Selective Inhibitors of the Mutant B-Raf Pathway: Discovery of A Potent and Orally Bioavailable Aminoisoquinoline.

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Supporting Information

Synthetic Procedures and Compound Characterization Data

LC-MS Methods:

Samples were run on an Agilent model-1100 LC-MSD system with an Agilent Technologies SB-C18 (3.0 μM) reverse phase column (3.0 x 50 mm) at 40 °C. The flow rate was constant at 1.5 mL/min. The mobile phase used a mixture of solvent A (H_2O/0.1% TFA) and solvent B (MeCN/0.1% TFA) with a 3.6 min time period for a gradient from 5% to 95% solvent B. The gradient was followed by a 0.5 min period to return to 5% solvent B and a 2.5 min 5% solvent B re-equilibration (flush) of the column. Integrated HPLC purities are reported at 215 nM.

NMR Spectra:

All NMR spectra were run on a Varian (Varian, Palo Alto, CA) series Mercury 300 MHz instrument or a Bruker (Bruker, Bilerica, MA) series 400MHz instrument. Where so characterized, all observed protons are reported as parts-per-million (ppm) downfield from tetramethylsilane (TMS) or other internal reference in the appropriate solvent indicated.

4-Methyl-3-(3-(pyrimidin-4-yl)pyridin-2-ylamino)-N-(3-(trifluoromethyl)phenyl)benzamide (2)

A mixture of 4-methyl-3-(3-(pyrimidin-4-yl)pyridin-2-ylamino)benzoic acid[1] (1.000 g, 3265 μmol), DIPEA (684 μl, 3917 μmol), and 3-(trifluoromethyl)benzenamine (489 μl, 3917 μmol) in DMF (10 mL) was treated with HATU (1490 mg, 3917 μmol) and heated at 80 °C for 16 h after which time the reaction had gone to completion. The DMF was removed in vacuo and the resulting oil was diluted with DCM (50 mL). The product crystallized from solution and was collected by filtration, washing with a small amount of DCM. 4-Methyl-3-(3-(pyrimidin-4-yl)pyridin-2-ylamino)-N-(3-(trifluoromethyl)phenyl)benzamide (800 mg, 55% yield) was obtained as a crystalline yellow solid. ^{1}H NMR (300 MHz, d_6-DMSO) δ 11.78 (s, 1 H); 10.49 (s, 1 H); 9.40 (d, J=1.13 Hz, 1 H); 8.96 (d, J=5.65 Hz, 1 H); 8.81 (d, J=1.51 Hz, 1 H); 8.54 (dd, J=7.91, 1.70 Hz, 1 H); 8.36 (dd, J=4.71, 1.70 Hz, 1 H); 8.22 - 8.33 (m, 2 H); 8.01 - 8.11 (m, 1 H); 7.53 - 7.68 (m, 2 H); 7.44 (d, J=8.10 Hz, 2 H); 7.02 (dd, J=7.82, 4.80 Hz, 1 H); 2.48 (s, 3 H). HPLC purity >99%. MS (ESI, +ve ion) *m/z* 450.1, $(M+H)^+$.

4-Methyl-3-(2-(methylamino)quinazolin-6-yl)-N-(3-(trifluoromethyl)phenyl)benzamide (3)

A mixture of 3-iodo-4-methyl-N-(3-(trifluoromethyl)phenyl)benzamide (0.750 g, 1.85 mmol), N-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinazolin-2-amine (0.581 g, 2.04 mmol), dichlorobis(triphenyl-phosphine)palladium (II) (0.130 g, 0.185 mmol), and sodium carbonate hydrate (0.459 g, 3.70 mmol) in 10:1 DMF:Water (4.0 mL) was heated at 140 °C for 25 min in a microwave reactor. The reaction mixture was diluted with CH_2Cl_2 and water and extracted with CH_2Cl_2.The combined organic extracts were washed with brine, dried with magnesium sulfate and concentrated under vacuum. The resulting residue was purified by flash chromatgraphy (0.5 - 6% MeOH in DCM) to give 4-methyl-3-(2-(methylamino)quinazolin-6-yl)-N-(3-(trifluoromethyl)phenyl)benzamide (0.715 g, 89% yield). ^{1}H NMR (400 MHz, d_6-DMSO) δ 10.52 (s, 1 H); 9.16 (br. s., 1 H); 8.24 (s, 1 H); 8.08 (d, J=8.61 Hz, 1 H); 7.90 - 7.99 (m, 3 H); 7.86 (d, J=1.96 Hz, 1 H); 7.78 (dd, J=8.71, 2.05 Hz, 1 H); 7.55 - 7.63 (m, 2 H); 7.52 (d, J=8.02 Hz, 1 H); 7.39 - 7.47 (m, 2 H); 2.92 (d, J=4.89 Hz, 3 H); 2.37 (s, 3 H). HPLC purity >99%. MS (ESI, +ve ion) *m/z* 437.1, $(M+H)^+$.

6-Methylisoquinoline (5)

Aminoacetaldehyde dimethyl acetal (8.83 mL, 81.1 mmol) was added over 1 min to a stirred solution of p-tolualdehyde (9.88 mL, 81.1 mmol) in chloroform (150 mL) at 22 °C. An exotherm was noted. The reaction was heated to reflux (65 °C) and half the solvent was removed (to remove water). The heat was removed and the yellow solution was cooled to r.t. NMR showed the imine was formed smoothly, however, a trace of aldehyde could be observed. The yellow solution was diluted with chloroform to bring the volume back to ~100 mL, cooled to -3 °C and ethyl chloroformate (7.99 mL, 81.1 mmol) was added dropwise over 5 min followed by triethyl phosphite (17.4 mL, 97.3 mmol) over 10 minutes. The clear yellow solution was then allowed to warm to room temperature. A reflux condenser added to reaction vessel. After 23 h, titanium tetrachloride (35.6 mL, 324 mmol) was added very slowly (strong exotherm and white fumes observed) and the reaction began to gently reflux (50 °C). Color changed from yellow to dark red to dark brown. Once addition was complete, the dark brown solution was heated to reflux (52 °C) for 10.5 h. After allowing to cool to room temperature overnight, the dark brown solution was poured onto ice (filled a 2 L beaker with approximately 1 L of ice), the organic layer was separated off, and the aqueous layer was washed with dichloromethane (2 x 100 mL). The aqueous layer (now orange in color) was poured into a solution of potassium sodium tartrate tetrahydrate (183 g, 648 mmol) in water (300 mL), basified to

pH 9 with 28-30 % ammonium hydroxide (a white ppt crashed out) and then extracted with dichloromethane (3 x 200 mL). The organic layer was separated, dried over sodium sulfate, filtered and the solvent was evaporated in vacuo to yield 6-methylisoquinoline (9.02 g, 78% yield) as a light tan amorphous solid. MS (ESI, pos. ion) m/z: 144.1 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.56 (s, 3 H) 7.44 (dd, J=8.31, 1.27 Hz, 1 H) 7.57 (d, J=5.87 Hz, 1 H) 7.60 (s, 1 H) 7.87 (d, J=8.22 Hz, 1 H) 8.48 (d, J=5.67 Hz, 1 H) 9.19 (s, 1 H).

6-Methyl-5-nitroisoquinoline (6)

6-Methylisoquinoline (2.00 g, 14 mmol) was taken up in sulfuric acid (25.0 mL) and the mixture cooled down to 0 °C. The reaction was treated with potassium nitrate (2.8 g, 28 mmol) added in portions. After addition was complete, the reaction was stirred for 1.5 h at 0 °C. The reaction was poured onto crushed ice and basified with 5 N NaOH. The solid that precipitated was collected by suction filtration, washed with water and dried to give the product (2.5 g, 95%) as a tan solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.60 (s, 3 H), 7.54 (d, J=8.03 Hz, 1 H), 7.60 (d, J=6.02 Hz, 1 H), 8.06 (d, J=8.03 Hz, 1 H), 8.66 (d, J=6.02 Hz, 1 H), 9.30 (s, 1 H).

1-Chloro-6-methyl-5-nitroisoquinoline (7)

6-Methyl-5-nitroisoquinoline (32 g, 170 mmol) was dissolved in DCM (500 mL) and cooled in an ice-acetone bath to 0 °C. 3-Chloroperoxybenzoic acid (49.9 g, 289 mmol) (73%) was added in portions with vigorous stirring. After the addition, the reaction mixture was stirred at 0 °C for 4 h. Upon being warmed to room temperature, the reaction mixture was partitioned in DCM/NaOH (aq., 1 N). After multiple extractions, the organic layers were combined and washed with brine then dried over Na_2SO_4. Removal of the solvent in vacuo gave 6-methyl-5-nitroisoquinoline N-oxide as a yellow solid (23 g).

