

## Supporting Information

### Discovery of Diaminopyrimidine Carboxamide HPK1 Inhibitors as Preclinical Immunotherapy Tool Compounds

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## Synthetic chemistry

The compounds in the present invention can be prepared according to the following general schemes using appropriate materials, and are further exemplified by the subsequent specific examples. All reactions were stirred (mechanically, stir bar/stir plate, or shaken) and conducted under an ambient (air) atmosphere unless specifically stated otherwise. All temperatures are degrees Celsius (°C) unless otherwise noted. Ambient temperature is 15-25 °C. Most compounds were purified by reverse-phase preparative HPLC, MPLC on silica gel, recrystallization and/or trituration (suspension in a solvent followed by filtration of the solid). The course of the reactions was followed by thin layer chromatography (TLC) and/or LCMS and/or NMR and reaction times are given for illustration only. Intermediates were analyzed by NMR and/or TLC and/or LCMS. LC/HRMS (Xevo G2 Tof-XS; Column: Acquity UPLC BEH C18 1.7um; Flow rate: 0.7 ml/min; Column temperature: 40 Celsius, Injection Volume: 1 µL). NMR spectra were recorded on a Bruker 500MHz or 600MHz (1H, 500 or 600 MHz; 13C, 125 or 150 MHz) spectrometer. 1H and 13C NMR spectra were recorded using TMS as an internal reference. Chemical shifts are expressed in  $\delta$  (ppm), and J values are given in Hz. Liquid chromatography mass spectra (LCMS) were obtained from Agilent 1100-LC/MSD VL using mobile phase: 0.1% formic acid in water and acetonitrile; ionization was achieved either by positive or negative mode. Purity of final compounds was determined by analytical HPLC, which was carried out on an Agilent 1100 series, conditions: Agilent XDB C18 (50x3mm, 5 micron OR 100x3 mm, 5 micron); flow rate 1 mL/min; UV detection at 215 or 254 nm; linear gradient from 95:5% of 0.01% TFA in water: acetonitrile to 100% acetonitrile in 5.5 min.

## Reverse Phase Prep-HPLC Purification Methods

### Purification Method A - TFA modifier

Reverse-phase Prep-HPLC [Waters SunFire OBD C18, 19 mm X 150 mm(5 µm); gradient elution, MeCN/H<sub>2</sub>O/0.1% TFA]. Electrospray (ESI) Mass-triggered fraction collected was employed using positive ion polarity scanning to monitor for the target mass.

HPLC Gradient:

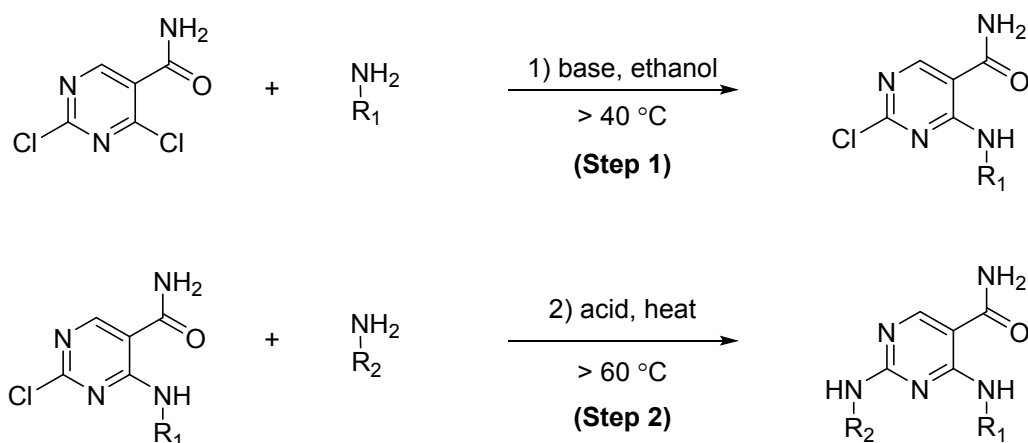
| Time (min) | % Acetonitrile | Mobile Phase Flowrate (mL/Min) | Modifier Flowrate (mL/min) |
|------------|----------------|--------------------------------|----------------------------|
| 0.0        | 2              | 25                             | 0.25                       |
| 3.0        | 2              | 35                             | 0.35                       |
| 33.0       | 95             | 35                             | 0.35                       |
| 33.1       | 100            | 40                             | 0.4                        |
| 36.1       | 100            | 50                             | 0.5                        |
| 36.8/end   | 2              | 25                             | 0.2                        |

#### Purification Method B - NH<sub>4</sub>OH modifier

Reverse-phase Prep-HPLC [Waters XBridge OBD C18, 19 mm X 150 mm(5 µm); gradient elution, MeCN/H<sub>2</sub>O/0.1% NH<sub>4</sub>OH]. Electrospray (ESI) Mass-triggered fraction collected was employed using positive ion polarity scanning to monitor for the target mass

| Time (min) | % Acetonitrile | Mobile Phase Flowrate (mL/Min) | Modifier Flowrate (mL/min) |
|------------|----------------|--------------------------------|----------------------------|
| 0.0        | 2              | 25                             | 0.25                       |
| 3.0        | 2              | 35                             | 0.35                       |
| 33.0       | 95             | 35                             | 0.35                       |
| 33.1       | 100            | 40                             | 0.4                        |
| 36.1       | 100            | 50                             | 0.5                        |
| 36.8/end   | 2              | 25                             | 0.2                        |

## General Synthetic Scheme



The below general procedures refer to compounds that were prepared using telescoped library techniques. The final purification following ‘Step 2’ uses reverse phase mass-directed HPLC according to ‘Purification Method A’ or “Purification Method B’ (vida supra).

## General Procedures

### General Procedure 1 (Step 1 of General Synthetic Scheme)

To a stirred solution of the R<sub>1</sub>-amine (1 equiv.) and 2,4-dichloropyrimidine-5-carboxamide (1 equiv.) was added Hunig’s base (2 equiv.) in one portion in Ethanol (0.4 – 0.3 M). The mixture was capped and stirred at 50 °C for 3 h. After this time the reaction was partitioned between DCM and water. The separated organic layer was washed with brine, dried over MgSO<sub>4</sub>, filtered, and evaporated under vacuum. The product was carried forward crude, unless otherwise stated (e.g., purified by silica gel column chromatography or reverse phase HPLC to give the title compound).

### General Procedure 2 (Step 2 of General Synthetic Scheme)

To a stirred solution of the 2-chloro-4-(amino)pyrimidine-5-carboxamide (1 equiv.) was added the R<sub>2</sub>-amine (1.1 equiv.) in 2-methoxymethanol (0.3 M). HCl in Ethanol (1.25M, 2.5 equiv.) was added via syringe and the mixture was stirred at 110 °C for 3 h. Upon completion as observed by LCMS analysis, the reaction was partitioned between DCM and water. The separated organic

layer was washed with brine, dried over MgSO<sub>4</sub>, filtered, and evaporated under vacuum. The product was purified by silica gel column chromatography or reverse phase HPLC to give the title compound.

### **General Procedure 3 (Step 1 of General Synthetic Scheme)**

Fresh LiHMDS (1M THF) (2 equiv.) was added via syringe to a solution containing the 2,6-disubstituted aniline (1 equiv.) and 2,4-dichloropyrimidine-5-carboxamide (1 equiv.) in dry THF (0.3M) and the mixture was stirred at 50 °C for 2 h. Upon completion, the mixture was cooled, and the solvent was evaporated. The resulting residue was diluted with DCM and quenched with sat. aq NH<sub>4</sub>Cl and mixed thoroughly. The aqueous layer was extracted with DCM (x 2), filtered through a phase separator, and concentrated to afford the desired 2-chloro-4-[amino]pyrimidine-5-carboxamide as white solid and carried forward crude without purification.

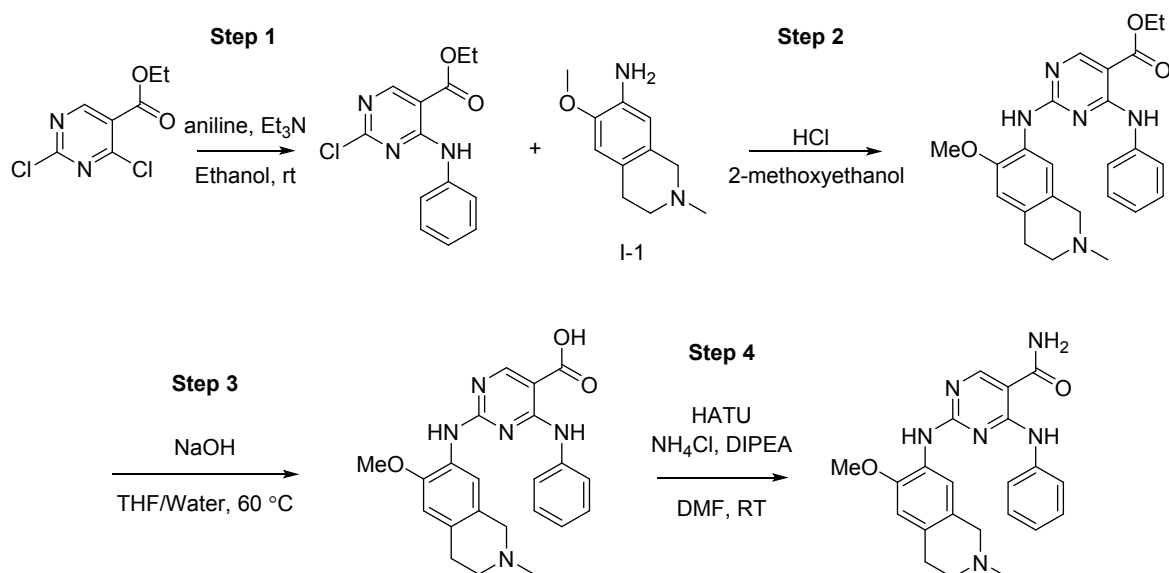
### **General Procedure 4 (step 2 of General Synthetic Scheme)**

To a stirred solution of the 2-chloro-4-(amino)pyrimidine-5-carboxamide (1 equiv.) was added the R<sup>2</sup>-amine (1.5 equiv.) in 2-methoxymethanol (0.3 M). Tosic acid (2.0 equiv.) was added and the mixture was stirred at 110 °C for 3 h. Upon completion as observed by LCMS analysis, the reaction was partitioned between DCM and water. The separated organic layer was washed with brine, dried over MgSO<sub>4</sub>, filtered, and evaporated under vacuum. The product was purified by silica gel column chromatography or reverse phase HPLC to give the title compound.

### **General Procedure 5 (Step 2 of General Synthetic Scheme)**

A microwave vial was charged with the 2-chloro-4-(amino)pyrimidine-5-carboxamide (1 equiv.) and 6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-amine (1 equiv.). A stir bar was added followed by ethanol (0.15 M) and 2 mL HCl (1 equiv.). Argon was blown over the mixture and the vial was capped and heated in the microwave at 140 °C between 15 and 60 min. Precipitate formed when cooled to room temperature. The mixture was filtered with EtOH and washed with diethyl ether to afford the title compound.

## Compound Examples



### 2-((6-Methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yl)amino)-4-(phenylamino)pyrimidine-5-carboxamide (1)

**Step 1.** Aniline (4.36 mL, 48.2 mmol) was added to a solution of ethyl 2,4-dichloropyrimidine-5-carboxylate (10.1 g, 45.9 mmol) and triethylamine (12.8 mL, 92 mmol) in ethanol (92 mL) at 0 °C. After the addition the reaction was allowed to reach room temperature and stirred overnight. Water was added (200 mL) and a solid precipitated. The solid was filtered and dried under a stream of nitrogen. This ethyl 2-chloro-4-(phenylamino)pyrimidine-5-carboxylate which was used without further purification. MS (ESI)  $m/z$  calc'd for  $\text{C}_{13}\text{H}_{13}\text{ClN}_3\text{O}_2$   $[\text{M}+\text{H}]^+$  278, found 278.

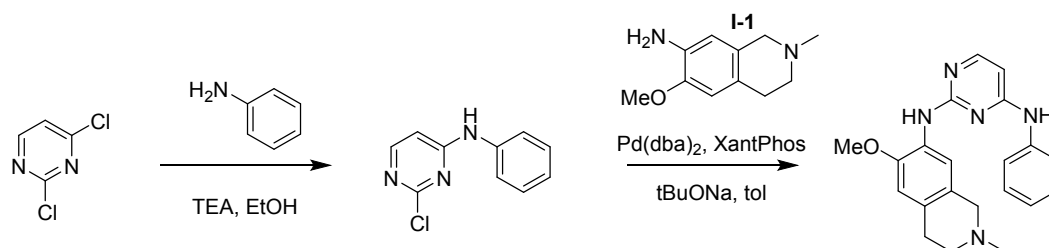
**Step 2.** A 500 mL 3-necked round bottomed flask with reflux condenser was charged with 6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-amine (**I-1**, 7.37 g, 38.3 mmol) and ethyl 2-chloro-4-(phenylamino)pyrimidine-5-carboxylate (9.68 g, 34.9 mmol). 2-methoxyethanol (100 mL) and was added followed by HCl (70 mL, 87 mmol, 1.25 M in ethanol) and the reaction was stirred at 110 °C overnight. The reaction was cooled in an ice bath and neutralized with  $\text{NaOH}_{(\text{aq})}$  (approx. 50 mL, litmus test pH 7), solid began precipitate, distilled water (250 mL) was added and the reaction was stirred for 10 minutes. The solid was filtered off and dried under a stream of nitrogen. This provided ethyl 2-((6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yl)amino)-

4-(phenylamino)pyrimidine-5-carboxylate which was used without further purification. MS (ESI)  $m/z$  calc'd for  $C_{24}H_{28}N_5O_3$   $[M+H]^+$  434, found 434.

Step 3. A 20 mL Biotage microwave vial was charged with ethyl 2-((6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yl)amino)-4-(phenylamino)pyrimidine-5-carboxylate (1 g, 2 mmol). THF/water (14 mL, 1:1) was added followed by solid sodium hydroxide (0.48 g, 12 mmol). The reaction was heated to 60 °C and stirred for 3 d. The reaction was cooled, neutralized with  $HCl_{(aq)}$  (2 M, litmus test), concentrated to remove the organic solvent. The solid was filtered, dried by azeotroping with isopropanol (3 x 50 mL) and further dried under high vacuum. This provided 2-((6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yl)amino)-4-(phenylamino)pyrimidine-5-carboxylic acid which was used without further purification. MS (ESI)  $m/z$  calc'd for  $C_{22}H_{24}N_5O_3$   $[M+H]^+$  406, found 406.

Step 4. A 40 mL vial was charged with 2-((6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yl)amino)-4-(phenylamino)pyrimidine-5-carboxylic acid (0.77 g, 1.89 mmol), DMF (5 mL) was added followed by DIPEA (0.99 mL, 5.7 mmol) and HATU (1.44 g, 3.78 mmol) at RT. Additional DMF (10 mL) was added followed by ammonium chloride (0.20 g, 3.78 mmol) and the reaction was stirred at room temperature overnight. The contents of the reaction were transferred to an Erlenmeyer flask and water (150 mL) was added, the solution was stirred until a precipitate had formed. The solid was collected by filtration and the filter cake was slurried with MeOH (5 mL) and filtered, the process was repeated (x 2) and the solid was dried overnight under a stream of nitrogen. This provided 2-((6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yl)amino)-4-(phenylamino)pyrimidine-5-carboxamide (331 mg, 45%) as a colorless solid. MS (ESI)  $m/z$  calc'd for  $C_{22}H_{25}N_6O_2$   $[M+H]^+$  405, found 405.  **$^1H$  NMR** (499 MHz,  $DMSO-d_6$ )  $\delta$  11.55 (s, 1H), 10.69 (s, 1H), 8.68 (s, 1H), 8.38 (s, 1H), 8.06 (s, 1H), 7.65 (s, 1H), 7.60 – 7.52 (m, 2H), 7.48 – 7.29 (m, 3H), 7.14 – 7.03 (m, 1H), 6.94 (s, 1H), 4.24 – 4.02 (m, 2H), 3.81 (s, 3H), 3.59 – 3.20 (m, 2H), 3.17 – 2.98 (m, 2H), 2.88 (s, 3H).  **$^{13}C$  NMR** (126 MHz,  $DMSO-d_6$ )  $\delta$  169.6, 160.5, 160.1, 158.6, 150.4, 139.1, 129.5, 127.2, 127.0, 123.8, 121.7, 120.4, 120.2, 111.3, 100.4, 56.3, 53.8, 50.7, 42.5, 25.5. **HRMS** ( $m/z$ ) calc'd for  $C_{22}H_{25}N_6O_2$   $[M+H]^+$  405.2039, found 405.2025. HPLC (Ascentis Express C18 100X3 mm column, 45C, 2  $\mu$ L injection, 215 nm) ( $CH_3CN$  in 0.1% TFA):  $t_R$  = 1.84 min (97.3% purity).

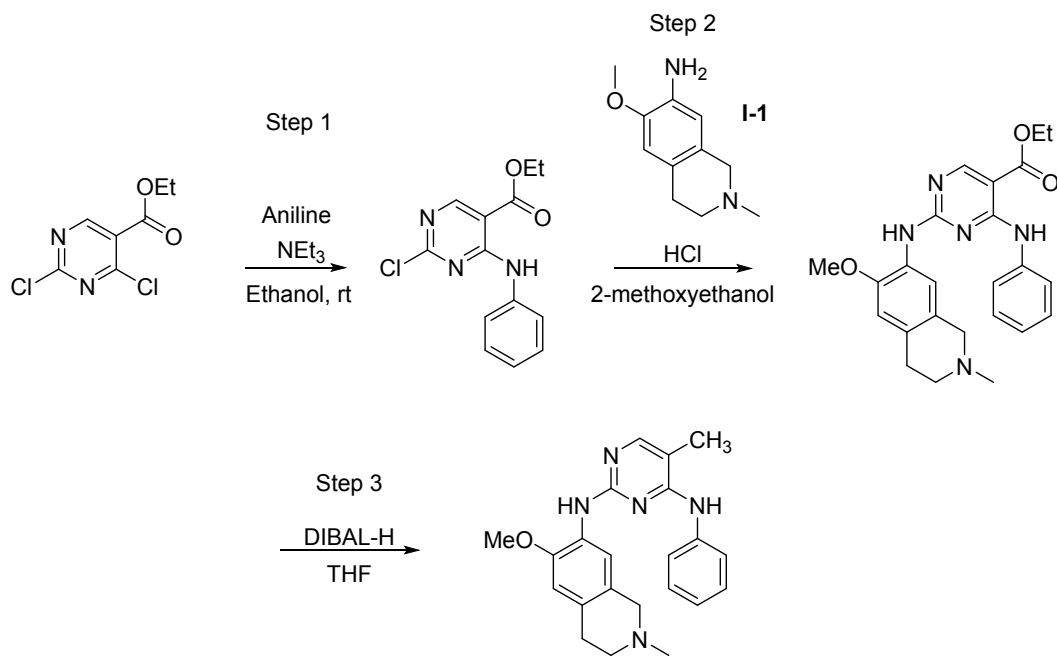




**N2-(6-Methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yl)-N4-phenylpyrimidine-2,4-diamine (2)**

Step 1: A mixture of 2, 4-dichloropyrimidine (881 mg, 5.91 mmol) and aniline (550 mg, 5.91 mmol) was added TEA (1.648 mL, 11.82 mmol) in Ethanol (8.0 mL). The mixture was stirred at 100 °C for 12 h. LCMS showed desired product formed. Solvent was removed under reduced pressure. The residue was purified by silica gel (ISCO; 20 g 50-90% EtOAc/Pet.ether) to give crude product. The crude product was further purified by reversed MPLC (Biotage; 20 g Agela, C18, Eluent of 0%~30% MeCN\H<sub>2</sub>O (0.5% TFA) gradient @ 30 mL/min) to give 2-chloro-N-phenylpyrimidin-4-amine (185 mg, 11 % yield) as a white solid. MS (ESI): *m/z* calc'd for C<sub>10</sub>H<sub>9</sub>ClN<sub>3</sub> [M+H]<sup>+</sup> 206, found 206.

Step 2: A mixture of 2-chloro-N-phenylpyrimidin-4-amine (29 mg, 0.142 mmol), 6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-amine **I-1** (41 mg, 0.213 mmol), Xantphos (16.43 mg, 0.028 mmol) and t-BuONa (27.3 mg, 0.284 mmol) in Toluene (2.0 mL) was degassed and backfilled with N<sub>2</sub> (three times), Pd<sub>2</sub>(dba)<sub>3</sub> (13.00 mg, 0.014 mmol) was added under N<sub>2</sub>. The mixture was heated to 100 °C for 18 h. After cooling to room temperature, LCMS showed the desired product. The mixture was diluted with water (10 mL) and washed with EtOAc (5 mL x 3). The organic layers were separated, combined, washed with brine (5 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The residue was purified by Prep-HPLC (Condition water (0.1% TFA)-ACN Begin B 12 End B 42 Gradient Time (min) 11; 100% B Hold Time (min) 1.1; FlowRate (ml/min) 40; Injections 1) to give N<sub>2</sub>-(6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yl)-N<sub>4</sub>-phenylpyrimidine-2,4-diamine (33 mg, 65% yield) as a colorless solid. MS (ESI): *m/z* calc'd for C<sub>21</sub>H<sub>24</sub>N<sub>5</sub>O [M+H]<sup>+</sup> 362, found 362. <sup>1</sup>H NMR (500 MHz, METHANOL-*d*<sub>4</sub>): δ 7.86 (br d, *J* = 7.0 Hz, 1H), 7.52 - 7.71 (m, 3H), 7.44 (t, *J* = 8.0 Hz, 2H), 7.30 (t, *J* = 7.0 Hz, 1H), 7.02 (s, 1H), 6.42 (d, *J* = 7.5 Hz, 1H), 4.01 - 4.31 (m, 2H), 3.90 (s, 3H), 3.63 - 3.82 (m, 1H), 3.35 - 3.56 (m, 1H), 3.15 - 3.27 (m, 2H), 3.05 (s, 3H).



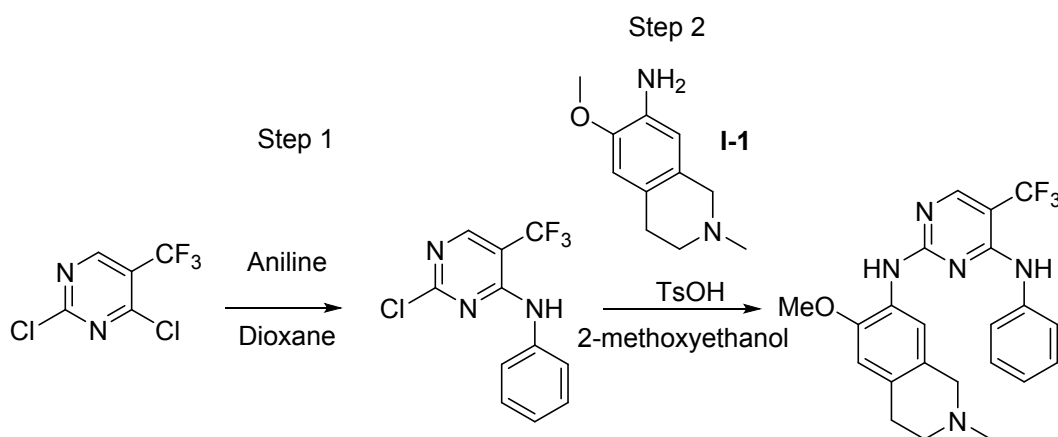
**N2-(6-Methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yl)-5-methyl-N4-phenylpyrimidine-2,4-diamine (3)**

Step 1: Aniline (4.36 mL, 48.2 mmol) was added to a solution of ethyl 2,4-dichloropyrimidine-5-carboxylate (10.1 g, 45.9 mmol) and triethylamine (12.8 mL, 92 mmol) in ethanol (92 mL) at 0 °C. After the addition the reaction was allowed to reach room temperature and stirred overnight. Water was added (200 mL) and a solid precipitated. The solid was filtered and dried under a stream of nitrogen. This ethyl 2-chloro-4-(phenylamino)pyrimidine-5-carboxylate which was used without further purification. MS (ESI) *m/z* calc'd for C<sub>13</sub>H<sub>13</sub>ClN<sub>3</sub>O<sub>2</sub> [M+H]<sup>+</sup> 278 found 278.

Step 2: A 500 mL 3-necked round bottomed flask with reflux condenser was charged with 6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-amine (7.37 g, 38.3 mmol) and ethyl 2-chloro-4-(phenylamino)pyrimidine-5-carboxylate (9.68 g, 34.9 mmol). 2-methoxyethanol (100 mL) and was added followed by HCl (70 mL, 87 mmol, 1.25 M in ethanol) and the reaction was stirred at 110 °C overnight. The reaction was cooled in an ice bath and neutralized with NaOH<sub>(aq)</sub> (approx. 50 mL, litmus test pH 7), solid began precipitate, distilled water (250 mL) was added and the reaction was stirred for 10 minutes. The solid was filtered off and dried under a stream of

nitrogen. This provided ethyl 2-((6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yl)amino)-4-(phenylamino)pyrimidine-5-carboxylate which was used without further purification. MS (ESI)  $m/z$  calc'd for  $C_{24}H_{28}N_5O_3$   $[M+H]^+$  434 found 434.

**Step 3:** DIBAL-H (0.288 ml, 0.288 mmol, 1M in hexanes) was added to a solution of ethyl 2-((6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yl)amino)-4-(phenylamino)pyrimidine-5-carboxylate (25 mg, 0.058 mmol) in THF (5 mL) at room temperature and the reaction was stirred for 72 h. The reaction was quenched with HCl (2 M aqueous, 1 mL) and concentrated. The crude was purified by preparative HPLC Reverse phase (C-18), eluting with Acetonitrile/Water + 0.1% TFA. This provided (2-((6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yl)amino)-4-(phenylamino)pyrimidin-5-yl)methanol, TFA (5.3 mg, 10.48  $\mu$ mol, 18.18 % yield). MS (ESI)  $m/z$  calc'd for  $C_{22}H_{26}N_5O$   $[M+H]^+$  376 found 376.  $^1H$  NMR (499 MHz, DMSO- $d_6$ )  $\delta$  9.88 (s, 1H), 9.58 (br s, 1H), 7.93 (s, 1H), 7.56 – 7.49 (m, 3H), 7.49 – 7.41 (m, 2H), 7.34 – 7.25 (m, 1H), 6.97 (s, 1H), 4.04 – 3.91 (m, 2H), 3.83 (s, 3H), 3.72 – 3.21 (m, 2H, overlapping with broad  $H_2O$  signal), 3.13 – 2.96 (m, 2H), 2.93 (s, 3H), 2.16 (s, 3H).

