

## Supporting Information

### Discovery of a Pyrrolopyrimidine (JH-II-127), a Highly Potent, Selective and Brain Penetrant LRRK2 Inhibitor

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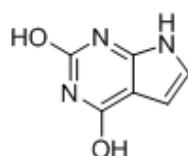
## Experimental Procedures and Compounds Characterization

### General

All reactions were monitored by thin layer chromatography (TLC) with 0.25 mm E. Merck pre-coated silica gel plates (60 F<sub>254</sub>) and Waters LCMS system (Waters 2489 UV/Visible Detector, Waters 3100 Mass, Waters 515 HPLC pump, Waters 2545 Binary Gradient Module, Waters Reagent Manager, Waters 2767 Sample Manager) using SunFire™ C18 column (4.6 x 50 mm, 5 µm particle size): solvent gradient = 100% A at 0 min, 1% A at 5 min; solvent A = 0.035% TFA in Water; solvent B = 0.035% TFA in MeOH; flow rate : 2.5 mL/min. Purification of reaction products was carried out by flash chromatography using CombiFlash®Rf with Teledyne Isco RediSep®Rf High Performance Gold or Silicycle SiliaSep™ High Performance columns (4 g, 12 g, 24 g, 40 g or 80 g). The purity of all

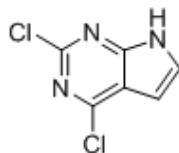
compounds was over 95% and was analyzed with Waters LCMS system.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were obtained using a Varian Inova-400 (400 MHz for  $^1\text{H}$ , and 75 MHz for  $^{13}\text{C}$ ) spectrometer. Chemical shifts are reported relative to chloroform ( $\delta = 7.24$ ) for  $^1\text{H}$  NMR or dimethyl sulfoxide ( $\delta = 2.50$ ) for  $^1\text{H}$  NMR and dimethyl sulfoxide ( $\delta = 39.51$ ) for  $^{13}\text{C}$  NMR. Data are reported as (*br* = broad, *s* = singlet, *d* = doublet, *t* = triplet, *q* = quartet, *m* = multiplet).

## Synthesis of JH-II-127 (18)



### 7H-pyrrolo[2,3-d]pyrimidine-2,4-diol

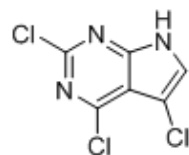
To a suspended solution of 6-aminouracil (12.7 g, 100 mmol) and sodium acetate (8.2 g, 100 mmol) in  $\text{H}_2\text{O}$  (100 mL) at a temperature of 70-75 °C was added a solution of chloroacetaldehyde (50% in water, 23.6 g, 150 mmol). The resulting reaction mixture was stirred at 80 °C for 20min and then cooled to room temperature. The resulting solid was collected by filtration, washed with water and acetone, and dried *in vacuo* to give the title compound as a light-brown solid (14.74 g, 98% yield).  $^1\text{H}$  NMR (DMSO-*d*<sub>6</sub>, 400 MHz)  $\delta$  11.38 (br, 1H), 11.10 (br, 1H), 10.46 (s, 1H), 6.53 (s, 1H), 6.18 (s, 1H), MS *m/z* 152.43 [M+1].



### 2,4-dichloro-7H-pyrrolo[2,3-d]pyrimidine (25)

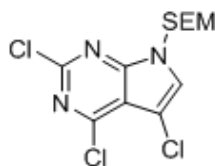
$\text{POCl}_3$  (229 mmol) was slowly added at room temperature to a suspension of 7H-pyrrolo[2,3-d]pyrimidine-2,4-diol 11.5 g (76 mmol) in toluene (60 mL). The reaction mixture was heated to 70 °C and 26.5 mL of DIPEA (153 mmol) was added drop-wise over a period of two hours, at which time the reaction temperature was increased to 106 °C and the mixture stirred overnight. After cooling to room temperature, the reaction mixture was poured into a mixture of 200 mL EtOAc and 300 mL ice-water and then filtered through celite. The aqueous layer was extracted with EtOAc (3x200 mL) and the combined organic layers were washed with

brine, decolored with activated carbon, filtered through celite and concentrated to give the title compound. MS  $m/z$  189.64 [M+1]



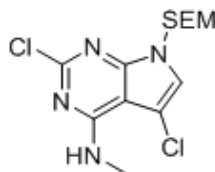
**2,4,5-trichloro-7H-pyrrolo[2,3-d]pyrimidine (26)**

To a solution of 2,4-Dichloro-7H-pyrrolo[2,3-d]pyrimidine (2.0 g, 10.64 mmol) in DCM/THF (15mL/6mL) was added NCS (1.70g, 12.76 mmol). The mixture was heated to 90 °C under microwave irradiation for 2.5 hours. The solvent was removed in vacuo and the crude product was purified by flash column chromatography using a 9:1 v/v Hexane:Ethyl acetate as solvent to afford the title compound 2.2 g (93% yield) as a white crystalline solid. MS  $m/z$  223.48 [M+1]