Phosphorous oxychloride (2.15 mL, 23.0 mmol) was added dropwise to a solution of 6-methyl-5-nitroisoquinoline N-oxide (0.940 g, 4.60 mmol) in 1,2-dichloroethane (40.0 mL). The mixture was heated to 70 °C for 3 h to afford an off-white suspension. The mixture was concentrated and the residue was partitioned between dichloromethane and water. The aqueous phase was separated and extracted with dichloromethane. The combined organic phases were washed with saturated aqueous sodium bicarbonate solution, brine, dried over anhydrous sodium sulfate,

filtered, and concentrated to afford 1-chloro-6-methyl-5-nitroisoquinoline (0.880 g, 86% yield) as an off-white solid. ^{1}H NMR (400 MHz, d6-DMSO) δ 8.47 (dd, 2 H), 7.92 (d, 1 H), 7.67 (d, 1 H), 2.56 (s, 3 H).

6-Methyl-5-nitro-N-(3-(trifluoromethyl)phenyl)isoquinolin-1-amine

1-Chloro-6-methyl-5-nitroisoquinoline (0.25 g, 1.1 mmol) and 3-(trifluoromethyl)benzenamine (0.17 mL, 1.3 mmol) were added to a microwave tube containing 3 mL of isopropanol. The tube was capped and heated at 180 °C for 1500 sec. The volatiles were removed in vacuo. The residue was taken up in DCM and washed with sat'd $NaHCO_3$. The organic layer was dried with sodium sulfate and purified by column chromatography on silica gel using a gradient of 10 to 40% of ethyl acetate in hexanes to give the product (310 mg, 79%) as an orange solid. ^{1}H NMR (400 MHz, d6-DMSO) δ 9.71 (s, 1 H), 8.71 (d, 1 H), 8.30 (s, 1 H), 8.21 (m, 2 H), 7.72 (d, 1 H), 7.59 (m, 1 H), 7.34 (d, 1 H), 6.93 (d, 1 H), 2.50 (s, 3 H).

6-Methyl-N^1-(3-(trifluoromethyl)phenyl)isoquinoline-1,5-diamine (9a)

A mixture of 6-methyl-5-nitro-N-(3-(trifluoromethyl)phenyl)isoquinolin-1-amine hydrochloride (7.68 g, 20 mmol), EtOH (150 mL), and tin(II) chloride dihydrate (23 g, 100 mmol) in a 500 mL round-bottomed flask was stirred in a 70 °C oil bath under N_2 and under a reflux condenser for 18 h. The reaction mixture was allowed to cool to room temperature (22 °C) and concentrated in vacuo to ~50-100 mL volume as a thick yellow oil. The oil was added to a 1:1 mixture of ice and 5 N NaOH (300 mL total volume) to afford a milky suspension which was extracted with EtOAc (3 x 200 mL). The combined extracts were washed with water (100 mL), satd NaCl (70 mL), dried over Na_2SO_4, filtered and concentrated in vacuo to give the product (8.2 g crude) as a dark red viscous oil. The crude product was adsorbed onto a plug of silica gel and chromatographed through a Redi-Sep® pre-packed silica gel column (120 g) eluting with a gradient of 10% to 30% EtOAc in hexane to provide a red solid. The solid was washed with 20% DCM/hexane and air-dried to afford 6-methyl-N^1-(3-(trifluoromethyl)phenyl)isoquinoline-1,5-diamine (**9a**) (4.2 g, 66% yield) as a pale pink solid; MS (ESI, pos. ion) m/z: 318 $[M+H]^+$. ^{1}H NMR (400 MHz, d6-DMSO) δ 2.27 (s, 3 H), 5.51 (s, 2 H), 7.25 (d, J=7.53 Hz, 1 H), 7.28 (d, J=8.53 Hz, 1 H), 7.41 - 7.57 (m, 2 H), 7.65 (d, J=8.53 Hz, 1 H), 7.92 (d, J=6.02 Hz, 1 H), 8.22 (d, J=8.03 Hz, 1 H), 8.36 (s, 1 H), 9.16 (s, 1 H).

N-(4-chlorophenyl)-6-methyl-5-nitroisoquinolin-1-amine hydrochloride

A mixture of 4-chlorobenzenamine (0.630 g, 4.94 mmol) and 1-chloro-6-methyl-5-nitroisoquinoline (1.000 g, 4.49 mmol) was suspended in isopropyl alcohol (12 mL) and heated in a microwave at 170 °C for 16 min. The resulting suspensions was cooled and the product was filtered off, washing with a small volume of IPA. N-(4-chlorophenyl)-6-methyl-5-nitroisoquinolin-1-amine hydrochloride (1.32 g, 84% yield) was obtained as a yellow crystalline solid. ^{1}H NMR (400 MHz, d6-DMSO) δ 10.8 (bs, 1 H), 8.92 (d, 1 H), 7.90 (d, 1 H), 7.82 (d, 1 H), 7.79 (d, 2 H), 7.51 (d, 2 H), 6.91 (d, 1 H), 5.7 (bs, 1 H), 2.51 (s, 3 H).

N^1-(4-chlorophenyl)-6-methylisoquinoline-1,5-diamine (9b)

A mixture of N-(4-chlorophenyl)-6-methyl-5-nitroisoquinolin-1-amine hydrochloride (5.28 g, 15.1 mmol) and tin (II) chloride (14.3 g, 75.4 mmol) in ethanol (100 mL) was heated at 75 °C for 16 h. The dark solution was concentrated, diluted with EtOAc (200 mL), washed with 5 N aqueous NaOH (250 mL) (re-extracted with a further 100 mL of EtOAc – a thick ppt crashed out of the aqueous layer shortly after extraction), and the combined organic layers (dark red) were dried (Na_2SO_4) and concentrated. The residue was dissolved in DCM (50 mL), scratched with a spatula until product started crytallizing, and then refrigerated. The product was filtered off, washing with a small volume of DCM. The filtrate was concentrated and suspended in DCM (10 mL), and a second crop was filtered off washing with a small volume of DCM. The solids were combined to give N^1-(4-chlorophenyl)-6-methylisoquinoline-1,5-diamine (3.07 g, 72% yield) as a brick red solid. ^{1}H NMR (400 MHz, d6-DMSO) δ 8.95 (s, 1 H), 7.95 (d, 2 H), 7.87 (s, 1 H), 7.62 (d, 1 H), 7.41 (d, 1 H), 7.32 (d, 2 H), 7.26 (d, 1 H), 5.50 (s, 2 H), 2.23 (s, 3 H).

6-Methyl-N^5-(3-(pyrimidin-4-yl)pyridin-2-yl)-N^1-(3-(trifluoromethyl)phenyl)isoquinoline-1,5-diamine (12a)

6-Methyl-N^1-(3-(trifluoromethyl)phenyl)naphthalene-1,5-diamine (**9a**) (0.100 g, 0.30 mmol), 4-(2-chloropyridin-3-yl)pyrimidine (0.057 g, 0.30 mmol), dicyclohexylphosphino-N,N-dimethylaminobiphenyl (0.0094 g, 0.024 mmol), $Pd_2(dba)_3$ (0.010 g, 0.012 mmol), were all placed in a sealed tube containing 5 ml of anhydrous THF. Lithium bis(trimethylsilyl)amide 1M THF (0.90 ml, 0.90 mmol) was then added to the mixture and nitrogen was bubbled into the reaction mixture for 5 min. The tube was capped and the reaction heated to 70 °C for 16 h. The reaction was allowed to cool down to Rt and quenched with methanol. The volatiles were removed in vacuo. The residue

was taken up in ethyl acetate and washed (2x) with an aqueous saturated solution of sodium bicarbonate, then with water and then brine. The organic layer was then dried with sodium sulfate and the purified by column chromatography on silica gel using a gradient of 20 to 60 % EtOAc in hexanes to give the product (80 mg, 56%) as a yellow solid. ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.13 (s, 1 H); 9.47 (s, 1 H); 9.33 (s, 1 H); 8.95 (d, J=5.67 Hz, 1 H); 8.40 - 8.49 (m, 2 H); 8.37 (s, 1 H); 8.21 - 8.31 (m, 2 H); 8.01 - 8.08 (m, 1 H); 7.96 (d, J=6.06 Hz, 1 H); 7.63 (d, J=8.61 Hz, 1 H); 7.50 - 7.58 (m, 1 H); 7.29 (d, J=7.63 Hz, 1 H); 7.15 (d, J=5.87 Hz, 1 H); 6.85 (dd, J=7.73, 4.79 Hz, 1 H); 2.35 (s, 3 H). HPLC purity >99%. MS (ESI, +ve ion) *m/z* 473.1, $(M+H)^+$.