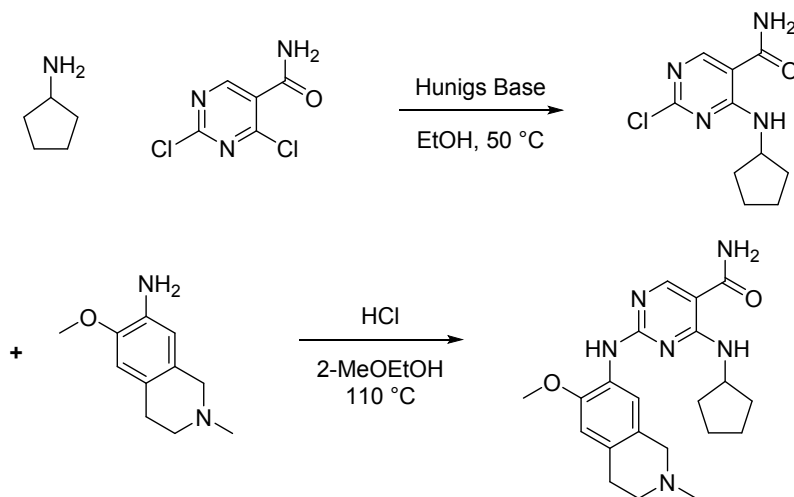


**N2-(6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yl)-N4-phenyl-5-(trifluoromethyl)pyrimidine-2,4-diamine (4)**

**Step 1:** Aniline (0.412 ml, 4.55 mmol) was added to a solution of 2,4-dichloro-5-(trifluoromethyl)pyrimidine (494 mg, 2.277 mmol) in Dioxane at 0 °C. The reaction was allowed to reach room temperature and stirred overnight. The reaction was concentrated and the crude mixture was purified by preparative HPLC Reverse phase (C-18), eluting with Acetonitrile/Water

+ 0.1% TFA. This provided 2-chloro-N-phenyl-5-(trifluoromethyl)pyrimidin-4-amine (300 mg, 1.10 mmol, 48 % yield). MS (ESI)  $m/z$  calc'd for  $C_{11}H_8ClF_3N_3$   $[M+H]^+$  274 found 274.

**Step 2:** Tosic Acid (28.5 mg, 0.150 mmol) was added to a solution of 6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-amine (**I-1**, 21.2 mg, 0.110 mmol) and 2-chloro-N-phenyl-5-(trifluoromethyl)pyrimidin-4-amine (27.4 mg, 0.1 mmol) in 2-methoxyethanol (1 mL) and the reaction was stirred at 110 °C overnight. The crude was concentrated purified by preparative HPLC Reverse phase (C-18), eluting with Acetonitrile/Water + 0.1% TFA. This provided N2-(6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yl)-N4-phenyl-5-(trifluoromethyl)pyrimidine-2,4-diamine (48 mg, 89% yield). MS (ESI)  $m/z$  calc'd for  $C_{22}H_{23}F_3N_5O$   $[M+H]^+$  430 found 430.  $^1H$  NMR (499 MHz, DMSO- $d_6$ )  $\delta$  10.00 (s, 1H), 8.87 (s, 1H), 8.35 (d,  $J$  = 13.7 Hz, 2H), 7.50 – 7.35 (m, 4H), 7.27 (t,  $J$  = 6.9 Hz, 1H), 6.90 (s, 1H), 3.98 – 3.86 (m, 2H), 3.81 (s, 3H), 3.67 – 3.59 (m, 1H), 3.33 – 3.23 (m, 1H), 3.12 – 2.97 (m, 2H), 2.93 (s, 3H).

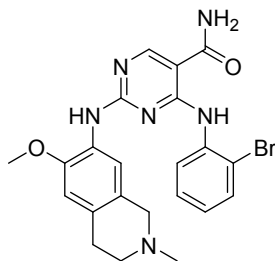


#### 4-(Cyclopentylamino)-2-((6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yl)amino)pyrimidine-5-carboxamide (**5**)

**Step 1.** To a 2 dram vial with a stirbar, N-Ethyl-N-isopropylpropan-2-amine (41  $\mu$ L, 0.234 mmol) was added to a solution of cyclopentyl (14 mg, 0.156 mmol) and 2,4-dichloropyrimidine-5-carboxamide (30 mg, 0.156 mmol) in Ethanol (446  $\mu$ L). The mixture was capped and stirred at 50 °C for 3 h. The mixture was diluted with DCM (2 mL) and water (2 mL), capped, and stirred

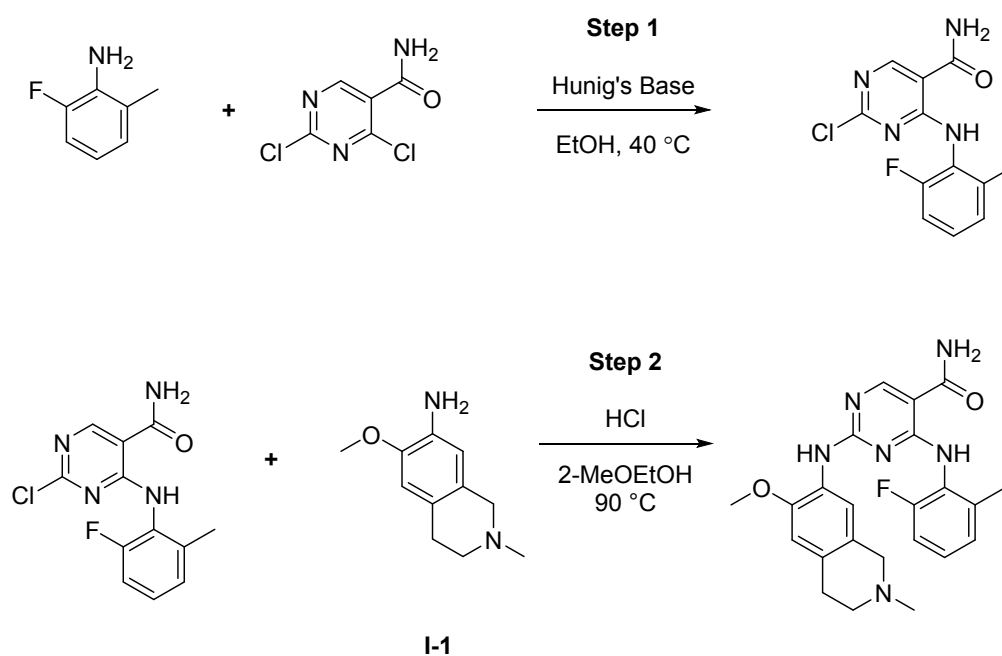
vigorously for 5 min. The mixture was filtered through a phase separator and the organics were concentrated to afford 2-chloro-4-(cyclopentylamino)pyrimidine-5-carboxamide as a yellow oil. The compound was sufficiently pure and carried on crude to the subsequent reaction. MS (ESI):  $m/z$  calc'd for  $C_{10}H_{14}ClN_4O$   $[M+H]^+$  241, found 241.

**Step 2.** 2-Chloro-4-(cyclopentylamino)pyrimidine-5-carboxamide (21 mg, 0.086 mmol) was added to a vial containing 6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-amine (I-1) (18.10 mg, 0.094 mmol) in 2-Methoxymethanol (245  $\mu$ l). HCl in Ethanol (1.25M) (171  $\mu$ l, 0.214 mmol) was added via syringe and the mixture was stirred at 110 °C for 3 h. Upon completion as observed by LCMS analysis, the mixture was cooled, diluted with DMA (2 mL), filtered, and purified by reverse phase preparative HPLC (Purification Method A), to afford 4-(cyclopentylamino)-2-((6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yl)amino)pyrimidine-5-carboxamide (28 mg, 80%) as a colorless solid. MS (ESI):  $m/z$  calc'd for  $C_{21}H_{29}N_6O_2$   $[M+H]^+$  397, found 397.  $^1H$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  10.46 (d,  $J$  = 58.6 Hz, 1H), 9.80 (s, 1H), 9.10 (br s, 1H), 8.58 (d,  $J$  = 34.1 Hz, 1H), 8.00 (d,  $J$  = 112.7 Hz, 2H), 7.54 (s, 1H), 7.01 (s, 1H), 4.38 (d,  $J$  = 14.4 Hz, 1H), 4.34 - 4.26 (m, 1H), 4.26 - 4.16 (m, 1H), 3.87 (s, 3H), 3.65 (s, 1H), 3.33 (s, 1H), 3.16 (d,  $J$  = 6.0 Hz, 1H), 3.03 (d,  $J$  = 17.1 Hz, 1H), 2.93 (s, 3H), 2.05 - 1.93 (m, 2H), 1.78 - 1.60 (m, 3H), 1.60 - 1.47 (m, 2H). HPLC (Ascentis Express C18 100 X 3 mm column, 45C, 2 $\mu$ l injection, 215 nm) ( $CH_3CN$  in 0.1% TFA):  $t_R$  = 1.41 min (95% purity).



**4-((2-Bromophenyl)amino)-2-((6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yl)amino)pyrimidine-5-carboxamide (6).** The preparation of 4-((2-bromophenyl)amino)-2-((6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yl)amino)pyrimidine-5-carboxamide was carried out according to *General Procedure 1*, using 2-bromoaniline (0.448 g, 2.6 mmol). The

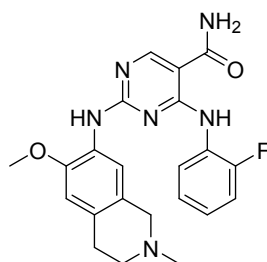
product from Step 1 was used crude as the starting material in Step 2, following *General Procedure 4* and Purification Method B, for preparation of the title compound (380 mg, 76%) as a colorless solid. MS (ESI):  $m/z$  calc'd for  $C_{22}H_{24}BrN_6O_2$   $[M+H]^+$   $[^{79}Br]^+$  383, found 383.  $^1H$  NMR (499 MHz, DMSO- $d_6$ )  $\delta$  11.67 (s, 1H), 8.71 (s, 1H), 8.35 (s, 1H), 8.21 (s, 1H), 8.03 (s, 1H), 7.67 (d,  $J$  = 7.9 Hz, 1H), 7.41 (s, 2H), 7.26 (s, 1H), 7.04 (t,  $J$  = 7.6 Hz, 1H), 6.78 (s, 1H), 3.77 (s, 3H), 3.27 (s, 2H), 2.80 (s, 2H), 2.58 (s, 3H), 2.34 (s, 3H). HPLC (Ascentis Express C18 100X3 mm column, 45C, 2uL injection, 215 nm) ( $CH_3CN$  in 0.1% TFA):  $t_R$  = 2.11 min (95% purity).



**4-((2-Fluoro-6-methylphenyl)amino)-2-((6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yl)amino)pyrimidine-5-carboxamide (7)**

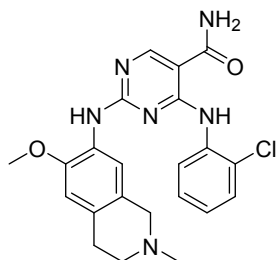
Step 1. *N*-Ethyl-*N*-isopropylpropan-2-amine (54.6  $\mu$ L, 0.313 mmol) was added to a solution of 2-fluoro-6-methylaniline (27.4 mg, 0.219 mmol) and 2,4-dichloropyrimidine-5-carboxamide (40 mg, 0.208 mmol) in Ethanol (595  $\mu$ L) and the mixture was stirred for 2 h at 40 °C. The mixture was cooled, diluted with DCM (2 mL) and water (2mL), and stirred vigorously for 5 min. The organics were filtered through a phase separator and concentrated to afford 2-chloro-4-((2-fluoro-6-methylphenyl)amino)pyrimidine-5-carboxamide and a colorless solid (58 mg, 99%). MS (ESI):  $m/z$  calc'd for  $C_{12}H_{11}ClFN_4O$   $[M+H]^+$  281, found 281.

**Step 2.** 2-Chloro-4-((2-fluoro-6-methylphenyl)amino)pyrimidine-5-carboxamide (40 mg, 0.143 mmol) was added to a vial containing 6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-amine (30.1 mg, 0.157 mmol) in 2-Methoxyethanol (407  $\mu$ l). Hydrogen chloride (1.25M in EtOH) (342  $\mu$ l, 0.428 mmol) was added via syringe and the mixture was stirred at 90 °C for 3 h. The residue was cooled, dissolved in MeOH/DMA, filtered, and was purified by HPLC Purification Method B to give 4-((2-fluoro-6-methylphenyl)amino)-2-((6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yl)amino)pyrimidine-5-carboxamide (8.8 mg, 14 % yield) as a colorless solid following dry down. MS (ESI):  $m/z$  calc'd for  $C_{23}H_{26}FN_6O_2$   $[M+H]^+$  437, found 437.  $^1H$  NMR (499 MHz, DMSO- $d_6$ )  $\delta$  10.68 (s, 1H), 8.67 (s, 1H), 8.03 (s, 1H), 7.76 (s, 1H), 7.40 (s, 2H), 7.30 (q,  $J$  = 6.3 Hz, 1H), 7.25 - 7.14 (m, 2H), 6.68 (s, 1H), 3.79 (s, 3H), 2.96 (s, 2H), 2.69 (s, 2H), 2.48 (s, 3H), 2.32 (s, 3H), 2.21 (s, 3H).  $^{13}C$  NMR (126 MHz, DMSO- $d_6$ )  $\delta$  169.6, 160.5, 160.1, 158.6, 150.4, 139.1, 129.5, 127.1, 123.8, 121.7, 111.2, 56.3, 53.8, 50.7, 42.5, 25.5. **HRMS** ( $m/z$ ) calc'd for  $C_{23}H_{26}FN_6O_2$   $[M+H]^+$  437.2101, found 437.2089. HPLC (Ascentis Express C18 100 X 3 mm column, 45C, 2 $\mu$ L injection, 215 nm) ( $CH_3CN$  in 0.1% TFA):  $t_R$  = 1.95 min (100% purity).

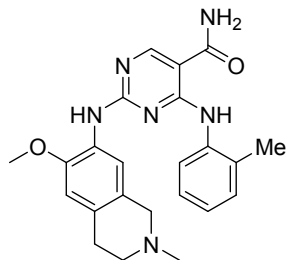


**4-(2-Fluoroanilino)-2-[(6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-2-ium-7-yl)amino]pyrimidine-5-carboxamide, HCl (8).** A microwave vial was charged with 2-chloro-4-((2-fluorophenyl)amino)pyrimidine-5-carboxamide (80 mg, 0.30 mmol) and 6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-amine (58 mg, 0.3 mmol). A stir bar was added followed by ethanol (2 mL) and HCl (75  $\mu$ l, 0.30 mmol). Argon was blown over the mixture and the vial was capped and heated to 120 °C for 2 h. Precipitate formed when cooled to room temperature. The mixture was filtered with EtOH, and washed with diethyl ether to afford 4-((2-fluorophenyl)amino)-2-((6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yl)amino)pyrimidine-5-carboxamide, HCl (60 mg, 44% yield) as a white solid. MS (ESI):  $m/z$  calc'd

for  $C_{22}H_{24}FN_6O_2$   $[M+H]^+$  423, found 423. HPLC (Ascentis Express C18 50X3 mm column, 45C, 2 $\mu$ L injection, 215 nm) ( $CH_3CN$  in 0.1% TFA):  $t_R$  = 2.13 min (100% purity).



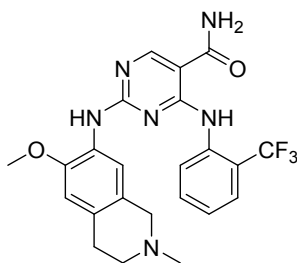
**4-(2-Chloroanilino)-2-[(6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-2-ium-7-yl)amino]pyrimidine-5-carboxamide, HCl (9).** A microwave vial was charged with 2-chloro-4-((2-chlorophenyl)amino)pyrimidine-5-carboxamide (85 mg, 0.30 mmol) and 6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-amine (58 mg, 0.3 mmol). A stir bar was added followed by ethanol (2 mL) and HCl (75  $\mu$ L, 0.30 mmol). Argon was blown over the mixture and the vial was capped and heated in the microwave at 160  $^{\circ}C$  for 15 min. Usual precipitate did not form. The mixture was concentrated and recrystallized from ethanol overnight (15 h) and filtered to afford 4-((2-chlorophenyl)amino)-2-((6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yl)amino)pyrimidine-5-carboxamide, HCl (22 mg, 15 % yield) as a white solid. MS (ESI):  $m/z$  calc'd for  $C_{22}H_{24}ClN_6O_2$   $[M+H]^+$  439, found 439. HPLC (Ascentis Express C18 50X3 mm column, 45C, 2 $\mu$ L injection, 215 nm) ( $CH_3CN$  in 0.1% TFA):  $t_R$  = 2.23 min (98% purity).



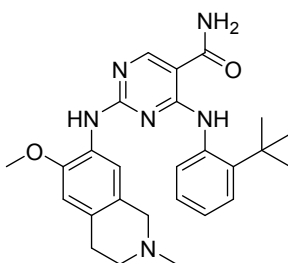
**2-[(6-Methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-2-ium-7-yl)amino]-4-(2-methylanilino)pyrimidine-5-carboxamide, TFA (10).** The preparation of 2-[(6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-2-ium-7-yl)amino]-4-(2-methylanilino)pyrimidine-5-



carboxamide was carried out according to *General Procedure 1*, using 2-methylaniline (0.15 mmol). The product from Step 1 was used crude as the starting material in Step 2, following *General Procedure 4* and Purification Method A, for preparation of the title compound (52 mg, 82%) as a colorless solid. MS (ESI):  $m/z$  calc'd for  $C_{23}H_{27}N_6O_2$   $[M+H]^+$  419, found 419. UPLC 2 min (Sunfire C18 50X3 mm column, 45C, 1  $\mu$ L injection, 215 nm) ( $CH_3CN$  in 0.1% TFA):  $t_R$  = 0.72 min (100% purity).

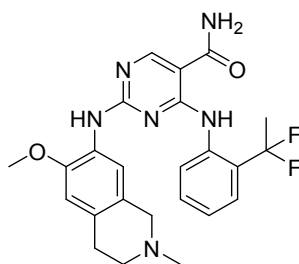


**2-[(6-Methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)amino]-4-[2-(trifluoromethyl)anilino]pyrimidine-5-carboxamide, TFA (11).** The preparation of 2-[(6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)amino]-4-[2-(trifluoromethyl)anilino]pyrimidine-5-carboxamide was carried out according to *General Procedure 1*, using 2-trifluoromethylaniline (0.15 mmol). The product from Step 1 was used crude as the starting material in Step 2, following *General Procedure 4* and Purification Method A, for preparation of the title compound (42 mg, 59%) as a colorless solid. MS (ESI):  $m/z$  calc'd for  $C_{23}H_{24}F_3N_6O_2$   $[M+H]^+$  473, found 473. UPLC 2 min (Sunfire C18 50X3 mm column, 45C, 1  $\mu$ L injection, 215 nm) ( $CH_3CN$  in 0.1% TFA):  $t_R$  = 0.78 min (100% purity).

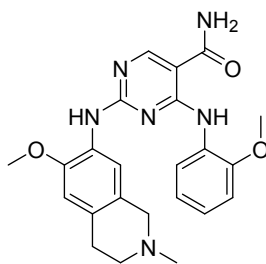


**4-(2-tert-Butylanilino)-2-[(6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)amino]pyrimidine-5-carboxamide, TFA (12).** The preparation of 4-(2-tert-butylanilino)-2-[(6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)amino]pyrimidine-5-carboxamide was carried out according to *General Procedure 1*, using 2-(1,1-difluoroethyl)aniline (0.15 mmol).

The product from Step 1 was used crude as the starting material in Step 2, following *General Procedure 4* and Purification Method A, for preparation of the title compound (77 mg, 98%) as a colorless solid. MS (ESI):  $m/z$  calc'd for  $C_{26}H_{33}N_6O_2$   $[M+H]^+$  461, found 462. UPLC 2 min (Sunfire C18 50X3 mm column, 45C, 1  $\mu$ L injection, 215 nm) ( $CH_3CN$  in 0.1% TFA):  $t_R$  = 0.82 min (97% purity).

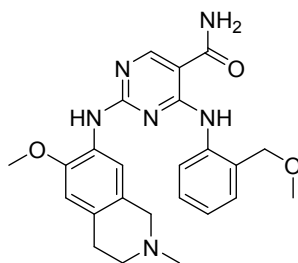


**4-[2-(1,1-Difluoroethyl)anilino]-2-[(6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-2-ium-7-yl)amino]pyrimidine-5-carboxamide, TFA (13).** The preparation of 4-[2-(1,1-difluoroethyl)anilino]-2-[(6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-2-ium-7-yl)amino]pyrimidine-5-carboxamide was carried out according to *General Procedure 1*, using 2-aniline (0.15 mmol). The product from Step 1 was used crude as the starting material in Step 2, following *General Procedure 4* and Purification Method A, for preparation of the title compound (4 mg, 8%) as a colorless solid. MS (ESI):  $m/z$  calc'd for  $C_{24}H_{27}F_2N_6O_2$   $[M+H]^+$  469, found 469. HPLC (Ascentis Express C18 100X3 mm column, 45C, 1  $\mu$ L injection, 215 nm) ( $CH_3CN$  in 0.1% TFA):  $t_R$  = 2.11 min (99% purity).

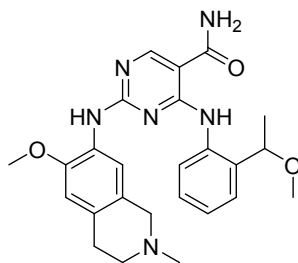


**4-(2-Methoxyanilino)-2-[(6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-2-ium-7-yl)amino]pyrimidine-5-carboxamide, HCl (14).** A microwave vial was charged with 2-chloro-4-((2-methoxyphenyl)amino)pyrimidine-5-carboxamide (44 mg, 0.156 mmol) and 6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-amine (30 mg, 0.156 mmol). A stir bar was added followed by ethanol (1 mL) and HCl (39  $\mu$ L, 0.156 mmol). Argon was blown over the mixture and

the vial was capped and heated to 120 °C for 2 h. Precipitate formed when cooled to room temperature. The mixture was filtered with EtOH, and washed with diethyl ether to afford 4-(2-methoxyanilino)-2-[(6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-2-ium-7-yl)amino]pyrimidine-5-carboxamide, HCl (11 mg, 15% yield) as a white solid. MS (ESI):  $m/z$  calc'd for  $C_{23}H_{27}N_6O_3$   $[M+H]^+$  435, found 435. HPLC (Ascentis Express C18 50X3 mm column, 45C, 2 $\mu$ L injection, 215 nm) ( $CH_3CN$  in 0.1% TFA):  $t_R$  = 2.02 min (99% purity).



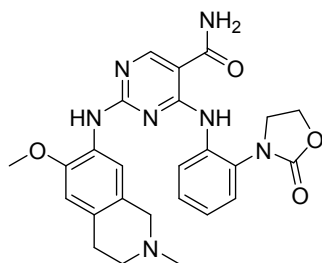
**4-[2-(methoxymethyl)anilino]-2-[(6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-2-ium-7-yl)amino]pyrimidine-5-carboxamide, HCl (15).** A microwave vial was charged with 2-chloro-4-((2-(methoxymethyl)phenyl)amino)pyrimidine-5-carboxamide (11 mg, 0.038 mmol) and 6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-amine (7 mg, 0.038 mmol). A stir bar was added followed by ethanol (0.5 mL) and HCl (15  $\mu$ L mmol). Argon was blown over the mixture and the vial was capped and heated to 120 °C for 2 h. Precipitate formed when cooled to room temperature. The mixture was filtered with EtOH, and washed with diethyl ether to afford 4-(2-methoxyanilino)-2-[(6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-2-ium-7-yl)amino]pyrimidine-5-carboxamide, HCl (2.3 mg, 13% yield) as a white solid. MS (ESI):  $m/z$  calc'd for  $C_{24}H_{29}N_6O_3$   $[M+H]^+$  449, found 449. HPLC (Ascentis Express C18 50X3 mm column, 45C, 2 $\mu$ L injection, 215 nm) ( $CH_3CN$  in 0.1% TFA):  $t_R$  = 1.89 min (98% purity).



**4-[2-(1-Methoxyethyl)anilino]-2-[(6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-2-ium-7-yl)amino]pyrimidine-5-carboxamide, TFA (16).** The preparation of 4-[2-(1-

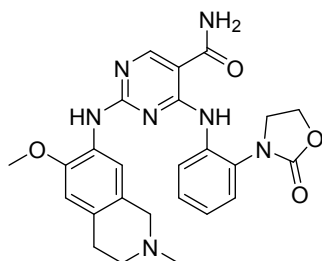
methoxyethyl)anilino]-2-[(6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)amino]pyrimidine-5-carboxamide was carried out according to *General Procedure 1*, using 2-(1-methoxyethyl)aniline (0.15 mmol). The product from Step 1 was used crude as the starting material in Step 2, following *General Procedure 4* and Purification Method A, for preparation of the title compound (73 mg, 99%) as a colorless solid. MS (ESI):  $m/z$  calc'd for  $C_{25}H_{31}N_6O_3$   $[M+H]^+$  463, found 463. UPLC 2 min (Sunfire C18 50X3 mm column, 45C, 1  $\mu$ L injection, 215 nm) ( $CH_3CN$  in 0.1% TFA):  $t_R$  = 0.73 min (10% purity).

**4-[2-[(1S)-1-Methoxyethyl]anilino]-2-[(6-methoxy-2-methyl-3,4-dihydro-1H-isoquinolin-7-yl)amino]pyrimidine-5-carboxamide (17).** The preparation of 4-[2-[(1S)-1-Methoxyethyl]anilino]-2-[(6-methoxy-2-methyl-3,4-dihydro-1H-isoquinolin-7-yl)amino]pyrimidine-5-carboxamide was carried out according to *General Procedure 1*, using intermediate **I-2** (998 mg, 6.6 mmol). The product from Step 1 (50 mg, 0.163 mmol) was used as the starting material in Step 2, following *General Procedure 4* and Purification Method B, to isolate the title compound (11 mg, 12%) as a colorless solid. MS (ESI):  $m/z$  calc'd for  $C_{25}H_{31}N_6O_3$   $[M+H]^+$  463, found 463. HPLC (Sunfire C18 50X3 mm column, 45C, 1  $\mu$ L injection, 215 nm) ( $CH_3CN$  in 0.1% TFA):  $t_R$  = 2.04 min (100% purity).