**2,4,5-trichloro-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine (27)**

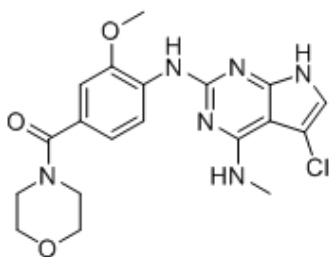
To a solution of 2,4,5-trichloro-7H-pyrrolo[2,3-d]pyrimidine (2.0 g, 8.99 mmol) in *N,N*-dimethylformamide (30 mL) was added sodium hydride 60% (432 mg, 10.78 mmol) at 0 °C. The reaction mixture was allowed to stir for 30 min. after which time 2-(trimethylsilyl)ethoxymethyl chloride (1.91 mL, 10.78 mmol) was added. The reaction mixture was further stirred at room temperature for a period of 3 hours after which time water was added and stirred for 20 minutes. The resulting precipitate was filtered and dried to yield the title compound 2.85 g (90% yield) as a brown solid. MS  $m/z$  353.84 [M+1]



**2,5-dichloro-N-methyl-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (28)**

To a solution 2,4,5-trichloro-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine (1 g, 2.8 mmol) in MeOH (15 mL) was added a solution of Methylamine 33%

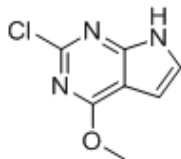
in MeOH (0.29 mL, 3.11 mmol). The mixture was heated to 70 °C for 1 hour followed by cooling to room temperature at which time the solvent was removed *in vacuo* and the crude product was purified by flash column chromatography using a 9:1 v/v Hexane:Ethyl acetate as solvent to afford the title compound 916 mg (93% yield) as a white solid. MS *m/z* 348.08 [M+1]



**(4-((5-chloro-4-(methylamino)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)amino)-3-methoxyphenyl)(morpholino)methanone (18)**

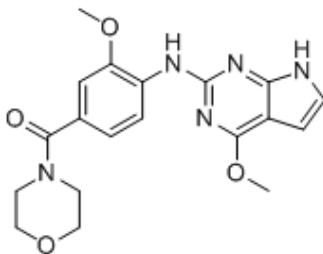
To a solution of 2,5-dichloro-N-methyl-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (100 mg, 0.28 mmol) in *sec*-BuOH (5 mL) was added (4-amino-3-methoxyphenyl)(morpholino)methanone (82 mg, 0.35 mmol) and K<sub>2</sub>CO<sub>3</sub> (77.4 mg, 0.56 mmol). The reaction mixture was degassed for 5 min and then Pd<sub>2</sub>(dba)<sub>3</sub> (15.4 mg, 0.017 mmol) and 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (12 mg, 0.025 mmol) were added. The reaction flask was stirred at 90 °C for 6 hr. After cooling to room temperature, the reaction mixture was filtered through a pad of celite and partitioned between ethyl acetate and water. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic extracts were washed with brine, dried over MgSO<sub>4</sub>, filtered and concentrated. The crude product was dissolved in DCM (10 mL) and TFA (2mL) was added and stirred at 50 °C for 3 hours at which time the solvent was removed *in vacuo*. The crude material was dissolved in THF (10 mL) and a saturated solution of NaHCO<sub>3</sub> (10 mL) was added and stirred for 6 hr. The mixture was extracted with ethyl acetate and the combined organic extracts were washed with brine, dried over MgSO<sub>4</sub>, filtered and concentrated. Purification by HPLC gave the title compound 68 mg (58% yield) as a brown solid. <sup>1</sup>H NMR 400 MHz (DMSO-*d*<sub>6</sub>) δ 11.43 (br, 1H), 8.62 (*d*, *J* = 4 Hz, 1H), 7.44 (*s*, 1H), 7.03 (*m*, 1H), 6.6 (*m*, 1H), 3.95 (*s*, 3H), 3.62 – 3.5 (*m*, 8H), 2.48 (*s*, 3H); <sup>13</sup>C NMR 100 MHz (DMSO-*d*<sub>6</sub>) δ 169.65, 156.91, 150.37, 146.98, 131.83, 127.29, 120.39, 116.70, 116.43, 110.00, 102.03, 95.63, 66.59, 56.42, 28.17; MS *m/z*: 417.34 [M+1].

**Synthesis of (4-((5-chloro-4-methoxy-7H-pyrrolo[2,3-d]pyrimidin-2-yl)amino)-3-methoxyphenyl)(morpholino)methanone (9)**



**2-chloro-4-methoxy-7H-pyrrolo[2,3-d]pyrimidine**

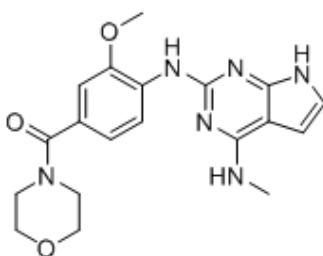
To a solution of 2-chloro-4-methoxy-7H-pyrrolo[2,3-d]pyrimidine (1 g, 5.4 mmol) in MeOH (15 mL) was added a solution of Methylamine 33% in MeOH (0.56 mL, 5.9 mmol). The mixture was heated to 70 °C for 1 hour followed by cooling to room temperature at which time the solvent was removed *in vacuo* and the crude product was purified by flash column chromatography using a 9:1 v/v Hexane:Ethyl acetate as solvent to afford the title compound 965 mg (95% yield) as a white solid. MS *m/z* 184.08 [M+1]



