl salt of the title compound **9** as a light yellow solid (84 mg, 70%). ¹H NMR (400 MHz, CD₃OD) δ 8.74 (d, *J* = 1.7 Hz, 1H), 8.39 (s, 1H), 8.12 (dd, *J* =

8.5, 2.1 Hz, 1H), 7.97 (d, $J = 8.5$ Hz, 1H), 4.31–4.19 (m, 2H), 4.16 (ddd, $J = 14.4, 9.0, 3.9$ Hz, 1H), 3.74–3.38 (m, 9H), 3.28–3.11 (m, 2H), 2.97 (s, 3H), 2.17 (d, $J = 10.6$ Hz, 2H), 2.10–1.95 (m, 2H), 1.67 (dt, $J = 12.6, 7.6$ Hz, 2H), 1.59–1.36 (m, 6H), 1.01 (t, $J = 7.4$ Hz, 3H). LC-MS (ESI⁺): $t_R = 3.702$ min, MS m/z 454.0 $[M+1]^+$, >98% purity.

***trans*-4-((2-(butylamino)-5-(5-(morpholinomethyl)pyridin-2-yl)pyrimidin-4-yl)amino)**

cyclohexanol (10) The title compound **10** (75 mg, 68%) was prepared according to general procedure C from morpholine (88 mg, 0.090 mL, 1.0 mmol) as a light yellow solid. ¹H NMR (400 MHz, CD₃OD) δ 8.78 (d, $J = 1.8$ Hz, 1H), 8.45 (s, 1H), 8.15 (dd, $J = 8.5, 2.3$ Hz, 1H), 8.04 (d, $J = 8.5$ Hz, 1H), 4.48 (s, 2H), 4.22–4.13 (m, 1H), 4.05 (s, 2H), 3.81 (s, 2H), 3.66 (dd, $J = 10.0, 4.9$ Hz, 1H), 3.46 (d, $J = 34.9$ Hz, 4H), 3.26–3.17 (m, 2H), 2.18 (d, $J = 11.2$ Hz, 2H), 2.08–1.97 (m, 2H), 1.68 (dd, $J = 8.2, 6.0$ Hz, 2H), 1.59–1.39 (m, 6H), 1.01 (t, $J = 7.4$ Hz, 3H). LC-MS (ESI⁺): $t_R = 3.769$ min, MS m/z 441.0 $[M+1]^+$, >98% purity.

(2-Chloro-4-((*trans*-4-hydroxycyclohexyl)amino)pyrimidin-5-yl)boronic acid (IVa) A mixture of *trans*-4-((5-bromo-2-chloropyrimidin-4-yl)amino)cyclohexanol (5.50 g, 17.9 mmol), bis(pinacolato)diboron (6.83 g, 26.9 mmol), Pd(dppf)₂Cl₂ (732 mg, 0.90 mmol) and KOAc (5.28 g, 53.8 mmol) in anhydrous dioxane (125 mL) was thoroughly degassed and heated at 90°C under N₂ atmosphere for 75 min. The hot mixture was then filtered through a pad of celite and concentrated. The residue was purified by an ISCO silica gel column followed by a reversed ISCO silica gel column to provide the titled compound **IVa** (1.84 g, 38%). ¹H NMR (400 MHz, cd₃od) δ 7.98 (s, 1H), 3.99–3.90 (m, 1H), 3.64–3.56 (m, 1H), 2.07–2.00 (m, 2H), 2.00–1.91 (m, 2H), 1.48–1.34 (m, 4H); LC-MS (ESI⁺): MS m/z 272.30 $[M+H]^+$, >98% purity.

4-((6-Chloropyridin-3-yl)sulfonyl)morpholine A solution of 2-chloropyridine-5-sulfonyl chloride (212 mg, 1.0 mmol) in CH₂Cl₂ (10 mL) was added morpholine (87.1 mg, 1.0 mmol) at

0°C. The resulting mixture was stirred at 0°C for 10 min and then was added diisopropylethylamine (194 mg, 1.5 mmol) dropwisely. The mixture was stirred at 0°C for 2h, diluted with EtOAc (15 mL), and washed with water (2x). The organic layer was dried (Na₂SO₄) and concentrated. The residue was purified by an ISCO silica gel column to afford the title compound (246 mg, 94%) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ 8.73–8.65 (m, 1H), 7.94 (dd, *J* = 8.3, 2.5 Hz, 1H), 7.49 (dd, *J* = 8.3, 0.6 Hz, 1H), 3.74–3.63 (m, 5H), 2.99 (dd, *J* = 5.6, 3.9 Hz, 4H); ¹³C NMR (101 MHz, cdcl₃) δ 155.9, 148.7, 137.9, 131.1, 124.9, 65.9, 45.8. LC-MS (ESI+): MS *m/z* 263.30 [M+H]⁺, >97% purity.

***trans*-4-((2-(Butylamino)-5-(3-(morpholinomethyl)pyridin-2-yl)pyrimidin-4-yl)amino)**

cyclohexan-1-ol (11) (General Procedure D) A mixture of **IVa** (45 mg, 0.17 mmol), 4-((2-bromopyridin-3-yl)methyl)morpholine (85 mg, 0.33 mmol), Pd₂(dba)₃ (7.6 mg, 0.0080 mmol), PCy₃ (6.9 mg, 0.025 mmol), K₃PO₄ (0.33 mL, 1.0 M solution in deionized water) and butylamine (50 mg, 0.66 mmol) in a 10 mL microwave tube with dioxane (2.5 mL) was degassed and heated at 120°C under N₂ atmosphere and microwave irradiation for 20 min. The reaction mixture was diluted with water and extracted with EtOAc (3x). The combined organic layer was dried (MgSO₄) and concentrated. The residue was purified by prep-HPLC to afford the title compound **11** (47.1 mg, 65%) as a white solid. ¹H NMR (400 MHz, CD₃OD) δ 8.76–8.71 (m, 1H), 8.25–8.19 (m, 1H), 7.76–7.71 (m, 1H), 7.64–7.57 (m, 1H), 4.41–4.33 (m, 2H), 4.20–4.07 (m, 1H), 3.91–3.74 (m, 4H), 3.55–3.45 (m, 2H), 3.24–3.08 (m, 4H), 2.16–2.03 (m, 1H), 2.00–1.90 (m, 3H), 1.73–1.63 (m, 2H), 1.51–1.42 (m, 2H), 1.41–1.29 (m, 3H), 1.00 (t, *J* = 7.4 Hz, 3H); LC-MS (ESI+): MS *m/z* 441.30 [M+H]⁺, >95% purity.

***trans*-4-((2-(Butylamino)-5-(4-(morpholinomethyl)pyridin-2-yl)pyrimidin-4-yl)amino)**

cyclohexanol (12) The title compound **12** (54.5 mg, 75%) was prepared according to general

procedure D from 4-((2-bromopyridin-4-yl)methyl)morpholine (85.2 mg, 0.33 mmol) as a white solid. ^1H NMR (400 MHz, CD_3OD) δ 8.72–8.66 (m, 1H), 8.42–8.34 (m, 1H), 8.04 (s, 1H), 7.52–7.48 (m, 1H), 4.43 (s, 2H), 4.19–4.09 (m, 1H), 3.93–3.83 (m, 3H), 3.70–3.60 (m, 1H), 3.56–3.42 (m, 2H), 3.37–3.30 (m, 3H), 2.20–2.11 (m, 2H), 2.07–1.95 (m, 2H), 1.70–1.62 (m, 2H), 1.55–1.36 (m, 5H), 0.99 (t, $J = 7.4$ Hz, 3H); MS m/z 441.30 $[\text{M}+\text{H}]^+$, >95% purity.

***trans*-4-((2-(Butylamino)-5-(6-(morpholinomethyl)pyridin-2-yl)pyrimidin-4-yl)amino)**

cyclohexanol (13) The title compound **13** (62 mg, 56%) was prepared according to general procedure C from morpholine (88 mg, 0.090 mL, 1.0 mmol) as a light yellow solid. ^1H NMR (400 MHz, CD_3OD) δ 8.30 (s, 1H), 8.07 (t, $J = 7.9$ Hz, 1H), 7.93 (d, $J = 8.1$ Hz, 1H), 7.72 (d, $J = 7.5$ Hz, 1H), 4.56 (s, 2H), 4.15 (td, $J = 7.5, 3.7$ Hz, 1H), 3.96 (d, $J = 59.6$ Hz, 4H), 3.71–3.62 (m, 1H), 3.51 (s, 6H), 2.17 (d, $J = 10.8$ Hz, 2H), 2.05 (d, $J = 10.5$ Hz, 2H), 1.66 (dt, $J = 22.6, 11.0$ Hz, 4H), 1.50–1.36 (m, 4H), 1.01 (t, $J = 7.4$ Hz, 3H). LC-MS (ESI+): $t_R = 3.872$ min, MS m/z 441.0 $[\text{M}+1]^+$, >98% purity.