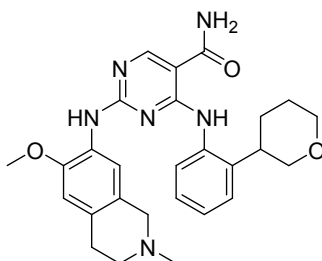


**2-[(6-Methoxy-2-methyl-3,4-dihydro-1H-isoquinolin-7-yl)amino]-4-[2-(2-oxooxazolidin-3-yl)anilino]pyrimidine-5-carboxamide (18).** The preparation of 2-[(6-methoxy-2-methyl-3,4-dihydro-1H-isoquinolin-7-yl)amino]-4-[2-(2-oxooxazolidin-3-yl)anilino]pyrimidine-5-carboxamide was carried out according to *General Procedure 1*, using 3-(2-aminophenyl)oxazolidin-2-one (0.25 mmol). The product from Step 1 was used crude as the starting material in Step 2, following *General Procedure 2* and Purification Method B, for preparation of the title compound (38 mg, 39%) as a colorless solid. MS (ESI):  $m/z$  calc'd for

$C_{25}H_{28}N_7O_4$   $[M+H]^+$  490, found 490. UPLC 2 min (Sunfire C18 50X3 mm column, 45C, 1  $\mu$ L injection, 215 nm) ( $CH_3CN$  in 0.1% TFA):  $t_R$  = 0.62 min (100% purity).

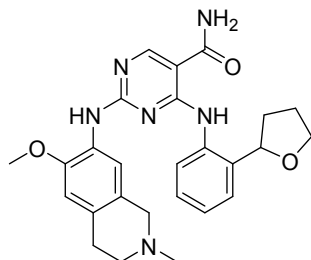


**2-[(6-Methoxy-2-methyl-3,4-dihydro-1H-isoquinolin-7-yl)amino]-4-(2-sulfamoylanilino)pyrimidine-5-carboxamide (19).** The preparation of 2-[(6-methoxy-2-methyl-3,4-dihydro-1H-isoquinolin-7-yl)amino]-4-(2-sulfamoylanilino)pyrimidine-5-carboxamide was carried out according to *General Procedure 1*, using 3-(2-aminophenyl)oxazolidin-2-one (0.25 mmol). The product from Step 1 was used crude as the starting material in Step 2, following *General Procedure 2* and Purification Method B, for preparation of the title compound (8 mg, 8%) as a colorless solid. MS (ESI):  $m/z$  calc'd for  $C_{22}H_{26}N_7O_4S$   $[M+H]^+$  484, found 484. UPLC 2 min (Sunfire C18 50X3 mm column, 45C, 1  $\mu$ L injection, 215 nm) ( $CH_3CN$  in 0.1% TFA):  $t_R$  = 0.59 min (100% purity).

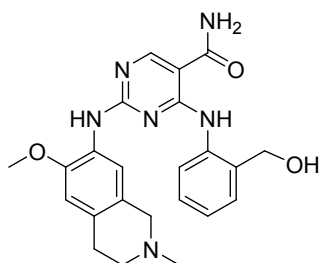


**2-[(6-Methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-2-ium-7-yl)amino]-4-(2-tetrahydropyran-3-ylanilino)pyrimidine-5-carboxamide, TFA (20).** A vial was charged with compound **6** (20 mg, 0.041 mmol),  $[Ni(dtbbpy)(H_2O)_4]Cl_2 \cdot 1$  (1.945 mg, 4.14  $\mu$ mol), TBAI (3.82 mg, 10.34  $\mu$ mol), zinc (8.12 mg, 0.124 mmol) and lastly 3-bromotetrahydro-2H-pyran (13.7 mg, 0.083 mmol), to which a stir bar and DMA (414  $\mu$ L) was quickly added, capped, flushed 3x with argon and heated to 80  $^{\circ}C$ . After 3 h LCMS aliquot suggested product and the mixture was diluted to 2 mL with DMSO, filtered, and submitted for purification using Purification Method A. 2-[(6-Methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yl)amino]-4-[(2-(tetrahydro-2H-pyran-3-

yl)phenyl)amino)pyrimidine-5-carboxamide, TFA (3.6 mg, 14 % yield) was isolated as a white solid. MS (ESI):  $m/z$  calc'd for  $C_{27}H_{33}N_6O_3$   $[M+H]^+$  489, found 489. HPLC (Sunfire C18 50X3 mm column, 45C, 1  $\mu$ L injection, 215 nm) ( $CH_3CN$  in 0.1% TFA):  $t_R$  = 2.2 min (99% purity).

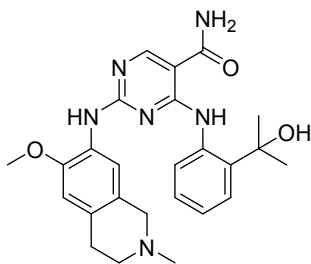


**2-[(6-Methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)amino]-4-(2-tetrahydrofuran-2-ylanilino)pyrimidine-5-carboxamide, HCl (21).** The preparation of 2-[(6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)amino]-4-(2-tetrahydrofuran-2-ylanilino)pyrimidine-5-carboxamide was carried out according to *General Procedure 1*, using 2-(tetrahydrofuran-2-yl)aniline (1.45 mmol). The product from Step 1 was used crude as the starting material in Step 2, following *General Procedure 5*, for preparation of the title compound (16 mg, 21%) as a colorless solid. MS (ESI):  $m/z$  calc'd for  $C_{26}H_{31}N_6O_3$   $[M+H]^+$  475, found 475. HPLC (Sunfire C18 50X3 mm column, 45C, 1  $\mu$ L injection, 215 nm) ( $CH_3CN$  in 0.1% TFA):  $t_R$  = 2.19 min (92% purity).

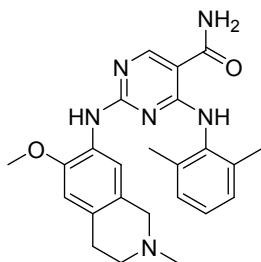


**4-[2-(Hydroxymethyl)anilino]-2-[(6-methoxy-2-methyl-3,4-dihydro-1H-isoquinolin-7-yl)amino]pyrimidine-5-carboxamide (22).** The preparation of 4-[2-(hydroxymethyl)anilino]-2-[(6-methoxy-2-methyl-3,4-dihydro-1H-isoquinolin-7-yl)amino]pyrimidine-5-carboxamide was carried out according to *General Procedure 1*, using (2-aminophenyl)methanol (2.6 mmol). The product from Step 1 was purified on silica gel chromatography (5-15% MeOH in DCM) to afford the title compound (581 mg, 80% yield). 2-Chloro-4-[(2-(hydroxymethyl)phenyl)amino]pyrimidine-5-carboxamide (60 mg, 0.215 mmol) was used as the

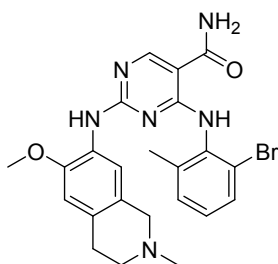
starting material in Step 2, following *General Procedure 2*, and purified using Purification Method B to afford the title compound (15 mg, 16%) as a colorless solid. MS (ESI):  $m/z$  calc'd for  $C_{23}H_{27}N_6O_3$   $[M+H]^+$  435, found 435.  $^1H$  NMR (499 MHz, DMSO- $d_6$ )  $\delta$  11.22 (s, 1H), 8.66 (s, 1H), 8.01 – 7.95 (m, 2H), 7.88 – 7.73 (m, 1H), 7.52 (s, 1H), 7.47 (d,  $J$  = 7.5 Hz, 1H), 7.33 (s, 1H), 7.27 – 7.20 (m, 1H), 7.17 (t,  $J$  = 7.1 Hz, 1H), 6.74 (s, 1H), 5.19 (t,  $J$  = 5.1 Hz, 1H), 4.50 (d,  $J$  = 4.8 Hz, 2H), 3.79 (s, 3H), 3.20 (s, 2H), 2.77 (t,  $J$  = 5.7 Hz, 2H), 2.59 (d,  $J$  = 5.2 Hz, 2H), 2.35 (s, 3H).  $^{13}C$  NMR: 13C NMR (126 MHz, DMSO)  $\delta$  169.5, 160.7, 160.3, 158.5, 149.9, 148.2, 136.4, 134.9, 128.9, 128.0, 127.6, 126.8, 126.0, 124.5, 119.1, 110.8, 100.3, 60.4, 57.8, 56.2, 52.9, 46.2, 29.1. HRMS (ESI):  $m/z$  calc'd for  $C_{23}H_{27}N_6O_3$   $[M+H]^+$  435.2144, found 435.2141. HPLC (Sunfire C18 50X3 mm column, 45C, 1  $\mu$ L injection, 215 nm) ( $CH_3CN$  in 0.1% TFA):  $t_R$  = 0.89 min (94% purity).



**4-[2-(1-Hydroxy-1-methyl-ethyl)anilino]-2-[(6-methoxy-2-methyl-3,4-dihydro-1H-isoquinolin-7-yl)amino]pyrimidine-5-carboxamide (23).** To a solution of methyl 2-((5-carbamoyl-2-((6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yl)amino)pyrimidin-4-yl)amino)benzoate (50 mg, 0.11 mmol) in THF (2 mL), cooled to 0 °C by ice bath, was added methylmagnesium chloride (180  $\mu$ L, 0.541 mmol) dropwise via syringe. The mixture was allowed to warm to RT over 12 h. The reaction mixture was diluted in EtOAc and quenched with of sat. aq.  $NH_4Cl$  (10 mL), and the aqueous layers were extracted with EtOAc (2x). The combined organics were dried over  $Na_2SO_4$ , filtered, and concentrated in vacuo to dryness. The crude material was purified by Purification Method B to give 4-((2-(2-hydroxypropan-2-yl)phenyl)amino)-2-((6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yl)amino)pyrimidine-5-carboxamide (4.4 mg, 9 % yield) as light yellow solid. MS (ESI):  $m/z$  calc'd for  $C_{25}H_{31}N_6O_3$   $[M+H]^+$  463, found 463. HPLC (Sunfire C18 50X3 mm column, 45C, 1  $\mu$ L injection, 215 nm) ( $CH_3CN$  in 0.1% TFA):  $t_R$  = 1.17 min (98% purity).

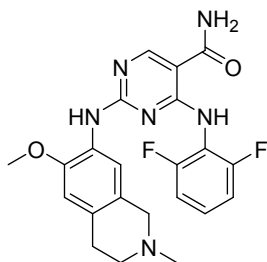


**4-(2,6-Dimethylanilino)-2-[(6-methoxy-2-methyl-3,4-dihydro-1H-isoquinolin-7-yl)amino]pyrimidine-5-carboxamide (24).** The preparation of 4-(2,6-dimethylanilino)-2-[(6-methoxy-2-methyl-3,4-dihydro-1H-isoquinolin-7-yl)amino]pyrimidine-5-carboxamide was carried out according to *General Procedure 3*, using 2,6-dimethylaniline (25 mg, 0.21 mmol). The crude product from Step 1 (58 mg, 0.21 mmol) was used as the starting material in Step 2, following *General Procedure 2* and Purification Method B, to isolate the title compound (10 mg, 13%) as a colorless solid. MS (ESI):  $m/z$  calc'd for  $C_{24}H_{29}N_6O_2$   $[M+H]^+$  433, found 433. HPLC (Sunfire C18 50X3 mm column, 45C, 1  $\mu$ L injection, 215 nm) ( $CH_3CN$  in 0.1% TFA):  $t_R$  = 1.95 min (100% purity).

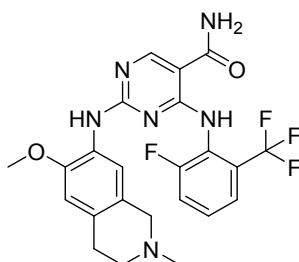


**4-(2-Bromo-6-methyl-anilino)-2-[(6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-2-ium-7-yl)amino]pyrimidine-5-carboxamide, TFA (25).** The preparation of 4-(2,6-dimethylanilino)-2-[(6-methoxy-2-methyl-3,4-dihydro-1H-isoquinolin-7-yl)amino]pyrimidine-5-carboxamide was carried out according to *General Procedure 1*, using 2-bromo-6-methylaniline (242 mg, 1.3 mmol). The crude product from Step 1 (102 mg, 0.30 mmol) was used as the starting material in Step 2, following *General Procedure 5* and Purification Method A, to isolate the title compound (48 mg, 26%) as a colorless solid TFA salt. MS (ESI):  $m/z$  calc'd for  $C_{23}H_{26}BrN_6O_2$   $[M+H]^+$   $[^{79}Br]^+$  497, found 497. HPLC (Sunfire C18 50X3 mm column, 45C, 1  $\mu$ L injection, 215 nm) ( $CH_3CN$  in 0.1% TFA):  $t_R$  = 2.09 min (87% purity).



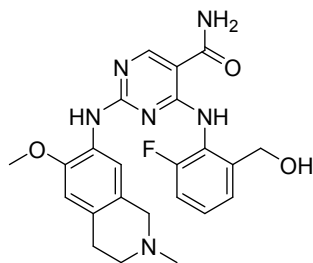


**4-(2,6-Difluoroanilino)-2-[(6-methoxy-2-methyl-3,4-dihydro-1H-isoquinolin-7-yl)amino]pyrimidine-5-carboxamide (26).** The preparation of 4-(2,6-difluoroanilino)-2-[(6-methoxy-2-methyl-3,4-dihydro-1H-isoquinolin-7-yl)amino]pyrimidine-5-carboxamide was carried out according to *General Procedure 3*, using 2,6-fluoroaniline (33 mg, 0.25 mmol). The crude product from Step 1 (74 mg, 0.25 mmol) was used as the starting material in Step 2, following *General Procedure 2* and Purification Method B, to isolate the title compound (3.6 mg, 3%) as a colorless solid. MS (ESI):  $m/z$  calc'd for  $C_{22}H_{23}F_2N_6O_2$   $[M+H]^+$  441, found 441. HPLC (Sunfire C18 50X3 mm column, 45C, 1  $\mu$ L injection, 215 nm) ( $CH_3CN$  in 0.1% TFA):  $t_R$  = 1.46 min (100% purity).



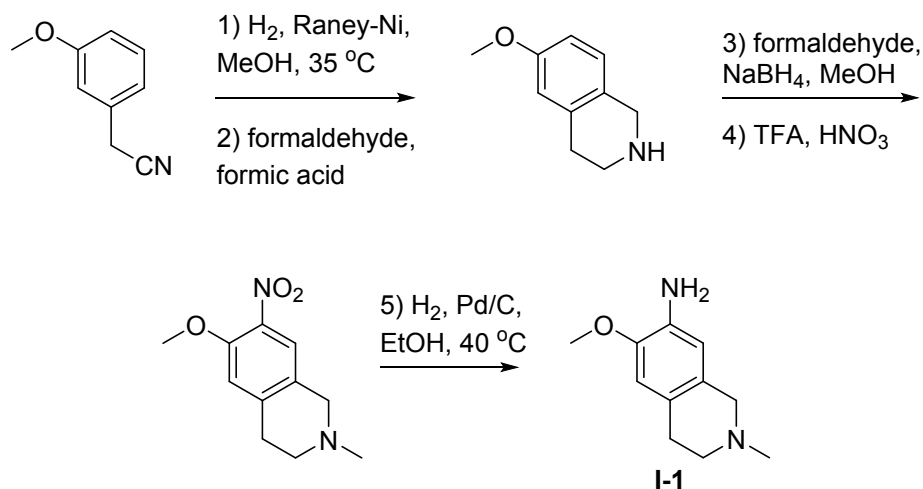
**4-[2-Fluoro-6-(trifluoromethyl)anilino]-2-[(6-methoxy-2-methyl-3,4-dihydro-1H-isoquinolin-7-yl)amino]pyrimidine-5-carboxamide (27).** The preparation of 4-[2-fluoro-6-(trifluoromethyl)anilino]-2-[(6-methoxy-2-methyl-3,4-dihydro-1H-isoquinolin-7-yl)amino]pyrimidine-5-carboxamide was carried out according to *General Procedure 3*, using 2-fluoro-6-(trifluoromethyl)aniline (249 mg, 1.4 mmol). The product from Step 1 (25 mg, 0.082 mmol) was used as the starting material in Step 2, following *General Procedure 2* and Purification Method B, to isolate the title compound (6.2 mg, 17%) as a colorless solid. MS (ESI):  $m/z$  calc'd for  $C_{23}H_{23}F_4N_6O_2$   $[M+H]^+$  491, found 491.  $^1H$  NMR (499 MHz, DMSO- $d_6$ )  $\delta$  11.07 (s, 1H), 8.72 (s, 1H), 8.09 (s, 1H), 7.89 (s, 1H), 7.83 - 7.68 (m, 2H), 7.63 (q,  $J$  = 8.0 Hz, 1H), 7.48 (s, 1H), 7.30 (s, 1H), 6.68 (s, 1H), 3.78 (s, 3H), 2.97 (s, 2H), 2.69 (d,  $J$  = 5.5 Hz, 2H), 2.49 (d,  $J$  = 5.6 Hz, 2H), 2.33 (s, 3H).

HPLC (Sunfire C18 50X3 mm column, 45C, 1 uL injection, 215 nm) (CH<sub>3</sub>CN in 0.1% TFA): t<sub>R</sub> = 1.64 min (100% purity). **<sup>13</sup>C NMR** (126 MHz, DMSO-d<sub>6</sub>) δ 169.8, 161.8, 159.9, 159.8, 158.5, 157.8, 147.4 (d, *J* = 13.9 Hz), 129.3, 129.3, 128.1 (t), 126.6, 126.0, 125.1 (d, *J* = 18.0 Hz), 122.6 (m), 121.4, 121.2, 110.7, 100.2, 58.0, 56.3, 52.7, 46.2, 29.0. **HRMS** (ESI): *m/z* calc'd for C<sub>23</sub>H<sub>23</sub>F<sub>4</sub>N<sub>6</sub>O<sub>2</sub> [M+H]<sup>+</sup> 491.1818, found 491.1813. HPLC (Sunfire C18 50X3 mm column, 45C, 1 uL injection, 215 nm) (CH<sub>3</sub>CN in 0.1% TFA): t<sub>R</sub> = 1.64 min (100% purity).



**4-[2-Fluoro-6-(hydroxymethyl)anilino]-2-[(6-methoxy-2-methyl-3,4-dihydro-1H-isoquinolin-7-yl)amino]pyrimidine-5-carboxamide (28).** The preparation of 4-[2-fluoro-6-(hydroxymethyl)anilino]-2-[(6-methoxy-2-methyl-3,4-dihydro-1H-isoquinolin-7-yl)amino]pyrimidine-5-carboxamide was carried out according to *General Procedure 1*, using (2-amino-3-fluorophenyl)methanol (20 mg, 0.104 mmol). The crude product from Step 1 (31 mg, 0.104 mmol) was used as the starting material in Step 2, following *General Procedure 2* and Purification Method B, to isolate the title compound (8.1 mg, 18%) as a colorless solid. MS (ESI): *m/z* calc'd for C<sub>23</sub>H<sub>26</sub>FN<sub>6</sub>O<sub>3</sub> [M+H]<sup>+</sup> 453, found 453. HPLC (Sunfire C18 50X3 mm column, 45C, 1 uL injection, 215 nm) (CH<sub>3</sub>CN in 0.1% TFA): t<sub>R</sub> = 1.00 min (98% purity).

## Synthesis of Intermediates



### Intermediate I-1. 6-Methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-amine

**Step 1.** A 20-L pressure reactor was charged with Raney-Ni (200 g), MeOH (10 L) and 2-(3-methoxyphenyl)acetonitrile (1000 g, 6.80 mol). The resulting solution was stirred overnight at 35 °C under an atmosphere of  $\text{H}_2$  (30 atm). The reaction vessel was purged with  $\text{N}_2$  (g) and the mixture was filtered and concentrated in vacuo, affording of 2-(3-methoxyphenyl)ethanamine (740 g, 72% yield) as a tan oil.

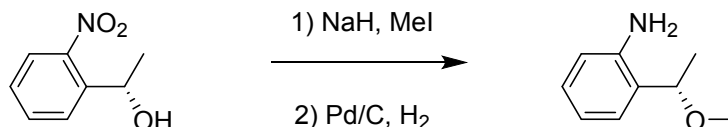
**Step 2.** A 20-L 4-necked round-bottom flask was charged with 2-(3-methoxyphenyl)ethanamine (740 g, 4.89 mol), formic acid (7.4 L),  $\text{H}_2\text{O}$  (740 mL), formaldehyde (151 g, 5.04 mol) under an atmosphere of nitrogen. The resulting solution was stirred overnight (16 h) and concentrated in vacuo. To this mixture, was added acetyl chloride (370 mL) in MeOH (6 L) and the mixture stirred at RT for 30 minutes. The mixture was concentrated in vacuo and then triturated in the presence of EtOAc (1 L) to provide the desired product, 6-methoxy-1,2,3,4-tetrahydroisoquinoline (370 g, 46% yield) as a tan solid upon vacuum filtration.

**Step 3.** A 5-L 4-necked round-bottom flask was charged with 6-methoxy-1,2,3,4-tetrahydroisoquinoline (370 g, 2.27 mol), MeOH (4.0 L) and formaldehyde (1.1 L) was added slowly at 0 °C. The resulting solution was stirred for 15 min, then  $\text{NaBH}_4$  (300 g, 7.93 mol) was

added in portions at 0 °C. The resulting solution was stirred for 3 h, and quenched with water (1 L). The resulting mixture was stirred for 30 min, then concentrated under reduced pressure. The resulting mixture was diluted with water (5 L) and extracted with DCM (3 x 3 L). The combined organic layers were concentrated in vacuo, giving 6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (260 g) as a tan solid.

**Step 4.** A 5-L 4-necked round-bottom flask was charged with TFA (2.0 L), 6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (260 g, 1.47 mol), and HNO<sub>3</sub> (150 g, 2.38 mmol). The resulting solution was stirred for 1 h and concentrated in vacuo. The residue was diluted with water (3 L), adding 4 N NaOH to adjust the pH to 10. The resulting solution was extracted with DCM (3 x 3 L) and concentrated. The residue was purified by chromatography on SiO<sub>2</sub> (5% MeOH/DCM) to provide 6-methoxy-2-methyl-7-nitro-1,2,3,4-tetrahydroisoquinoline (130 g) as an orange solid.

**Step 5.** A 3-L 4-necked round-bottom flask was charged with 6-methoxy-2-methyl-7-nitro-1,2,3,4-tetrahydroisoquinoline (130 g, 593 mmol), Pd/C (20 g, 188 mmol), MeOH (1.5 L). The flask was evacuated and flushed three times with nitrogen, followed by flushing with hydrogen gas. The mixture was stirred 3 h at 40 °C under an atmosphere of hydrogen (balloon). The mixture was filtered and the filtrate concentrated in vacuo, providing 6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-amine (97 g, 85% yield) as a tan solid. MS (EI) *m/z* calc'd for C<sub>11</sub>H<sub>17</sub>N<sub>2</sub>O [M+H]<sup>+</sup> 193, found 193. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 6.53 (s, 1 H), 6.39 (s, 1 H), 3.83 (s, 3 H), 3.62 (bs, 2 H), 3.46 (d, *J* = 1.2 Hz, 2 H), 2.83 (m, 2 H), 2.67 (m, 2 H), 2.45 (s, 3 H).



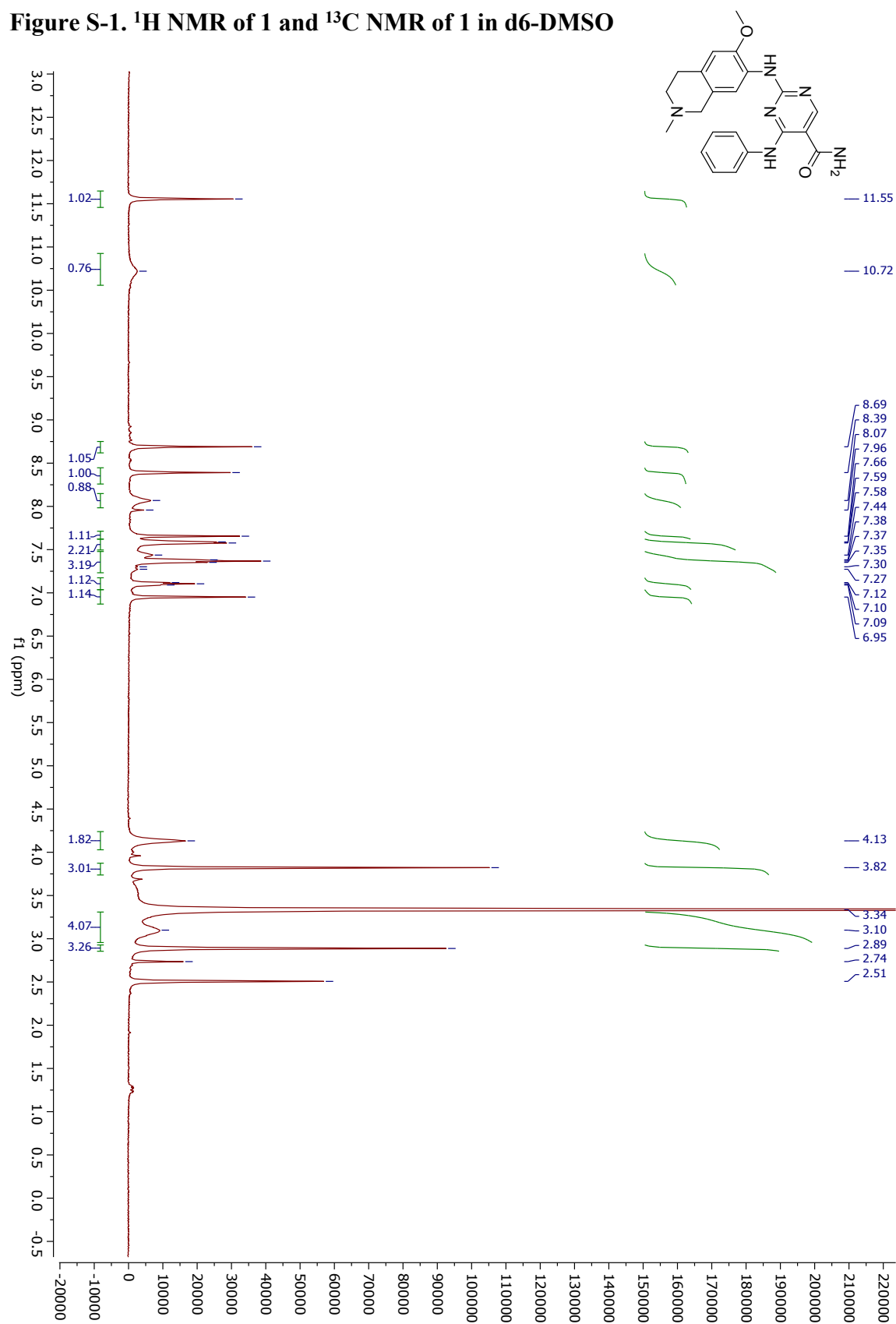
#### Intermediate I-2. (S)-2-(1-methoxyethyl)aniline (I-2)

**Step 1:** A 100 mL flask was charged with (S)-1-(2-nitrophenyl)ethan-1-ol (2.38 g, 14.24 mmol) and sodium hydride (0.85 g, 21.4 mmol) in THF (28.5 ml) at 0 °C and the mixture was allowed to warm to RT over 30 min. Methyl iodide (2.67 ml, 42.7 mmol) was added via syringe and stirred for 2 h. The mixture was quenched with sat. aq. ammonium chloride and extracted with diethyl ether,

dried ( $\text{MgSO}_4$ ), filtered, and concentrated to afford (*S*)-1-(1-methoxyethyl)-2-nitrobenzene. This material was carried forward directly onto aniline reduction.