N^1-(4-chlorophenyl)-6-methyl-N^5-(3-(pyrimidin-4-yl)pyridin-2-yl)isoquinoline-1,5-diamine (12b)

A 0.02 M stock solution of $Pd_2(dba)_3$ [183 mg, 0.2 mmol] and Xantphos [231 mg, 0.4 mmol] was prepared in an oven-dried flask under Ar in 1,4-dioxane (10 mL). The mixture was vacuumed and purged with Ar three times, then sonicated for 2 min. After stirring at room temperature for 10 min the stock solution was ready for use.

A Radley Carousel reactor vessel was charged with 4-(2-chloropyridin-3-yl)pyrimidine (150 mg, 0.80 mmol) and N^1-(4-chlorophenyl)-6-methylisoquinoline-1,5-diamine (190 mg, 0.67 mmol) and dissolved in anhydrous 1,4-dioxane (7 mL). The mixture was purged with Ar, then 0.05 eq of the catalyst stock solution was added via syringe with stirring at room temperature. The reaction mixture was treated with LiHMDS (1.6 mL of 1.0 M solution in THF) added dropwise, and then stirred at 85 °C under Ar for 14 h. The reaction mixture was quenched by the addition of 1 N HCl (5 mL) and stirred 10 min at room temperature. Satd $NaHCO_3$ (30 mL) was added and extracted with 3:1 $CHCl_3$:IPA (2 x 50 mL). The organic extracts were washed with satd NaCl (30 mL), dried over Na_2SO_4, and concentrated in vacuo. The product was purifed by silica gel chromatography (10-50% EtOAc/DCM) to give a yellow solid (85 mg, 29%). ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.13 (s, 1 H); 9.33 (s, 1 H); 9.28 (s, 1 H); 8.94 (d, J=5.52 Hz, 1 H); 8.45 (d, J=7.53 Hz, 1 H); 8.40 (d, J=8.53 Hz, 1 H); 8.29 (d, J=5.52 Hz, 1 H); 8.04 (d, J=3.51 Hz, 1 H); 7.96 (d, J=8.53 Hz, 2 H); 7.91 (d, J=6.02 Hz, 1 H); 7.60 (d, J=8.53 Hz, 1 H); 7.36 (d, J=8.53 Hz, 2 H); 7.10 (d, J=5.52 Hz, 1 H); 6.81 - 6.88 (m, 1 H); 2.34 (s, 3 H). HPLC purity >99%. MS (ESI, +ve ion) *m/z* 439.5, $(M+H)^+$.

N^1-(4-chlorophenyl)-6-methyl-N^5-(3-(6-(methylamino)pyrimidin-4-yl)pyridin-2-yl)isoquinoline-1,5-diamine (13)

A mixture of 6-(2-fluoropyridin-3-yl)-N-methylpyrimidin-4-amine (52.30 mg, 256.1 µmol) and N^1-(4-chlorophenyl)-6-methylisoquinoline-1,5-diamine (70.69 mg, 249.1 µmol) was suspended in THF (5 mL) and treated with 1.0 M LiHMDS in THF (1.5 mL). The resulting dark solution was stirred at 45 °C for 1 h, cooled, and added dropwise to 2 M HCl (6 mL) in water (100 mL). The resulting mixture was heated to reflux to aid dissolution and filtered. The cooled stirred filtrate was basified by dropwise addition of saturated sodium bicarbonate solution resulting in a pale yellow precipitate which was collected by filtration, washing with water. The solid was air dried. Recrystallization from a small volume of $CHCl_3$ gave N^1-(4-chlorophenyl)-6-methyl-N^5-(3-(6-(methylamino)pyrimidin-4-yl)pyridin-2-yl)isoquinoline-1,5-diamine (84 mg, 72%) as a pale yellow solid. ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.09 (br. s., 1 H); 9.26 (s, 1 H); 8.59 (br. s., 1 H); 8.36 (d, J=8.80 Hz, 1 H); 8.05 (br. s., 0 H); 7.87 - 7.96 (m, 4 H); 7.49 - 7.63 (m, 2 H); 7.30 - 7.40 (m, 2 H); 7.14 (d, J=6.06 Hz, 1 H); 6.99 (d, J=0.98 Hz, 1 H); 6.78 (dd, J=7.73, 4.79 Hz, 1 H); 2.90 (d, J=4.89 Hz, 3 H); 2.33 (s, 3 H). HPLC purity >99%. MS (ESI, +ve ion) *m/z* 468.1, $(M+H)^+$.

4-(6-Methyl-5-(3-(6-(methylamino)pyrimidin-4-yl)pyridin-2-ylamino)isoquinolin-1-ylamino)benzonitrile (14)

A mixture of 4-(5-amino-6-methylisoquinolin-1-ylamino)benzonitrile (0.60 g, 2.2 mmol) and 6-(2-fluoropyridin-3-yl)-N-methylpyrimidin-4-amine (0.49 g, 2.4 mmol) in anhydrous 1,4-dioxane (40 mL) was stirred at room temperature under Ar and treated dropwise with lithium bis(trimethylsilyl)amide (1.0 M solution in tetrahydrofuran; 14 mL, 14 mmol). The resulting suspension was stirred for 3 h min at 45 °C. The mixture was treated with 5 N aq HCl (10 mL) and stirred at room temperature for 10 min. The dark solution was concentrated in vacuo to ~50 mL then added slowly to satd $NaHCO_3$ (150 mL) to afford a tan precipitate. The solid was collected by suction filtration and re-dissolved in EtOAc (400 mL). The solution was washed with water (100 mL), satd NaCl (100 mL), dried over Na_2SO_4, filtered and concentrated in vacuo to afford 1.3 g crude. The crude product was adsorbed onto a plug of silica gel and chromatographed through a Redi-Sep® pre-packed silica gel column (120 g), eluting with a gradient of 20% to 80% EtOAc in CH_2Cl_2 over 20 min to provide 4-(6-methyl-5-(3-(6-(methylamino)pyrimidin-4-yl)pyridin-2-ylamino)isoquinolin-1-ylamino)benzonitrile (0.61 g, 61% yield) as a tan solid. ^{1}H NMR (400 MHz, d6-DMSO) δ 11.09 (br. s., 1 H); 9.63 (s, 1 H); 8.60 (br. s., 1 H); 8.38 (d, J=8.61 Hz, 1

H); 8.12 (d, J=9.00 Hz, 2 H); 8.05 (br. s., 1 H); 8.02 (d, J=6.06 Hz, 1 H); 7.95 (dd, J=4.69, 1.37 Hz, 1 H); 7.74 (d, J=8.80 Hz, 2 H); 7.62 (d, J=8.80 Hz, 1 H); 7.55 (br. s., 1 H); 7.27 (d, J=5.87 Hz, 1 H); 7.00 (s, 1 H); 6.79 (dd, J=7.73, 4.79 Hz, 1 H); 2.91 (d, J=4.69 Hz, 3 H); 2.35 (s, 3 H). HPLC purity >99%. MS (ESI, +ve ion) *m/z* 459.6, $(M+H)^+$.

N1-(3-Ethynylphenyl)-6-methyl-N5-(3-(6-(methylamino)pyrimidin-4-yl)pyridin-2-yl)isoquinoline-1,5-diamine (15)

A 12 L round bottom flask with open flange head, equipped with overhead mechanical stirrer and temperature probe was assembled and purged with nitrogen. The flask was charged with N1-chloro-N1-(3-ethynylphenyl)-6-methylisoquinoline-1,5-diamine (131.31 g, 424 mmol), 6-(2-fluoropyridin-3-yl)-N-methylpyrimidin-4-amine (95 g, 466 mmol) and THF (600 mL). The pink slurry was cooled in an ice-water bath and lithium bis(trimethylsilyl)amide, 1.0M in THF (2543 mL, 2543 mmol) was added via large teflon cannula and the mixture was stirred for 5.5 h. The reaction mixture was quenched by addition of water (600 mL). Two homogeneous layers (aqueous and organic) were observed, but when this was poured into separating funnel a lot of solid crashed out which obscured the interface. The layers were separated as best as possible. An interphase of organic and aqueous containing a lot of solid was filtered. The filter cake was shown to contain the desired product. The filtrate was combined with the organic extracts. The biphasic filtrate was separated and the aqueous fraction was extracted with isopropyl acetate (3x). These organic extracts were combined with the previous organic extracts and filter cake and concentrated under vacuum to a dark brown solid. Purification by SFC gave 130 g of product (67%). ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.05 (br. s., 1 H); 9.23 (s, 1 H); 8.61 (br. s., 1 H); 8.37 (d, J=8.53 Hz, 1 H); 8.12 (s, 1 H); 8.02 (br. s., 1 H); 7.90 - 7.98 (m, 3 H); 7.58 (d, J=8.53 Hz, 2 H); 7.33 (t, J=8.03 Hz, 1 H); 7.15 (d, J=6.02 Hz, 1 H); 7.08 (d, J=7.53 Hz, 1 H); 6.99 (s, 1 H); 6.79 (dd, J=7.53, 5.02 Hz, 1 H); 4.13 (s, 1 H); 2.91 (d, J=5.02 Hz, 3 H); 2.34 (s, 3 H). HPLC purity >99%. MS (ESI, +ve ion) *m/z* 457.8, $(M+H)^+$.