***trans*-4-((2-(Butylamino)-5-(5-((4,4-difluoropiperidin-1-yl)methyl)pyridin-2-yl)pyrimidin-4-yl)amino)cyclohexanol (14)** The title compound **14** (38 mg, 40%) was prepared according to general procedure C from 4,4-difluoropiperidine (122 mg, 1.0 mmol) as a light yellow solid. ^1H NMR (400 MHz, CD_3OD) δ 8.78 (d, $J = 2.0$ Hz, 1H), 8.43 (s, 1H), 8.16 (dd, $J = 8.5, 2.3$ Hz, 1H), 8.01 (d, $J = 8.5$ Hz, 1H), 4.50 (s, 2H), 4.19–4.10 (m, 1H), 3.75–3.69 (m, 1H), 3.68–3.62 (m, 2H), 3.57–3.41 (m, 4H), 2.47–2.34 (m, 4H), 2.15 (d, $J = 12.5$ Hz, 2H), 2.05–1.96 (m, 2H), 1.70–1.60 (m, 2H), 1.46 (ddd, $J = 15.6, 14.9, 7.4$ Hz, 6H), 0.99 (t, $J = 7.4$ Hz, 3H). LC-MS (ESI+): $t_R = 4.246$ min, MS m/z 475.0 $[\text{M}+1]^+$, >98% purity.

***trans*-4-((2-(Butylamino)-5-(5-((dimethylamino)methyl)pyridin-2-yl)pyrimidin-4-yl)amino)**

cyclohexanol (15) The title compound **15** (65 mg, 69%) was prepared according to general

procedure C from dimethylamine (0.50 mL, a 2.0 N solution in methanol, 1.0 mmol) as a light yellow solid. ¹H NMR (400 MHz, CD₃OD) δ 8.75 (d, *J* = 1.5 Hz, 1H), 8.44 (s, 1H), 8.16–8.07 (m, 1H), 8.03 (d, *J* = 8.5 Hz, 1H), 4.43 (s, 2H), 4.15 (dd, *J* = 12.3, 8.4 Hz, 1H), 3.69–3.62 (m, 1H), 3.48 (d, *J* = 10.6 Hz, 2H), 2.90 (s, 6H), 2.16 (d, *J* = 10.2 Hz, 2H), 2.01 (d, *J* = 11.0 Hz, 2H), 1.72–1.61 (m, 2H), 1.56–1.35 (m, 6H), 0.99 (t, *J* = 7.4 Hz, 3H). LC-MS (ESI⁺): *t*_R = 3.270 min, MS *m/z* 399.0 [M+1]⁺; LC-MS: >98% purity.

***trans*-4-((2-(Butylamino)-5-(5-(morpholinosulfonyl)pyridin-2-yl)pyrimidin-4-yl)amino)**

cyclohexanol (16) A mixture of 2,4-dichloropyrimidine-5-boronic acid pinacol ester (55 mg, 0.20 mmol), *trans*-4-aminocyclohexanol (23 mg, 0.20 mmol), K₂CO₃ (42 mg, 0.30 mmol) in THF (2.0 mL) was heated at 80°C under microwave irradiation for 15 min. Then 4-((6-chloropyridin-3-yl)sulfonyl)morpholine (52.5 mg, 0.20 mmol), Pd(PPh₃)₄ (23.1 mg, 0.02 mmol), K₂CO₃ (41.5 mg, 0.30 mmol), butylamine (116.8 mg, 1.60 mmol) and water (0.5 mL) were added sequentially. The resulting mixture was heated at 150°C under microwave irradiation for 30 min, diluted with EtOAc (15 mL), and washed with water (2x). The organic layer was dried (Na₂SO₄) and concentrated. The residue was purified by ISCO and prep-HPLC to afford the title compound **16** (27.4 mg, 28%) as a white solid (and compound **6** (25.7 mg, 49%) as a side product). ¹H NMR (400 MHz, CDCl₃) δ 10.65 (d, *J* = 7.1 Hz, 1H), 9.46 (t, *J* = 5.4 Hz, 1H), 8.87 (d, *J* = 2.2 Hz, 1H), 8.31 (s, 1H), 8.10 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.77 (d, *J* = 8.7 Hz, 1H), 4.19–4.09 (m, 1H), 3.84–3.68 (m, 5H), 3.51–3.44 (m, 2H), 3.15–3.06 (m, 4H), 2.86–2.66 (m, 2H), 2.28–2.15 (m, 2H), 2.13–2.01 (m, 2H), 1.72–1.61 (m, 2H), 1.56–1.36 (m, 6H), 0.96 (t, *J* = 7.4 Hz, 3H); ¹³C NMR (101 MHz, cdcl₃) δ 159.4, 156.9, 153.1, 146.6, 142.8, 136.8, 130.2, 118.9, 103.4, 69.2, 66.0, 49.7, 45.8, 41.3, 33.3, 31.0, 29.5, 20.1, 13.7. LC-MS (ESI⁺): MS *m/z* 491.30 [M+H]⁺, >96% purity.

6-(2-Butylamino-4-((*trans*-4-hydroxycyclohexylamino)pyrimidin-5-yl)-*N*-(1-methylpiperidin-4-yl)nicotinamide (17) A solution of 4-((5-(5-(1,3-dioxolan-2-yl)pyridin-2-yl)-2-(butylamino)pyrimidin-4-yl)amino)cyclohexanol (124 mg, 0.30 mmol) (prepared according to general procedure B) was dissolved in a 4.0 N HCl solution (10 mL) and heated at 100°C for 30 min. The mixture was quenched at room temperature with a 4.0 N KOH solution (11 mL), extracted with a mixture of CH₂Cl₂ and *i*-propyl alcohol (9:1). The organic layer was dried (MgSO₄) and concentrated. The residue was dissolved in formic acid (95%, 2.3 mL) and was added hydrogen peroxide urea adduct (113 mg, 1.2 mmol) at room temperature. The mixture was stirred at room temperature until the completion of reaction based on TLC analysis. The excess formic acid was removed under reduced pressure and the residue was dissolved in anhydrous DMF (10 mL). The solution was added 1-methylpiperidin-4-amine (172 mg, 1.50 mmol), DIEA (233 mg, 1.80 mmol) and TBTU (482 mg, 1.50 mmol) and stirred at room temperature overnight. The reaction was then treated with a saturated NaHCO₃ solution for 2 h. CH₂Cl₂ was added and the organic layer was dried (MgSO₄) and concentrated. The residue was purified by prep-HPLC to afford the title compound **17** (99 mg, 84% for three steps.). ¹H NMR (400 MHz, CD₃OD) δ 9.02 (d, *J* = 2.2 Hz, 1H), 8.43 (s, 1H), 8.31 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.99 (d, *J* = 8.5 Hz, 1H), 4.22–4.11 (m, 2H), 3.98 (d, *J* = 6.8 Hz, 1H), 3.72–3.62 (m, 2H), 3.58 (dd, *J* = 15.5, 6.3 Hz, 2H), 3.53–3.41 (m, 2H), 3.18 (dd, *J* = 13.3, 10.6 Hz, 2H), 2.88 (s, 3H), 2.26 (d, *J* = 14.9 Hz, 2H), 2.17 (d, *J* = 11.8 Hz, 2H), 2.01 (d, *J* = 14.1 Hz, 2H), 1.93 (dd, *J* = 12.2, 3.3 Hz, 2H), 1.71–1.58 (m, 2H), 1.59–1.40 (m, 6H), 0.99 (t, *J* = 7.4 Hz, 3H). LC-MS (ESI⁺): *t*_R = 3.608 min, MS *m/z* 482.0 [M+1]⁺, >95% purity.