Step 2: A 250 mL round bottom flask was charged with (*S*)-1-(1-methoxyethyl)-2-nitrobenzene (2.6 g, 14.4 mmol), MeOH (72 mL), flushed 3X with argon, and Pd-C (0.305 g, 2.87 mmol) was added in one portion. The mixture was flushed with a hydrogen balloon and stirred under hydrogen atmosphere at RT overnight. The mixture was purged with  $\text{N}_2$  and filtered over a bed of celite with EtOAc and the organics concentrated to afford (*S*)-2-(1-methoxyethyl)aniline (2.1 g, 99% yield) as a tan oil.  $^1\text{H}$  NMR (499 MHz, Chloroform- $d$ )  $\delta$  7.11 (td,  $J = 7.7, 1.5$  Hz, 1H), 7.01 (dd,  $J = 7.5, 1.3$  Hz, 1H), 6.72 (td,  $J = 7.4, 0.9$  Hz, 1H), 6.67 (d,  $J = 7.9$  Hz, 1H), 4.42 (q,  $J = 6.7$  Hz, 1H), 3.50 (s, 2H), 3.30 (s, 3H), 1.56 (d,  $J = 6.7$  Hz, 3H).

Figure S-1.  $^1\text{H}$  NMR of 1 and  $^{13}\text{C}$  NMR of 1 in  $\text{d}_6\text{-DMSO}$



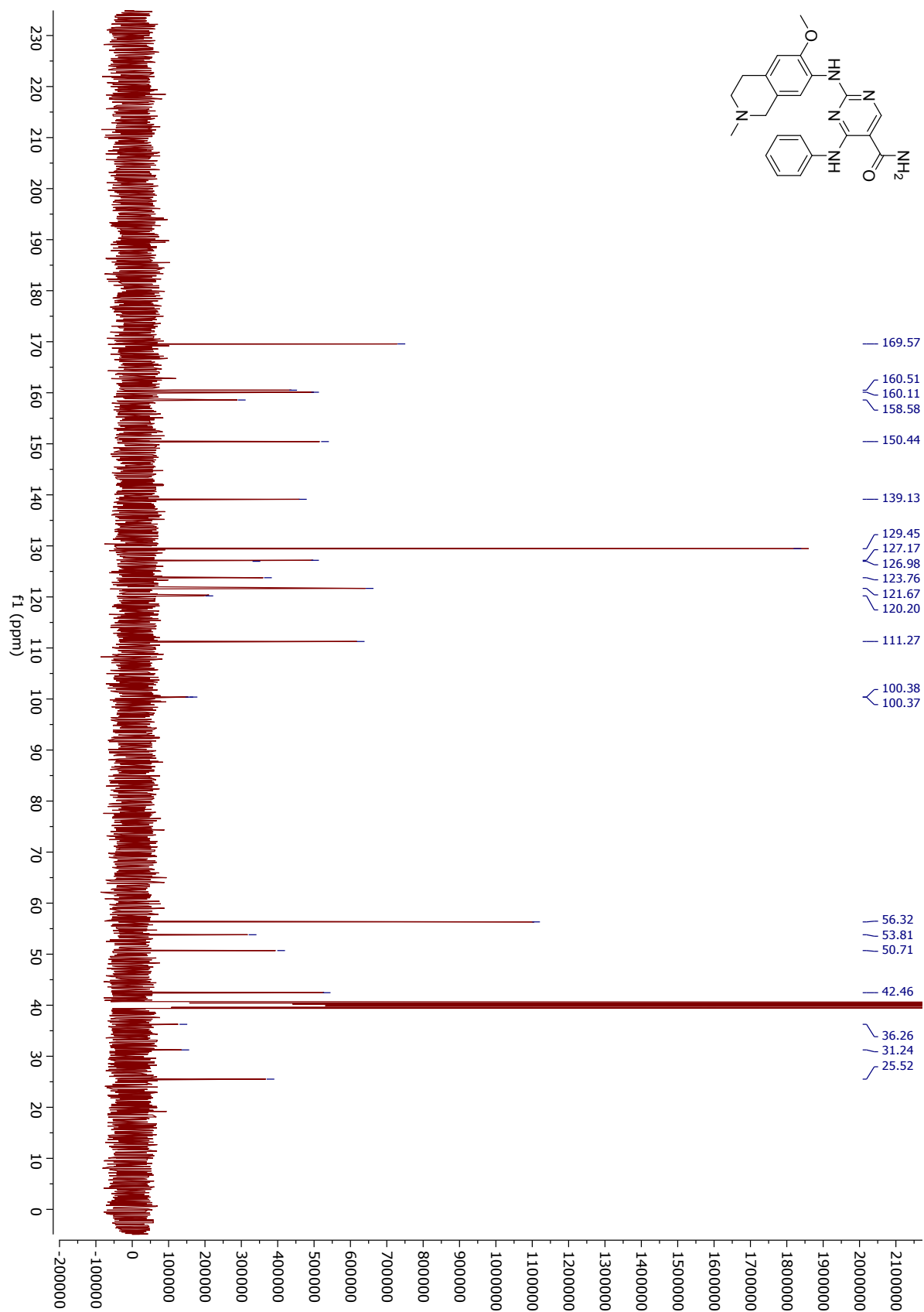
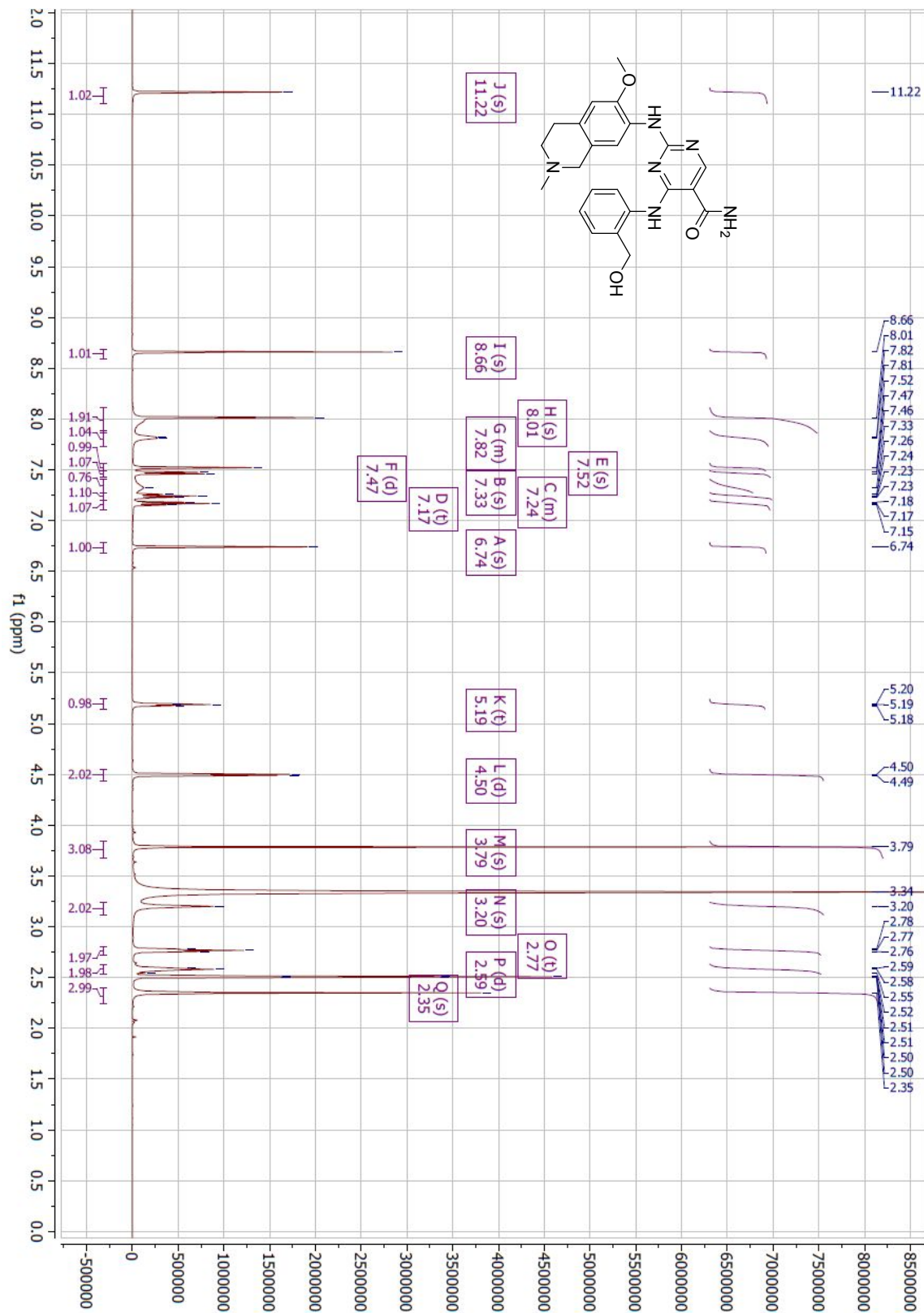


Figure S-2.  $^1\text{H}$  NMR of 22 and  $^{13}\text{C}$  NMR of 22 in  $\text{d}_6\text{-DMSO}$





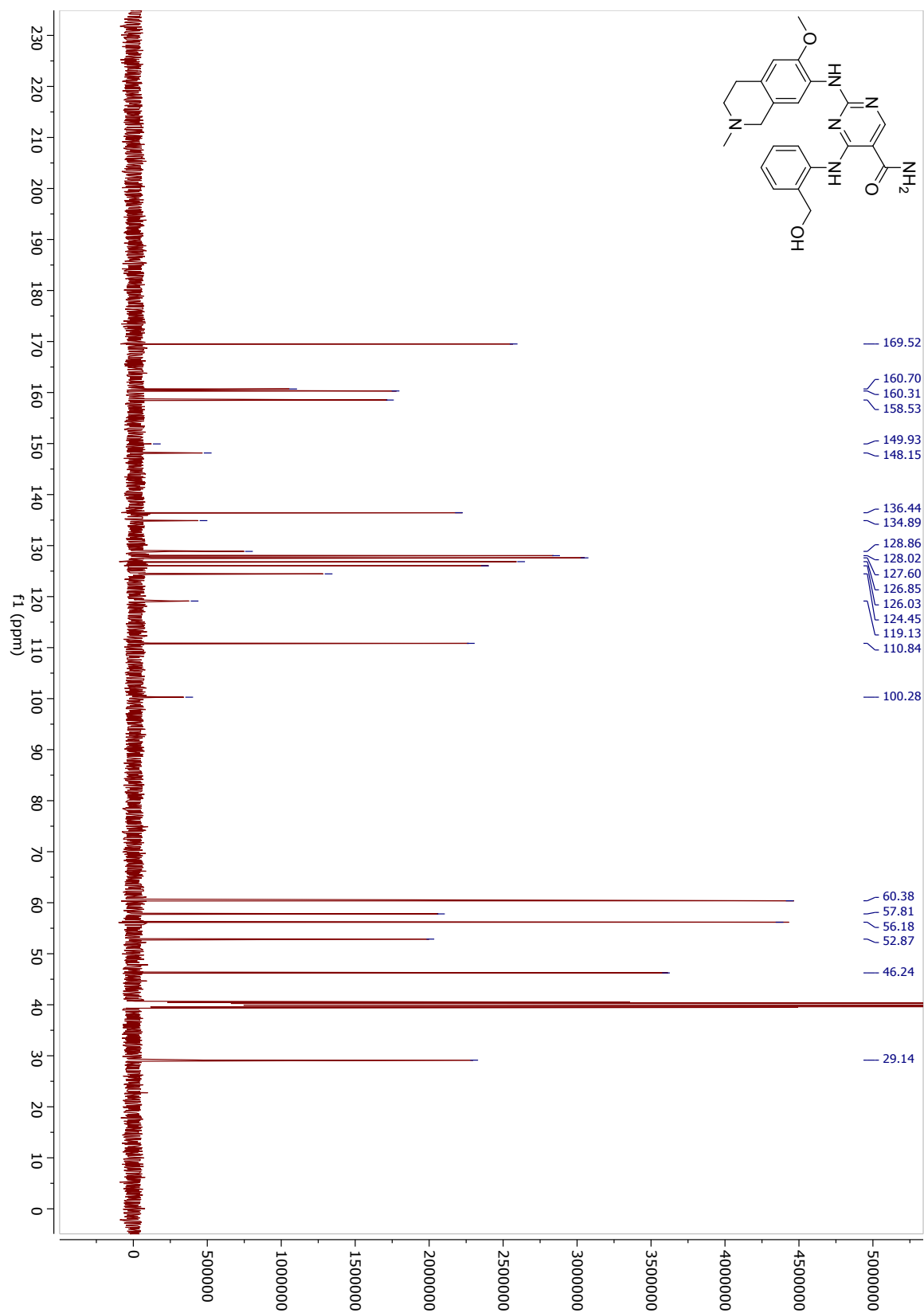
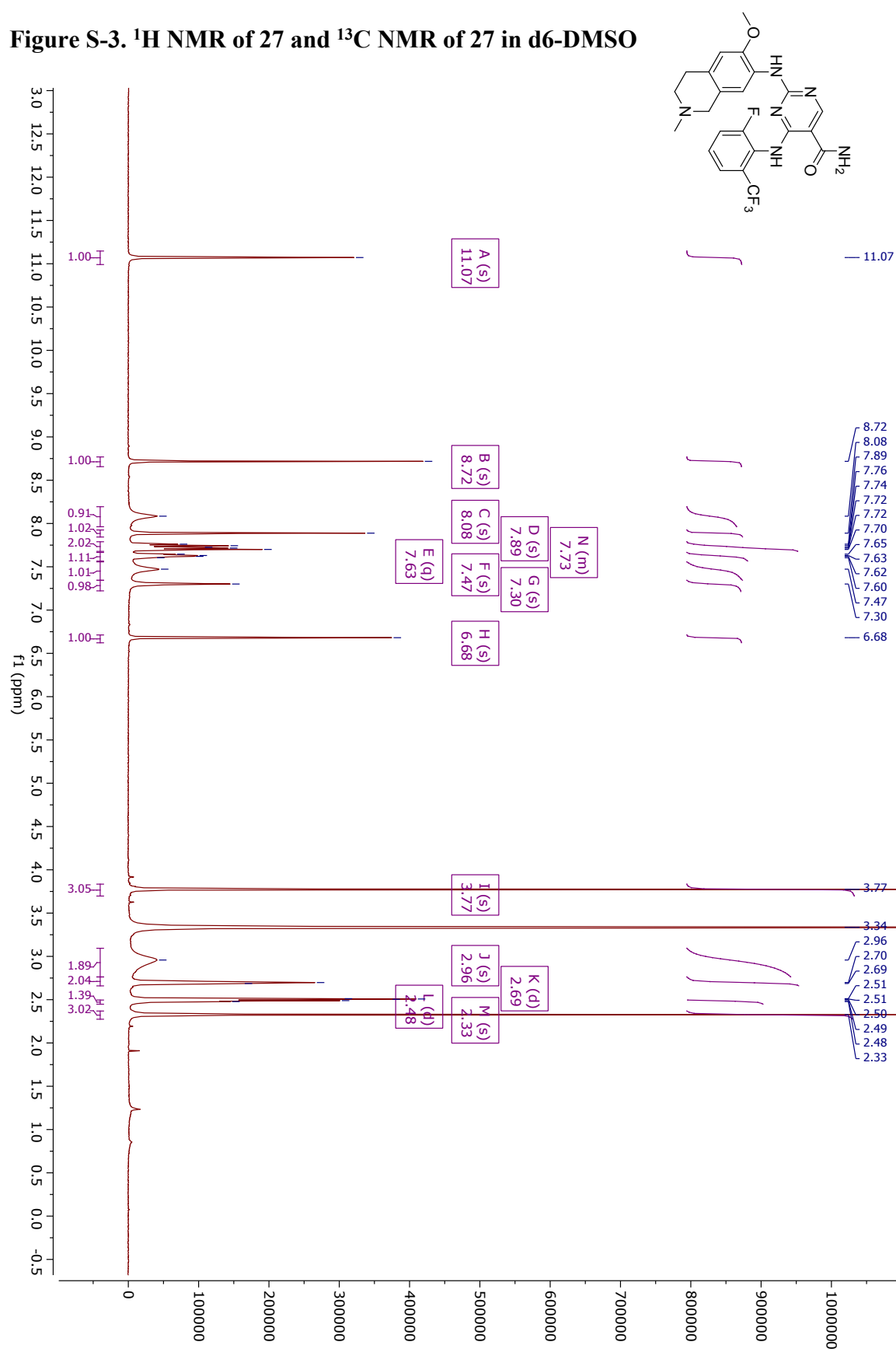
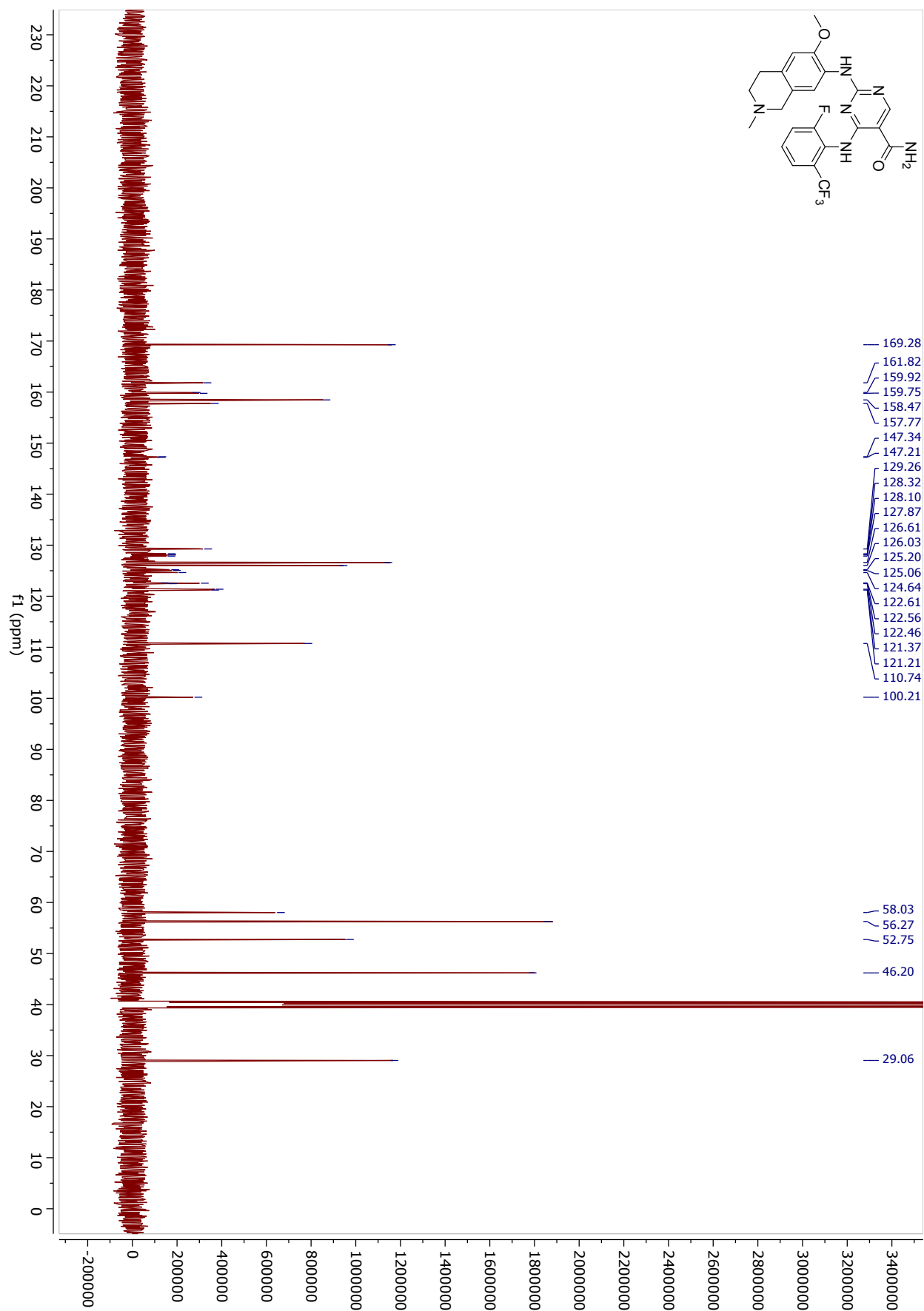


Figure S-3.  $^1\text{H}$  NMR of 27 and  $^{13}\text{C}$  NMR of 27 in  $\text{d}_6\text{-DMSO}$





## Assay Materials and Methods<sup>2</sup>

### *Commercial Reagents*

Buffers and biochemical reagents were purchased from Sigma-Aldrich unless otherwise noted (St. Louis, MO). HPK1 kinase (catalytic domain residues 1-346 accession number NP\_009112.1 with a N-terminal DYKDDDDK tag) was purchased from Carna Biosciences, Inc. (Japan). MAP4K3 kinase (catalytic domain residues 1-380 accession number NP\_003609.2 with N-terminal GST tag) was purchased from ThermoFisher Scientific (Carlsbad, CA). Anti-phospho-SLP-76 (Ser376) (D7S1K) Rabbit mAb (Alexa Fluor<sup>®</sup> 647 Conjugate) was purchased from Cell Signaling Technology (Danvers, MA). Lance Ultra Ulight-p70 S6K (Thr 389) and Lance Ultra Europium-anti-phospho-p70 S6K (Thr389) antibody were purchased from Perkin Elmer (Waltham, MA). SLP-76 and phospho-SLP-76 Ser 376 antibodies, protease/phosphatase inhibitor cocktail, mouse phospho-SLP-76 (Ser376) ELISA kit, and PathScan<sup>®</sup> human phospho-SLP-76 (Ser376) sandwich 384-well ELISA kits were purchased from Cell Signaling Technology. AlphaLISA SureFire Ultra p-SLP-76 (Ser376) HV assay kit was purchased from PerkinElmer. Phospho-SLP-76 (Ser376) cellular kit was purchased from Cisbio (Bedford, MA). Human IL-2 ELISA plates were purchased from Meso Scale Diagnostics (MSD), LLC (Rockville, Maryland). Dynabeads<sup>™</sup> Human T-Activator CD3/CD28 and 384 channel reservoirs were purchased from Thermo Fisher Scientific. CellTiter-Glo<sup>®</sup> luminescent cell viability assay reagent was purchased from Promega (Madison, WI). 384-well tissue culture plates were purchased from Corning. RPMI GlutMax media, non-essential amino acids and sodium pyruvate were purchased from Life Technologies (Carlsbad, CA). Hamilton Robotics CORE TIPS were purchased from Hamilton. Echo LDV source plates were purchased from Labcyte (San Jose, CA). Single molecule arrays (SiMoA) related reagents were purchased from Quanterix Corporation (Billerica, MA).

## ***Cells***

Cryopreserved human PBMC were purchased from Cellular Technology Limited (Shaker Heights, OH). Jurkat cells were purchased from ATCC (Manassas, VA). HPK1 deficient Jurkat knock out clones were created by Thermo Fisher Scientific (Waltham, MA). Mouse spleen T cells were isolated from C57BL/6 mice.

## **HPK1 Biochemical Assay**

All assay solutions were prepared in assay buffer consisting of 50 mM HEPES (pH 7.5), 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.01% Brij-35, 0.05 % BSA and 0.5 mM TCEP. Assay solutions were dispensed into a black 384-well assay plate (Corning 3820) using a BioRPTer (Beckman Coulter). HPK1 enzyme solution (5 µL, at one and a half times its final concentration of 75 pM) was added to all wells and the kinase reaction initiated by addition of 2.5 µL of substrate solution (at three times the final concentration of 10 nM SLP76 protein and 10 µM ATP, or at other concentrations as indicated in figure legends). Following incubation at room temperature for one hour, reactions were quenched by addition of 2.5 µL of stop and detection solution (at four times the final concentrations of 10 mM EDTA, 0.75 nM LANCE Eu-W1024 Anti-6xHis Ab and 0.75 nM Phospho-SLP-76 (Ser376) (D7S1K) XP Rabbit mAb (AF 647 Conjugate)). After an additional one-hour of incubation at room temperature, the TR-FRET signal was measured on an Envision (Perkin Elmer) plate reader set for 320 nm excitation and dual emission detection at 615nm (Eu) and 665nm (AF647).