**(3-methoxy-4-((4-methoxy-7H-pyrrolo[2,3-d]pyrimidin-2-yl)amino)phenyl)(morpholino)methanone (9)**

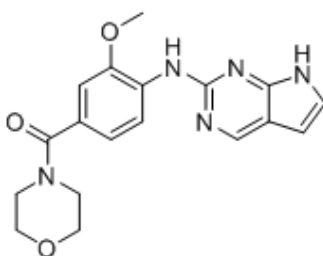
To a solution of 2-chloro-4-methoxy-7H-pyrrolo[2,3-d]pyrimidine (100 mg, 0.54 mmol) in *sec*-BuOH (5 mL) was added (4-amino-3-methoxyphenyl)(morpholino)methanone (154 mg, 0.65 mmol) and K<sub>2</sub>CO<sub>3</sub> (150 mg, 1.08 mmol). The reaction mixture was degassed for 5 min and then Pd<sub>2</sub>(dba)<sub>3</sub> (30 mg, 0.032 mmol) and 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (23 mg, 0.049 mmol) were added. The reaction flask was stirred at 110 °C for 8 hr. After cooling to room temperature, the reaction mixture was filtered through a pad of celite and partitioned between ethyl acetate and water. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic extracts were washed with brine, dried over MgSO<sub>4</sub>, filtered and concentrated. Purification by HPLC gave the title compound 64 mg (31% yield) as a brown solid. <sup>1</sup>H NMR 400 MHz (DMSO-*d*<sub>6</sub>) δ 11.58 (br, 1H), 8.58 (d, *J* = 7 Hz, 1H), 7.67 (m, 1H), 7.07 – 6.99 (m, 2H), 6.34 – 6.31 (m, 1H), 4.01 (s, 1H), 3.92 (s, 3H), 2.52 (s, 1H), 2.51 – 2.46 (m, 8H) MS *m/z* : 384.58

(M + 1).



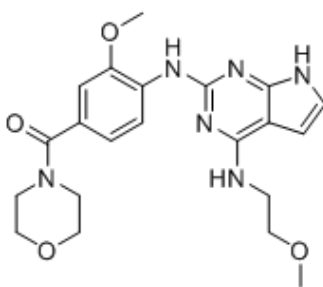
**(3-methoxy-4-((4-(methylamino)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)amino)phenyl)(morpholino)methanone (8)**

$^1\text{H}$ NMR (400 MHz, DMSO)  $\delta$  11.96 (br, 1H), 8.48 (d,  $J$  = 4 Hz, 1H), 7.04 (s, 1H), 6.96 (m, 1H), 6.54 (s, 1H), 3.95 (s, 3H), 2.48 (s, 3H) 2.44 – 2.39 (m, 8H)



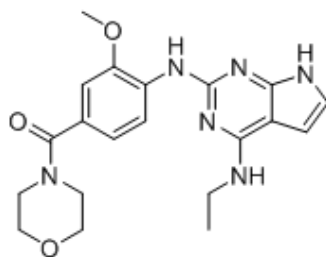
**(4-((7H-pyrrolo[2,3-d]pyrimidin-2-yl)amino)-3-methoxyphenyl)(morpholino)methanone (10)**

$^1\text{H}$  NMR 400 MHz (DMSO- $d_6$ )  $\delta$  12.05 (br, 1H), 8.81 (s, 1H), 8.56 (br, 1H), 8.38 (d,  $J$  = 7 Hz, 1H), 7.37 (m, 2H), 7.22 (m, 1H), 7.01 (m, 1H), 3.90 (s, 3H), 2.51 – 2.46 (m, 8H) MS  $m/z$  : 354.58 (M + 1).



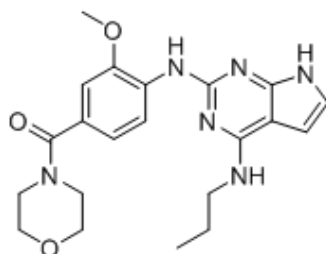
**(3-methoxy-4-((4-((2-methoxyethyl)amino)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)amino)phenyl)(morpholino)methanone (11)**

$^1\text{H}$ NMR (400 MHz, DMSO- $d_6$ )  $\delta$  11.87 (br, 1H), 8.40 (d,  $J$  = 9 Hz, 1H), 7.23 (s, 1H), 7.10 (m, 1H), 7.03 – 6.98 (m, 1H), 3.92 (s, 3H), 3.27 (s, 3H), 2.48 (m, 12H) MS  $m/z$  : 427.19 (M + 1).



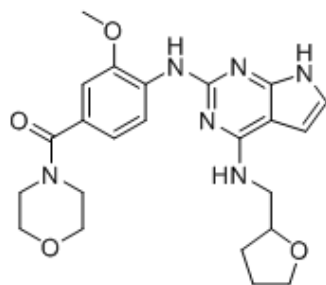
**(4-((4-(ethylamino)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)amino)-3-methoxyphenyl)(morpholino)methanone (12)**

$^1\text{H}$ NMR (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$  11.98 (br, 1H), 9.01 (m, 1H), 8.4 (m, 1H), 7.13 (s, 1H), 7.02 (m, 1H), 6.84 (m, 1H), 6.64 (s, 1H), 3.95 (s, 3H), 2.45 – 2.41 (m, 8H), 1.68 (q,  $J$  = 7 Hz, 2H), 0.88 (t,  $J$  = 7 Hz, 3H) MS  $m/z$  : 397.09 ( $M + 1$ ).