***N*-(6-(2-butylamin)-4-((*trans*-4-hydroxycyclohexylamino)pyrimidin-5-yl)pyridin-3-yl)-1-methylpiperidine-4-carboxamide (18)** A mixture of 4-((2-(butylamino)-5-(5-chloropyridin-2-

yl)pyrimidin-4-yl)amino)cyclohexanol (94 mg, 0.25 mmol) (prepared based on general procedure B from (5-chloropyridin-2-yl) trihydroxyborate lithium (3.246 g, 18.0 mmol, 3.0 eq.) as a brown solid (1.63 g, 73%). ¹H NMR (400 MHz, CD₃OD) δ 8.71–8.57 (m, 1H), 8.33 (s, 1H), 7.96 (dd, *J* = 8.8, 2.5 Hz, 1H), 7.89 (d, *J* = 8.8 Hz, 1H), 4.22–4.05 (m, 1H), 3.77–3.61 (m, 1H), 3.49 (s, 2H), 2.16 (d, *J* = 10.6 Hz, 2H), 2.08–1.95 (m, 2H), 1.67 (dt, *J* = 12.7, 7.6 Hz, 2H), 1.59–1.33 (m, 6H), 1.00 (t, *J* = 7.4 Hz, 3H). LC-MS (ESI⁺): *t*_R = 4.809 min, MS *m/z* 376.0 [M+1]⁺; LC-MS, >98% purity), 1-methylpiperidine-4-carboxamide (54 mg, 0.375 mmol), Pd₂dba₃ (4.6 mg, 0.005 mmol), di-tert-butyl(2',4',6'-triisopropyl-3,4,5,6-tetramethyl-[1,1'-biphenyl]-2-yl)phosphine (12 mg, 0.025 mmol), K₃PO₄ (80 mg, 0.375 mmol) in *t*-BuOH (2.0 mL) was heated at 110°C overnight. The solvent was removed under reduced pressure. The residue was purified by pre-HPLC to afford a TFA salt of the title compound **18**. The TFA salt was then dissolved in a 3.0 M HCl in MeOH (0.20 mL) and stirred at room temperature for 12 h. The solvent was removed under the reduced pressure to afford a HCl salt of compound **18** (105 mg, 71%). ¹H NMR (400 MHz, CD₃OD) δ 8.90 (d, *J* = 2.5 Hz, 1H), 8.26 (s, 1H), 8.19 (dd, *J* = 8.9, 2.5 Hz, 1H), 7.87 (d, *J* = 8.9 Hz, 1H), 4.14 (dd, *J* = 12.4, 8.5 Hz, 1H), 3.65 (ddd, *J* = 21.9, 14.7, 6.2 Hz, 2H), 3.52–3.40 (m, 2H), 3.12 (dd, *J* = 13.1, 10.5 Hz, 2H), 2.91 (s, 3H), 2.80–2.72 (m, 1H), 2.18 (t, *J* = 14.9 Hz, 4H), 2.03 (d, *J* = 11.5 Hz, 4H), 1.75–1.59 (m, 2H), 1.58–1.36 (m, 6H), 1.00 (t, *J* = 7.4 Hz, 3H). LC-MS (ESI⁺): *t*_R = 4.645 min, MS *m/z* 482.0 [M+1]⁺, >98% purity.

***trans*-4-((2-Butylamino-5-(5-(cyclohexylaminomethyl)pyridin-2-yl)pyrimidin-4-yl)amino)cyclohexanol (19)** The title compound **19** (80 mg, 71%) was prepared according to general procedure C from cyclohexylamine (100 mg, 1.0 mmol) as a light yellow solid. ¹H NMR (400 MHz, CD₃OD) δ 8.73 (d, *J* = 1.8 Hz, 1H), 8.41 (s, 1H), 8.09 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.99 (d, *J* = 8.5 Hz, 1H), 4.32 (s, 2H), 4.22–4.05 (m, 1H), 3.74–3.61 (m, 1H), 3.47 (m, 2H), 3.22–3.13 (m,

1H), 2.18 (dd, $J = 23.9, 11.4$ Hz, 4H), 2.00 (dd, $J = 13.3, 6.8$ Hz, 2H), 1.90 (d, $J = 12.6$ Hz, 2H), 1.67 (m, 4H), 1.56–1.31 (m, 8H), 1.32–1.19 (m, 2H), 1.04–0.92 (t, $J = 4$ Hz, 3H). LC-MS (ESI⁺): $t_R = 3.722$ min, MS m/z 453.0 $[M+1]^+$, >98% purity.

***trans*-4-((2-Butylamino-5-(5-(cyclopropylaminomethyl)pyridin-2-yl)pyrimidin-4-yl)**

amino)cyclohexanol (20) The title compound **20** (78 mg, 75%) was prepared according to general procedure C from cyclopropylamine (58 mg, 1.0 mmol) to afford a light yellow solid. ¹H NMR (400 MHz, CD₃OD) δ 9.11 (s, 1H), 8.69 (s, 1H), 8.23 (d, $J = 26.9$ Hz, 2H), 4.62 (s, 2H), 4.13 (s, 1H), 3.71–3.43 (m, 3H), 2.94 (s, 1H), 2.22–1.89 (m, 4H), 1.67 (s, 2H), 1.59–1.31 (m, 6H), 1.11 (s, 2H), 0.98 (t, $J = 6.9$ Hz, 5H). LC-MS (ESI⁺): $t_R = 3.402$ min, MS m/z 411.0 $[M+1]^+$, >98% purity.

***trans*-4-((2-Butylamino-5-(5-((2-hydroxyethylamino)methyl)pyridin-2-yl)pyrimidin-4-yl)**

amino)cyclohexanol (21) The title compound **21** (67 mg, 64%) was prepared according to general procedure C from 2-aminoethanol (62 mg, 1.0 mmol) as a light yellow solid. ¹H NMR (400 MHz, CD₃OD) δ 8.75 (d, $J = 1.9$ Hz, 1H), 8.42 (s, 1H), 8.13 (dd, $J = 8.5, 2.3$ Hz, 1H), 8.01 (d, $J = 8.5$ Hz, 1H), 4.36 (s, 2H), 4.20–4.08 (m, 1H), 3.89–3.80 (m, 2H), 3.66 (d, $J = 4.0$ Hz, 1H), 3.49 (s, 2H), 3.23–3.14 (m, 2H), 2.16 (d, $J = 10.6$ Hz, 2H), 2.07–1.92 (m, 2H), 1.66 (dt, $J = 12.6, 7.6$ Hz, 2H), 1.59–1.32 (m, 6H), 0.99 (t, $J = 7.4$ Hz, 3H). LC-MS (ESI⁺): $t_R = 3.269$ min, MS m/z 415.0 $[M+1]^+$, >98% purity.

***N*2-Butyl-*N*4-cyclohexyl-5-(pyridin-2-yl)pyrimidine-2,4-diamine (22)**

The title compound **22** (135 mg, 17% over three steps) was prepared according to general procedure A from cyclohexylamine (208 mg, 2.1 mmol) as a light yellow solid. ¹H NMR (400 MHz, CD₃OD) δ 8.65–8.52 (m, 1H), 8.28 (s, 1H), 7.91 (dt, $J = 18.9, 8.1$ Hz, 2H), 7.50–7.34 (m, 1H), 4.20 (d, $J = 3.6$ Hz, 1H), 3.46 (s, 2H), 2.02 (s, 2H), 1.80 (s, 2H), 1.65 (dt, $J = 14.8, 7.3$ Hz, 3H), 1.43 (td, $J =$

14.8, 7.4 Hz, 7H), 0.98 (t, $J = 7.4$ Hz, 3H). LC-MS (ESI+): $t_R = 5.309$ min, MS m/z 326.0 $[M+1]^+$, >98% purity.