6-Methyl-N5-(3-(6-(methylamino)pyrimidin-4-yl)pyridin-2-yl)-N1-(2-methylbenzo[d]thiazol-5-yl)isoquinoline-1,5-diamine (16)

A mixture of 1-chloro-6-methyl-N-(3-(6-(methylamino)pyrimidin-4-yl)pyridin-2-yl)isoquinolin-5-amine (752 mg, 2.00 mmol), 2-methylbenzo[d]thiazol-5-amine (360 mg, 2.20 mmol), tris(dibenzylideneacetone)dipalladium (0) (73

mg, 0.08 mmol) and DavePhos (63 mg, 0.16 mmol) was suspended in THF (6 mL) and the mixture was sonicated for 5 min to give a homogeneous suspension. LiHMDS (1.0 M solution in THF; 9.0 mL) was added and the mixture was sonicated again for 5 min, and then stirred for 16 h. The reaction mixture was partitioned between DCM/NaHCO3 (aq., sat.). The organic layer was reduced in volume and loaded on silica gel and purified via a flash column (2M NH_3-MeOH/DCM=0-6%) on an ISCO system. The desired fractions were combined and concentrated in vacuo. The residue was triturated with EtOAc to give 300 mg (30% yield) of the desired product as a fluffy yellow solid. ^{1}H NMR (300 MHz, d_4-MeOH) δ 8.56 (s, 1 H); 8.31 (d, J=1.70 Hz, 1 H); 8.25 (d, J=8.67 Hz, 1 H); 8.11 (br. s., 1 H); 7.89 (dd, J=4.90, 1.51 Hz, 1 H); 7.83 (d, J=7.54 Hz, 2 H); 7.62 - 7.69 (m, 1 H); 7.56 (d, J=8.67 Hz, 1 H); 7.25 (d, J=6.22 Hz, 1 H); 6.97 (s, 1 H); 6.79 (dd, J=7.54, 4.90 Hz, 1 H); 2.99 (s, 3 H); 2.82 (s, 3 H); 2.39 (s, 3 H). HPLC purity >99%. MS (ESI, +ve ion) *m/z* 505.2, $(M+H)^+$.

6-Methyl-5-nitroisoquinolin-1(2H)-one

1-Chloro-6-methyl-5-nitroisoquinoline (50 g, 225 mmol) was suspended in THF (500 mL, 10 mL/g) and treated with 5 N aq HCl (500 mL, 10 mL/g). The suspension was stirred vigorously in a 2 L Morton Flask under a reflux condenser and heated with a heating mantle to reflux overnight (14 h). The resulting suspension was allowed to cool to room temperature (22 °C). The solid was removed by suction filtration and the filtrate set aside. The solid was washed with water (100 mL), Et_2O (2 x 100 mL) and hexane (100 mL), then air-dried to afford 40 g as a light yellow powder. The reserved filtrate was concentrated in vacuo to a volume of ~ 500 mL to afford a second crop of product. The second crop was washed with water (100 mL), Et_2O (2 x 100 mL) and hexane (100 mL), then air-dried to afford 4 g as an orange powder. A total of 44 g (87% yield) of the title compound were isolated in this fashion. ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.65 (br s, 1 H); 8.30 (d, J=8.53 Hz, 1 H); 7.55 (d, J=8.53 Hz, 1 H); 7.29 - 7.42 (m, 1 H); 6.22 (d, J=7.53 Hz, 1 H); 2.42 (s, 3 H). MS (ESI, +ve ion) *m/z* 205, $(M+H)^+$.

5-Amino-6-methylisoquinolin-1(2H)-one

A solution of 6-methyl-5-nitroisoquinolin-1(2H)-one (16.4 g, 80.3 mmol) in EtOH (150 mL/g) was treated with palladium, 10 wt. % on activated carbon (2.11 g). The reaction mixture was heated to 80 °C and the starting material dissolved. The reaction was stirred under H_2 for 16 h. The reaction mixture was then filtered through a pad of celite warm, and washed with excess EtOH and DMF. The filtrate was concentrated and the resulting residue

was dissolved in a mininal amount of DMF. This solution was then poured into ice water. The resulting ppt was collected by filtration and dried in a vacuum oven to give 5-amino-6-methylisoquinolin-1(2H)-one (13.2 g, 94% yield). ^{1}H NMR (400 MHz, d_6-DMSO) δ 10.95 (s, 1 H); 7.37 (d, J=8.03 Hz, 1 H); 7.09 (d, J=8.03 Hz, 1 H); 6.97 - 7.03 (m, 1 H); 6.75 (d, J=7.53 Hz, 1 H); 5.28 (br s, 2 H); 2.19 (s, 3 H). MS (ESI, +ve ion) *m/z* 175, $(M+H)^+$.

5-Iodo-6-methylisoquinolin-1(2H)-one (17)

A suspension of 5-amino-6-methylisoquinolin-1(2H)-one (13.719 g, 78.75 mmol) in conc. HCl (200 mL) was treated drpwise with sodium nitrate (6.520 g, 94.51 mmol) in 50 mL water at 0 °C. The reaction mixture was stirred for 30 min and then potassium iodide (39.22 g, 236.3 mmol) in water (50 ml) was added. The reaction mixture was stirred at 70 °C for 2 h. The reaction mixture was filtered and washed with excess water. The resulting brown solid was then stirred in a saturated solution of sodium sulfite for 30 min. The resulting yellow ppt was collected by filtration and dried in vacuum oven overnight to give 5-iodo-6-methylisoquinolin-1(2H)-one (19.80 g, 88% yield) as a yellow solid. ^{1}H NMR (300 MHz, d_6-DMSO) δ 11.44 (s, 1 H); 8.10 (d, J=8.18 Hz, 1 H); 7.45 (d, J=8.18 Hz, 1 H); 7.29 (dd, J=7.31, 5.99 Hz, 1 H); 6.70 (d, J=7.45 Hz, 1 H); 2.57 (s, 3 H). MS (ESI, +ve ion) *m/z* 285.9, $(M+H)^+$.

6-Methyl-5-(2-(methylamino)quinazolin-6-yl)isoquinolin-1(2H)-one

A clear 80 mL microwave vessel was charged with N-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinazolin-2-amine (2.40 g, 8.42 mmol) , 5-iodo-6-methylisoquinolin-1(2H)-one (2.00 g, 7.02 mmol), tetrakis(triphenylphosphine) palladium(0) (0.811 g, 0.702 mmol), 2 M aqueous sodium carbonate (10.5 mL, 21.0 mmol) and 12.0 mL of dioxane. The mixture was capped and heated in a CEM microwave reactor for 15 min at 160 °C with the PowerMax set at 150 W. The reaction was then partioned between water and chloroform, adding methanol for solubility. The organic layer was dried with sodium sulfate and purified by column chromatography on silica gel using a gradient of 2 to 10% MeOH in DCM to give 6-methyl-5-(2-(methylamino)quinazolin-6-yl)isoquinolin-1(2H)-one (1.15 g, 52% yield) as a light yellow solid. ^{1}H NMR (300 MHz, d6-DMSO) δ 11.24 (d, J=5.70 Hz, 1 H); 9.12 (s, 1 H); 8.17 (d, J=8.33 Hz, 1 H); 7.67 (d, J=1.75 Hz, 1 H); 7.61 (d, J=8.49 Hz, 1 H); 7.53 (dd, J=8.50, 1.75 Hz, 3 H); 7.47 (d, J=8.33 Hz, 1 H); 7.40 - 7.46 (m, 1 H); 7.00 - 7.08 (m, 1 H); 5.90 (d, J=7.31 Hz, 1 H); 2.93 (d, J=4.68 Hz, 3 H); 2.18 (s, 3 H). MS (ESI, +ve ion) *m/z* 317.2, $(M+H)^+$.