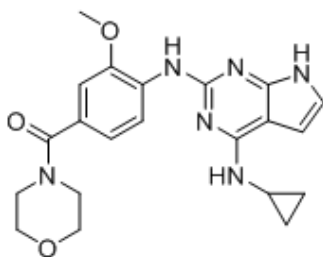
**(3-methoxy-4-((4-(propylamino)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)amino)phenyl)(morpholino)methanone (13)**

$^1\text{H}$ NMR (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$  11.91 (br, 1H), 8.43 (d,  $J$  = 4 Hz, 1H), 7.81 (m, 1H), 7.10 (s, 1H), 7.0 (m, 1H), 6.61 (s, 1H), 6.48 (m, 1H), 3.98 (s, 3H), 2.47 (s, 1H), 2.45 – 2.41 (m, 8H), 1.38 – 1.01 (m, 4H), 0.88 (t,  $J$  = 7 Hz, 3H) MS  $m/z$  : 411.33 ( $M + 1$ ).



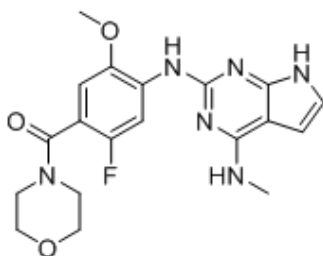
**(3-methoxy-4-((4-(((tetrahydrofuran-2-yl)methyl)amino)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)amino)phenyl)(morpholino)methanone (14)**

$^1\text{H}$ NMR (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$  7.22 (s, 1H), 7.16 (d,  $J$  = 9 Hz, 1H), 7.23 (s, 1H), 4.01 (s, 3H), 3.14 (d,  $J$  = 4 Hz 3H), 2.63 – 2.6 (m, 12H) MS  $m/z$  : 453.07 ( $M + 1$ ).



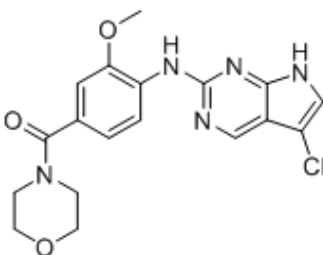
**(4-((4-(cyclopropylamino)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)amino)-3-methoxyphenyl)(morpholino)methanone (15)**

$^1\text{H}$ NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  12.06 (br, 1H), 8.56 (d,  $J = 4$  Hz, 1H), 7.98 (m, 1H), 7.08 (s, 1H), 6.64 (s, 1H), 4.01 (s, 3H), 2.56 (s, 3H) 2.48 (m, 8H), 0.99 (m, 1H), 0.81 – 0.55 (m, 4H) MS  $m/z$  : 409.21 ( $M + 1$ ).



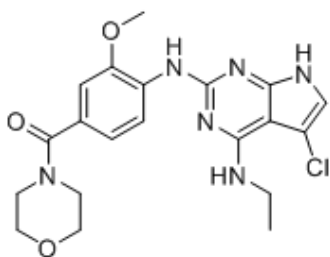
**(2-fluoro-5-methoxy-4-((4-(methylamino)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)amino)phenyl)(morpholino)methanone (16)**

$^1\text{H}$ NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  11.94 (br, 1H), 8.26 (s, 1H), 7.64 (s, 1H), 6.96 (m, 1H), 6.54 (s, 1H), 3.95 (s, 3H), 2.48 (s, 3H) 2.44 – 2.39 (m, 8H) MS  $m/z$  : 401.36 ( $M + 1$ ).



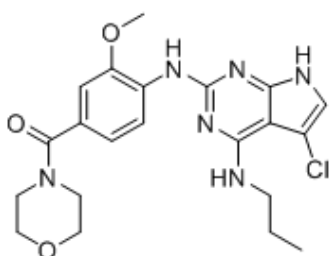
**(4-((5-chloro-7H-pyrrolo[2,3-d]pyrimidin-2-yl)amino)-3-methoxyphenyl)(morpholino)methanone (17)**

$^1\text{H}$  NMR 400 MHz ( $\text{DMSO}-d_6$ )  $\delta$  12.05 (br, 1H), 8.81 (s, 1H), 8.56 (br, 1H), 8.38 (d,  $J = 7$  Hz, 1H), 7.37 (m, 2H), 7.22 (m, 1H), 6.88 (s, 1H), 3.92 (s, 3H), 2.50 – 2.48 (m, 8H) MS  $m/z$  : 388.27 ( $M + 1$ ).



**(4-((5-chloro-4-(ethylamino)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)amino)-3-methoxyphenyl)(morpholino)methanone (19)**

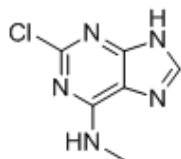
$^1\text{H}$  NMR 400 MHz (DMSO- $d_6$ )  $\delta$  12.01 (br, 1H), 8.62 (*d*,  $J$  = 4 Hz, 1H), 7.68 (br, 1H), 7.42 (*s*, 1H), 6.98 (*m*, 1H), 6.6 (*m*, 1H), 4.01 (*q*,  $J$  = 7 Hz, 2H), 3.91 (*s*, 3H), 2.50 – 2.46 (*m*, 8H), 1.2 (*m*, 3H); MS  $m/z$ : 431.17 [ $M+1$ ].



**(4-((5-chloro-4-(propylamino)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)amino)-3-methoxyphenyl)(morpholino)methanone (20)**

$^1\text{H}$  NMR 400 MHz (DMSO- $d_6$ )  $\delta$  11.48 (br, 1H), 8.59 (*m*, 1H), 7.0 (*m*, 2H), 3.91 (*s*, 3H), 2.50 – 2.46 (*m*, 8H), 1.65 (*m*, 5H), 0.95 (*m*, 2H); MS  $m/z$ : 445.25 [ $M+1$ ].