## ***Compound Preparation and Dilution for Biochemical Inhibition Assays***

Compounds were solvated as 10 mM stocks in DMSO and were further diluted to 1 mM or 100 µM as needed for the concentration range to be tested. Compounds were serially diluted 3-fold in Echo LDV plates using a Hamilton STAR Plus liquid handler and dispensed into assay plates using an Echo acoustic dispenser (Labcyte). For inhibition assays, compounds were preincubated with either HPK1 or MAP4K3 for 30 minutes prior to initiation by addition of ATP and peptide/protein substrate. After 60-120 minutes (HPK1, MAP4K3, respectively) of incubation at

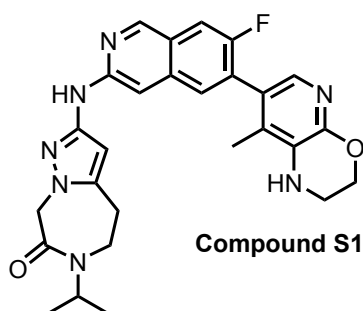
room temperature the reactions were quenched as described above. TR-FRET signal was normalized relative to control wells containing DMSO only or no kinase.

### **Human pSLP-76 Enzyme-Linked Immunosorbent Assay (ELISA)**

Human PBMC were thawed, counted, and incubated in RPMI GlutMax supplemented with NEAA, sodium pyruvate and 10% fetal bovine serum overnight in a 37°C incubator with 5% CO<sub>2</sub>. The next day, cells were counted again and seeded on 384-well tissue culture plates at a density of 60000 per well in a volume of 10µL with columns 1 and 24 as no cell blank controls. Intermediate plates containing 2x compounds were made by acoustic transfer (Echo, Labctyte) 100nL of compound and addition of 50 µL media, followed by a brief spin and 1 minute of shaking at 1200 rpm. A Hamilton liquid dispenser was used to add 10 µL of fresh media containing 2x compounds to the cell plate, followed by a brief spin to collect liquid at the bottom of the plate. Plates were incubated in a 37 °C incubator supplemented with 5% CO<sub>2</sub> for one hour. T cells were activated by adding 10 µL of anti-CD3/CD28 dynabeads (beads: cells=2:1) to the plate and 10 µL of media was used as a negative control. After a 30-second shake at 1200 rpm followed by a brief spin, plates were incubated in a 37 °C incubator with 5% CO<sub>2</sub> for 25 minutes. Using the Hamilton liquid dispenser, 10 µL of 4x lysis buffer containing protease/phosphatase inhibitor was added, followed by a brief centrifugation and 10 minutes of shaking at 1000 rpm. Plates were frozen immediately in minus 80 °C freezer. Plates were thawed the following day on 37 °C heat blocks, followed by a spin at 3500 rpm at 4 °C for 5 minutes. Cell lysates (20 µL) were transferred to a 384-well pSLP-76 ELISA plates and incubated for 2h at room temperature with constant shaking at 1000 rpm. After washing plates 6 times with 1x wash buffer, 20 µL of detection antibody was added and incubated for 1 hour at room temperature with constant shaking at 1000 rpm. After washing plates 6 times with 1x wash buffer, 20 µL of HRP-conjugated secondary antibody was added and incubated for 1 hour at room temperature with constant shaking at 1000rpm. After washing plates 6 times with 1x wash buffer, 20 µL of TMB substrate solution was added for blue color development and 20 µL of stop solution was added. Plates were read at 450nm using media only wells (no cells) as a reference blank.

## Human IL-2 assay

Human PBMC cells were thawed, seeded and treated with HPK1 inhibitors identically as mentioned in pSLP-76 ELISA assay above. The major difference is that cells were incubated with anti-CD3/CD28 dynabeads overnight for 16-18 hours in a 37 °C incubator with 5% CO<sub>2</sub>. Supernatants taken from the cell plates were diluted 8-fold with 1x PBS. Diluted supernatants (10 µL) were transferred to human IL-2 MSD ELISA plates and the assay was performed according to the manufacturer's instructions.

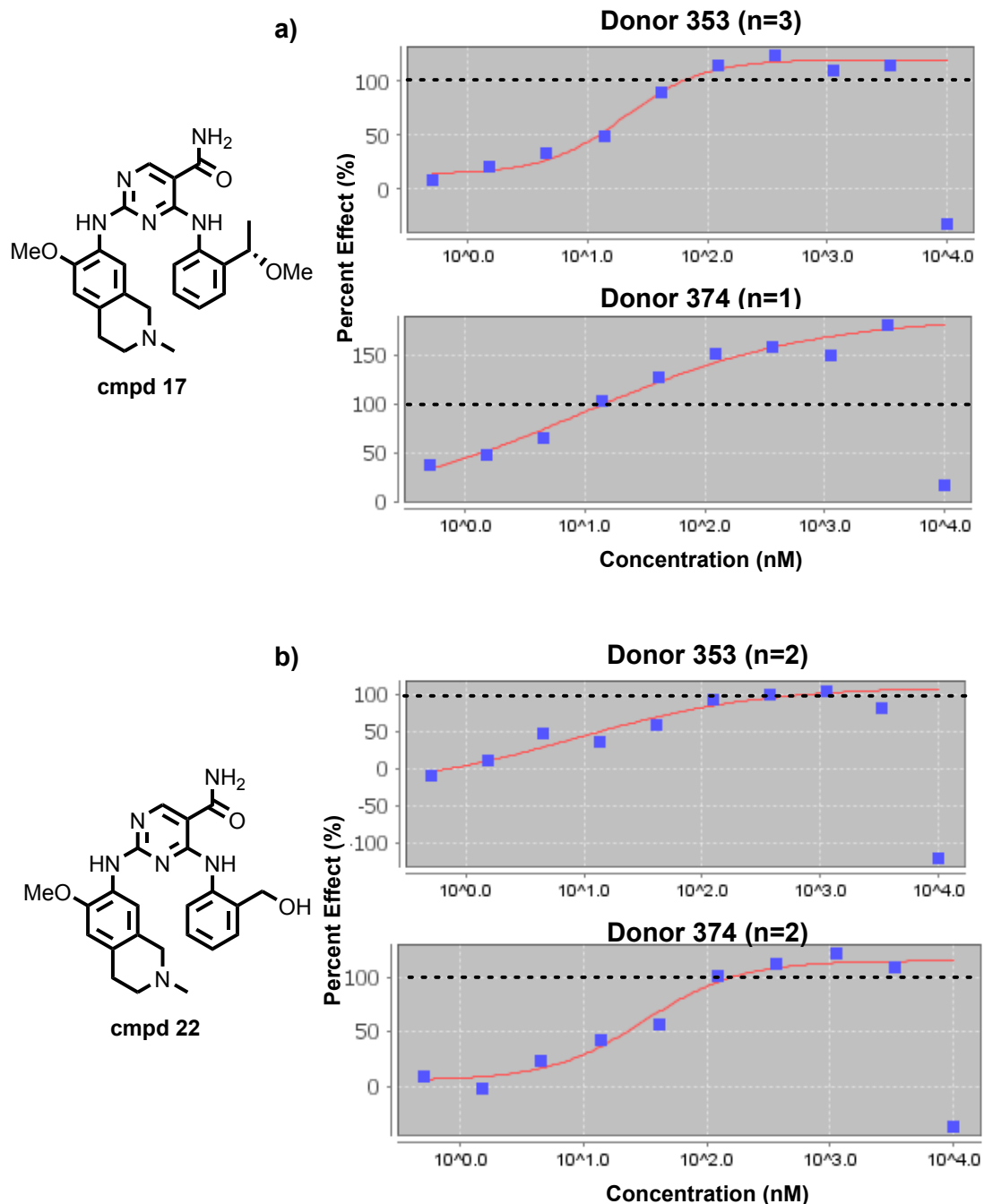


Compound **S1** derived from existing literature (Genentech):

Chan, B., *et. al.* ISOQUINOLINES AS INHIBITORS OF HPK1. *PCT Patent Publication WO 2018183964 A1* **2018**. (Genentech)

Additional work utilizing compound **S1**: Lacey, B. M., *et. al.* Development of High-Throughput Assays for Evaluation of Hematopoietic Progenitor Kinase 1 Inhibitors. *SLAS Discov. Adv. Sci. Drug Discov.* **August 2020**. <https://doi.org/10.1177/2472555220952071>.

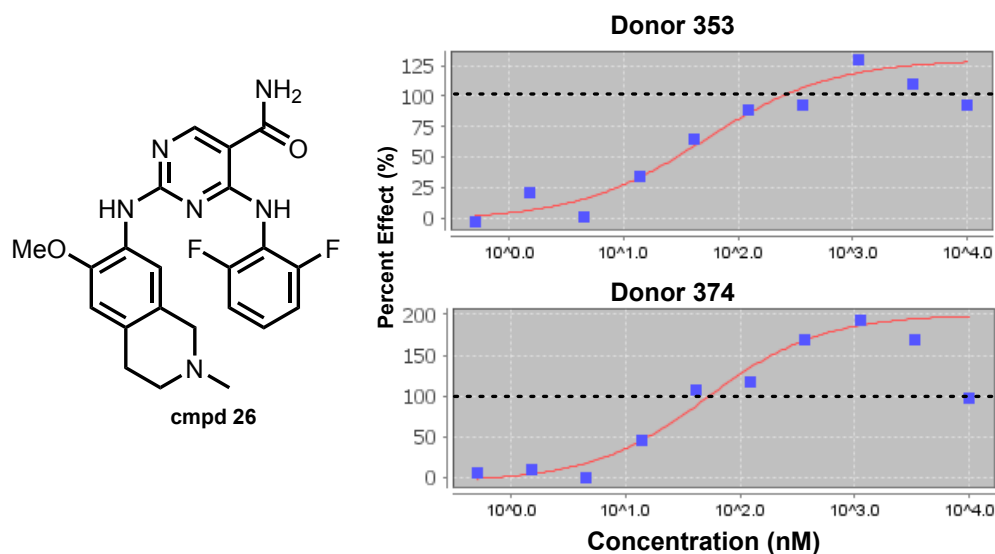
### Human PBMC IL-2 Curve Shapes for Compounds 17 and 22



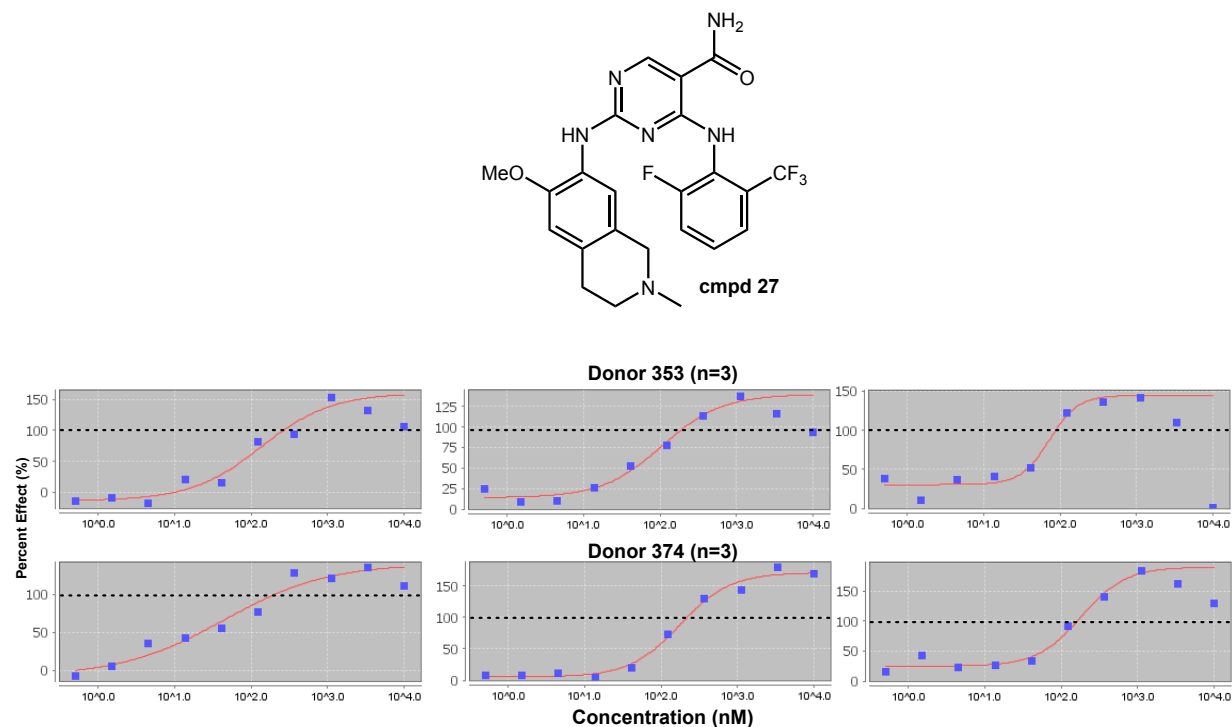
**Figure S-4.** a) IL-2 functional response curve for compound **17** in PBMC Donor 353 (n=2) and Donor 374 (n=2). b) the IL-2 functional response curve for compound **22** in PBMC Donor 353 (n=2) and Donor 374 (n=2). Standard benchmark “100% Effect” vs. control **S1** (dotted line) presents a qualitative assessment of elevated or sustained IL-2 responses when inhibitor is present.



### Human PBMC IL-2 Curve Shapes for Compound 26



### Human PBMC IL-2 Curve Shapes for Compound 27

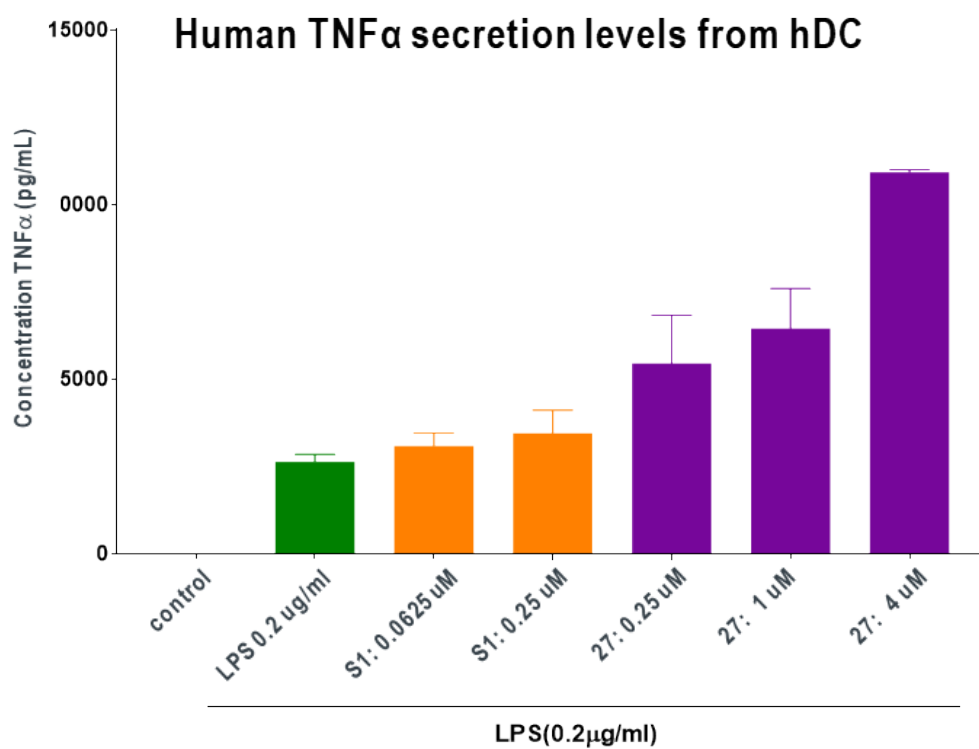
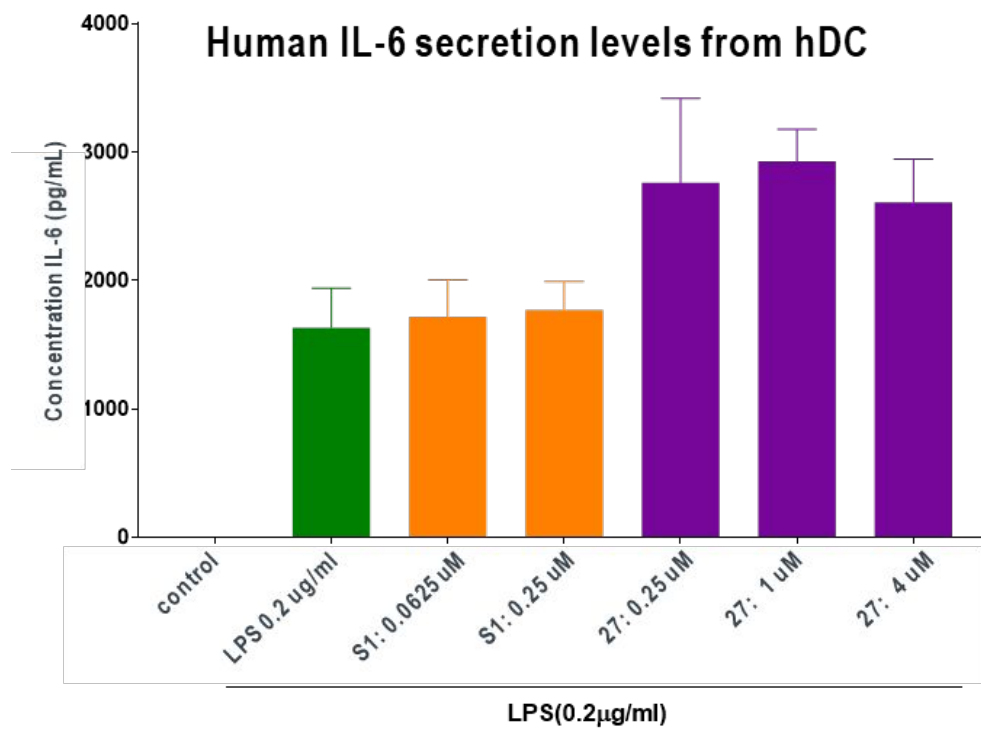


**Figure S-5.** IL-2 functional response curves for compound **27** in PBMC Donor 353 (n=3) and 374 (n=3). Peak shapes varied between donors and between runs, although this compounds consistently expressed elevated and sustained IL-2 curve shapes, suggesting a sustained IL-2 secretion through the highest concentration examined. Further, max% inhibition at the maximum dose was elevated compared to many compounds, consistently >100% effect at 10  $\mu$ M, most notably in Donor 374. Standard benchmark “100%

Effect” vs. control **S1** (dotted line) presents a qualitative assessment of elevated or sustained IL-2 responses when inhibitor is present.

### **Dendritic cell activation and analysis**

Mouse bone marrow cells were harvested from the femurs of C57/BL6 mice and cultured in cRPMI with recombinant 20 ng/mL mouse GM-CSF and 20 ng/mL mouse IL-4 for 6 days to generate immature DCs. Human DCs purchased from HemaCare and immature mouse DCs generated in house were pretreated with Compound 1 for 1 hour and then stimulated with LPS. After 18 to 24 hours of incubation, media were collected for cytokine measurement, and cells were stained with fluorescence-labeled antibodies against CD11c, mI-A/I-E, mCD40, mCD86, hCD80, hCD83 and hCD86 for flow cytometry analysis.



**Figure S-6.** IL-6 and TNF $\alpha$  secretion levels for compound **27** as compared to **S1**.

## Protein Crystallography

### *Crystallography data for Compound 1, 6, & 27*

#### Crystallographic Data Collection and Refinement Statistics

|  | HPK1 / 1                             | HPK1 / 6                             | HPK1 / 27                            |
|--|--------------------------------------|--------------------------------------|--------------------------------------|
| <b>PDB code</b>                                      | 7M0K                                 | 7M0L                                 | 7M0M                                 |
| <b>Data Collection and Processing</b>                |                                      |                                      |                                      |
| X-ray source   | SLS PXII                             | SLS PXII                             | DLS I03                              |
| Detector   | PILATUS 6M                           | PILATUS 6M                           | PILATUS 6M                           |
| Wavelength (Å)                                       | 1.0000                               | 1.0001                               | 0.9762                               |
| Temperature (K)                                      | 100                                  | 100                                  | 100                                  |
| Space group  | <i>P</i> 1                           | <i>P</i> 1                           | <i>P</i> 1                           |
| Cell: a, b, c (Å), $\alpha$ , $\beta$ , $\gamma$ (°) | 45.1, 76.6, 89.2<br>90.0, 97.0, 90.0 | 44.9, 76.7, 88.4<br>90.0, 97.8, 90.0 | 51.2, 57.0, 63.5<br>86.1, 86.6, 67.1 |
| Resolution (Å)*                                      | 80-2.01 (2.26-2.01)                  | 88-2.43 (2.68-2.43)                  | 63-1.93 (2.18-1.93)                  |
| Unique Reflections                                   | 74 742 (21 707)                      | 42 306 (10 850)                      | 48 216 (14 591)                      |
| Multiplicity   | 2.2 (2.2)                            | 2.2 (2.1)                            | 2.2 (2.3)                            |
| Completeness (%)                                     | 94.7 (92.6)                          | 96.1 (96.2)                          | 97.3 (96.1)                          |
| $R_{\text{sym}}$ (%)                                 | 3.4 (44.8)                           | 4.3 (43.3)                           | 3.9 (44.2)                           |
| $R_{\text{meas}}$ (%)                                | 4.5 (59.0)                           | 5.7 (56.3)                           | 5.1 (57.6)                           |
| Mean(I)/sd   | 12.37 (2.15)                         | 12.89 (2.99)                         | 13.66 (2.52)                         |
| <b>Refinement</b>                                    |                                      |                                      |                                      |
| Resolution (Å)                                       | 89-2.01                              | 88-2.43                              | 63-1.93                              |
| No. reflections (work / test)                        | 72 002 / 1 704                       | 41 326 / 980                         | 46 005 / 2 211                       |
| $R_{\text{cryst}} / R_{\text{free}}$ (%)             | 24.8 / 27.8                          | 24.6 / 29.3                          | 18.6 / 22.0                          |
| Total No. Atoms                                      |                                      |                                      |                                      |
| Protein  | 9 146                                | 9 102                                | 4 670                                |
| Water  | 215                                  | 72                                   | 350                                  |
| Ligand   | 120                                  | 124                                  | 70                                   |
| Deviation from Ideality                              |                                      |                                      |                                      |
| Bond lengths (Å)                                     | 0.011                                | 0.012                                | 0.011                                |
| Bond Angles (°)                                      | 1.47                                 | 1.57                                 | 1.59                                 |
| Ramachandran Analysis                                |                                      |                                      |                                      |
| Most favored (%)                                     | 92.5                                 | 90.3                                 | 91.6                                 |
| Additionally allowed (%)                             | 7.5                                  | 9.4                                  | 8.4                                  |
| Generously allowed (%)                               | 0.0                                  | 0.3                                  | 0.0                                  |
| Disallowed (%)                                       | 0.0                                  | 0.0                                  | 0.0                                  |

\* Values in parentheses indicate highest resolution shell

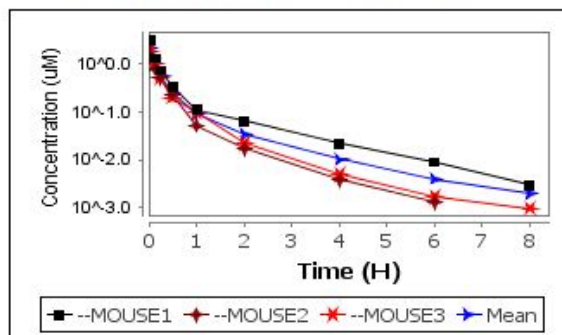
Crystal structures were obtained commercially from Proteros biostructures GmbH, Martinsried, Germany

## In vivo ADME

IV/PO mouse PK for compound **22**. 1 mg IV/10 mg PO

Route IV  
Dose 1 mg/kg  
Dose Vol 1 mL/kg  
Gender MALE  
Formulation  
DMSO/PROPYLENE GLYCOL/H<sub>2</sub>O - 23/57/20

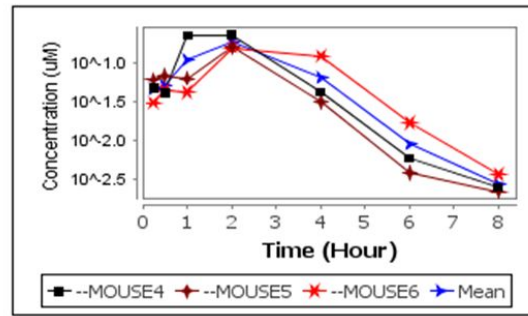
in 1 mg/kg IV Dosed MOUSE C57BL/6



|                   |           | Subject<br>MOUSE1 |       | Subject<br>MOUSE2 |       | Subject<br>MOUSE3 |       | Mean<br>n= |
|-------------------|-----------|-------------------|-------|-------------------|-------|-------------------|-------|------------|
|                   |           | (0-∞)             | (0-x) | (0-∞)             | (0-x) | (0-∞)             | (0-x) | 3          |
| AUC               | uM-h      | 0.871             | 0.865 | 0.450             | 0.448 | 0.528             | 0.526 | 0.616      |
| %AUC Extrap       | %         | 0.69              |       | 0.44              |       | 0.38              |       | 0.54       |
| Cl                | mL/min/kg | 44.0              |       | 85.2              |       | 72.6              |       | 67.3       |
| Vd                | L/kg      | 2.33              |       | 2.58              |       | 2.58              |       | 2.50       |
| MRT               | h         | 0.881             |       | 0.504             |       | 0.592             |       | 0.659      |
| t 1/2             | h         | 1.390             |       | 1.090             |       | 1.720             |       | 1.400      |
| Regression Points | h         | 4 6 8             |       | 2 4 6             |       | 4 6 8             |       |            |

in 10 mg/kg PO Dosed MOUSE C57BL/6

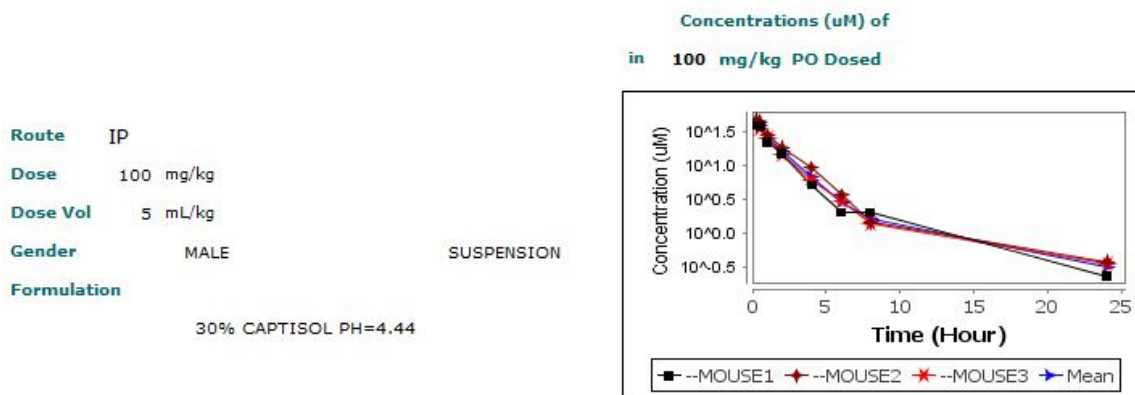
Route PO  
Dose 10 mg/kg  
Dose Vol 10 mL/kg  
Gender MALE SOLUTION  
Formulation  
DMSO/PROPYLENE GLYCOL/H2O - 23/57/20



|             |      | Subject |       | Subject |       | Subject |       | Mean  |
|-------------|------|---------|-------|---------|-------|---------|-------|-------|
|             |      | MOUSE4  |       | MOUSE5  |       | MOUSE6  |       | n=    |
|             |      | (0-∞)   | (0-x) | (0-∞)   | (0-x) | (0-∞)   | (0-x) | 3     |
| AUC         | uM-h | 0.574   | 0.571 | 0.359   | 0.356 | 0.535   | 0.531 | 0.489 |
| %AUC Extrap | %    | 0.52    |       | 0.84    |       | 0.75    |       | 0.67  |
| Cmax        | uM   | 0.23    |       | 0.16    |       | 0.15    |       | 0.18  |
| Tmax        | h    | 2.00    |       | 2.00    |       | 2.00    |       | 2.00  |
| F           | %    | 9.310   | 9.310 | 5.830   | 5.810 | 8.670   | 8.660 | 7.937 |