N4-(trans-4-Aminocyclohexyl)-N2-butyl-5-(pyridin-2-yl)pyrimidine-2,4-diamine (23) The title compound **23** (99 mg, 11% over four steps) was prepared according to general procedure **A** from *trans*-cyclohexane-1,4-diamine (240 mg, 2.1 mmol) as a light yellow solid. ^1H NMR (400 MHz, CD_3OD) δ 8.59 (d, $J = 4.9$ Hz, 1H), 8.32 (s, 1H), 7.91 (ddd, $J = 20.9, 13.7, 5.0$ Hz, 1H), 7.47–7.30 (m, 1H), 4.15 (d, $J = 4.3$ Hz, 2H), 3.47 (dd, $J = 7.0, 3.8$ Hz, 2H), 3.20 (s, 2H), 2.27 (s, 2H), 2.16 (s, 2H), 1.66 (dd, $J = 14.5, 7.2$ Hz, 2H), 1.59 (dd, $J = 16.5, 7.4$ Hz, 2H), 1.44 (td, $J = 14.8, 7.3$ Hz, 2H), 0.98 (t, $J = 7.4$ Hz, 3H). LC-MS (ESI+): $t_R = 3.381$ min, MS m/z 341.0 $[M+1]^+$, >98% purity.

N4-(trans-4-(Aminomethyl)cyclohexyl)-N2-butyl-5-(pyridin-2-yl)pyrimidine-2,4-diamine (24) The title compound **24** (65 mg, 7% over four steps) was prepared according to general procedure **A** from *tert*-butyl (4-aminocyclohexylmethyl)carbamate (479 mg, 2.1 mmol) as a light yellow solid. ^1H NMR (400 MHz, CD_3OD) δ 8.66 (d, $J = 5.1$ Hz, 1H), 8.27 (s, 1H), 8.07 (t, $J = 7.9$ Hz, 1H), 7.94 (d, $J = 8.1$ Hz, 1H), 7.58–7.47 (m, 1H), 4.22–4.04 (m, 1H), 3.75–3.53 (m, 1H), 3.48 (s, 2H), 2.84 (d, $J = 7.0$ Hz, 2H), 2.22 (d, $J = 11.0$ Hz, 2H), 1.95 (d, $J = 12.5$ Hz, 2H), 1.68 (ddd, $J = 22.3, 10.9, 5.5$ Hz, 3H), 1.59–1.33 (m, 4H), 1.22 (dd, $J = 25.7, 12.1$ Hz, 2H), 0.98 (t, $J = 7.4$ Hz, 3H). LC-MS (ESI+): $t_R = 3.462$ min, MS m/z 355.0 $[M+1]^+$, >98% purity.

N4-(4-Aminophenyl)-N2-butyl-5-(pyridin-2-yl)pyrimidine-2,4-diamine (25) The title compound **25** (222 mg, 25% over four steps) was prepared according to general procedure **A** from *tert*-butyl (4-aminophenyl)carbamate (437 mg, 2.1 mmol) as a light yellow solid. ^1H NMR (400 MHz, CD_3OD) δ 8.72 (d, $J = 4.9$ Hz, 1H), 8.55 (s, 1H), 7.98 (dd, $J = 15.0, 5.8$ Hz, 4H),

7.47 (dd, $J = 8.8, 4.0$ Hz, 3H), 3.53–3.38 (m, 2H), 1.68–1.59 (m, 2H), 1.41 (d, $J = 7.0$ Hz, 2H), 1.28–1.13 (m, 2H), 0.95 (t, $J = 8.0$ Hz, 3H). LC-MS (ESI+): $t_R = 4.388$ min, MS m/z 335.0 $[M+1]^+$, >98% purity.

N2-Butyl-N4-(piperidin-4-yl)-5-(pyridin-2-yl)pyrimidine-2,4-diamine (26) The title compound **26** (79 mg, 9% over four steps) was prepared according to general procedure **A** from *tert*-butyl 4-aminopiperidine-1-carboxylate (420 mg, 2.1 mmol) as a light yellow solid. ^1H NMR (400 MHz, CD_3OD) δ 8.63 (d, $J = 5.0$ Hz, 1H), 8.38 (s, 1H), 8.07–7.88 (m, 2H), 7.47–7.40 (m, 1H), 4.47 (s, 1H), 3.72–3.55 (m, 1H), 3.47 (dd, $J = 13.4, 4.0$ Hz, 3H), 3.21 (d, $J = 11.5$ Hz, 2H), 2.36 (d, $J = 11.1$ Hz, 2H), 1.93 (dd, $J = 21.0, 10.4$ Hz, 2H), 1.66 (dt, $J = 15.0, 7.4$ Hz, 2H), 1.45 (dd, $J = 15.0, 7.4$ Hz, 2H), 0.99 (t, $J = 7.3$ Hz, 3H). LC-MS (ESI+): $t_R = 3.029$ min, MS m/z 327.0 $[M+1]^+$, >98% purity.

N2-Butyl-N4-(piperidin-4-ylmethyl)-5-(pyridin-2-yl)pyrimidine-2,4-diamine (27) The title compound **27** (45 mg, 5% over four steps) was prepared according to general procedure **A** from *tert*-butyl 4-(aminomethyl)piperidine-1-carboxylate (450 mg, 2.1 mmol) as a light yellow solid. ^1H NMR (400 MHz, CD_3OD) δ 8.66 (d, $J = 5.0$ Hz, 1H), 8.30 (s, 1H), 8.01 (t, $J = 7.7$ Hz, 1H), 7.92 (d, $J = 8.2$ Hz, 1H), 7.55–7.40 (m, 1H), 3.67 (t, $J = 16.8$ Hz, 3H), 3.50 (s, 1H), 3.42 (d, $J = 12.7$ Hz, 2H), 2.99 (t, $J = 11.7$ Hz, 2H), 2.11 (d, $J = 3.7$ Hz, 1H), 2.02 (d, $J = 13.8$ Hz, 2H), 1.71–1.61 (m, 2H), 1.54 (d, $J = 13.2$ Hz, 2H), 1.45 (dd, $J = 15.0, 7.5$ Hz, 2H), 0.99 (t, $J = 7.4$ Hz, 3H). LC-MS (ESI+): $t_R = 3.276$ min, MS m/z 341.0 $[M+1]^+$, >98% purity.

(1R,3R)-3-((2-Butylamino-5-(pyridin-2-yl)pyrimidin-4-yl)amino)cyclopentanol (28) The title compound **28** (160 mg, 20% over three steps) was prepared according to general procedure **A** from *(1R, 3R)*-3-aminocyclopentanol (213 mg, 2.1 mmol) as a light yellow solid. ^1H NMR (400

MHz, CD₃OD) δ 8.74 (d, J = 5.2 Hz, 1H), 8.25 (t, J = 7.5 Hz, 1H), 8.18 (s, 1H), 8.01 (d, J = 8.1 Hz, 1H), 7.76–7.58 (m, 1H), 4.82–4.73 (m, 1H), 4.37 (dt, J = 8.5, 2.9 Hz, 1H), 3.54 (d, J = 12.6 Hz, 2H), 2.38–2.23 (m, 1H), 2.19–2.11 (m, 1H), 2.10–2.01 (m, 1H), 1.86–1.77 (m, 1H), 1.67 (dt, J = 14.8, 7.2 Hz, 4H), 1.45 (dq, J = 14.5, 7.4 Hz, 2H), 0.98 (t, J = 7.4 Hz, 3H). LC-MS (ESI⁺): t_R = 4.433 min, MS m/z 328.0 [M+1]⁺, >98% purity.

***trans*-3-((2-Butylamino-5-(pyridin-2-yl)pyrimidin-4-yl)amino)cyclobutanol (29)** The title compound **29** (162 mg, 21% over three steps) was prepared according to general procedure **A** from *trans*-3-aminocyclobutanol (183 mg, 2.1 mmol) as a light yellow solid. ¹H NMR (400 MHz, CD₃OD) δ 8.66 (d, J = 5.1 Hz, 1H), 8.27 (s, 1H), 7.98 (d, J = 8.2 Hz, 1H), 7.90 (d, J = 8.2 Hz, 1H), 7.57–7.37 (m, 1H), 4.77–4.64 (m, 1H), 4.53–4.41 (m, 1H), 3.75–3.55 (m, 1H), 3.49 (s, 2H), 2.43 (t, J = 6.2 Hz, 4H), 1.64 (dd, J = 14.8, 7.5 Hz, 2H), 1.44 (dd, J = 15.0, 7.4 Hz, 2H), 0.98 (t, J = 7.4 Hz, 3H). LC-MS (ESI⁺): t_R = 4.274 min, MS m/z 314.0 [M+1]⁺, >98% purity.