6-(1-Chloro-6-methylisoquinolin-5-yl)-N-methylquinazolin-2-amine

6-Methyl-5-(2-(methylamino)quinazolin-6-yl)isoquinolin-1(2H)-one (13.20 g, 41.7 mmol) was treated with phosphorus oxychloride (15.0 mL) and the was mixture heated to 100 °C resulting in a clear solution. Stirring was continue for 2 h. The reaction was then cooled down to RT and the volatiles removed in vacuo. Residual $POCl_3$ was removed by azeotroping (2x) with toluene. Crushed ice was added to the flask. The mixutre was allowed to stirr for 30 min. The solid that formed was collected by suction filtration and dried in a vacuum oven. The product was purified by flash chromatography on silica gel using 2 to 8% MeOH in DCM to give 6-(1-chloro-6-methylisoquinolin-5-yl)-N-methylquinazolin-2-amine (11.4 g, 82% yield) as a yellow solid. ^{1}H NMR (300 MHz, d6-DMSO) δ 9.14 (s, 1 H); 8.28 (d, J=8.77 Hz, 1 H); 8.17 (d, J=5.99 Hz, 1 H); 7.83 (d, J=8.77 Hz, 1 H); 7.74 (d, J=1.46 Hz, 1 H); 7.65 (d, J=8.50 Hz, 1 H); 7.59 (dd, J=8.50, 1.85 Hz, 1 H); 7.49 (d, J=4.24 Hz, 1 H); 7.23 (d, J=5.85 Hz, 1 H); 2.95 (d, J=4.68 Hz, 3 H); 2.31 (s, 3 H). MS (ESI, +ve ion) *m/z* 335.1, $(M+H)^+$.

6-(1-(4-Chlorophenylamino)-6-methylisoquinolin-5-yl)-N-methylquinazolin-2-amine (18)

A mixture of $Pd_2(dba)_3$ (0.0049 g, 0.0054 mmol), sodium 2-methylpropan-2-olate (0.017 g, 0.18 mmol), 4-chlorobenzenamine (0.023 g, 0.18 mmol), 6-(1-chloro-6-methylisoquinolin-5-yl)-N-methylquinazolin-2-amine (0.060 g, 0.18 mmol), DavePhos (0.0051 g, 0.011 mmol) and 1,4-dioxane (2.5 mL) in a 5 mL microwave vessel was stirred at 120 °C for 1.5 h. The mixture was diluted with EtOAc, washed with 5% brine (2X), dried over Na_2SO_4, filtered and concentrated. Silica gel chromatography with 5% MeOH/CH_2Cl_2 gave 6-(1-(4-chlorophenylamino)-6-methylisoquinolin-5-yl)-N-methylquinazolin-2-amine (0.045 g, 59% yield). ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.31 (s, 1 H); 9.14 (br. s., 1 H); 8.51 (d, J=8.80 Hz, 1 H); 7.91 - 7.99 (m, 2 H); 7.87 (d, J=6.06 Hz, 1 H); 7.70 (d, J=1.76 Hz, 1 H); 7.59 - 7.65 (m, 2 H); 7.55 (dd, J=8.61, 1.96 Hz, 1 H); 7.43 (br. s., 1 H); 7.31 - 7.38 (m, 2 H); 6.58 (d, J=6.06 Hz, 1 H); 2.94 (d, J=4.89 Hz, 3 H); 2.25 (s, 3 H). HPLC purity = 95.6%. MS (ESI, +ve ion) *m/z* 426.0, $(M+H)^+$.

6-(1-(3-tert-Butyl-1-methyl-1H-pyrazol-5-ylamino)-6-methylisoquinolin-5-yl)-N-methylquinazolin-2-amine (19)

A clear 80 mL microwave vessel was charged with 6-(1-chloro-6-methylisoquinolin-5-yl)-N-methylquinazolin-2-amine (3.300 g, 9.9 mmol), Davephos (0.16 g, 0.39 mmol), 5-amino-3-tert-butyl-1-methyl pyrazole (1.7 g, 11

mmol), $Pd_2(dba)_3$ (0.18 g, 0.20 mmol) and 30 ml of dioxane. Nitrogen was bubbled into the reaction for 10 mins and lithium bis(trimethylsilyl)amide, 1.0 M in THF (20 mL, 20 mmol) was added. The reaction was heated to 150 °C in a CEM microwave reactor for 10 min with the PowerMax set at 120 W. The volatiles were removed under vacuum and the residue was taken up in DCM, preadsorbed onto silica gel and purified by column chromatography on silica gel using a gradient of 40 to 100% of EtOAc in hexanes and then 0 to 10% MeOH in EtOAc. The pure fractions were combined and concentrated under vacuum. The residue was disolved in warm ether. Upon cooling a solid precipitated out. It was filtered off, washed with cold ether and dried to give 2.7 g (61%) of the desired produt as a light yellow solid. ^{1}H NMR (300 MHz, d_6-DMSO) δ 9.04 - 9.21 (m, 2 H); 8.38 (d, J=8.62 Hz, 1 H); 7.76 (d, J=5.99 Hz, 1 H); 7.68 (d, J=1.32 Hz, 1 H); 7.50 - 7.64 (m, 3 H); 7.43 (br. s., 1 H); 6.51 (d, J=5.99 Hz, 1 H); 6.04 (s, 1 H); 3.54 (s, 3 H); 2.94 (d, J=4.82 Hz, 3 H); 2.25 (s, 3 H); 1.26 (s, 9 H). HPLC purity >99%. MS (ESI, +ve ion) *m/z* 451.9, $(M+H)^+$.

3,3-Dimethyl-6-(6-methyl-5-(2-(methylamino)quinazolin-6-yl)isoquinolin-1-ylamino)indolin-2-one (20)

6-(1-Chloro-6-methylisoquinolin-5-yl)-N-methylquinazolin-2-amine (0.150 g, 0.45 mmol), 6-amino-3,3-dimethylindolin-2-one (0.087 g, 0.49 mmol), tris(dibenzylideneacetone)dipalladium (0) (0.021 g, 0.022 mmol) and 2-dicyclohexylphosphino-2'-(N,N-dimethylamino)biphenyl (0.018 g, 0.045 mmol) were all placed in a clear microwave vial along with 3 mL of dioxane. LiHMDS, 1.0 M in THF (1.1 mL, 1.1 mmol) was added and the vial was capped. The reaction was heated in the the PersonalChemistry SmithSynthesizer to 150 °C for 10 min. The reaction was diluted with water and extracted with ethyl acetate. The organic layer was washed (2x) with an aqueous saturated solution of sodium bicarbonate, then with water and then brine. The organic layer was then dried with sodium sulfate and concentrated under reduced pressure. The residue was purified by preparative HPLC using a gradient of 5%MeCN 0.1% TFA to 95% MeCN 0.1% TFA in water 0.1% TFA. The pure fractions were neutralized with ammonium hydroxide and the volatiles were removed under reduced pressure. The solid that crashed out of the aqueous layer was filtered off, washed with with water and dried in a vacuum oven at 45 degrees to give 3,3-dimethyl-6-(6-methyl-5-(2-(methylamino)quinazolin-6-yl)isoquinolin-1-ylamino)indolin-2-one (0.084 g, 40% yield) as a light yellow solid. ^{1}H NMR (300 MHz, d_6-DMSO) δ 10.31 (s, 1 H); 9.23 (br. s., 1 H); 9.14 (s, 1 H); 8.52 (d, J=8.62 Hz, 1 H); 7.83 (d, J=5.99 Hz, 1 H); 7.70 (d, J=1.61 Hz, 1 H); 7.52 - 7.66 (m, 4 H); 7.41 - 7.48

(m, 1 H); 7.31 - 7.40 (m, 1 H); 7.20 (d, J=8.04 Hz, 1 H); 6.54 (d, J=6.14 Hz, 1 H); 2.91 - 2.99 (m, 3 H); 2.25 (s, 3 H); 1.25 (s, 6 H). HPLC purity >99%. MS (ESI, +ve ion) *m/z* 475.2, $(M+H)^+$.

6-Chloro-9-(tetrahydro-2H-pyran-2-yl)-9H-purine (22)

A suspension of 6-chloro-9H-purine (25.36 g, 164 mmol) (Alfa Aesar) and 4-methylbenzenesulfonic acid (0.565 g, 3.28 mmol) in EtOAc (250 mL) was treated with 3,4-dihydro-2H-pyran (44.9 mL, 492 mmol). The mixture was heated at 90 °C and the solid slowly dissolved over 1 h. The flask was removed from the oil bath and the cloudy yellow solution was filtered and concentrated in vacuo.

The pale yellow residue was dissolved in DCM and purified by flash chromatography (50% EtOAc / hexane) (1 L silica / 4 L solvent) to give 6-chloro-9-(tetrahydro-2H-pyran-2-yl)-9H-purine (38.90 g, 99% yield) as a colorless oil which slowly crystallized. ^{1}H NMR (400 MHz, d6-DMSO) δ 8.91 (s, 1 H), 8.82 (s, 1 H), 5.80 (d, 1 H), 4.04 (m, 1 H), 3.75 (m, 1 H), 2.35 (m, 1 H), 2.01 (m, 2 H), 1.76 (m, 1 H), 1.62 (m, 2 H).