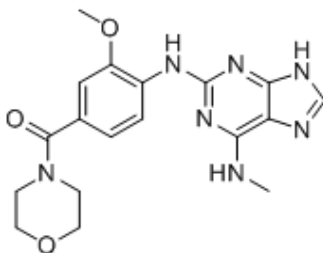
**Synthesis of (3-methoxy-4-((6-(methylamino)-9H-purin-2-yl)amino)phenyl)(morpholino)methanone (21)**



**2-chloro-N-methyl-9H-purin-6-amine**

To a solution of 2,6-dichloro-9H-purine (1 g, 5.29 mmol) in MeOH (15 mL) was added a solution of Methylamine 33% in MeOH (0.55 mL, 5.89 mmol). The mixture was heated to 70 °C for 1 hour followed by cooling to room temperature. The resulting precipitate was filtered, washed with ice-cold MeOH and dried to afford the title compound 880 mg (92% yield) as a

white solid. MS  $m/z$  184.73 [M+1]

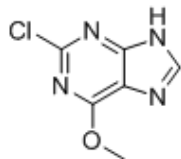


**(3-methoxy-4-((6-(methylamino)-9H-purin-2-yl)amino)phenyl)(morpholino)methanone (21)**

To a solution of 2-chloro-N-methyl-9H-purin-6-amine (100 mg, 0.54 mmol) in *sec*-BuOH (5 mL) was added (4-amino-3-methoxyphenyl)(morpholino)methanone (154 mg, 0.65 mmol) and  $K_2CO_3$  (150 mg, 1.08 mmol). The reaction mixture was degassed for 5 min and then  $Pd_2(dba)_3$  (30 mg, 0.032 mmol) and 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (23 mg, 0.049 mmol) were added. The reaction flask was stirred at 110 °C for 8 hr. After cooling to room temperature, the reaction mixture was filtered through a pad of celite and partitioned between ethyl acetate and water. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic extracts were washed with brine, dried over  $MgSO_4$ , filtered and concentrated. Purification by HPLC gave the title compound 70 mg (35% yield) as a light-brown solid.  $^1H$ NMR (400 MHz, DMSO)  $\delta$  8.39 (d,  $J$  = 9 Hz, 1H), 8.06 (s, 1H), 7.06 – 7.02 (m, 1H), 6.98 (d,  $J$  = 3Hz, 1H), 3.85 (s, 1H), 2.47 (s, 3H), 2.45 – 2.51 (m, 8H), MS  $m/z$  384.19 [M+1]

**Synthesis of (3-methoxy-4-((6-methoxy-9H-purin-2-yl)amino)phenyl)(morpholino)methanone (22)**

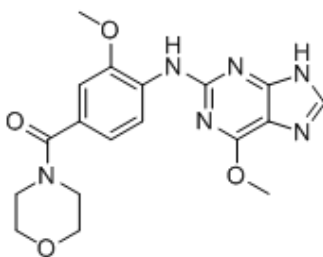
**2-chloro-6-methoxy-9H-purine**



**2-chloro-6-methoxy-9H-purine**

To a solution of 2,6-dichloro-9H-purine (1 g, 5.29 mmol) in MeOH (15 mL) was added a

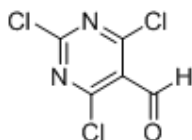
solution of NaOMe 25% in MeOH (1.26 mL, 5.89 mmol). The mixture was heated to 80 °C for 1 hour followed by cooling to room temperature. The resulting precipitate was filtered, washed with ice-cold MeOH and dried to afford the title compound 920 mg (94% yield) as a white solid. MS  $m/z$  185.36 [M+1]



**(3-methoxy-4-((6-methoxy-9H-purin-2-yl)amino)phenyl)(morpholino)methanone (22)**

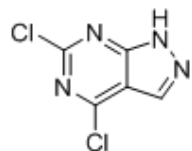
To a solution of 2-chloro-6-methoxy-9H-purine (100 mg, 0.54 mmol) in *sec*-BuOH (5 mL) was added (4-amino-3-methoxyphenyl)(morpholino)methanone (154 mg, 0.65 mmol) and  $K_2CO_3$  (150 mg, 1.08 mmol). The reaction mixture was degassed for 5 min and then  $Pd_2(dba)_3$  (30 mg, 0.032 mmol) and 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (23 mg, 0.049 mmol) were added. The reaction flask was stirred at 110 °C for 8 hr. After cooling to room temperature, the reaction mixture was filtered through a pad of celite and partitioned between ethyl acetate and water. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic extracts were washed with brine, dried over  $MgSO_4$ , filtered and concentrated. Purification by HPLC gave the title compound 50 mg (24% yield) as a light-brown solid.  $^1H$ NMR (400 MHz, DMSO)  $\delta$  8.46 (d,  $J$  = 9 Hz, 1H), 8.01 (s, 1H), 7.89 (s, 1H), 7.04 (m, 1H), 4.03 (s, 3H), 3.96 (s, 3H), 2.48 – 2.40 (m, 8H), MS  $m/z$  385.62 [M+1]

**Synthesis of (3-methoxy-4-((4-(methylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-yl)amino)phenyl)(morpholino)methanone (23)**



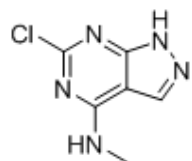
#### 2,4,6-trichloropyrimidine-5-carbaldehyde