3-((2-Butylamino-5-(pyridin-2-yl)pyrimidin-4-yl)amino)propan-1-ol (30) The title compound **30** (150 mg, 20% over three steps) was prepared according to general procedure **A** from 3-aminopropan-1-ol (158 mg, 2.1 mmol) as a light yellow solid. ¹H NMR (400 MHz, CD₃OD) δ 8.76 (d, J = 5.2 Hz, 1H), 8.26 (t, J = 7.6 Hz, 1H), 8.17 (s, 1H), 8.00 (d, J = 8.1 Hz, 1H), 7.79–7.63 (m, 1H), 3.75–3.58 (m, 4H), 3.61–3.44 (m, 2H), 1.97–1.83 (m, 2H), 1.66 (dt, J = 14.9, 7.3 Hz, 2H), 1.44 (dq, J = 14.5, 7.3 Hz, 2H), 0.98 (t, J = 7.4 Hz, 3H). LC-MS (ESI⁺): t_R = 5.309 min, MS m/z 326.0 [M+1]⁺; LC-MS (ESI⁺): t_R = 4.195 min, MS m/z 302.0 [M+1]⁺, >98% purity.

4-((2-Butylamino-5-(pyridin-2-yl)pyrimidin-4-yl)amino)butan-1-ol (31) The title compound **31** (210 mg, 27% over three steps) was prepared according to general procedure **A** from 4-

aminopropan-1-ol (188 mg, 2.1 mmol) as a light yellow solid. ^1H NMR (400 MHz, CD_3OD) δ 8.62 (d, $J = 5.0$ Hz, 1H), 8.25 (s, 1H), 8.01–7.91 (m, 1H), 7.86 (d, $J = 8.2$ Hz, 1H), 7.44–7.34 (m, 1H), 3.67 (dd, $J = 8.9, 5.0$ Hz, 2H), 3.60 (t, $J = 6.4$ Hz, 2H), 3.48 (d, $J = 8.0$ Hz, 2H), 1.84–1.72 (m, 2H), 1.71–1.56 (m, 4H), 1.43 (dd, $J = 15.0, 7.5$ Hz, 2H), 0.97 (t, $J = 7.4$ Hz, 3H). LC-MS (ESI $^+$): $t_{\text{R}} = 4.436$ min, MS m/z 316.0 $[\text{M}+1]^+$, >98% purity.

***N*-butyl-4-(piperazin-1-yl)-5-(pyridin-2-yl)pyrimidin-2-amine (32)** The title compound **32** (68 mg, 8% over four steps) was prepared according to general procedure A from *tert*-butyl piperazine-1-carboxylate (392 mg, 2.1 mmol) as a light yellow solid. ^1H NMR (400 MHz, CD_3OD) δ 8.67 (dd, $J = 4.3, 0.8$ Hz, 1H), 8.05 (td, $J = 7.8, 1.7$ Hz, 1H), 7.95 (s, 1H), 7.68 (d, $J = 7.8$ Hz, 1H), 7.53 (dd, $J = 7.6, 5.1$ Hz, 1H), 3.71 (s, 4H), 3.50 (s, 2H), 3.24 (s, 4H), 1.65 (dt, $J = 14.9, 7.2$ Hz, 2H), 1.43 (dd, $J = 15.0, 7.5$ Hz, 2H), 0.97 (t, $J = 7.4$ Hz, 3H). LC-MS (ESI $^+$): $t_{\text{R}} = 2.731$ min, MS m/z 313.0 $[\text{M}+1]^+$, >98% purity.

***trans*-4-((5-Bromo-2-(methylthio)pyrimidin-4-yl)amino)cyclohexanol (Va)** A solution of 2,4-dichloro-5-iodopyrimidine (2.6 g, 10 mmol) and 4-aminocyclohexanol (1.27 g, 11 mmol) in i -PrOH (60 mL) was stirred at room temperature overnight, then diluted with CH_2Cl_2 and washed by brine. The organic layer was dried (Na_2SO_4) and concentrated. The residue was purified by an ISCO silica gel column to afford the title compound **Va** as a white solid (3.19 g, >99%). ^1H NMR (400 MHz, CDCl_3) δ 8.04 (s, 1H), 5.10 (d, $J = 7.2$ Hz, 1H), 3.97 (dtd, $J = 11.1, 7.3, 3.8$ Hz, 1H), 3.74–3.59 (m, 1H), 2.13 (d, $J = 12.4$ Hz, 2H), 2.02 (d, $J = 12.4$ Hz, 2H), 1.58 (d, $J = 25.3$ Hz, 1H), 1.44 (ddd, $J = 23.2, 12.9, 3.2$ Hz, 2H), 1.30 (ddd, $J = 24.2, 12.8, 3.1$ Hz, 2H). ^{13}C NMR (101 MHz, cdcl_3) δ 170.33 (s), 156.85 (s), 155.07 (s), 99.73 (s), 69.70 (s), 49.15 (s), 33.78 (s), 30.41 (s), 14.34 (d, $J = 3.7$ Hz). LC-MS (ESI $^+$): $t_{\text{R}} = 5.104$ min, MS m/z 318.0 $[\text{M}+1]^+$, >98% purity.

***trans*-4-((2-Methylthio-5-(pyridin-2-yl)pyrimidin-4-yl)amino)cyclohexanol (33)** A solution of **Va** (2.18 g, 6.36 mmol) in a mixture of DMF/H₂O (16.0 mL/4.0 mL) was added 2-pyridinyl trihydroxyborate lithium (2.80 g, 19.1 mmol), copper bromide (183 mg, 1.27 mmol), potassium carbonate (2.63 g, 19.1 mmol) were and PdCl₂(dppf) (519 mg, 0.64 mmol) at room temperature. The reaction mixture was heated at 120°C for 30 min, and then diluted with EtOAc (15 mL) after cooled to room temperature. The resulting suspension was filtered through a short pad of celite, which was washed with ethyl acetate. The filtrate was washed with water (2x30 mL), dried (MgSO₄), and concentrated. The residue was purified by the flash chromatography to afford the title compound **33** as a white solid (1.06 g, 53%). ¹H NMR (400 MHz, CD₃OD) δ 8.58–8.49 (m, 1H), 8.43 (s, 1H), 7.85 (ddd, *J* = 10.0, 9.6, 5.0 Hz, 2H), 7.34–7.22 (m, 1H), 4.09 (dd, *J* = 9.4, 5.3 Hz, 1H), 3.69–3.58 (m, 1H), 2.52 (s, 3H), 2.19–2.11 (m, 2H), 1.98 (dd, *J* = 10.7, 7.7 Hz, 2H), 1.50–1.32 (m, 3H). ¹³C NMR (101 MHz, cd₃od) δ 170.96 (s), 158.47 (s), 154.56 (s), 152.22 (s), 147.21 (s), 137.25 (s), 121.55 (s), 119.78 (s), 108.55 (s), 68.89 (s), 33.02 (s), 29.75 (s), 12.66 (d, *J* = 3.6 Hz). LC-MS: LC-MS (ESI+): *t*_R = 4.934 min, MS *m/z* 317.2 [M+1]⁺, >98% purity.

***trans*-4-((2-Methylamino-5-(pyridin-2-yl)pyrimidin-4-yl)amino)cyclohexanol (34)** (General Procedure E) A solution of **33** (95mg, 0.5 mmol) in THF (5.0 mL) was added mCPBA (130 mg, 0.75 mmol). The reaction mixture was stirred at room temperature for 2.0 h. Then methylamine (2 mmol) was added. The reaction mixture was heated under microwave irradiation at 150°C for 30 min. The solvent was removed under reduced pressure. The residue was purified on ISCO to afford the title compound **34** as a light brown solid (120 mg, 80%). ¹H NMR (400 MHz, CD₃OD) δ 8.73 (d, *J* = 4.5 Hz, 1H), 8.27–8.15 (m, 2H), 8.00 (d, *J* = 8.1 Hz, 1H), 7.71–7.58 (m, 1H), 4.18 (s, 1H), 3.68–3.56 (m, 1H), 3.05 (s, 3H), 2.12 (s, 2H), 2.01 (d, *J* = 10.6 Hz, 2H), 1.46 (dd, *J* = 23.8, 12.8 Hz, 4H). LC-MS (ESI+): *t*_R = 3.914 min, MS *m/z* 300.0 [M+1]⁺, >98% purity.