6-(2-Fluoropyridin-3-yl)-9-(tetrahydro-2H-pyran-2-yl)-9H-purine (23)

A solution of 6-chloro-9-(tetrahydro-2H-pyran-2-yl)-9H-purine (33.06 g, 139 mmol) in ethanol (560 mL) was sequentially treated with water (80 mL), 2-fluoropyridin-3-ylboronic acid (25.4 g, 180 mmol), potassium acetate (29.9 g, 305 mmol) and bis(di-tert-butyl-(4-dimethylaminophenyl)phosphine)dichloropalladium(II) (A-Phos) (1.47 g, 2.1 mmol). The stirred mixture was degassed (alternating vacuum / nitrogen) and heated under nitrogen at 80 °C for 2 h. The mixture was cooled and concentrated to give a sticky solid which was extracted into EtOAc (500 mL) from water (400 mL). The aqueous layer was extracted with EtOAc (200 mL) and the combined organic extracts were dried ($MgSO_4$), filtered through celite, and concentrated. The crude product was dissolved in a minimum volume of DCM and purified by flash chromatography (50% -> 75% -> 100% EtOAc / hexane) to give 6-(2-fluoropyridin-3-yl)-9-(tetrahydro-2H-pyran-2-yl)-9H-purine (40.0 g, 96% yield) as an off-white solid. ^{1}H NMR (400 MHz, d6-DMSO) δ 9.11 (s, 1 H), 8.91 (s, 1 H), 8.58 (m, 1 H), 8.49 (s, 1 H), 7.62 (m, 1 H), 5.85 (d, 1 H), 4.05 (m, 1 H), 3.75 (m, 1 H), 2.38 (m, 1 H), 2.05 (m, 2 H), 1.79 (m, 1 H), 1.61 (m, 2 H).

N^1-(4-Chlorophenyl)-6-methyl-N^5-(3-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-yl)pyridin-2-yl)isoquinoline-1,5-diamine (24)

6-(2-fluoropyridin-3-yl)-9-(tetrahydro-2H-pyran-2-yl)-9H-purine (3.273 g, 10.94 mmol) and N1-(4-chlorophenyl)-6-methylisoquinoline-1,5-diamine (3.103 g, 10.94 mmol) were dissolved in anhydrous THF (30 mL) under nitrogen. [Gentle heating was applied with a heat gun to aid dissolution]. The deep red solution was cooled in an ice/water bath and treated dropwise with LiHMDS (55 mL of a 1.0 M solution in THF, 5 equiv.). The resulting deep orange/red solution was stirred for 1 h (ice bath cooling). The deep orange/red reaction mixture was quenched with dropwise addition of water (2 mL - ice bath cooling) resulting in a light orange solution containing a white solid in suspension. The mixture was concentrated to a give yellow solid which was suspended in EtOAc (500 mL), dried ($MgSO_4$) and filtered through a plug of celite to give a yellow solution. The solution was concentrated, suspended in Et2O and dried to give N^1-(4-chlorophenyl)-6-methyl-N^5-(3-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-yl)pyridin-2-yl)isoquinoline-1,5-diamine (6.157 g, 100% yield) as a yellow solid (97% pure by LCMS). ^{1}H NMR (400 MHz, d6-DMSO) δ 11.69 (s, 1 H), 9.65 (d, 1 H), 9.27 (s, 1 H), 9.11 (s, 1 H), 8.98 (s, 1 H), 8.41 (d, 1 H), 8.06 (d, 1 H), 7.95 (d, 2 H), 7.90 (d, 1 H), 7.60 (d, 1 H), 7.34 (d, 2 H), 7.13 (d, 1 H), 6.91 (dd, 1 H), 5.90 (d, 1 H), 4.06 (m, 1 H), 3.75 (m, 1 H), 2.38 (m, 1 H), 2.35 (s, 3 H), 2.05 (m, 1 H), 1.81 (m, 1 H), 1.62 (m, 2 H).

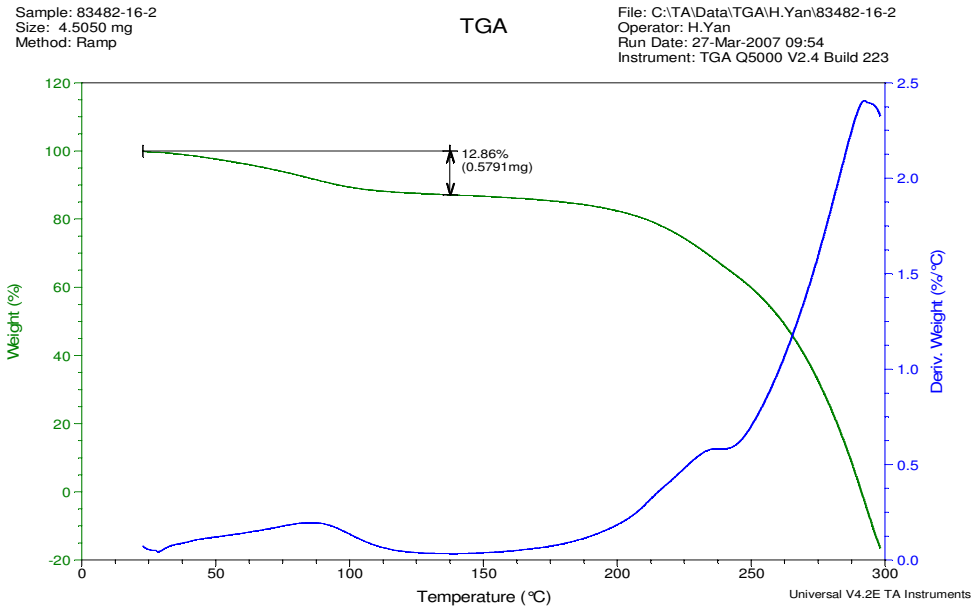
N^5-(3-(9H-Purin-6-yl)pyridin-2-yl)-N^1-(4-chlorophenyl)-6-methylisoquinoline-1,5-diamine dihydrochloride hydrate (1)

N^1-(4-chlorophenyl)-6-methyl-N5-(3-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-yl)pyridin-2-yl)isoquinoline-1,5-diamine (6.137 g, 11 mmol) was suspended in 0.5 M aqueous HCl (200 mL, 100 mmol) and heated to reflux. The bulk of the solid dissolved to give a yellow solution. LCMS analysis indicated complete removal of the THP protecting group in a clean conversion. The hot solution was filtered, washing with boiling water (2 x 20 mL). The resulting solution was cooled in an ice bath and product crystallised from solution as a yellow solid. N^5-(3-(9H-purin-6-yl)pyridin-2-yl)-N^1-(4-chlorophenyl)-6-methylisoquinoline-1,5-diamine dihydrochloride hydrate (4.516 g, 73% yield) was collected by filtration and dried under vacuum. HCl determination by ion chromatography indicated a dihydrochloride salt. ^{1}H NMR (400 MHz, d_6-DMSO) δ 12.13 (br. s., 1 H); 11.58 (br. s., 1 H); 9.79 (d, J=7.04 Hz, 1 H); 9.08 (s, 1 H); 8.82 (d, J=8.61 Hz, 1 H); 8.78 (s, 1 H); 8.06 (dd, J=5.09, 1.76 Hz, 1 H); 7.89 (d,

J=8.80 Hz, 1 H); 7.62 - 7.70 (m, 4 H); 7.51 (d, J=7.24 Hz, 1 H); 7.24 (d, J=7.04 Hz, 1 H); 7.05 (dd, J=7.63, 5.09 Hz, 1 H); 2.47 (s, 3 H). HPLC purity >99%. MS (ESI, +ve ion) *m/z* 479.2, $(M+H)^+$.