DMF (12 mL) was added drop-wise to POCl<sub>3</sub> (75 mL, 800 mmol) at 0 °C, followed by barbituric acid (15 g, 118 mmol) was added portion-wise to the mixture. The mixture was stirred at 120 °C for 16 hr. Excess POCl<sub>3</sub> was removed *in vacuo* and the resulting residue was gradually poured into ice water. The mixture was extracted with DCM and the organic layer was washed with sat. NaHCO<sub>3</sub> solution, dried and concentrated to give the title compound as a yellow solid, yield 19.0 g, 77%. <sup>1</sup>HNMR (CDCl<sub>3</sub>, 400MHz): δ 10.45 (s, 1H). MS *m/z* 212.81 [M+1]



#### 4,6-dichloro-1H-pyrazolo[3,4-d]pyrimidine

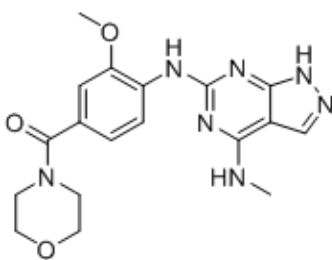
To a solution of 2,4,6-trichloropyrimidine-5-carbaldehyde (19.0 g, 990 mmol) in methanol (300 mL) was added drop-wise a solution of hydrazine monohydrate (4.8 mL) in methanol (80 mL) at 0 °C followed by drop-wise addition of triethylamine (13 mL) in methanol (80 mL) at 0 °C. The mixture was stirred at the same temperature for 30 min. The solvent was removed and the residue was purified by flash chromatography to afford the title compound (10.3 g, 62% yield) as a yellow solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400MHz): δ 11.56 (br, 1H), 8.43 (s, 1H). MS *m/z* 190.68 [M+1]



#### 6-chloro-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine

To a solution of 4,6-dichloro-1H-pyrazolo[3,4-d]pyrimidine (1 g, 5.29 mmol) in MeOH (15 mL) was added a solution of Methylamine 33% in MeOH (0.55 mL, 5.89 mmol). The mixture was heated to 70 °C for 1 hour followed by cooling to room temperature. The resulting precipitate was filtered, washed with ice-cold MeOH and dried to afford the title

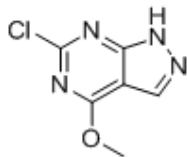
compound 874 mg (90% yield) as a white solid. MS  $m/z$  184.73 [M+1]



**3-methoxy-4-((4-(methylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-yl)amino)phenyl(morpholino)methanone (23)**

To a solution of 6-chloro-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (100 mg, 0.54 mmol) in *sec*-BuOH (5 mL) was added (4-amino-3-methoxyphenyl)(morpholino)methanone (154 mg, 0.65 mmol) and  $K_2CO_3$  (150 mg, 1.08 mmol). The reaction mixture was degassed for 5 min and then  $Pd_2(dba)_3$  (30 mg, 0.032 mmol) and 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (23 mg, 0.049 mmol) were added. The reaction flask was stirred at 110 °C for 8 hr. After cooling to room temperature, the reaction mixture was filtered through a pad of celite and partitioned between ethyl acetate and water. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic extracts were washed with brine, dried over  $MgSO_4$ , filtered and concentrated. Purification by HPLC gave the title compound 64 mg (31% yield) as a brown solid.  $^1H$  NMR 400 MHz ( $CD_3OD$ )  $\delta$  8.18 (*m*, 1H), 8.10 (*br*, 1H), 8.02 (*m*, 1H), 7.83 (*m*, 1H), 7.58 (*m*, 1H), 7.20 (*m*, 1H), 6.86 (*m*, 1H), 4.05 (*m*, 1H), 3.97 (*s*, 3H), 3.80 (*m*, 2H), 3.50 (*m*, 2H), 2.20 (*m*, 2H), 1.98 (*m*, 2H), 1.18 (*d*,  $J = 6.6$  Hz, 6H), MS  $m/z$  : 384.63 (M + 1).

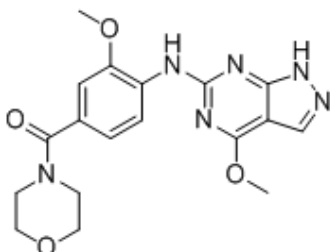
**Synthesis of (3-methoxy-4-((4-methoxy-1H-pyrazolo[3,4-d]pyrimidin-6-yl)amino)phenyl(morpholino)methanone (24)**



**6-chloro-4-methoxy-1H-pyrazolo[3,4-d]pyrimidine**

To a solution of 6-chloro-4-methoxy-1H-pyrazolo[3,4-d]pyrimidine (1 g, 5.29 mmol) in

MeOH (15 mL) was added a solution of NaOMe 25% in MeOH (1.26 mL, 5.89 mmol). The mixture was heated to 80 °C for 1 hour followed by cooling to room temperature. The resulting precipitate was filtered, washed with ice-cold MeOH and dried to afford the title compound 910 mg (92% yield) as a white solid. MS  $m/z$  185.46 [M+1]