***trans*-4-((2-Ethylamino-5-(pyridin-2-yl)pyrimidin-4-yl)amino)cyclohexanol (35)** The title compound **35** (98 mg, 85%) was prepared according to general procedure E from ethylamine (0.60 mL, 2.0 *N* solution in methanol, 1.2 mmol) as a light yellow solid. ¹H NMR (400 MHz, CD₃OD) δ 8.82–8.70 (m, 1H), 8.32–8.15 (m, 2H), 8.01 (d, *J* = 8.1 Hz, 1H), 7.76–7.64 (m, 1H), 4.23–4.10 (m, 1H), 3.69–3.55 (m, 1H), 3.53 (s, 2H), 2.12 (d, *J* = 11.5 Hz, 2H), 2.01 (d, *J* = 11.3 Hz, 2H), 1.58–1.36 (m, 4H), 1.30 (t, *J* = 7.2 Hz, 3H). LC-MS (ESI⁺): *t_R* = 4.052 min, MS *m/z* 314.0 [M+1]⁺, >98% purity.

***trans*-4-((2-Propylamino-5-(pyridin-2-yl)pyrimidin-4-yl)amino)cyclohexanol (36)** The title compound **36** (112 mg, 93%) was prepared according to general procedure E from 1-propylamine (71 mg, 1.2 mmol) as a light yellow solid. ¹H NMR (400 MHz, CD₃OD) δ 8.67 (d, *J* = 4.9 Hz, 1H), 8.24 (s, 1H), 8.10 (t, *J* = 7.7 Hz, 1H), 7.95 (d, *J* = 8.2 Hz, 1H), 7.61–7.46 (m, 1H), 4.20–4.07 (m, 1H), 3.71–3.55 (m, 1H), 3.44 (s, 2H), 2.12 (d, *J* = 11.9 Hz, 2H), 2.07–1.91 (m, 2H), 1.80–1.61 (m, 2H), 1.59–1.35 (m, 4H), 1.01 (t, *J* = 7.4 Hz, 3H). LC-MS (ESI⁺): *t_R* = 4.242 min, MS *m/z* 328.0 [M+1]⁺, >98% purity.

***trans*-4-((2-Pentylamino-5-(pyridin-2-yl)pyrimidin-4-yl)amino)cyclohexanol (37)** The title compound **37** (104 mg, 81%) was prepared according to general procedure E from pentan-1-amine (105 mg, 1.2 mmol) as a light yellow solid. ¹H NMR (400 MHz, CD₃OD) δ 8.66 (d, *J* = 4.9 Hz, 1H), 8.25 (s, 1H), 8.05 (d, *J* = 7.8 Hz, 1H), 7.93 (d, *J* = 8.2 Hz, 1H), 7.58–7.46 (m, 1H), 4.22–3.98 (m, 1H), 3.68–3.53 (m, 1H), 3.48 (s, 2H), 2.13 (d, *J* = 11.7 Hz, 2H), 2.00 (d, *J* = 11.0 Hz, 2H), 1.75–1.62 (m, 2H), 1.43 (ddd, *J* = 16.6, 14.1, 9.7 Hz, 8H), 0.94 (t, *J* = 6.9 Hz, 3H). LC-MS (ESI⁺): *t_R* = 4.691 min, MS *m/z* 356.0 [M+1]⁺, >98% purity.

***trans*-4-((2-Pentan-2-ylamino-5-(pyridin-2-yl)pyrimidin-4-yl)amino)cyclohexanol (38)** The title compound **38** (92 mg, 72%) was prepared according to general procedure E from pentan-2-amine (105 mg, 1.2 mmol) as a light yellow solid. ¹H NMR (400 MHz, CD₃OD) δ 8.67 (s, 1H), 8.23 (s, 1H), 8.11 (d, *J* = 7.4 Hz, 1H), 7.95 (d, *J* = 7.1 Hz, 1H), 7.56 (s, 1H), 4.24–4.07 (m, 2H), 3.69–3.58 (m, 1H), 2.12 (d, *J* = 12.4 Hz, 2H), 2.00 (d, *J* = 9.9 Hz, 2H), 1.72–1.35 (m, 8H), 1.29 (d, *J* = 6.6 Hz, 3H), 0.97 (t, *J* = 7.3 Hz, 3H). LC-MS (ESI⁺): *t*_R = 4.452 min, MS *m/z* 356.0 [M+1]⁺, >98% purity.

***trans*-4-((2-Butyl(methyl)amino-5-(pyridin-2-yl)pyrimidin-4-yl)amino)cyclohexanol (39)** The title compound **39** (84 mg, 65%) was prepared according to general procedure E from methylbutylamine (105 mg, 1.2 mmol) as a light yellow solid. ¹H NMR (400 MHz, CD₃OD) δ 8.77 (d, *J* = 5.1 Hz, 1H), 8.30 (td, *J* = 7.9, 1.5 Hz, 1H), 8.16 (s, 1H), 8.05 (d, *J* = 8.1 Hz, 1H), 7.75 (dd, *J* = 7.1, 5.9 Hz, 1H), 4.09 (dd, *J* = 12.4, 8.8 Hz, 1H), 3.71 (s, 2H), 3.59 (ddd, *J* = 9.8, 9.2, 4.1 Hz, 1H), 3.24 (s, 3H), 2.17–2.04 (m, 2H), 2.04–1.97 (m, 2H), 1.70 (dt, *J* = 15.2, 7.6 Hz, 2H), 1.61–1.32 (m, 6H), 1.01 (t, *J* = 7.4 Hz, 3H). LC-MS (ESI⁺): *t*_R = 4.381 min, MS *m/z* 356.0 [M+1]⁺, >98% purity.

***trans*-4-((2-Piperidine-1-yl-5-(pyridin-2-yl)pyrimidin-4-yl)amino)cyclohexanol (40)** The title compound **40** (103 mg, 80%) was prepared according to general procedure E from piperidine (103 mg, 1.2 mmol) as a light yellow solid. ¹H NMR (400 MHz, CD₃OD) δ 8.66 (d, *J* = 5.1 Hz, 1H), 8.21 (s, 1H), 8.12–8.01 (m, 1H), 7.96 (d, *J* = 8.1 Hz, 1H), 7.53 (dd, *J* = 7.4, 5.3 Hz, 1H), 4.08 (td, *J* = 10.2, 4.8 Hz, 1H), 3.79 (s, 4H), 3.66–3.58 (m, 1H), 2.13 (d, *J* = 11.1 Hz, 2H), 2.05–1.92 (m, 2H), 1.84–1.69 (m, 6H), 1.56–1.36 (m, 4H). LC-MS (ESI⁺): *t*_R = 4.269 min, MS *m/z* 354.0 [M+1]⁺, >98% purity.

***trans*-4-((2-Cyclohexylamino-5-(pyridin-2-yl)pyrimidin-4-yl)amino)cyclohexanol (41)** The title compound **41** (99 mg, 75%) was prepared according to general procedure E from cyclohexylamine (119 mg, 1.2 mmol) as a light yellow solid. ¹H NMR (400 MHz, CD₃OD) δ 8.62 (d, *J* = 5.0 Hz, 1H), 8.28 (s, 1H), 8.09–7.92 (m, 1H), 7.89 (d, *J* = 8.2 Hz, 1H), 7.44 (dd, *J* = 7.0, 5.5 Hz, 1H), 4.09 (t, *J* = 10.3 Hz, 1H), 3.89 (s, 1H), 3.65 (tt, *J* = 8.4, 4.1 Hz, 1H), 2.15 (d, *J* = 11.2 Hz, 2H), 2.02 (d, *J* = 11.9 Hz, 4H), 1.94–1.78 (m, 2H), 1.69 (d, *J* = 12.5 Hz, 1H), 1.62–1.20 (m, 9H). LC-MS (ESI⁺): *t*_R = 4.495 min, MS *m/z* 368.0 [M+1]⁺, >98% purity.