Compound **22** dosed 100 mpk PO with 1.5 mpk ABT (IP):

Study Notes: 15 minutes after dosing animals with **22** body temperatures decreased and activity decreased. The animals were recovered at 24 hours but did not return to normal. A homogenous opaque suspension with fine particles observed for IP. 1.50 mpk ABT was pre-dosed by 2 hours and the dose volume was 5 ml/kg. The vehicle was 0.5% methocel for ABT and clear solution was obtained.



|             |      | Subject |        | Subject |         | Subject |        | Mean    |
|-------------|------|---------|--------|---------|---------|---------|--------|---------|
|             |      | MOUSE1  |        | MOUSE2  |         | MOUSE3  |        | n=      |
|             |      | (0-∞)   | (0-x)  | (0-∞)   | (0-x)   | (0-∞)   | (0-x)  | 3       |
| AUC         | uM-h | 91.800  | 89.900 | 117.000 | 114.000 | 96.600  | 93.300 | 101.800 |
| %AUC Extrap | %    | 2.07    |        | 2.56    |         | 3.42    |        | 2.68    |
| Cmax        | uM   |         |        |         |         |         |        |         |
| Tmax        | h    |         |        |         |         |         |        |         |
| F           | %    |         |        |         |         |         |        |         |

Export -Avg

Export - Subject



## Supplemental Data Tables

### *Full Kinone panel for Compound 1, in ascending order of off-target potencies*

**Kinome Selectivity Counterscreen:** Screening performed at Invitrogen (Thermofisher), 3-point titrations. MAP4K3 screening was tested in the internal LanthaScreen binding assay and MAP4K4 and MAP4K5 screening were tested in the internal Z'-Lyte kinase assay kit.

| Enzyme               | Efficient<br>IC <sub>50</sub> (μM) | Percent<br>Inhibition | Median<br>Result Dose |
|----------------------|------------------------------------|-----------------------|-----------------------|
| CSF1R                | < 0.001                            | 89.49                 | 0.01                  |
| STK22D               | 0.004021                           | 68.76                 | 0.01                  |
| LRRK2 (G2019S)       | 0.005564                           | 59.71                 | 0.01                  |
| STK4                 | 0.00707                            | 53.95                 | 0.01                  |
| LRRK2                | 0.00723                            | 55.46                 | 0.01                  |
| STK3                 | 0.008521                           | 50.01                 | 0.01                  |
| STK25                | 0.01015                            | 48.56                 | 0.01                  |
| MST4                 | 0.01039                            | 46.71                 | 0.01                  |
| STK24                | 0.01112                            | 46.3                  | 0.01                  |
| PRKD1                | 0.01282                            | 42.97                 | 0.01                  |
| TNIK                 | 0.01335                            |                       |                       |
| FES                  | 0.01613                            | 39.6                  | 0.01                  |
| NUAK1                | 0.01649                            | 40.91                 | 0.01                  |
| TYK2                 | 0.01699                            | 37.15                 | 0.01                  |
| TAOK2                | 0.01706                            | 37.34                 | 0.01                  |
| PRKD2                | 0.02007                            | 30.86                 | 0.01                  |
| PRKCN                | 0.02039                            | 33.79                 | 0.01                  |
| PTK2                 | 0.02328                            | 24.04                 | 0.01                  |
| EGFR(T790M<br>L858R) | 0.02655                            | 27.93                 | 0.01                  |

|                         |         |       |     |
|-------------------------|---------|-------|-----|
| PAK7                    | 0.03193 | 73.78 | 0.1 |
| EGFR(T790M)             | 0.03323 | 71.84 | 0.1 |
| RPS6KA2                 | 0.0347  | 72.6  | 0.1 |
| FER                     | 0.04081 | 69.82 | 0.1 |
| PTK2B                   | 0.04775 | 63.98 | 0.1 |
| JAK2                    | 0.05261 | 63.5  | 0.1 |
| JAK3                    | 0.05662 | 62.65 | 0.1 |
| PAK4                    | 0.05666 | 61.9  | 0.1 |
| FGR                     | 0.05938 | 61.17 | 0.1 |
| RPS6KA3                 | 0.06156 | 55.08 | 0.1 |
| INSRR                   | 0.07655 | 54.26 | 0.1 |
| STK22B                  | 0.08203 | 53.16 | 0.1 |
| JAK2 JH1 JH2 MT         | 0.08207 | 53.74 | 0.1 |
| YES1                    | 0.09064 | 50.15 | 0.1 |
| PRKCG                   | 0.09239 | 50.62 | 0.1 |
| RPS6KA1                 | 0.09691 | 50.32 | 0.1 |
| JAK1                    | 0.09834 | 50.44 | 0.1 |
| IGF1R                   | 0.1009  | 50.66 | 0.1 |
| LYNB                    | 0.1055  | 48.67 | 0.1 |
| JAK2 JH1 JH2<br>(V617F) | 0.1105  | 48.03 | 0.1 |
| FLT3(D835Y)             | 0.1208  | 45.6  | 0.1 |
| PDGFRA(V561D)           | 0.1326  | 43.1  | 0.1 |
| NEK4                    | 0.1421  | 42.7  | 0.1 |
| FRK                     | 0.1476  | 42.14 | 0.1 |
| CLK2                    | 0.1638  | 40.55 | 0.1 |
| HCK                     | 0.1646  | 40.01 | 0.1 |
| INSR                    | 0.1649  | 37.83 | 0.1 |

|              |        |       |     |
|--------------|--------|-------|-----|
| DCAMKL2      | 0.1764 | 39.34 | 0.1 |
| LCK          | 0.1911 | 34.89 | 0.1 |
| DAPK3        | 0.1912 | 36.16 | 0.1 |
| LYN          | 0.1916 | 35.64 | 0.1 |
| MAPK8        | 0.2076 | 30.88 | 0.1 |
| PRKCD        | 0.2128 | 32.5  | 0.1 |
| NEK6         | 0.2161 | 37.08 | 0.1 |
| IRAK1        | 0.2206 | 34.19 | 0.1 |
| BLK          | 0.2257 | 30.33 | 0.1 |
| FGFR1        | 0.2346 | 33.17 | 0.1 |
| MARK3        | 0.2441 | 32.43 | 0.1 |
| RET(V804L)   | 0.2521 | 28.49 | 0.1 |
| MELK         | 0.2585 | 32.21 | 0.1 |
| PRKCA        | 0.2656 | 29.41 | 0.1 |
| MARK4        | 0.2696 | 26.78 | 0.1 |
| AXL          | 0.2836 | 28.57 | 0.1 |
| PAK2         | 0.2888 | 25.34 | 0.1 |
| CSNK1E       | 0.3035 | 28.55 | 0.1 |
| SNF1LK2      | 0.3037 | 28.16 | 0.1 |
| PRKCB2       | 0.3051 | 73.44 | 1   |
| PAK2         | 0.312  | 73.81 | 1   |
| FLT3         | 0.3128 | 69.47 | 1   |
| DAPK1        | 0.3423 | 27.96 | 0.1 |
| NEK2         | 0.3566 | 72.46 | 1   |
| FGFR3(K650E) | 0.379  | 70.94 | 1   |
| MAPK9        | 0.3795 | 69.82 | 1   |
| CAMK I ALPHA | 0.3863 | 72.15 | 1   |
| SRC N1       | 0.3866 | 70.5  | 1   |

|                             |        |       |   |
|-----------------------------|--------|-------|---|
| ABL1(G250E)                 | 0.3923 | 71.34 | 1 |
| AMPK ALPHA2<br>BETA1 GAMMA1 | 0.3938 | 69.05 | 1 |
| PAK1                        | 0.3996 | 62.73 | 1 |
| MUSK                        | 0.4068 | 73.79 | 1 |
| NEK9                        | 0.4176 | 69.51 | 1 |
| IRAK4                       | 0.4733 | 66.66 | 1 |
| CSNK1D                      | 0.4751 | 66.21 | 1 |
| FGFR2                       | 0.4835 | 65.03 | 1 |
| RET(Y791F)                  | 0.4942 | 64.41 | 1 |
| AMPK ALPHA1<br>BETA1 GAMMA1 | 0.5291 | 67.14 | 1 |
| MAPK10                      | 0.5354 | 58.06 | 1 |
| PDGFRB                      | 0.5363 | 57.25 | 1 |
| PDGFRA                      | 0.5467 | 66.57 | 1 |
| RET                         | 0.5546 | 58.35 | 1 |
| ROS1                        | 0.5674 | 61.29 | 1 |
| SRC                         | 0.5829 | 60.03 | 1 |
| KIT                         | 0.6137 | 55    | 1 |
| PRKCH                       | 0.6437 | 56.62 | 1 |
| TBK1                        | 0.6439 | 55.82 | 1 |
| PDGFRA(D842V)               | 0.6459 | 59.77 | 1 |
| ABL1(Y253F)                 | 0.6546 | 57.31 | 1 |
| MARK1                       | 0.6844 | 54.56 | 1 |
| LTK                         | 0.7112 | 52.95 | 1 |

|              |        |       |   |
|--------------|--------|-------|---|
| CDC42BPA     | 0.7315 | 54.54 | 1 |
| CHEK1        | 0.7775 | 54.04 | 1 |
| CHEK2        | 0.7838 | 53.35 | 1 |
| NEK1         | 0.7841 | 55.71 | 1 |
| FYN          | 0.8016 | 54.77 | 1 |
| RPS6KA6      | 0.8019 | 53.67 | 1 |
| ERBB4        | 0.8052 | 53.42 | 1 |
| MAP3K9       | 0.8193 | 52.51 | 1 |
| CLK1         | 0.8422 | 53.29 | 1 |
| PLK1         | 0.8973 | 50.66 | 1 |
| PRKCB1       | 0.9111 | 51.26 | 1 |
| BTK          | 0.9286 | 51.28 | 1 |
| ABL1(E255K)  | 0.9623 | 47.96 | 1 |
| MARK2        | 0.9904 | 49.32 | 1 |
| CDK7         | 1      | 50.04 | 1 |
| PTK6         | 1.021  | 50.01 | 1 |
| PAK3         | 1.057  | 51.3  | 1 |
| CAMK I DELTA | 1.079  | 48.48 | 1 |
| ABL1         | 1.144  | 47.79 | 1 |
| GRK7         | 1.17   | 45.59 | 1 |
| ITK          | 1.223  | 44.06 | 1 |
| CDC42BPB     | 1.252  | 44.91 | 1 |
| ABL2(ARG)    | 1.402  | 42.17 | 1 |
| PRKCQ        | 1.458  | 45.37 | 1 |
| PHK GAMMA1   | 1.516  | 39.73 | 1 |
| CSNK1G2      | 1.617  | 38.15 | 1 |
| SYK          | 1.677  | 38.55 | 1 |

|               |       |       |   |
|---------------|-------|-------|---|
| BRAF(V599E)   | 1.7   | 34.88 | 1 |
| NTRK3         | 1.719 | 36.54 | 1 |
| CSNK1A1       | 1.812 | 34.8  | 1 |
| PKN1          | 1.886 | 34.93 | 1 |
| EGFR(L861Q)   | 1.911 | 35.92 | 1 |
| ROCK1         | 1.913 | 35.03 | 1 |
| ABL1(T315I)   | 1.927 | 34.22 | 1 |
| TXK           | 1.972 | 37.13 | 1 |
| CAMK II DELTA | 2.005 | 33.42 | 1 |
| DYRK1A        | 2.141 | 33    | 1 |
| AURKB         | 2.155 | 34.28 | 1 |
| EGFR(L858R)   | 2.27  | 31.36 | 1 |
| PRKCE         | 2.308 | 29.39 | 1 |
| ROCK2         | 2.325 | 29.93 | 1 |
| NTRK1         | 2.471 | 30.76 | 1 |
| EGFR          | 2.489 | 30.6  | 1 |
| SRMS          | 2.535 | 28.8  | 1 |
| MAP2K2        | 2.71  | 25.25 | 1 |
| TYRO3         | 2.756 | 27.05 | 1 |
| FGFR3         | 2.825 | 30.47 | 1 |
| IKK_EPSILON   | 2.883 | 25.44 | 1 |
| PDK1          | 2.939 | 29.88 | 1 |
| CSK           | 2.952 | 24.65 | 1 |
| MAP2K6        | 3.071 | 26.31 | 1 |
| NTRK2         | 3.1   | 24.91 | 1 |
| IKKA          | 3.185 | 30.62 | 1 |
| DYRK1B        | 3.199 | 24.12 | 1 |

|                       |       |         |      |
|-----------------------|-------|---------|------|
| FGFR4                 | 3.428 | 22.85   | 1    |
| SGK                   | 3.509 | 22.09   | 1    |
| BRAF                  | 3.62  | 20.98   | 1    |
| GRK6                  | 3.659 | 21.56   | 1    |
| RAF1( Y340D<br>Y341D) | 3.724 | 21.48   | 1    |
| KIT(T670I)            | 3.745 | 24.12   | 1    |
| CSNK1G3               | 3.8   | 20.33   | 1    |
| EEF2K                 | 3.887 | 21.42   | 1    |
| ERBB2                 | 3.954 | 21.17   | 1    |
| PRKX                  | 4.341 | 24.75   | 1    |
| ADRBK1                | 10    | 17.13   | 1    |
| ADRBK2                | 10    | 7.147   | 1    |
| AKT1 ALPHA            | 10    | 4.369   | 0.01 |
| AKT2 BETA             | 10    | 8.911   | 1    |
| AKT2 GAMMA            | 10    | 8.007   | 1    |
| ALK4                  | 10    | -0.772  | 0.1  |
| AURKA                 | 10    | 6.016   | 1    |
| AURKC                 | 10    | 12.66   | 1    |
| BMX                   | 10    | 17.2    | 1    |
| BRSK1                 | 10    | 7.57    | 1    |
| CAMK II ALPHA         | 10    | 7.969   | 1    |
| CAMK II BETA          | 10    | 12.11   | 1    |
| CAMK4                 | 10    | -0.4773 | 1    |
| CDK1                  | 10    | 10.11   | 1    |
| CDK2                  | 10    | 13.5    | 1    |
| CDK5/P25              | 10    | 8.675   | 1    |

|                 |    |       |      |
|-----------------|----|-------|------|
| CDK5/P35        | 10 | 10.48 | 1    |
| CDK9(CYCLIN T1) | 10 | 22.6  | 1    |
| CLK3            | 10 | 6.37  | 0.01 |
| CSNK1G1         | 10 | 12.25 | 1    |
| CSNK2A1         | 10 | 5.967 | 1    |
| CSNK2A2         | 10 | 13.18 | 1    |
| DNA-PK          | 10 | 13.81 | 1    |
| DYRK3           | 10 | 7.455 | 0.1  |
| DYRK4           | 10 | 1.355 | 0.01 |
| EPHA1           | 10 | 6.453 | 0.1  |
| EPHA2           | 10 | 18.51 | 1    |
| EPHA3           | 10 | 2.96  | 0.01 |
| EPHA4           | 10 | 4.167 | 0.01 |
| EPHA5           | 10 | 16.58 | 1    |
| EPHA8           | 10 | 17.26 | 0.01 |
| EPHB1           | 10 | 8.069 | 1    |
| EPHB2           | 10 | 13.06 | 1    |
| EPHB3           | 10 |       |      |
| EPHB4           | 10 | 15.39 | 1    |
| FLT1            | 10 | 5.15  | 0.01 |
| FLT4            | 10 | 18.17 | 1    |
| FRAP1(MTOR)     | 10 | 1.562 | 0.1  |
| GRK4            | 10 | 15.27 | 1    |
| GRK5            | 10 | 11.04 | 1    |
| GSG2            | 10 |       |      |
| GSK3A           | 10 | 14.5  | 1    |
| GSK3B           | 10 | 17.78 | 0.1  |
| HIPK1           | 10 | 11.06 | 1    |
| HIPK2           | 10 | 16.57 | 1    |



|  |    |       |      |
|--|----|-------|------|
| HIPK3 (YAK1)                               | 10 | 13.51 | 1    |
| HIPK4                                      | 10 | 19.05 | 1    |
| IKK_BETA                                   | 10 | 11.91 | 1    |
| KDR  | 10 | 16.21 | 1    |
| MAP2K1                                     | 10 | 16.1  | 1    |
| MAP3K8                                     | 10 | 1.935 | 1    |
| MAPK1                                      | 10 | 6.05  | 0.1  |
| MAPK11                                     | 10 | 10.1  | 1    |
| MAPK12                                     | 10 | 18.47 | 1    |
| MAPK13                                     | 10 | 15.28 | 1    |
| MAPK14 (P38<br>ALPHA/INACTIVE<br>MAPKAPK2) | 10 | 12.93 | 1    |
| MAPK14 ALPHA                               | 10 | 2.829 | 0.01 |
| MAPK3(ERK1)                                | 10 | 16.69 | 1    |
| MAPKAPK2                                   | 10 | 2.015 | 1    |
| MAPKAPK3                                   | 10 | 5.359 | 1    |
| MAPKAPK5                                   | 10 | 6.519 | 1    |
| MATK                                       | 10 | 5.354 | 0.01 |
| MERTK                                      | 10 | 10.28 | 1    |
| MET  | 10 | 14.77 | 1    |
| MET(M1250T)                                | 10 | 10.1  | 1    |
| MKNK1                                      | 10 | 13.52 | 1    |
| MST1R                                      | 10 | 11.48 | 0.01 |
| MYLK2                                      | 10 | 11.49 | 1    |

|                |    |        |      |
|----------------|----|--------|------|
| PASK           | 10 | 19.8   | 1    |
| PDGFRA(T674I)  | 10 | 4.972  | 0.1  |
| PHKG2          | 10 | 24.48  | 1    |
| PI4KA          | 10 | 3.501  | 0.1  |
| PI4KB          | 10 | 12.08  | 1    |
| PIK3C2 ALPHA   | 10 | 2.361  | 1    |
| PIK3C2 BETA    | 10 | 11.88  | 0.1  |
| PIK3C3         | 10 | 8.011  | 1    |
| PIK3CG         | 10 | 5.852  | 1    |
| PIK3P110(P85)  | 10 | 9.959  | 0.1  |
| PIK3P110D/P85A | 10 | 9.654  | 0.01 |
| PIM1           | 10 | 8.477  | 0.1  |
| PIM2           | 10 | 10.73  | 1    |
| PKG2           | 10 | 6.099  | 1    |
| PLK2           | 10 | 14.74  | 1    |
| PLK3           | 10 | 14.49  | 0.1  |
| PRKACA         | 10 | 17.68  | 1    |
| PRKCI          | 10 | 7.44   | 1    |
| PRKCZ          | 10 | 7.093  | 0.01 |
| PRKG1          | 10 | 16.73  | 1    |
| RPS6KA4        | 10 | 8.054  | 1    |
| RPS6KA5        | 10 | 8.323  | 1    |
| RPS6KB1        | 10 | 11.21  | 1    |
| SGK2           | 10 | 15.19  | 1    |
| SGKL           | 10 | 11.41  | 1    |
| SPHK1          | 10 | 0.0036 | 1    |
| SPHK2          | 10 | 0.2078 | 0.01 |
| SRPK1          | 10 | 8.011  | 0.01 |

|       |    |       |      |
|-------|----|-------|------|
| SRPK2 | 10 | 2.884 | 0.1  |
| STK23 | 10 | 4.376 | 0.01 |
| TEK   | 10 | 7.407 | 0.01 |
| ZAP70 | 10 | 7.443 | 1    |
| EPHB3 |    | 2.645 | 0.01 |
| GSG2  |    | 11.94 | 1    |
| TNIK  |    | 45.5  | 0.01 |

*Full Kinone panel for Compound 7, in ascending order of off-target potencies*

| Enzyme               | Efficient<br>IC <sub>50</sub> (μM) | Percent<br>Inhibition | Median Result<br>Dose |
|----------------------|------------------------------------|-----------------------|-----------------------|
| LRRK2                | 0.004705                           |                       |                       |
| TAOK2                | 0.004984                           | 59.13                 | 0.01                  |
| LRRK2 (G2019S)       | 0.005409                           | 64.26                 | 0.01                  |
| MST4                 | 0.005839                           | 54.05                 | 0.01                  |
| STK4                 | 0.006247                           | 54.28                 | 0.01                  |
| STK25                | 0.008539                           | 52.83                 | 0.01                  |
| STK3                 | 0.009                              | 48.92                 | 0.01                  |
| STK24                | 0.01078                            | 45.97                 | 0.01                  |
| TYK2                 | 0.01101                            | 47.83                 | 0.01                  |
| NUAK1                | 0.01137                            | 48.45                 | 0.01                  |
| STK22D               | 0.02095                            | 36.36                 | 0.01                  |
| CSF1R                | 0.02315                            | 34.1                  | 0.01                  |
| PTK2                 | 0.04429                            | 69.75                 | 0.1                   |
| JAK3                 | 0.05794                            | 62.53                 | 0.1                   |
| PRKCN                | 0.0843                             | 44.7                  | 0.1                   |
| EGFR(T790M<br>L858R) | 0.09405                            | 52.11                 | 0.1                   |
| JAK2                 | 0.09858                            | 48.42                 | 0.1                   |
| PRKD1                | 0.1035                             | 50.07                 | 0.1                   |
| LYNB                 | 0.1159                             | 46.18                 | 0.1                   |
| TNIK                 | 0.1216                             | 44.91                 | 0.1                   |
| JAK1                 | 0.1218                             | 50.05                 | 0.1                   |
| PRKD2                | 0.1342                             | 43.43                 | 0.1                   |
| FGR                  | 0.1353                             | 42.32                 | 0.1                   |
| DCAMKL2              | 0.1583                             | 40.16                 | 0.1                   |

|                             |        |       |     |
|-----------------------------|--------|-------|-----|
| MARK3                       | 0.1677 | 38.91 | 0.1 |
| RPS6KA2                     | 0.1809 | 38.82 | 0.1 |
| RPS6KA3                     | 0.1823 | 38.94 | 0.1 |
| EGFR(T790M)                 | 0.1896 | 34.72 | 0.1 |
| JAK2 JH1 JH2<br>(V617F)     | 0.2012 | 34.06 | 0.1 |
| MARK4                       | 0.215  | 31.34 | 0.1 |
| JAK2 JH1 JH2 MT             | 0.2253 | 29.88 | 0.1 |
| HCK                         | 0.2638 | 72.17 | 1   |
| FER                         | 0.2661 | 30.13 | 0.1 |
| LYN                         | 0.2682 | 31.21 | 0.1 |
| RPS6KA1                     | 0.281  | 28.31 | 0.1 |
| PRKCD                       | 0.2913 | 27.3  | 0.1 |
| FES                         | 0.2993 | 29.12 | 0.1 |
| LCK                         | 0.3255 | 71.45 | 1   |
| BLK                         | 0.3499 | 69.63 | 1   |
| LCK                         | 0.3531 | 68.87 | 1   |
| PRKCG                       | 0.36   | 77.11 | 1   |
| AMPK ALPHA1<br>BETA1 GAMMA1 | 0.3903 | 71.46 | 1   |
| SNF1LK2                     | 0.4011 | 66.54 | 1   |
| PAK7                        | 0.4062 | 69.04 | 1   |
| FGFR1                       | 0.427  | 58.18 | 1   |
| YES1                        | 0.4329 | 65.59 | 1   |
| PRKCA                       | 0.4857 |       |     |
| PRKCB2                      | 0.4868 | 65.17 | 1   |
| PTK2B                       | 0.4923 | 63.75 | 1   |

|                             |        |       |   |
|-----------------------------|--------|-------|---|
| AMPK ALPHA2<br>BETA1 GAMMA1 | 0.5584 | 62.1  | 1 |
| CDC42BPA                    | 0.5662 | 61.73 | 1 |
| MARK2                       | 0.5763 | 59.81 | 1 |
| FRK                         | 0.6    | 60.39 | 1 |
| PDGFRA(V561D)               | 0.6442 | 58.54 | 1 |
| PAK4                        | 0.6611 | 56.62 | 1 |
| STK22B                      | 0.6692 | 59.63 | 1 |
| MARK1                       | 0.6835 | 56.73 | 1 |
| ROCK1                       | 0.7321 | 53.66 | 1 |
| PAK2                        | 0.8384 | 53.02 | 1 |
| FLT3(D835Y)                 | 0.8849 | 50.66 | 1 |
| PRKCQ                       | 0.9139 | 54.61 | 1 |
| CLK2                        | 0.9189 | 50.29 | 1 |
| CDC42BPB                    | 1.022  | 50.76 | 1 |
| ABL1(G250E)                 | 1.261  | 45.73 | 1 |
| AURKB                       | 1.52   | 42.07 | 1 |
| MAPK12                      | 1.527  | 39.38 | 1 |
| ABL1(Y253F)                 | 1.549  | 39.58 | 1 |
| CAMK II DELTA               | 1.555  | 38.75 | 1 |
| ABL1(E255K)                 | 1.618  | 38.28 | 1 |
| CAMK I ALPHA                | 1.736  | 37.71 | 1 |
| FYN                         | 1.758  | 37.38 | 1 |
| FLT3                        | 1.799  | 41.03 | 1 |
| ROCK2                       | 1.817  | 35.91 | 1 |