Compound 1 Determination of HCl content by IC

TGA:

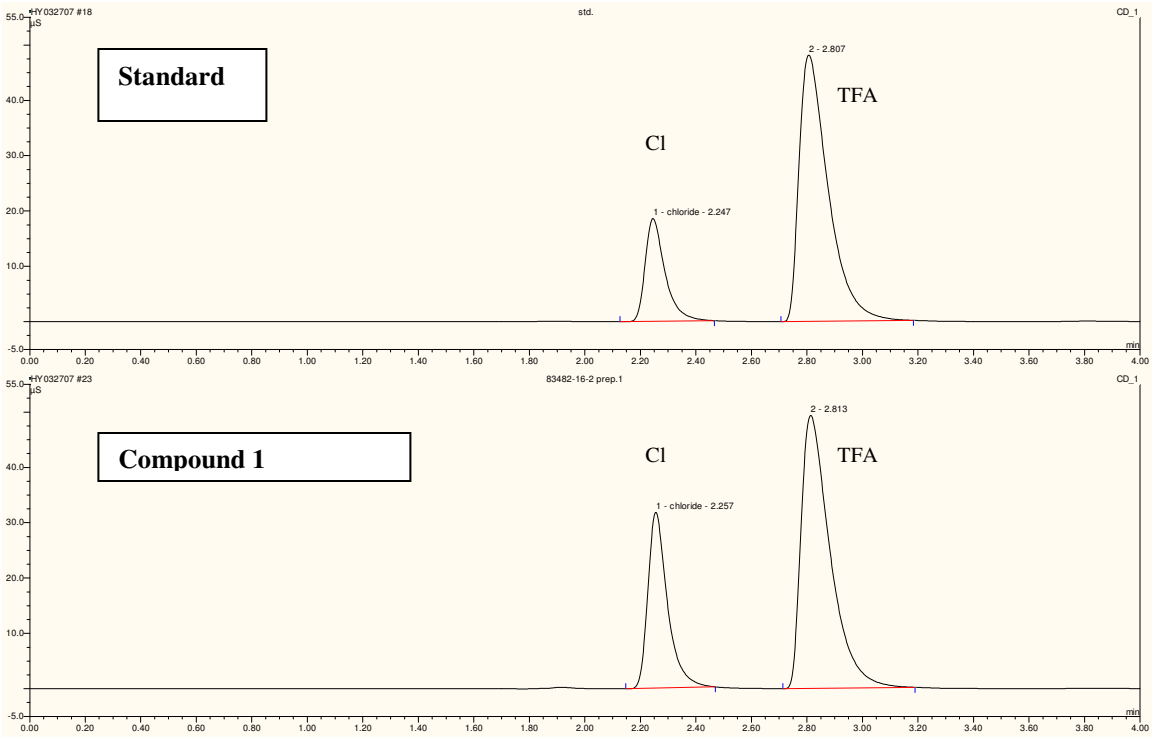


IC:

Table 1. HCl Contents Determined by IC

Sample ID	Wt (mg)*	HCl (%)	Molar ratio HCl : Compound **1**	Report
83482-16-2 prep.1	6.227	13.96	2.13 : 1.0	2.1 : 1.0
83482-16-2 prep.2	6.741	13.95	2.13 : 1.0	

*Sample weight after corrected with the %total volatile by TGA



Co-Crystal Structure Determination of B-Raf with Compound 1. B-Raf(433-726)V600E with an N-terminal His_6 affinity tag, His_6B-Raf, and the co-chaperone p50Cdc37 were cloned into the pFASTBac Dual vector (Invitrogen) and co-expressed by baculovirus-mediated infection of insect cells (Hi-5; Invitrogen), essentially as described.[2] The protein was purified by immobilized metal affinity chromatography on Ni-NTA Superflow (Qiagen, Inc.), followed by cation exchange chromatography on Source 15S (GE Healthcare Life Sciences). 0.2 M NDSB-256 was included in buffers for the final column to minimize protein aggregation and precipitation. Purified protein was concentrated to ~2 mg/ml using an Ultrafree-0.5 concentrator (Millipore) and compound **1** was added to a concentration of 0.4 mM. Crystals were grown at room temperature by vapor diffusion in hanging drops consisting of 0.8 μL of protein solution mixed with an equal volume of well solution (17% (w/v) PEG 8000, 75 mM sodium succinate, 0.1 M Tris, pH 8.6). For data collection, crystals were transferred sequentially into mother liquor containing 5-30% ethylene glycol before flash-cooling in liquid nitrogen. X-ray diffraction data were collected at beamline 5.0.2 of the Advanced Light Source at Lawrence Berkeley National Laboratories using an ADSC Quantum 315 detector and λ = 1.000 Å. Data were indexed and scaled using the HKL suite of programs.[3] B-Raf crystals belonged to space group $P4_12_12$ with two molecules per asymmetric unit. Unit cell dimensions were a = b =93.5 Å and c = 166.7 Å. Data collection statistics appear in Table S1.

The structure was solved by molecular replacement using EPMR[4] and the coordinates of wild-type B-Raf + sorafenib (PDB code 1UWH) as a search model.[2] Refinements were performed with REFMAC5[5] as implemented in CCP4[6] and model building was done with COOT.[7] The final model contained two protein molecules, two inhibitor molecules, and 79 water molecules and had good geometry with 89.6% and 9.9% of the residues located in the most favored and additionally allowed regions of the Ramachandran plot, respectively. Data refinement statistics appear in Table S1.

Table S1. Data collection and refinement statistics

	B-RAF + Compound **1**
Data Collection	
Resolution (Å)	30-2.70 (2.80-2.70)
Total reflections	117105
Unique reflections	19744
Completeness (%)	93.8 (68.7)
R_{merge}	0.133 (0.370)
$I/\sigma(I)$	15.3 (2.0)
Refinement	
Reflections used	18168
R_{cryst}	0.201
R_{free}	0.264
Average B-value ($Å^2$)	44.6
Number of protein atoms	4085
Number of ligand atoms	70
Number of solvent atoms	79
r.m.s.d. bonds (Å)	0.008
r.m.s.d. angles (°)	1.13

Kinase Counterscreening Data for Compound 1

Ambit KinomeScan Kinase Profiling (1 μM test concentration):-

Ambit Gene Symbol	Percent of Control	Call a Hit
AAK1	92	No
ABL1	51	No
ABL1(E255K)	80	No
ABL1(F317I)	86	No
ABL1(F317L)	79	No
ABL1(H396P)	84	No
ABL1(M351T)	45	No
ABL1(Q252H)	71	No
ABL1(T315I)	72	No
ABL1(Y253F)	61	No
ABL2	42	No
ACVR1	100	No
ACVR1B	82	No
ACVR2A	100	No
ACVR2B	100	No
ACVRL1	92	No
ADCK3	100	No
ADCK4	77	No
AKT1	100	No
AKT2	88	No
AKT3	96	No
ALK	96	No
AMPK-alpha1	89	No
AMPK-alpha2	96	No
ANKK1	100	No
ARK5	100	No
AURKA	100	No
AURKB	85	No
AURKC	86	No
AXL	95	No
BIKE	70	No
BLK	75	No
BMPR1A	100	No
BMPR1B	96	No
BMPR2	100	No
BMX	91	No
BRAF	2.2	Yes
BRAF(V600E)	2.6	Yes
BRSK1	91	No
BRSK2	87	No
BTK	91	No
CAMK1	100	No
CAMK1D	100	No
CAMK1G	100	No
CAMK2A	100	No
CAMK2B	100	No
CAMK2D	79	No
CAMK2G	98	No
CAMK4	96	No
CAMKK1	96	No
CAMKK2	98	No
CDC2L1	42	No
CDC2L2	33	Yes
CDK11	100	No
CDK2	100	No
CDK3	84	No
CDK5	100	No
CDK7	100	No
CDK8	100	No
CDK9	97	No
CDKL2	100	No
CHEK1	100	No
CHEK2	100	No
CIT	64	No
CLK1	100	No
CLK2	100	No
CLK3	94	No
CLK4	100	No
CSF1R	0.25	Yes
CSK	100	No
CSNK1A1L	95	No
CSNK1D	99	No
CSNK1E	99	No
CSNK1G1	91	No
CSNK1G2	100	No
CSNK1G3	100	No
CSNK2A1	100	No
CSNK2A2	100	No
DAPK1	90	No
DAPK2	100	No
DAPK3	100	No
DCAMKL1	80	No
DCAMKL2	100	No
DCAMKL3	100	No
DDR1 dyscoidin domain recept	0.1	Yes
DDR2	6.6	Yes
DLK	76	No
DMPK	100	No
DMPK2	99	No
DRAK1	100	No
DRAK2	96	No
DYRK1B	100	No
EGFR	100	No