***trans*-4-((2-Benzylamino-5-(pyridin-2-yl)pyrimidin-4-yl)amino)cyclohexanol (42)** The title compound **42** (112 mg, 83%) was prepared according to general procedure E from phenylmethanamine (129 mg, 1.2 mmol) as a light yellow solid. ¹H NMR (400 MHz, CD₃OD) δ 8.64 (d, *J* = 5.0 Hz, 1H), 8.28 (s, 1H), 8.07 (td, *J* = 8.0, 1.6 Hz, 1H), 7.93 (d, *J* = 8.2 Hz, 1H), 7.53 (dd, *J* = 7.4, 5.3 Hz, 1H), 7.41–7.31 (m, 4H), 7.26 (t, *J* = 6.8 Hz, 1H), 4.65 (s, 2H), 3.99 (s, 1H), 3.57 (s, 1H), 1.93 (d, *J* = 5.2 Hz, 4H), 1.47–1.31 (m, 4H). LC-MS (ESI⁺): *t*_R = 4.325 min, MS *m/z* 376.0 [M+1]⁺, >98% purity.

***trans*-4-((2-Phenethylamino-5-(pyridin-2-yl)pyrimidin-4-yl)amino)cyclohexanol (43)** The title compound **43** (110 mg, 79%) was prepared according to general procedure E from 2-phenylethanamine (146 mg, 1.2 mmol) as a light yellow solid. ¹H NMR (400 MHz, CD₃OD) δ 8.66 (d, *J* = 5.1 Hz, 1H), 8.24 (s, 1H), 8.07 (dd, *J* = 11.1, 4.7 Hz, 1H), 7.92 (d, *J* = 8.2 Hz, 1H), 7.54 (dd, *J* = 7.1, 5.6 Hz, 1H), 7.35–7.16 (m, 5H), 4.12 (d, *J* = 3.9 Hz, 1H), 3.73 (s, 2H), 3.68–3.56 (m, 1H), 2.96 (t, *J* = 7.3 Hz, 2H), 2.13 (d, *J* = 10.0 Hz, 2H), 2.01 (d, *J* = 10.0 Hz, 2H), 1.59–1.38 (m, 4H). LC-MS (ESI⁺): *t*_R = 4.595 min, MS *m/z* 390.0 [M+1]⁺, >98% purity.

***trans*-4-((2-(2-Hydroxyethyl)amino-5-(pyridin-2-yl)pyrimidin-4-yl)amino)cyclohexanol (44)**

The title compound **44** (109 mg, 90%) was prepared according to general procedure E from 2-aminoethanol (74 mg, 1.2 mmol) as a light yellow solid. ¹H NMR (400 MHz, CD₃OD) δ 8.74 (d, *J* = 5.3 Hz, 1H), 8.28–8.18 (m, 2H), 8.00 (d, *J* = 8.1 Hz, 1H), 7.80–7.64 (m, 1H), 4.22–4.05 (m, 1H), 3.77 (t, *J* = 5.4 Hz, 2H), 3.61 (dq, *J* = 15.8, 5.4 Hz, 3H), 2.10 (d, *J* = 9.9 Hz, 2H), 1.99 (d, *J* = 10.0 Hz, 2H), 1.44 (q, *J* = 11.8 Hz, 4H). LC-MS (ESI⁺): *t*_R = 3.729 min, MS *m/z* 330.0 [M+1]⁺, >96% purity.

***trans*-4-((2-(3-Hydroxypropyl)amino-5-(pyridin-2-yl)pyrimidin-4-yl)amino)cyclohexanol (45)**

The title compound **45** (103 mg, 85%) was prepared according to general procedure E from 3-aminopropan-1-ol (91 mg, 1.2 mmol) as a light yellow solid. ¹H NMR (400 MHz, CD₃OD) δ 8.70 (d, *J* = 4.8 Hz, 1H), 8.23 (s, 1H), 8.17 (t, *J* = 7.6 Hz, 1H), 7.97 (d, *J* = 8.1 Hz, 1H), 7.68–7.58 (m, 1H), 4.20–4.10 (m, 1H), 3.68 (t, *J* = 6.1 Hz, 2H), 3.64–3.53 (m, 2H), 2.12 (d, *J* = 10.1 Hz, 2H), 1.99 (d, *J* = 10.2 Hz, 2H), 1.95–1.80 (m, 2H), 1.45 (dd, *J* = 21.7, 11.6 Hz, 4H). LC-MS (ESI⁺): *t*_R = 3.930 min, MS *m/z* 330.0 [M+1]⁺, >98% purity.

***trans*-4-((2-(4-Hydroxybutyl)amino-5-(pyridin-2-yl)pyrimidin-4-yl)amino)cyclohexanol (46)**

The title compound **46** (111 mg, 86%) was prepared according to general procedure E from 4-aminobutan-1-ol (107 mg, 1.2 mmol) as a light yellow solid. ¹H NMR (400 MHz, CD₃OD) δ 8.74 (dd, *J* = 23.7, 5.1 Hz, 1H), 8.31 (t, *J* = 7.7 Hz, 1H), 8.18 (s, 1H), 8.05 (t, *J* = 12.3 Hz, 1H), 7.79–7.67 (m, 1H), 4.20–4.01 (m, 1H), 3.61 (t, *J* = 6.3 Hz, 2H), 3.53 (s, 2H), 2.08 (t, *J* = 12.5 Hz, 2H), 2.00 (d, *J* = 10.3 Hz, 2H), 1.83–1.71 (m, 2H), 1.63 (dt, *J* = 13.0, 6.3 Hz, 2H), 1.53–1.35 (m, 4H). LC-MS (ESI⁺): *t*_R = 3.928 min, MS *m/z* 358.0 [M+1]⁺, >98% purity.

Selectivity Profiling for 10 (UNC2250) (Carna Biosciences)

Compound preparation: Test compound was dissolved in and diluted with dimethylsulfoxide (DMSO) to achieve 100-fold higher concentration as specified for the compound. The solution was further diluted 25-fold with assay buffer to make the final test compound solution. Reference compounds for assay control were prepared similarly.

Assay reagents and procedures

Off-chip Mobility Shift Assay (MSA)

- 1) 5 uL of 4x compound solution, 5 uL of 4x Substrate/ATP/Metal solution, and 10 uL of 2x kinase solution were prepared with assay buffer (20 mM HEPES, 0.01% Triton X-100, 2 mM DTT, pH7.5) and mixed and incubated in a polypropylene 384 well microplate for 1 or 5 hour(s) at room temperature, depending on kinase)
- 2) 60 uL of Termination Buffer (QuickScout Screening Assist MSA; Cama Biosciences) was added to the well.
- 3) The reaction mixture was applied to a LabChip3000 system (Caliper Life Science), and the product and substrate peptide peaks were separated and quantitated.
- 4) The kinase reaction was evaluated by the product ratio calculated from peak heights of product(P) and substrate(S) peptides ($P/(P+S)$).

Data Analysis

The readout value of reaction control (complete reaction mixture) was set as 0% inhibition, and the readout value of background (Enzyme(-)) was set as 100% inhibition, then the percent inhibition of each test solution was calculated.

Table 1. % Inhibition Data of UNC2250.

Kinase	UNC2250 @ 180 nM
ABL	10.4
CSK	-5.7

EGFR	-5.2
FGFR1	62.6
IGF1R	12.4
ITK	24.8
JAK3	24.7
KDR	65.8
LCK	30.4
MER	97.7
MET	12.6
PDGFR α	93.7
SRC	11.0
SYK	-2.0
TRKA	97.6
AKT1	-4.8
AurA	34.3
CaMK4	-6.4
CDK2/CycA2	1.0
DAPK1	0.2
DYRK1B	8.0
Erk2	-7.9
IKK β	-7.3
IRAK4	-3.9
JNK2	-2.1
MST1	32.8
p38 α	-5.6
p70S6K	35.1
PKC α	3.2
ROCK1	0.3

X-Ray Crystallography

Protein Expression and Purification

A pET28 expression vector containing the coding sequence for the Mer catalytic domain² was transformed into Rosetta BL21(DE3)pLysS competent cells (Novagen). Cells were grown at 37°C with shaking until the OD₆₀₀ reached ~0.8 at which time the temperature was lowered to 15°C and expression was induced by adding 0.2 mM IPTG and continuing shaking overnight.