|              |       |       |   |
|--------------|-------|-------|---|
| MELK         | 1.866 | 33.65 | 1 |
| MAP3K9       | 1.976 | 36.87 | 1 |
| PAK2         | 2     | 33.19 | 1 |
| SRC N1       | 2.001 | 33.71 | 1 |
| CHEK2        | 2.03  | 34.89 | 1 |
| CAMK I DELTA | 2.077 | 32.76 | 1 |
| PRKCB1       | 2.099 | 35.05 | 1 |
| KIT          | 2.111 | 35.94 | 1 |
| IGF1R        | 2.178 | 32.31 | 1 |
| ABL1         | 2.198 | 31.64 | 1 |
| CDK7         | 2.229 |       |   |
| IRAK4        | 2.31  | 36.55 | 1 |
| FGFR3(K650E) | 2.367 | 29.85 | 1 |
| RPS6KA6      | 2.372 | 29.91 | 1 |
| SRC          | 2.425 | 32.18 | 1 |
| INSRR        | 2.463 | 28.94 | 1 |
| PAK3         | 2.469 | 30.79 | 1 |
| ABL2(ARG)    | 2.533 | 29.34 | 1 |
| FGFR2        | 2.602 | 29.16 | 1 |
| MAP2K2       | 2.664 | 24.35 | 1 |
| PDGFRB       | 2.752 | 26.76 | 1 |
| PKN1         | 2.828 | 33.68 | 1 |
| MAP3K8       | 2.835 | 24.02 | 1 |
| ERBB4        | 2.864 | 27.35 | 1 |
| PAK1         | 2.864 | 31.86 | 1 |
| PDGFRA       | 2.903 | 26.57 | 1 |
| LTK          | 3.003 | 28.49 | 1 |
| PRKCH        | 3.015 | 26.09 | 1 |
| EEF2K        | 3.026 | 24.92 | 1 |

|                       |       |       |      |
|-----------------------|-------|-------|------|
| IRAK1                 | 3.054 | 29.12 | 1    |
| ROS1                  | 3.068 | 26.37 | 1    |
| GRK7                  | 3.069 | 24.02 | 1    |
| MAPK8                 | 3.215 | 22.72 | 1    |
| INSR                  | 3.216 | 26.81 | 1    |
| PRKCE                 | 3.594 | 24.19 | 1    |
| SGK                   | 3.708 | 23.08 | 1    |
| TBK1                  | 3.71  | 21.08 | 1    |
| RAF1( Y340D<br>Y341D) | 5.885 | 20.25 | 0.01 |
| ABL1(T315I)           | 10    | 18.28 | 1    |
| ADRBK1                | 10    | 11.72 | 1    |
| ADRBK2                | 10    | 12.47 | 1    |
| AKT1 ALPHA            | 10    | 9.159 | 0.01 |
| AKT2 BETA             | 10    | 3.192 | 0.01 |
| AKT2 GAMMA            | 10    | 4.871 | 1    |
| ALK4                  | 10    | 6.857 | 0.1  |
| AURKA                 | 10    | 10.04 | 1    |
| AURKC                 | 10    | 20.33 | 0.01 |
| AXL                   | 10    | 9.397 | 1    |
| BMX                   | 10    | 13    | 1    |
| BRAF                  | 10    | 11.17 | 1    |
| BRAF(V599E)           | 10    | 10.13 | 1    |
| BRSK1                 | 10    | 13.13 | 1    |
| BTK                   | 10    | 4.631 | 1    |
| CAMK II ALPHA         | 10    | 2.566 | 1    |
| CAMK II BETA          | 10    | 14.9  | 1    |



|                 |    |       |      |
|-----------------|----|-------|------|
| CAMK4           | 10 | -2.03 | 1    |
| CDK1            | 10 | 3.17  | 1    |
| CDK2            | 10 | 11.26 | 1    |
| CDK5/P25        | 10 | 9.369 | 1    |
| CDK5/P35        | 10 | 9.196 | 1    |
| CDK9(CYCLIN T1) | 10 | 13.82 | 1    |
| CHEK1           | 10 | 19.45 | 1    |
| CLK1            | 10 | 7.395 | 1    |
| CLK3            | 10 | 3.627 | 1    |
| CSK             | 10 | 7.274 | 0.01 |
| CSNK1A1         | 10 | 5.407 | 1    |
| CSNK1D          | 10 | 8.093 | 0.1  |
| CSNK1E          | 10 | 15.22 | 1    |
| CSNK1G1         | 10 | 8.139 | 1    |
| CSNK1G2         | 10 | 12.76 | 1    |
| CSNK1G3         | 10 | 9.813 | 1    |
| CSNK2A1         | 10 | 3.593 | 0.01 |
| CSNK2A2         | 10 | 6.202 | 0.1  |
| DAPK1           | 10 | 15.29 | 1    |
| DAPK3           | 10 | 10.09 | 1    |
| DNA-PK          | 10 | 11.69 | 1    |
| DYRK1A          | 10 | 7.226 | 0.01 |
| DYRK1B          | 10 | 7.242 | 1    |
| DYRK3           | 10 | 4.611 | 0.1  |
| DYRK4           | 10 | 4.609 | 1    |
| EGFR            | 10 | 14.4  | 1    |
| EGFR(L858R)     | 10 | 8.544 | 1    |
| EGFR(L861Q)     | 10 | 16.9  | 1    |

|              |    |       |      |
|--------------|----|-------|------|
| EPHA1        | 10 | 5.441 | 1    |
| EPHA2        | 10 | 1.743 | 1    |
| EPHA3        | 10 | 5.1   | 0.01 |
| EPHA4        | 10 | 7.278 | 1    |
| EPHA5        | 10 | 14.63 | 1    |
| EPHA8        | 10 | 7.47  | 1    |
| EPHB1        | 10 | 7.728 | 1    |
| EPHB2        | 10 | 7.3   | 1    |
| EPHB3        | 10 | 3.642 | 1    |
| EPHB4        | 10 | 9.038 | 1    |
| ERBB2        | 10 | 2.58  | 1    |
| FGFR3        | 10 | 13.35 | 1    |
| FGFR4        | 10 | 15.9  | 1    |
| FLT1         | 10 | 6.804 | 0.01 |
| FLT4         | 10 | 11.1  | 1    |
| FRAP1(MTOR)  | 10 | 3.892 | 1    |
| GRK4         | 10 | 7.572 | 1    |
| GRK5         | 10 | 9.327 | 1    |
| GRK6         | 10 | 9.943 | 1    |
| GSG2         | 10 | 9.424 | 1    |
| GSK3A        | 10 | 8.71  | 1    |
| GSK3B        | 10 | 4.729 | 1    |
| HIPK1        | 10 | 3.352 | 0.1  |
| HIPK2        | 10 | 4.021 | 1    |
| HIPK3 (YAK1) | 10 | 1.95  | 0.01 |
| HIPK4        | 10 | 4.462 | 1    |
| IKKA         | 10 | 13.5  | 0.1  |
| IKK_BETA     | 10 | 9.556 | 1    |

|  |    |       |      |
|--|----|-------|------|
| IKK_EPSILON                                | 10 | 10.44 | 1    |
| ITK  | 10 | 13.01 | 0.1  |
| KDR  | 10 | 12.2  | 1    |
| KIT(T670I)                                 | 10 | 15.27 | 1    |
| MAP2K1                                     | 10 | 14.07 | 1    |
| MAP2K6                                     | 10 |       |      |
| MAPK1                                      | 10 | 7.213 | 1    |
| MAPK10                                     | 10 | 14.03 | 1    |
| MAPK11                                     | 10 | 16.5  | 1    |
| MAPK13                                     | 10 | 13.79 | 1    |
| MAPK14 (P38<br>ALPHA/INACTIVE<br>MAPKAPK2) | 10 | 14.57 | 1    |
| MAPK14 ALPHA                               | 10 | 17.22 | 1    |
| MAPK3(ERK1)                                | 10 | 8.104 | 1    |
| MAPK9                                      | 10 | 15.04 | 1    |
| MAPKAPK2                                   | 10 |       |      |
| MAPKAPK3                                   | 10 | 13.86 | 1    |
| MAPKAPK5                                   | 10 | 4.079 | 0.01 |
| MATK                                       | 10 | 10.4  | 1    |
| MERTK                                      | 10 | 7.209 | 0.01 |
| MET  | 10 | 5.091 | 1    |
| MET(M1250T)                                | 10 | 3.956 | 1    |
| MKNK1                                      | 10 | 7.436 | 0.1  |
| MST1R                                      | 10 | 5.839 | 1    |

|                |    |       |      |
|----------------|----|-------|------|
| MUSK           | 10 | 16.38 | 0.01 |
| MYLK2          | 10 | 0.919 | 0.1  |
| NEK1           | 10 | 4.563 | 1    |
| NEK2           | 10 | 9.466 | 1    |
| NEK4           | 10 | 19.81 | 1    |
| NEK6           | 10 | 6.174 | 1    |
| NEK9           | 10 | 1.477 | 0.01 |
| NTRK1          | 10 | 18.05 | 0.01 |
| NTRK2          | 10 | 14.4  | 1    |
| NTRK3          | 10 | 13.53 | 1    |
| PASK           | 10 | 13.48 | 1    |
| PDGFRA(D842V)  | 10 | 17.43 | 1    |
| PDGFRA(T674I)  | 10 | 10.85 | 1    |
| PDK1           | 10 | 15.93 | 1    |
| PHK GAMMA1     | 10 | 17.6  | 1    |
| PHKG2          | 10 | 5.673 | 1    |
| PI4KA          | 10 | 9.135 | 0.1  |
| PI4KB          | 10 | 10.26 | 0.01 |
| PIK3C2 ALPHA   | 10 | 5.144 | 0.01 |
| PIK3C2 BETA    | 10 | 3.279 | 1    |
| PIK3C3         | 10 | 2.268 | 1    |
| PIK3CG         | 10 |       |      |
| PIK3P110(P85)  | 10 | 15.35 | 0.1  |
| PIK3P110D/P85A | 10 | 1.412 | 0.1  |
| PIM1           | 10 | 4.319 | 0.01 |
| PIM2           | 10 | 4.013 | 0.01 |

|            |    |       |      |
|------------|----|-------|------|
| PKG2       | 10 | 7.845 | 1    |
| PLK1       | 10 | 9.274 | 1    |
| PLK2       | 10 | 5.493 | 0.01 |
| PLK3       | 10 | 10.81 | 1    |
| PRKACA     | 10 | 9.776 | 1    |
| PRKCI      | 10 | 5.291 | 0.01 |
| PRKCZ      | 10 | 17.11 | 0.01 |
| PRKG1      | 10 | 11.42 | 1    |
| PRKX       | 10 | 13.5  | 1    |
| PTK6       | 10 | 13.56 | 1    |
| RET        | 10 | 10.91 | 1    |
| RET(V804L) | 10 | 14.7  | 1    |
| RET(Y791F) | 10 | 9.197 | 1    |
| RPS6KA4    | 10 | 21.67 | 1    |
| RPS6KA5    | 10 | 13.7  | 1    |
| RPS6KB1    | 10 | 5.622 | 1    |
| SGK2       | 10 | 10.96 | 1    |
| SGKL       | 10 | 8.493 | 1    |
| SPHK1      | 10 | 12.98 | 0.1  |
| SPHK2      | 10 | 2.438 | 0.1  |
| SRMS       | 10 | 19.81 | 1    |
| SRPK1      | 10 | 2.643 | 0.01 |
| SRPK2      | 10 | 4.576 | 0.1  |
| STK23      | 10 | 1.513 | 0.1  |
| SYK        | 10 | 5.621 | 0.01 |
| TEK        | 10 | 16.17 | 1    |
| TXK        | 10 | 8.81  | 0.1  |
| TYRO3      | 10 | 20.82 | 1    |
| ZAP70      | 10 | 9.519 | 0.01 |

|          |  |       |      |
|----------|--|-------|------|
| CDK7     |  | 38.83 | 1    |
| LRRK2    |  | 61.02 | 0.01 |
| MAP2K6   |  | 21.01 | 1    |
| MAPKAPK2 |  | 4.577 | 1    |
| PIK3CG   |  | 1.403 | 0.1  |
| PRKCA    |  | 65.73 | 1    |
|          |  |       |      |

*Full Kinone panel for Compound 22, in ascending order of off-target potencies*

| Enzyme               | Efficient<br>IC <sub>50</sub> (μM) | Percent<br>Inhibition | Median<br>Result Dose |
|----------------------|------------------------------------|-----------------------|-----------------------|
| STK22D               | 0.002509                           | 73.59                 | 0.01                  |
| LRRK2                | 0.003584                           | 69.13                 | 0.01                  |
| LRRK2 (G2019S)       | 0.003644                           | 68.88                 | 0.01                  |
| STK3                 | 0.00702                            | 46.44                 | 0.01                  |
| STK4                 | 0.008045                           | 50.63                 | 0.01                  |
| STK25                | 0.01112                            | 55.91                 | 0.01                  |
| STK24                | 0.01154                            |                       |                       |
| NUAK1                | 0.01237                            | 49.02                 | 0.01                  |
| MST4                 | 0.014                              | 39.58                 | 0.01                  |
| TAOK2                | 0.01408                            | 50.32                 | 0.01                  |
| PRKD1                | 0.01924                            | 40.16                 | 0.01                  |
| PRKCN                | 0.01949                            | 40.32                 | 0.01                  |
| JAK3                 | 0.01994                            | 34.05                 | 0.01                  |
| FES                  | 0.02022                            | 45.73                 | 0.01                  |
| EGFR(T790M<br>L858R) | 0.02433                            | 38.14                 | 0.01                  |
| FER                  | 0.02565                            | 28.49                 | 0.01                  |
| JAK3                 | 0.02587                            |                       |                       |
| JAK2                 | 0.02816                            | 31.58                 | 0.01                  |
| FGR                  | 0.02977                            | 30.52                 | 0.01                  |
| FER                  | 0.03467                            | 75.33                 | 0.1                   |
| RPS6KA2              | 0.0349                             | 35.25                 | 0.01                  |
| EGFR(T790M)          | 0.03564                            | 68.03                 | 0.1                   |
| PRKD2                | 0.03692                            | 71.14                 | 0.1                   |
| PAK7                 | 0.03808                            | 63.55                 | 0.1                   |

|                         |         |       |     |
|-------------------------|---------|-------|-----|
| PTK2                    | 0.04282 | 70.15 | 0.1 |
| TYK2                    | 0.04294 | 64.76 | 0.1 |
| RPS6KA1                 | 0.04695 | 58.05 | 0.1 |
| RPS6KA3                 | 0.05216 | 64.91 | 0.1 |
| STK22B                  | 0.05565 | 57.5  | 0.1 |
| LCK                     | 0.05627 | 58.02 | 0.1 |
| JAK2 JH1 JH2<br>(V617F) | 0.05722 | 60.31 | 0.1 |
| JAK2 JH1 JH2 MT         | 0.06128 | 54.68 | 0.1 |
| PRKCG                   | 0.06269 | 49.09 | 0.1 |
| JAK2 JH1 JH2<br>(V617F) | 0.06407 |       |     |
| LYNB                    | 0.07004 | 50.27 | 0.1 |
| PAK4                    | 0.07189 | 49.66 | 0.1 |
| FGR                     | 0.0763  | 56.13 | 0.1 |
| CSF1R                   | 0.07716 | 50.07 | 0.1 |
| YES1                    | 0.07934 | 47.41 | 0.1 |
| PTK2B                   | 0.09148 | 51.3  | 0.1 |
| BLK                     | 0.09999 | 50.39 | 0.1 |
| JAK1                    | 0.11    | 53.98 | 0.1 |
| HCK                     | 0.1211  | 52.17 | 0.1 |
| LCK                     | 0.1259  | 44.14 | 0.1 |
| CLK2                    | 0.1354  | 46.52 | 0.1 |
| FLT3(D835Y)             | 0.1531  | 51.32 | 0.1 |
| LYN                     | 0.1548  | 50.12 | 0.1 |
| FGFR1                   | 0.1554  | 52.17 | 0.1 |
| FRK                     | 0.1562  | 49.85 | 0.1 |
| TBK1                    | 0.1601  | 38.18 | 0.1 |
| INSR                    | 0.1623  | 43.07 | 0.1 |



|                             |        |       |     |
|-----------------------------|--------|-------|-----|
| MARK3                       | 0.1645 |       |     |
| IGF1R                       | 0.1668 | 44.42 | 0.1 |
| PAK2                        | 0.1758 | 41.13 | 0.1 |
| PDGFRA(V561D)               | 0.1884 | 41.05 | 0.1 |
| MARK4                       | 0.1983 |       |     |
| IRAK1                       | 0.2492 | 42.8  | 0.1 |
| DCAMKL2                     | 0.268  |       |     |
| SNF1LK2                     | 0.2824 |       |     |
| TBK1                        | 0.2932 | 28.07 | 0.1 |
| AMPK ALPHA1<br>BETA1 GAMMA1 | 0.3137 | 33.69 | 0.1 |
| PAK2                        | 0.3314 | 63.28 | 1   |
| PRKCD                       | 0.3573 | 68.25 | 1   |
| ABL1(G250E)                 | 0.3639 | 67.64 | 1   |
| PAK1                        | 0.3826 | 62.96 | 1   |
| PRKCB2                      | 0.3868 | 61.07 | 1   |
| NEK4                        | 0.3913 | 68.98 | 1   |
| SRC N1                      | 0.4048 | 63.22 | 1   |
| AMPK ALPHA2<br>BETA1 GAMMA1 | 0.4075 | 66.42 | 1   |
| CDC42BPA                    | 0.412  | 62.44 | 1   |
| IRAK4                       | 0.4209 |       |     |
| FGFR3(K650E)                | 0.4242 | 62.6  | 1   |
| DAPK3                       | 0.442  |       |     |
| SRC                         | 0.4495 | 58.08 | 1   |

|              |        |       |   |
|--------------|--------|-------|---|
| INSRR        | 0.466  |       |   |
| FGFR2        | 0.4685 |       |   |
| PRKCA        | 0.4762 |       |   |
| MARK1        | 0.5366 | 57.05 | 1 |
| CHEK2        | 0.5595 | 59.44 | 1 |
| FLT3         | 0.6232 | 54.25 | 1 |
| PTK6         | 0.6263 | 54.24 | 1 |
| MARK2        | 0.6528 | 56.39 | 1 |
| FYN          | 0.6973 | 51.74 | 1 |
| CDC42BPB     | 0.7328 |       |   |
| ERBB4        | 0.7418 |       |   |
| RET(V804L)   | 0.7434 | 55.03 | 1 |
| MELK         | 0.773  | 51.23 | 1 |
| AXL          | 0.7759 | 51.81 | 1 |
| CAMK I ALPHA | 0.7848 | 49.85 | 1 |
| RPS6KA6      | 0.818  | 47.11 | 1 |
| SYK          | 0.8184 | 41.41 | 1 |
| ABL1(Y253F)  | 0.8466 | 47.18 | 1 |
| NEK2         | 0.8613 | 50.2  | 1 |
| PRKCQ        | 0.8763 | 47.12 | 1 |
| ROCK1        | 0.8773 | 47.25 | 1 |
| PDGFRB       | 0.9419 |       |   |
| PDGFRA       | 0.9675 | 46.39 | 1 |
| LTK          | 1.056  | 47.97 | 1 |
| DAPK1        | 1.105  | 50.13 | 1 |
| MAP3K9       | 1.105  | 38.16 | 1 |
| RET          | 1.161  | 49.3  | 1 |
| ABL1(E255K)  | 1.17   | 52.06 | 1 |

|               |       |       |   |
|---------------|-------|-------|---|
| ABL1          | 1.19  | 52.31 | 1 |
| PKN1          | 1.221 | 46.28 | 1 |
| PRKCB1        | 1.241 |       |   |
| CHEK1         | 1.255 |       |   |
| RET(Y791F)    | 1.261 | 45.93 | 1 |
| NEK9          | 1.281 | 47.71 | 1 |
| ITK           | 1.337 | 42.75 | 1 |
| CLK1          | 1.34  | 59.19 | 1 |
| ABL2(ARG)     | 1.36  | 48.86 | 1 |
| BTK           | 1.387 | 42.69 | 1 |
| GRK7          | 1.438 | 42.34 | 1 |
| CAMK I DELTA  | 1.499 |       |   |
| NEK1          | 1.513 | 39.73 | 1 |
| PAK3          | 1.525 | 43.28 | 1 |
| PHK GAMMA1    | 1.578 | 48.99 | 1 |
| ROS1          | 1.613 | 44.12 | 1 |
| AURKB         | 1.65  | 45.16 | 1 |
| PDGFRA(D842V) | 1.712 | 40.74 | 1 |
| SRMS          | 1.783 | 36.02 | 1 |
| PRKCH         | 1.787 | 40.48 | 1 |
| DYRK1A        | 1.809 | 40.01 | 1 |
| ABL1(T315I)   | 1.811 | 38.82 | 1 |
| MAPK8         | 1.877 | 32.94 | 1 |
| MUSK          | 1.976 | 37.93 | 1 |
| DNA-PK        | 1.996 | 32.27 | 1 |
| FGFR3         | 2.012 |       |   |
| KIT           | 2.081 | 34.01 | 1 |

|               |       |       |     |
|---------------|-------|-------|-----|
| FGFR4         | 2.098 | 37.27 | 1   |
| EGFR(L861Q)   | 2.109 | 36.89 | 1   |
| IKK_EPSILON   | 2.289 |       |     |
| PRKX          | 2.462 | 31.65 | 1   |
| CAMK II DELTA | 2.484 |       |     |
| PRKCE         | 2.598 |       |     |
| ERBB2         | 2.639 | 33.39 | 1   |
| MAPK9         | 2.722 | 27.63 | 1   |
| PDK1          | 2.724 | 27.27 | 1   |
| ROCK2         | 2.866 | 34.35 | 1   |
| MAP2K2        | 2.867 | 25    | 1   |
| PI4KB         | 3.008 | 25.68 | 1   |
| MAPK10        | 3.057 | 23.52 | 1   |
| PLK1          | 3.088 | 27.94 | 1   |
| EGFR          | 3.095 | 30.04 | 1   |
| EGFR(L858R)   | 3.226 | 24.55 | 1   |
| MAP3K8        | 3.276 |       |     |
| GRK4          | 3.352 |       |     |
| NTRK1         | 3.355 | 25.24 | 1   |
| MAP3K8        | 3.413 | 20.9  | 1   |
| RPS6KB1       | 3.59  | 26.83 | 1   |
| NTRK3         | 3.629 | 23.53 | 1   |
| EEF2K         | 3.652 | 22.64 | 1   |
| CDK7          | 3.753 | 21.6  | 1   |
| GRK6          | 3.792 | 20.49 | 1   |
| RPS6KA5       | 3.826 | 22.04 | 1   |
| IKKA          | 3.917 | 20.44 | 1   |
| BRAF(V599E)   | 5.477 | 21.92 | 0.1 |

|                    |    |        |      |
|--------------------|----|--------|------|
| ADRBK1             | 10 |        |      |
| ADRBK2             | 10 |        |      |
| AKT1 ALPHA         | 10 |        |      |
| AKT2 BETA          | 10 |        |      |
| AKT2 GAMMA         | 10 | 6.959  | 0.01 |
| ALK4               | 10 | 5.726  | 0.1  |
| AURKA              | 10 | 15.7   | 1    |
| AURKC              | 10 |        |      |
| BMX                | 10 | 18.88  | 1    |
| BRAF               | 10 |        |      |
| BRSK1              | 10 |        |      |
| CAMK II ALPHA      | 10 | 9.085  | 1    |
| CAMK II BETA       | 10 |        |      |
| CAMK4              | 10 | -4.489 | 1    |
| CDK1               | 10 | 3.838  | 1    |
| CDK1               | 10 | 11.59  | 1    |
| CDK2               | 10 | 12.24  | 1    |
| CDK5/P25           | 10 | 5.946  | 1    |
| CDK5/P35           | 10 | 13.95  | 1    |
| CDK9(CYCLIN<br>T1) | 10 | 7.86   | 1    |
| CLK3               | 10 | 7.829  | 1    |
| CSK                | 10 | 15.77  | 1    |
| CSNK1A1            | 10 |        |      |
| CSNK1D             | 10 | 13.25  | 1    |
| CSNK1E             | 10 |        |      |
| CSNK1G1            | 10 |        |      |

|              |    |       |      |
|--------------|----|-------|------|
| CSNK1G2      | 10 | 6.46  | 1    |
| CSNK1G3      | 10 | 4.962 | 1    |
| CSNK2A1      | 10 |       |      |
| CSNK2A2      | 10 | 10.79 | 0.1  |
| DYRK1B       | 10 | 20.32 | 1    |
| DYRK3        | 10 |       |      |
| DYRK4        | 10 |       |      |
| EPHA1        | 10 |       |      |
| EPHA2        | 10 | 21.8  | 1    |
| EPHA3        | 10 | 11.63 | 1    |
| EPHA4        | 10 | 5.145 | 0.01 |
| EPHA5        | 10 | 13.21 | 1    |
| EPHA8        | 10 |       |      |
| EPHB1        | 10 |       |      |
| EPHB2        | 10 |       |      |
| EPHB3        | 10 |       |      |
| EPHB4        | 10 | 10.71 | 1    |
| FGFR4        | 10 |       |      |
| FLT1         | 10 |       |      |
| FLT4         | 10 | 17.67 | 1    |
| FRAP1(MTOR)  | 10 | 5.797 | 0.1  |
| GRK5         | 10 | 12.41 | 1    |
| GSG2         | 10 | 15.21 | 1    |
| GSK3A        | 10 | 7.159 | 1    |
| GSK3B        | 10 | 1.651 | 0.01 |
| GSK3B        | 10 | 4.056 | 1    |
| HIPK1        | 10 | 4.387 | 1    |
| HIPK2        | 10 |       |      |
| HIPK3 (YAK1) | 10 | 3.28  | 0.01 |