**(3-methoxy-4-((4-methoxy-1H-pyrazolo[3,4-d]pyrimidin-6-yl)amino)phenyl)(morpholino)methanone (24)**

To a solution of 6-chloro-4-methoxy-1H-pyrazolo[3,4-d]pyrimidine (100 mg, 0.54 mmol) in *sec*-BuOH (5 mL) was added (4-amino-3-methoxyphenyl)(morpholino)methanone (154 mg, 0.65 mmol) and K<sub>2</sub>CO<sub>3</sub> (150 mg, 1.08 mmol). The reaction mixture was degassed for 5 min and then Pd<sub>2</sub>(dba)<sub>3</sub> (30 mg, 0.032 mmol) and 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (23 mg, 0.049 mmol) were added. The reaction flask was stirred at 110 °C for 8 hr. After cooling to room temperature, the reaction mixture was filtered through a pad of celite and partitioned between ethyl acetate and water. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic extracts were washed with brine, dried over MgSO<sub>4</sub>, filtered and concentrated. Purification by HPLC gave the title compound 58 mg (28% yield) as a light-brown solid. <sup>1</sup>HNMR 400 MHz (DMSO)  $\delta$  13.31 (br, 1H), 8.39 (d,  $J$  = 4 Hz, 1H), 7.98 (d,  $J$  = 8 Hz, 1H), 7.01 (m, 2H), 4.05 (s, 3H), 3.98 (s, 3H), 2.46 (m, 8H), MS  $m/z$  : 385.23 (M + 1).

## Biological materials and methods

**Reagents and General methods.** Tissue-culture reagents were from Life Technologies. P81 phosphocellulose paper was from Whatman and [ $\gamma$ -<sup>32</sup>P]-ATP was from Perkin Elmer. Nictide and LRRKtide were synthesised by Pepceuticals. Protein G sepharose was from Amersham. DNA constructs used for transfection were purified from *Escherichia coli* DH5 $\alpha$

using Qiagen or Invitrogen plasmid Maxi kits according to the manufacturer's protocol. All DNA constructs were verified by DNA sequencing, which was performed by The Sequencing Service, School of Life Sciences, University of Dundee, Scotland, U.K., using DYEnamic ET terminator chemistry (Amersham Biosciences) on Applied Biosystems automated DNA sequencers.

**Cell culture, treatments and cell lysis.** HEK293 and Swiss 3T3 cells were cultured in DMEM (Dulbecco's Modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum), 2mM glutamine and 1× penicillin/streptomycin solution. HEK-293 T-Rex cell lines were cultured in DMEM supplemented with 10% (v/v) FBS and 2mM glutamine, 1× penicillin/streptomycin solution, 15 µg/ml blastocidin and 100 µg/ml hygromycin. T-Rex cultures were induced to express the indicated protein by inclusion of 0.1 µg/ml doxycycline in the culture medium for 24-48 hours. Human lymphoblastoid cells were maintained in RPMI 1640 with 10% FBS, 2 mM glutamine, 1× penicillin/streptomycin solution and were maintained at cell density of  $0.3 \times 10^6$ – $2 \times 10^6$  cells per ml. Epstein-Barr virus immortalized primary human lymphoblastoid cells from one control subject and one Parkinson's disease patient homozygous for the LRRK2[G2019S] mutation were kindly provided by Alastair Reith (GSK) and have been described previously <sup>1</sup>. For inhibitor experiments, JH-II-127 and/or LRRK2-IN-1 was dissolved in DMSO and utilised at the indicated concentrations. The concentration of DMSO in the culture media did not exceed 1%. Following treatment, cells were washed once with PBS and lysed with buffer containing 50 mM Tris/HCl, pH 7.5, 1 mM EGTA, 1 mM EDTA, 1 mM sodium orthovanadate, 10 mM sodium β-glycerophosphate, 50 mM NaF, 5 mM sodium pyrophosphate, 0.27 M sucrose, 1 mM benzamidine, 2 mM phenylmethanesulphonylfluoride (PMSF) and 1% Triton X-100. When not used immediately, all lysate supernatants were snap-frozen in liquid nitrogen and stored at –80 °C until use. Protein concentrations were determined following centrifugation of the lysate at 16,000 x g at 4 °C for 20 minutes using the Bradford method with BSA as the standard. Transient transfection of HEK293 cells was performed using the PEI method <sup>2</sup>.

**IC<sub>50</sub> determination.** Active GST-LRRK2 (1326-2527), GST-LRRK2[G2019S] (1326-2527), GST-LRRK2[A2016T] (1326-2527) and GST-LRRK2[A2016T+G2019S] (1326-2527) enzyme was purified with glutathione sepharose from HEK293 cell lysate 36 h following transient transfection of the appropriate cDNA constructs as described previously <sup>3</sup>. Peptide kinase assays, performed in triplicate, were set up in a total volume of 40 µl containing 0.5 µg LRRK2 kinase (which at approximately 10% purity gives a final concentration of 8 nM) in

50 mM Tris/HCl, pH 7.5, 0.1 mM EGTA, 10 mM MgCl<sub>2</sub>, 20 μM Nictide, 0.1 μM [ $\gamma$ -<sup>32</sup>P]ATP (~500 cpm/pmol) and the indicated concentrations of inhibitor dissolved in DMSO. After incubation for 15 min at 30 °C, reactions were terminated by spotting 35 μl of the reaction mix onto P81 phosphocellulose paper and immersion in 50 mM phosphoric acid. Samples were washed extensively and the incorporation of [ $\gamma$ -<sup>32</sup>P]ATP into Nictide was quantified by Cerenkov counting. IC<sub>50</sub> values were calculated with GraphPad Prism using non-linear regression analysis.