EGFR(E746-A750del)	86	No
EGFR(G719C)	92	No
EGFR(G719S)	92	No
EGFR(L747-E749del, A750P)	89	No
EGFR(L747-S752del, P753S)	94	No
EGFR(L747-T751del,Sins)	98	No
EGFR(L858R)	100	No
EGFR(L861Q)	86	No
EGFR(S752-I759del)	90	No
EPHA1	4.6	Yes
EPHA2	2.8	Yes
EPHA3	74	No
EPHA4	22	Yes
EPHA5	16	Yes
EPHA6	2.8	Yes
EPHA7	4.7	Yes
EPHA8	0.45	Yes
EPHB1	2	Yes
EPHB2	6.1	Yes
EPHB3	10	Yes
EPHB4	6.5	Yes
ERBB2	97	No
ERBB4	98	No
ERK1	100	No
ERK2	100	No
ERK3	98	No
ERK4	100	No
ERK5	95	No
ERK8	98	No
FER	78	No
FES	100	No
FGFR1	80	No
FGFR2	38	No
FGFR3	100	No
FGFR3(G697C)	100	No
FGFR4	100	No
FGR	100	No
FLT1	2.2	Yes
FLT3	62	No
FLT3(D835H)	99	No
FLT3(D835Y)	92	No
FLT3(ITD)	92	No
FLT3(K663Q)	75	No
FLT3(N841I)	100	No
FLT4	96	No
FRK (Fyn related Kinase)	17	Yes
FYN	82	No
GAK	92	No
GCN2(Kin.Dom.2,S808G)	100	No
GSK3A	100	No
GSK3B	100	No
HCK	53	No
HIPK1	99	No
IGF1R	94	No
IKK-alpha	100	No
IKK-beta	100	No
IKK-epsilon	73	No
INSR	86	No
INSRR	78	No
IRAK3	81	No
ITK	100	No
JAK1(Kin.Dom.1)	98	No
JAK1(Kin.Dom.2)	47	No
JAK2(Kin.Dom.2)	3.9	Yes
JAK3(Kin.Dom.2)	100	No
JNK1	2.7	Yes
JNK2	86	No
JNK3	86	No
KIT	28	Yes
KIT(D816V)	100	No
KIT(V559D)	36	No
KIT(V559D,T670I)	77	No
KIT(V559D,V654A)	100	No
LATS1	76	No
LATS2	85	No
LCK	1.6	Yes
LIMK1	55	No
LIMK2	98	No
LKB1	100	No
LOK	0	Yes
LTK	20	Yes
LYN	18	Yes
MAP3K3	40	No
MAP3K4	100	No
MAP3K5	100	No
MAP4K1	100	No
MAP4K2	100	No
MAP4K3	85	No
MAP4K4	95	No
MAP4K5	100	No
MAPKAPK2	100	No
MAPKAPK5	64	No
MARK1	97	No
MARK2	100	No
MARK3	100	No
MARK4	100	No
MEK1	100	No
MEK2	99	No
MEK3	100	No
MEK4	93	No
MEK6	96	No
MELK	100	No
MERTK	99	No
MET	73	No
MINK	80	No
MKNK1	100	No
MKNK2	100	No
MLCK	99	No
MLK1	100	No

MLK2	99	No	PKMYT1	85	No
MLK3	100	No	PKN1	100	No
MRCKA	87	No	PKN2	86	No
MRCKB	82	No	PLK1	100	No
MST1	88	No	PLK3	98	No
MST1R	97	No	PLK4	90	No
MST2	100	No	PRKCD	88	No
MST3	87	No	PRKCE	100	No
MST4	100	No	PRKCH	100	No
MUSK	66	No	PRKCQ	100	No
MYLK	76	No	PRKD1	100	No
MYLK2	100	No	PRKD2	100	No
MYO3A	99	No	PRKD3	100	No
MYO3B	68	No	PRKG1	93	No
NDR2	100	No	PRKG2	100	No
NEK1	100	No	PRKR	97	No
NEK2	90	No	PRKX	65	No
NEK5	98	No	PTK2	93	No
NEK6	100	No	PTK2B	40	No
NEK7	88	No	PTK6	13	Yes
NEK9	89	No	RAF1	0	Yes
NLK	83	No	RET	65	No
p38-alpha	91	No	RET(M918T)	100	No
p38-beta	99	No	RET(V804L)	100	No
p38-delta	100	No	RET(V804M)	96	No
p38-gamma	100	No	RIOK1	92	No
PAK1	76	No	RIOK2	100	No
PAK2	85	No	RIOK3	77	No
PAK3	87	No	RIPK1	92	No
PAK4	92	No	RIPK2	100	No
PAK6	100	No	RIPK4	64	No
PAK7/PAK5	90	No	ROCK2	100	No
PCTK1	98	No	ROS1	72	No
PCTK2	100	No	RPS6KA1(Kin.Dom.1)	94	No
PCTK3	95	No	RPS6KA1(Kin.Dom.2)	96	No
PDGFRA	88	No	RPS6KA2(Kin.Dom.1)	100	No
PDGFRB	78	No	RPS6KA2(Kin.Dom.2)	81	No
PDPK1	100	No	RPS6KA3(Kin.Dom.1)	91	No
PFTAIRE2	80	No	RPS6KA4(Kin.Dom.1)	100	No
PFTK1	100	No	RPS6KA4(Kin.Dom.2)	89	No
PHKG1	99	No	RPS6KA5(Kin.Dom.1)	90	No
PHKG2	100	No	RPS6KA5(Kin.Dom.2)	100	No
PIK3C2B	100	No	RPS6KA6(Kin.Dom.1)	92	No
PIK3CA	100	No	RPS6KA6(Kin.Dom.2)	86	No
PIK3CA(E545K)	100	No	SgK085	100	No
PIK3CB	100	No	SgK110	78	No
PIK3CD	100	No	SLK	40	No
PIK3CG	100	No	SNARK	100	No
PIM1	93	No	SNF1LK	75	No
PIM2	100	No	SNF1LK2	100	No
PIM3	100	No	SRC	73	No
PIP5K1A	95	No	SRMS	100	No
PIP5K2B	57	No	SRPK1	100	No
PKAC-alpha	100	No	SRPK2	100	No
PKAC-beta	94	No	SRPK3	100	No

STK16	100	No	TRKB	9.4	Yes
STK33	100	No	TRKC	2.2	Yes
STK35	92	No	TSSK1	71	No
STK36	92	No	TTK	100	No
SYK	86	No	TXK	100	No
TAK1	84	No	TYK2(Kin.Dom.1)	96	No
TAOK1	88	No	TYK2(Kin.Dom.2)	28	Yes
TAOK3	94	No	TYRO3	84	No
TEC	100	No	ULK1	82	No
TESK1	21	Yes	ULK2	100	No
TGFBR1	89	No	ULK3	100	No
TGFBR2	100	No	VEGFR2	29	Yes
TIE1	4.9	Yes	WEE1	92	No
TIE2	1.6	Yes	WEE2	97	No
TLK1	83	No	YANK2	95	No
TLK2	100	No	YANK3	84	No
TNIK	100	No	YES	52	No
TNK1	100	No	YSK1	100	No
TNK2	91	No	ZAK	87	No
TNNI3K	21	Yes	ZAP70	100	No
TRKA	15	Yes			

PD-Efficacy Data for Compounds in Figure 4.

Compound	Dose (mg/kg)	PD Assay pERK %Inh @ 6 h	A375 Tumor Growth %Inhibition / Regression	ED_{50} (mg/kg)	ED_{50} $AUC_{0\text{-}24h}$ (ng.h/mL)
1	1 (QD)	18	22	1.3 (QD)	2,734
	2 (QD)	69	90		
	5 (QD)	77	Reg. 85		
14	10 (BID)	21	50	11.5 (BID)	52,800
	17.5 (BID)	63	80		
	35 (BID)	78	Reg. 17		
15	5 (BID)	5	18	13 (BID)	9,414
	20 (BID)	40	78		
	35 (BID)	81	Reg. 54		
16	10 (BID)	30	42	11 (BID)	12,300
	20 (BID)	52	85		
	35 (BID)	66	Reg. 24		
19	30 (BID)	50	60	27 (BID)	N/A
	60 (BID)	67	90		
	100 (BID)	78	Reg. 45		
20	10 (BID)	60	85	4.7 (BID)	N/A
	20 (BID)	82	Reg. 55		
	35 (BID)	85	Reg. 75		

Efficacy studies using A375 SQ2 xenograft model:

The A375 SQ2 cell line was generated by performing two serial in vivo passages in CD1 nude mice to optimize for tumor take and growth. All Raf inhibitors were formulated in 2% HPMC, 1% Tween 80, pH 2.2. Formulation alone was used to dose animals in vehicle groups. Five million cells suspended in Matrigel (BD Bioscience) were injected subcutaneously in each CD1 nu/nu female mouse (0.2 mL/mouse). When tumor volume reached approximately 200 mm^3, mice were randomized into groups (n=10) and treatment started. Dosing occurred orally (PO), once (QD) or twice daily (BID) every day at the dose indicated in milligram per kilogram (mg/kg) and lasted for the duration of the experiment (14 days dosing). Tumor volume and body weight were measured in a blinded fashion twice per week using calipers and an animal scale, respectively. ED_{50} values were calculated from the linear regression of the percent tumor inhibition on the last day of the experiment versus the dose of compound used using GraphPad Prism 5.1 software.

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