|  |    |       |      |
|--|----|-------|------|
| HIPK4                                      | 10 | 16.8  | 1    |
| IKK_BETA                                   | 10 | 14.07 | 1    |
| KDR  | 10 | 20.43 | 1    |
| KIT(T670I)                                 | 10 | 5.024 | 1    |
| MAP2K1                                     | 10 | 12.62 | 1    |
| MAP2K2                                     | 10 | 15.44 | 1    |
| MAP2K6                                     | 10 |       |      |
| MAPK1                                      | 10 | 11.09 | 1    |
| MAPK11                                     | 10 | 8.79  | 0.1  |
| MAPK12                                     | 10 | 8.013 | 1    |
| MAPK13                                     | 10 | 4.596 | 1    |
| MAPK13                                     | 10 | 5.207 | 0.01 |
| MAPK14 (P38<br>ALPHA/INACTIVE<br>MAPKAPK2) | 10 |       |      |
| MAPK14 ALPHA                               | 10 | 11.02 | 1    |
| MAPK3(ERK1)                                | 10 | 13.01 | 1    |
| MAPKAPK2                                   | 10 | 9.962 | 0.01 |
| MAPKAPK3                                   | 10 | 7.843 | 1    |
| MAPKAPK5                                   | 10 | 12.25 | 1    |
| MATK                                       | 10 | 11.36 | 1    |
| MERTK                                      | 10 | 9.579 | 1    |
| MET  | 10 | 8.067 | 0.1  |
| MET(M1250T)                                | 10 | 6.332 | 1    |
| MKNK1                                      | 10 |       |      |

|                       |    |        |      |
|-----------------------|----|--------|------|
| MST1R                 | 10 | 8.491  | 0.1  |
| MYLK2                 | 10 | 7.81   | 1    |
| NEK6                  | 10 | 18.07  | 1    |
| NTRK2                 | 10 | 18.96  | 1    |
| PASK                  | 10 | 9.075  | 1    |
| PDGFRA(T674I)         | 10 | 8.402  | 0.01 |
| PHKG2                 | 10 | 9.706  | 0.1  |
| PI4KA                 | 10 | 9.77   | 0.1  |
| PIK3C2 ALPHA          | 10 | 3.874  | 0.1  |
| PIK3C2 BETA           | 10 | 7.826  | 0.1  |
| PIK3C3                | 10 | 7.85   | 0.1  |
| PIK3CG                | 10 | 7.665  | 1    |
| PIK3P110(P85)         | 10 | 7.23   | 0.01 |
| PIK3P110D/P85A        | 10 | 25.43  | 1    |
| PIM1                  | 10 | 5.296  | 0.1  |
| PIM2                  | 10 | 6.64   | 0.01 |
| PKG2                  | 10 | 3.06   | 1    |
| PLK2                  | 10 | 14.08  | 1    |
| PLK3                  | 10 | 0.9867 | 0.01 |
| PLK3                  | 10 | 9.605  | 0.01 |
| PRKACA                | 10 | 11.88  | 1    |
| PRKCI                 | 10 | 11.6   | 1    |
| PRKCZ                 | 10 | 22.25  | 0.1  |
| PRKG1                 | 10 | 11.88  | 1    |
| RAF1( Y340D<br>Y341D) | 10 | 13.37  | 1    |
| RPS6KA4               | 10 | 14.49  | 1    |
| SGK                   | 10 | 8.381  | 1    |



|               |    |       |      |
|---------------|----|-------|------|
| SGK2          | 10 | 14.02 | 1    |
| SGKL          | 10 | 8.611 | 0.1  |
| SPHK1         | 10 | 10.71 | 1    |
| SPHK2         | 10 |       |      |
| SRPK1         | 10 | 2.527 | 0.1  |
| SRPK2         | 10 | 3.239 | 1    |
| STK23         | 10 |       |      |
| TEK           | 10 | 11.92 | 0.01 |
| TXK           | 10 |       |      |
| TXK           | 10 | 9.787 | 1    |
| TYRO3         | 10 | 13.72 | 1    |
| ZAP70         | 10 | 9.402 | 0.1  |
| ADRBK1        |    | 5.088 | 1    |
| ADRBK2        |    | 4.989 | 0.01 |
| AKT1 ALPHA    |    | 4.871 | 1    |
| AKT2 BETA     |    | 14.06 | 1    |
| AURKC         |    | 14.77 | 0.01 |
| BRAF          |    | 16.72 | 0.1  |
| BRSK1         |    | 9.542 | 1    |
| CAMK I DELTA  |    | 42.45 | 1    |
| CAMK II BETA  |    | 18.05 | 0.1  |
| CAMK II DELTA |    | 44.84 | 1    |
| CDC42BPB      |    | 49.03 | 1    |
| CHEK1         |    | 49.02 | 1    |
| CSNK1A1       |    | 16    | 1    |
| CSNK1E        |    | 13.98 | 1    |
| CSNK1G1       |    | 8.14  | 0.1  |

|  |  |       |      |
|--|--|-------|------|
| CSNK2A1                                    |  | 7.848 | 0.01 |
| DAPK3                                      |  | 63.13 | 1    |
| DCAMKL2                                    |  | 37.44 | 0.1  |
| DYRK3                                      |  | 3.869 | 1    |
| DYRK4                                      |  | 4.872 | 0.1  |
| EPHA1                                      |  | 6.68  | 0.1  |
| EPHA8                                      |  | 21.82 | 0.01 |
| EPHB1                                      |  | 4.508 | 1    |
| EPHB2                                      |  | 9.76  | 1    |
| EPHB3                                      |  | 8.082 | 0.01 |
| ERBB4                                      |  | 46.44 | 1    |
| FGFR2                                      |  | 57.5  | 1    |
| FGFR3                                      |  | 51.89 | 1    |
| FGFR4                                      |  | 17.87 | 1    |
| FLT1                                       |  | 7.561 | 0.1  |
| GRK4                                       |  | 22.96 | 1    |
| HIPK2                                      |  | 6.436 | 1    |
| IKK_EPSILON                                |  | 32.78 | 1    |
| INSRR                                      |  | 58.81 | 1    |
| IRAK4                                      |  | 69.54 | 1    |
| JAK2 JH1 JH2<br>(V617F)                    |  | 62.51 | 0.1  |
| JAK3                                       |  | 37.05 | 0.01 |
| MAP2K6                                     |  | 17.2  | 1    |
| MAP3K8                                     |  | 23.27 | 0.1  |
| MAPK14 (P38<br>ALPHA/INACTIVE<br>MAPKAPK2) |  | 11.86 | 1    |

|         |  |       |      |
|---------|--|-------|------|
| MARK3   |  | 42.04 | 0.1  |
| MARK4   |  | 37.05 | 0.1  |
| MKNK1   |  | 5.007 | 0.01 |
| PDGFRB  |  | 52.78 | 1    |
| PLK3    |  | 3.881 | 0.1  |
| PRKCA   |  | 58.12 | 1    |
| PRKCB1  |  | 53.5  | 1    |
| PRKCE   |  | 26.81 | 1    |
| SNF1LK2 |  | 30.9  | 0.1  |
| SPHK2   |  | 8.384 | 1    |
| STK23   |  | 4.248 | 0.01 |
| STK24   |  | 57.1  | 0.01 |
| TXK     |  | 14.12 | 1    |

*Full Kinone panel for Compound 27, in ascending order of off-target potencies*

| Enzyme          | Efficient<br>IC <sub>50</sub> (μM) | Percent<br>Inhibition | Median Result<br>Dose |
|-----------------|------------------------------------|-----------------------|-----------------------|
| TAOK2           | 0.003478                           | 71.57                 | 0.01                  |
| NUAK1           | 0.003898                           | 71.83                 | 0.01                  |
| MST4            | 0.004261                           | 69.57                 | 0.01                  |
| LRRK2           | 0.005302                           | 63.36                 | 0.01                  |
| STK4            | 0.00569                            | 48.22                 | 0.01                  |
| LRRK2 (G2019S)  | 0.00574                            | 62.34                 | 0.01                  |
| STK3            | 0.005948                           | 61.3                  | 0.01                  |
| TYK2            | 0.01033                            | 48.48                 | 0.01                  |
| STK25           | 0.01043                            | 50.27                 | 0.01                  |
| STK24           | 0.01123                            | 48.6                  | 0.01                  |
| STK22D          | 0.02085                            | 34.88                 | 0.01                  |
| PTK2            | 0.02529                            | 20.4                  | 0.01                  |
| JAK3            | 0.03446                            | 68.6                  | 0.1                   |
| PRKCN           | 0.04782                            | 65.7                  | 0.1                   |
| JAK2            | 0.06616                            | 59.81                 | 0.1                   |
| DCAMKL2         | 0.07823                            | 54.66                 | 0.1                   |
| CSF1R           | 0.1029                             | 50.46                 | 0.1                   |
| MARK3           | 0.1153                             | 49.78                 | 0.1                   |
| LYNB            | 0.1209                             | 47.1                  | 0.1                   |
| FGR             | 0.1304                             | 46.12                 | 0.1                   |
| JAK2 JH1 JH2 MT | 0.1378                             | 42.56                 | 0.1                   |
| PRKD2           | 0.1403                             | 40.13                 | 0.1                   |
| PRKD1           | 0.148                              | 40.85                 | 0.1                   |

|                             |        |       |     |
|-----------------------------|--------|-------|-----|
| JAK2 JH1 JH2<br>(V617F)     | 0.16   | 41.11 | 0.1 |
| TNIK                        | 0.1609 | 40.2  | 0.1 |
| MARK4                       | 0.1626 | 39.5  | 0.1 |
| FGFR1                       | 0.166  | 39.58 | 0.1 |
| RPS6KA2                     | 0.1747 | 38.08 | 0.1 |
| LYN                         | 0.1782 | 37.02 | 0.1 |
| EGFR(T790M<br>L858R)        | 0.1871 | 37.97 | 0.1 |
| MARK2                       | 0.2634 | 28.96 | 0.1 |
| JAK1                        | 0.2702 | 35.69 | 0.1 |
| PRKCD                       | 0.2718 | 28.94 | 0.1 |
| AMPK ALPHA1<br>BETA1 GAMMA1 | 0.2787 | 28.68 | 0.1 |
| HCK                         | 0.2798 | 29.25 | 0.1 |
| RPS6KA3                     | 0.288  | 28.93 | 0.1 |
| BLK                         | 0.2958 | 71.64 | 1   |
| FER                         | 0.3005 | 23.25 | 0.1 |
| YES1                        | 0.336  | 71.22 | 1   |
| RPS6KA1                     | 0.3559 | 71.97 | 1   |
| LCK                         | 0.3678 | 71.66 | 1   |
| MARK1                       | 0.371  | 70.79 | 1   |
| LCK                         | 0.3817 | 71.07 | 1   |
| FES                         | 0.3926 | 66.65 | 1   |
| ROCK1                       | 0.4238 | 68.84 | 1   |
| EGFR(T790M)                 | 0.4261 | 68.1  | 1   |
| FRK                         | 0.4381 | 66.29 | 1   |

|                             |        |       |   |
|-----------------------------|--------|-------|---|
| AMPK ALPHA2<br>BETA1 GAMMA1 | 0.488  | 66.26 | 1 |
| SNF1LK2                     | 0.5203 | 65.32 | 1 |
| PAK7                        | 0.5491 | 63.01 | 1 |
| CDC42BPA                    | 0.5874 | 60.97 | 1 |
| PTK2B                       | 0.6686 | 57.9  | 1 |
| STK22B                      | 0.7121 | 55.51 | 1 |
| CLK2                        | 0.7412 | 52.64 | 1 |
| PRKCB2                      | 0.8104 | 54.29 | 1 |
| PAK2                        | 0.8146 | 54.55 | 1 |
| CAMK I ALPHA                | 0.8214 | 55.11 | 1 |
| PRKCA                       | 0.8585 | 48.93 | 1 |
| PRKCQ                       | 0.8914 | 47.4  | 1 |
| PAK4                        | 0.9486 | 50.43 | 1 |
| ABL1(G250E)                 | 1.03   | 48.47 | 1 |
| CDC42BPB                    | 1.089  | 50.06 | 1 |
| PRKCG                       | 1.097  |       |   |
| PRKCB1                      | 1.45   | 44.18 | 1 |
| CAMK I DELTA                | 1.608  |       |   |
| FLT3(D835Y)                 | 1.653  | 38.52 | 1 |
| ROCK2                       | 1.657  | 37.64 | 1 |
| SRC N1                      | 1.691  | 38.14 | 1 |
| FYN                         | 1.843  | 37.79 | 1 |
| FGFR3(K650E)                | 1.871  | 34.39 | 1 |
| SRC                         | 1.894  | 33.9  | 1 |

|               |       |       |      |
|---------------|-------|-------|------|
| FGFR2         | 1.93  | 37.12 | 1    |
| IRAK4         | 1.973 | 35.47 | 1    |
| CAMK II DELTA | 2.044 | 32.77 | 1    |
| PKN1          | 2.103 | 35.55 | 1    |
| AURKB         | 2.136 | 32.62 | 1    |
| ABL1(Y253F)   | 2.186 | 32.24 | 1    |
| PRKCH         | 2.414 | 29.14 | 1    |
| PRKCE         | 2.607 | 29.74 | 1    |
| PI4KB         | 2.748 | 35.53 | 1    |
| INSR          | 2.749 | 30.05 | 1    |
| CHEK1         | 2.87  | 29.82 | 1    |
| MELK          | 3.068 | 23.63 | 1    |
| LTK           | 3.072 | 26.07 | 1    |
| ABL1          | 3.166 | 24.7  | 1    |
| ABL2(ARG)     | 3.19  | 25.36 | 1    |
| KIT           | 3.228 | 23.49 | 1    |
| FGFR3         | 3.487 | 21.19 | 1    |
| ABL1(E255K)   | 3.521 | 21.66 | 1    |
| PDGFRA(V561D) | 3.533 | 25.04 | 1    |
| FLT3          | 3.647 | 23.28 | 1    |
| PDGFRB        | 3.692 | 24.23 | 1    |
| MAP3K9        | 3.854 | 23.32 | 1    |
| RPS6KA6       | 3.875 | 22.18 | 1    |
| ABL1(T315I)   | 10    | 13.05 | 1    |
| ADRBK1        | 10    | 3.07  | 0.01 |
| ADRBK2        | 10    |       |      |
| AKT1 ALPHA    | 10    | 9.298 | 0.1  |

|                    |    |       |      |
|--------------------|----|-------|------|
| AKT2 BETA          | 10 | 4.089 | 1    |
| AKT2 GAMMA         | 10 | 5.636 | 0.1  |
| ALK4               | 10 | 5.468 | 0.01 |
| AURKA              | 10 | 6.995 | 1    |
| AURKC              | 10 | 4.416 | 1    |
| AXL                | 10 | 7.215 | 1    |
| BMX                | 10 | 8.5   | 1    |
| BRAF               | 10 | 18.04 | 1    |
| BRAF(V599E)        | 10 | 16.23 | 0.01 |
| BRSK1              | 10 | 4.96  | 1    |
| BTK                | 10 | 8.787 | 1    |
| CAMK II ALPHA      | 10 | 7.796 | 1    |
| CAMK II BETA       | 10 | 5.526 | 1    |
| CAMK4              | 10 |       |      |
| CDK1               | 10 | 6.213 | 0.01 |
| CDK2               | 10 | 7.783 | 1    |
| CDK5/P25           | 10 | 4.281 | 1    |
| CDK5/P35           | 10 | 9.887 | 0.1  |
| CDK7               | 10 | 4.064 | 0.1  |
| CDK9(CYCLIN<br>T1) | 10 | 17.69 | 1    |
| CHEK2              | 10 | 22.18 | 1    |
| CLK1               | 10 | 7.252 | 1    |
| CLK3               | 10 | 4.659 | 0.1  |
| CSK                | 10 | 5.1   | 1    |
| CSNK1A1            | 10 | 5.734 | 1    |
| CSNK1D             | 10 | 2.252 | 0.1  |



|             |    |        |      |
|-------------|----|--------|------|
| CSNK1E      | 10 | 5.137  | 0.01 |
| CSNK1G1     | 10 | 4.067  | 1    |
| CSNK1G2     | 10 | 5.014  | 1    |
| CSNK1G3     | 10 | 1.4    | 1    |
| CSNK2A1     | 10 | 5.995  | 0.1  |
| CSNK2A2     | 10 | 7.945  | 0.1  |
| DAPK1       | 10 | 14.6   | 1    |
| DAPK3       | 10 | 5.991  | 1    |
| DNA-PK      | 10 | 10.94  | 1    |
| DYRK1A      | 10 | 9.755  | 1    |
| DYRK1B      | 10 | 4.929  | 1    |
| DYRK3       | 10 | 5.318  | 1    |
| DYRK4       | 10 | 1.893  | 1    |
| EEF2K       | 10 | 7.244  | 1    |
| EGFR        | 10 | 11.22  | 1    |
| EGFR(L858R) | 10 | 6.849  | 1    |
| EGFR(L861Q) | 10 | 8.978  | 1    |
| EPHA1       | 10 | 4.933  | 0.1  |
| EPHA2       | 10 | 4.067  | 0.01 |
| EPHA3       | 10 | 3.703  | 1    |
| EPHA4       | 10 | 2.109  | 0.01 |
| EPHA5       | 10 | 8.926  | 0.01 |
| EPHA8       | 10 | 0.4462 | 0.1  |
| EPHB1       | 10 | 2.901  | 1    |
| EPHB2       | 10 | 15.8   | 1    |
| EPHB3       | 10 | 1.161  | 1    |
| EPHB4       | 10 | 0.9491 | 0.01 |
| ERBB2       | 10 | -1.94  | 1    |
| ERBB4       | 10 | 5.211  | 1    |

|              |    |       |      |
|--------------|----|-------|------|
| FGFR4        | 10 | 11.85 | 1    |
| FLT1         | 10 | 5.75  | 0.01 |
| FLT4         | 10 | 5.091 | 1    |
| FRAP1(MTOR)  | 10 | 5.73  | 0.1  |
| GRK4         | 10 | 12.39 | 1    |
| GRK5         | 10 |       |      |
| GRK6         | 10 | 3.829 | 1    |
| GRK7         | 10 | 17.19 | 1    |
| GSG2         | 10 | 7.779 | 1    |
| GSK3A        | 10 | 1.722 | 1    |
| GSK3B        | 10 | 4.979 | 0.1  |
| HIPK1        | 10 | 2.885 | 1    |
| HIPK2        | 10 | 6.494 | 1    |
| HIPK3 (YAK1) | 10 | 3.505 | 1    |
| HIPK4        | 10 | 1.375 | 1    |
| IGF1R        | 10 | 18.9  | 1    |
| IKKA         | 10 | 1.305 | 0.01 |
| IKK_BETA     | 10 | 5.939 | 0.1  |
| IKK_EPSILON  | 10 | 5.38  | 0.1  |
| INSRR        | 10 |       |      |
| IRAK1        | 10 | 9.421 | 0.01 |
| ITK          | 10 | 8.521 | 1    |
| KDR          | 10 | 1.994 | 0.1  |
| KIT(T670I)   | 10 | 6.502 | 0.01 |
| MAP2K1       | 10 | 14.42 | 0.1  |
| MAP2K2       | 10 | 6.673 | 0.01 |
| MAP2K6       | 10 | 11.05 | 1    |
| MAP3K8       | 10 | 16.51 | 1    |

|  |    |         |      |
|--|----|---------|------|
| MAPK1                                      | 10 | 3.438   | 1    |
| MAPK10                                     | 10 | 6.078   | 1    |
| MAPK11                                     | 10 | 13.82   | 1    |
| MAPK12                                     | 10 | 20.23   | 1    |
| MAPK13                                     | 10 | 6.305   | 1    |
| MAPK14 (P38<br>ALPHA/INACTIVE<br>MAPKAPK2) | 10 | 1.682   | 0.1  |
| MAPK14 ALPHA                               | 10 | 8.633   | 1    |
| MAPK3(ERK1)                                | 10 | 4.863   | 0.1  |
| MAPK8                                      | 10 | 10.67   | 1    |
| MAPK9                                      | 10 | 8.2     | 1    |
| MAPKAPK2                                   | 10 | 24.16   | 0.01 |
| MAPKAPK3                                   | 10 | -1.14   | 0.01 |
| MAPKAPK5                                   | 10 | 7.075   | 1    |
| MATK                                       | 10 | 7.834   | 1    |
| MERTK                                      | 10 | 2.309   | 1    |
| MET  | 10 | 3.512   | 0.1  |
| MET(M1250T)                                | 10 | 4.833   | 0.1  |
| MKNK1                                      | 10 | 0.0568  | 0.01 |
| MST1R                                      | 10 | 1.963   | 0.1  |
| MUSK                                       | 10 | 14.28   | 1    |
| MYLK2                                      | 10 | 4.206   | 1    |
| NEK1                                       | 10 | -0.2089 | 1    |
| NEK2                                       | 10 | 2.778   | 1    |
| NEK4                                       | 10 | 12.06   | 1    |

|                |    |        |      |
|----------------|----|--------|------|
| NEK6           | 10 | 7.435  | 1    |
| NEK9           | 10 | 7.566  | 0.01 |
| NTRK1          | 10 | 16.6   | 1    |
| NTRK2          | 10 | 2.632  | 1    |
| NTRK3          | 10 | 8.438  | 1    |
| PAK1           | 10 | 15.78  | 1    |
| PAK2           | 10 | 19.91  | 1    |
| PAK3           | 10 | 5.175  | 1    |
| PASK           | 10 | 8.695  | 1    |
| PDGFRA         | 10 | 9.918  | 1    |
| PDGFRA(D842V)  | 10 | 4.73   | 1    |
| PDGFRA(T674I)  | 10 | 5.647  | 1    |
| PDK1           | 10 | 11.73  | 1    |
| PHK GAMMA1     | 10 | 7.466  | 1    |
| PHKG2          | 10 | -1.692 | 1    |
| PI4KA          | 10 | 10.33  | 0.01 |
| PIK3C2 ALPHA   | 10 | 4.343  | 1    |
| PIK3C2 BETA    | 10 | 5.348  | 0.01 |
| PIK3C3         | 10 | 11.23  | 0.1  |
| PIK3CG         | 10 | 0.9554 | 0.1  |
| PIK3P110(P85)  | 10 | 12.44  | 1    |
| PIK3P110D/P85A | 10 | 4.822  | 0.01 |
| PIM1           | 10 | 17.31  | 0.1  |
| PIM2           | 10 | 4.389  | 0.1  |
| PKG2           | 10 | 12.92  | 1    |
| PLK1           | 10 | 10.94  | 1    |

|                       |    |        |      |
|-----------------------|----|--------|------|
| PLK2                  | 10 | 3.122  | 1    |
| PLK3                  | 10 | 1.454  | 0.1  |
| PRKACA                | 10 | 7.582  | 0.01 |
| PRKCI                 | 10 | -1.581 | 0.1  |
| PRKCZ                 | 10 | 21.43  | 1    |
| PRKG1                 | 10 | 8.81   | 1    |
| PRKX                  | 10 | 12.68  | 1    |
| PTK6                  | 10 | 2.389  | 1    |
| RAF1( Y340D<br>Y341D) | 10 | 8.187  | 1    |
| RET                   | 10 | 9.473  | 1    |
| RET(V804L)            | 10 | 10.11  | 1    |
| RET(Y791F)            | 10 | 6.911  | 1    |
| ROS1                  | 10 | 22.02  | 1    |
| RPS6KA4               | 10 | 11.51  | 0.1  |
| RPS6KA5               | 10 | 11.8   | 1    |
| RPS6KB1               | 10 | 3.865  | 0.1  |
| SGK                   | 10 | 9.91   | 0.1  |
| SGK2                  | 10 | 4.737  | 0.1  |
| SGKL                  | 10 | 3.815  | 1    |
| SPHK1                 | 10 | 6.516  | 0.01 |
| SPHK2                 | 10 | 2.096  | 1    |
| SRMS                  | 10 | -1.448 | 0.1  |
| SRPK1                 | 10 | 2.838  | 1    |
| SRPK2                 | 10 | 1.496  | 1    |
| STK23                 | 10 | 5.42   | 1    |
| SYK                   | 10 | 8.429  | 1    |
| TBK1                  | 10 | 9.662  | 1    |
| TEK                   | 10 | 10.51  | 1    |

|              |    |          |      |
|--------------|----|----------|------|
| TXK          | 10 | 4.661    | 1    |
| TYRO3        | 10 | 19.85    | 1    |
| ZAP70        | 10 | 5.726    | 1    |
| ADRBK2       |    | 1.491    | 1    |
| CAMK I DELTA |    | 39.11    | 1    |
| CAMK4        |    | 7.621    | 0.01 |
| GRK5         |    | 9.00E-04 | 1    |
| INSRR        |    | 18.81    | 1    |
| PRKCG        |    | 47.4     | 1    |

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<sup>1</sup> *ACS Catal.* **2016**, 6, 8004. For additional examples: Mennie, K.M.; Vara, B.A.; Levi, S.M. Reductive sp<sup>3</sup>-sp<sup>2</sup> Coupling Reactions Enable Late-Stage Modification of Pharmaceuticals. *Org. Lett.* **2020**, 22, 556-559.

<sup>2</sup> Further details regarding related biochemical, target engagement, and IL-2 assay details:

Lacey, B. M.; Xu, Z.; Chai, X.; Laskey, J.; Fradera, X.; Mittal, P.; Mishra, S.; Piesvaux, J.; Saradjian, P.; Shaffer, L.; Vassileva, G.; Gerdt, C.; Wang, Y.; Ferguson, H.; Smith, D. M.; Ballard, J.; Wells, S.; Jain, R.; Mueller, U.; Addona, G.; Kariv, I.; Methot, J. L.; Bittinger, M.; Ranganath, S.; Mcleod, R.; Pasternak, A.; Miller, J. R.; Xu, H. Development of High-Throughput Assays for Evaluation of Hematopoietic Progenitor Kinase 1 Inhibitors. *SLAS Discov. Adv. Sci. Drug Discov.* August 2020. <https://doi.org/10.1177/2472555220952071>.