Cells were harvested by centrifugation and pellets were stored at -80 °C. Cell pellets were thawed and resuspended in 30ml of lysis buffer (50 mM sodium phosphate pH 7.2, 50 mM NaCl, 30 mM imidazole, 1X protease inhibitor cocktail (Roche)) per liter of culture. Cells were lysed on ice by sonication at 40% amplitude for a total of 15 minutes consisting of 10 second pulses followed by 50 second rest. The cell lysate was clarified by centrifugation and loaded onto a HisTrap FF column (GE Healthcare) that had been preequilibrated with 10 column volumes of binding buffer (50 mM sodium phosphate pH 7.2, 500 mM NaCl, 30 mM imidazole) using an AKTA FPLC (GE Healthcare). The column was washed with 15 column volumes of binding buffer and protein was eluted in a linear gradient to 100% elution buffer (50 mM sodium phosphate pH 7.2, 500 mM NaCl, 500 mM imidazole) over 20 column volumes. Peak fractions containing Mer were pooled and concentrated to 2.0 mL in Amicon Ultra-15 concentrators, 10,000 molecular weight cut-off (Millipore). Concentrated Mer was loaded onto a HiLoad 26/60 Superdex 200 prep grade column (GE Healthcare) that had been preequilibrated with 1.2 column volumes of sizing buffer (20 mM Tris pH 8.0, 500 mM NaCl, 2.0 mM β -mercaptoethanol) using an ATKA FPLC (GE Healthcare). Protein was eluted isocratically in sizing buffer over 1.3 column volumes at a flow rate of 2.0 mL/min collecting 3.0 mL fractions. Peak fractions were analyzed for purity by SDS-PAGE and those containing pure Mer were pooled and concentrated using Amicon Ultra-15 concentrators 10,000 molecular weight cut-off (Millipore) to a final concentration of 35-40 mg/mL.

Crystallization

Crystals of Mer in complex with **3** were obtained by vapor diffusion from sitting drops at 12 °C. Protein at 32.5 mg/mL in crystallization buffer (20 mM Tris pH 8.0, 500 mM sodium chloride, 2 mM β -mercaptoethanol) was incubated with **3** (dissolved in DMSO) to give a final

concentration of 2.5 mM and slowly rocked over night. This solution was mixed 1:1 with and equilibrated against crystallization solution containing 27-33% (v/v) Peg 400, 200 mM magnesium chloride, 100 mM Tris pH 8.5. Plate-like crystals grew to final dimensions of up to 1000 x 400 x 50 μm over 10 days. Prior to diffraction data collection, crystals were vitrified by plunging into liquid nitrogen. The Mer/**3** crystals displayed the symmetry of space group $P2_1$ with cell parameters $a = 51 \text{ \AA}$, $b = 91 \text{ \AA}$, $c = 70 \text{ \AA}$, $\beta = 99.81^\circ$, contained two molecules in the asymmetric unit, and diffracted X-rays to a minimum Bragg spacing of about 2.7 \AA .

Structure Determination

Data were collected at the Southeast Regional Collaborative Access Team (SER-CAT) 22-ID beamline at the Advanced Photon Source, Argonne National Laboratory at a wavelength of 0.97921 \AA and a temperature of 100K. Data were processed using the program HKL2000. The diffraction quality of our crystals was non-uniform: when the thin edge was exposed to X-rays, the resulting diffraction spots were radially smeared, which was accompanied by a loss of resolution and a somewhat reduced completeness in the high-resolution shells.

The structure of the Mer/**3** complex was determined by molecular replacement with the program Phaser³. The search model was generated from the coordinates of Mer in complex with ADP (PDB entry 3BRB) with all non-protein atoms removed. Refinement was carried out using the program Phenix³, interspersed with manual revisions of the model using the program Coot⁴. Refinement consisted of conjugate-gradient minimization and calculation of individual atomic displacement and translation/libration/screw (TLS) parameters. To avoid any model bias, coordinates for **3** were not included until the remainder of the model (including water molecules

and ions) was completed. Inclusion of high-resolution, albeit weaker, data increased the stability and convergence of the refinement process. For data collection and refinement statistics see Table 2.

The current model contains two molecules of the Mer kinase domain bound to compound **3**, 7 chloride ions, 1 magnesium ion, and 8 water molecules. Residues that could not be identified in the electron density were: chain A, 552-577, 621-639, 743-762, 773-774, 862-864; chain B, 552-576, 595-599, 608-612, 622-633, 658-665, 744-762, 862-864. The model exhibits excellent geometry as determined by MolProbity⁵. Ramachandran analysis identified 97.4% favored, 2.2% allowed, and 0.4% disallowed residues. The coordinates and structure factors have been deposited in the RCSB Protein Data Bank under accession number 4M3Q.

Table 2. Data collection and refinement statistics

Data collection	
Space group	P2 ₁
Cell dimensions <i>a</i> , <i>b</i> , <i>c</i> (Å), β (°)	50.9, 91.1, 69.7, 99.81
Resolution (Å)	34.74–2.71 (2.73–2.71)
<i>R</i> _{merge} (%) ^a	11.0 (57.0)
<i>I</i> / σ <i>I</i>	11.6 (2.3)
Cut-off (σ)	-3
Unique reflections	16,507 (422)
Completeness (%)	97.5 (100.0)
Redundancy	4.1 (4.2)
Wilson B-factor (Å ²)	29.2
Refinement	
Resolution (Å)	34.74–2.72 (2.82–2.72)

No. of reflections (work/free)	12,782/1,420 (786/88)
Cut-off (σ)	0
$R_{\text{work}} / R_{\text{free}}$	24.3/29.6 (22.8/27.6)
No. of atoms	
Protein	3,860
Compound 3	50
Ions	8
Water	7
B -factors (\AA^2)	
Protein	41.6
Compound 3	42.1
Ions	13.8
Water	36.6
R.m.s. deviations	
Bond lengths (\AA)	0.003
Bond angles ($^\circ$)	0.653
Ramachandran ^b	
Favored (%)	97.4
Generally Allowed (%)	2.2
Disallowed (%)	0.4
Missing residues	A: 552-577, 621-639, 743-762, 773-774, 862-864 B: 552-576, 595-599, 608-612, 622-633, 658-665, 744-762, 862-864

Values in parentheses denote highest resolution shell

^a $R_{\text{merge}} = 100 \sum_h \sum_i |I_{h,i} - \langle I_h \rangle| / \sum_h \sum_i I_{h,i}$, where the outer sum (h) is over the unique reflections and the inner sum (i) is over the set of independent observations of each unique reflection.

^bAs defined by the validation suite MolProbity⁵.

PK Study

A group of 9 male Swiss albino mice (group I) were dosed intravenously (IV) with solution formulation of **10** in 7.5 % (v/v) *N*-methyl pyrrolidone and 40% (v/v) PEG in water. Another group of 9 male Swiss albino mice (group II) were dosed orally (PO) with suspension formulation of **10** in 0.5% (w/v) NaCMC with 0.1% (v/v) Tween-80 in water. From each mouse, three blood samples (60 μ L) were collected from retro orbital plexus such that samples were obtained at 0, 0.08, 0.25, 0.5, 1, 2, 4, 8 & 24 h (iv) & 0, 0.25, 0.5, 1, 2, 4, 6, 8 & 24 h (po) post dose. At each time point blood samples were collected from three mice. Immediately after collection, plasma was harvested by centrifugation of blood and was stored below -70°C until analysis. All samples were processed for analysis by precipitation using Albendazole as internal standard and analyzed with partially validated LC/MS-MS method (LLOQ was 1.038 ng/mL). Pharmacokinetic parameters were calculated using the Non-compartmental analysis tool of WinNonlin® Enterprise software (version 5.2).

References

1. Chen, K.; Peterson, R.; Math, S. K.; LaMunyon, J. B.; Testa, C. A.; Cefalo, D. R., Lithium trihydroxy/triisopropoxy-2-pyridylborate salts (LTBS): synthesis, isolation, and use in modified Suzuki–Miyaura cross-coupling reactions. *Tetrahedron Letters* **2012**, *53* (36), 4873-4876.
2. Huang, X.; Finerty, P., Jr.; Walker, J. R.; Butler-Cole, C.; Vedadi, M.; Schapira, M.; Parker, S. A.; Turk, B. E.; Thompson, D. A.; Dhe-Paganon, S., Structural insights into the inhibited states of the Mer receptor tyrosine kinase. *J Struct Biol* **2009**, *165* (2), 88-96.
3. McCoy, A. J.; Grosse-Kunstleve, R. W.; Adams, P. D.; Winn, M. D.; Storoni, L. C.; Read, R. J., Phaser crystallographic software. *Journal of applied crystallography* **2007**, *40* (Pt 4), 658-674.