**Immunoblot procedures.** Cell lysates from Swiss 3T3 cells, human lymphoblastoid cells, GFP-LRRK2 expressing stable cell lines and mouse tissues were eluted in 65 μl 2x LDS sample buffer (Invitrogen) with final concentration of 1 μg/μl. Following heating at 70 °C for 10 min, 15 μl aliquots were resolved on 8 % SDS polyacrylamide gels and transferred to nitrocellulose membranes for detection of LRRK2 phosphorylated at Ser910, LRRK2 phosphorylated at Ser935 and total LRRK2, using purified rabbit monoclonal antibodies (LRRK2 phospho-serine 910 clone (LRRK2 $\alpha$  pS910 UDD1), LRRK2 phospho-serine 935 clone (LRRK2 $\alpha$  pS935 UDD2) and LRRK2 100-500 clone (LRRK2 (100 - 500) UDD3) ) in PBS with 0.1% sodium azide (Epitomics) at 0.1 μg/ml concentrations. Immunoblot films were scanned on an Epson 4990 scanner, and images were managed with Adobe Photoshop. Secondary antibodies coupled to horseradish peroxidase used for immunoblotting were obtained from Pierce.

**Pharmacodynamic (PD) study.** JH-II-127 (**18**) was dissolved in 5% 1-methyl-2-pyrrolidinone (NMP) /95% PEG 300 (Sigma) solution and administered by intraperitoneal injection into wild type male C57BL/6 mice at doses of 0, 3, 10, 30, 50 and 100 mg/kg and at 100 mg/kg LRRK2-IN-1 (**1**) as a comparative control. Control mice were treated with an equal volume of NMP/PEG solution. One hour after administration, mice were sacrificed by cervical dislocation and, kidney, spleen and brain tissues were rapidly dissected and snap-frozen in liquid nitrogen. Animal experiments were approved by the University of Dundee Ethics Committee and performed under a U.K. Home office project license.

## Dundee profiling<sup>4</sup>

JH-II-127 (**18**) was tested at 10 and 1 uM. Results show percent remaining enzymatic activity relative to the DMSO control.

<b>K</b>	<b>I1</b>	<b>K</b>	<b>I10</b>
TGFBR1	<b>128</b>	BTK	<b>126</b>
Aurora A	<b>124</b>	EPH-B1	<b>126</b>
MARK3	<b>120</b>	TGFBR1	<b>119</b>
EPH-B1	<b>119</b>	WNK1	<b>115</b>
PKBb	<b>118</b>	Aurora A	<b>112</b>
MELK	<b>118</b>	Lck	<b>111</b>
RSK2	<b>117</b>	PKC $\gamma$	<b>109</b>
p38g MAPK	<b>116</b>	MPSK1	<b>106</b>
BTK	<b>116</b>	PKBa	<b>105</b>
IKKe	<b>114</b>	PAK6	<b>105</b>
PKC $\gamma$	<b>113</b>	TLK1	<b>105</b>
TAO1	<b>113</b>	p38a MAPK	<b>104</b>
MPSK1	<b>113</b>	AMPK	<b>103</b>
ROCK 2	<b>113</b>	ABL	<b>103</b>
AMPK	<b>112</b>	PKBb	<b>103</b>
MAP4K5	<b>110</b>	NEK2a	<b>103</b>
ULK2	<b>110</b>	FGF-R1	<b>102</b>
Src	<b>110</b>	PAK2	<b>102</b>
PAK2	<b>110</b>	S6K1	<b>102</b>
CHK1	<b>110</b>	EF2K	<b>102</b>
BRSK2	<b>110</b>	p38d MAPK	<b>102</b>
SIK2	<b>109</b>	Src	<b>102</b>
MARK4	<b>109</b>	EPH-A4	<b>102</b>
MSK1	<b>109</b>	ROCK 2	<b>101</b>
IRAK1	<b>109</b>	EPH-A2	<b>101</b>
PDGFRA	<b>109</b>	PKA	<b>101</b>
WNK1	<b>108</b>	PKCa	<b>100</b>
ERK8	<b>108</b>	TAO1	<b>100</b>
p38b MAPK	<b>108</b>	VEG-FR	<b>100</b>
p38d MAPK	<b>107</b>	MSK1	<b>99</b>
EPH-A4	<b>107</b>	BRSK2	<b>98</b>
ERK1	<b>107</b>	BRSK1	<b>98</b>
SIK3	<b>107</b>	MARK4	<b>98</b>
TLK1	<b>106</b>	IKKe	<b>98</b>

ABL	106	TTBK1	98
S6K1	106	MST4	97
HIPK3	106	JAK2	97
CDK9-Cyclin T1	105	GSK3b	97
PINK	105	SIK3	96
EF2K	105	p38g MAPK	96
TTBK2	105	LKB1	96
VEG-FR	104	IKKb	95
TIE2	104	MST3	95
AMPK (hum)	104	TESK1	95
EPH-A2	104	ULK1	95
TTBK1	103	ULK2	95
PRK2	103	RSK2	94
ULK1	103	PKCz	94
LKB1	103	TIE2	94
MST4	103	PRK2	93
MST3	102	CHK1	93
PKCa	102	HIPK3	93
EIF2AK3	102	DDR2	93
MNK1	102	MAP4K5	92
PAK4	102	EPH-B3	92
RIPK2	102	AMPK (hum)	92
PAK6	102	CSK	92
CDK2-Cyclin A	102	PAK4	92
NEK6	101	IRAK1	92
PKBa	101	MARK3	92
ZAP70	101	SIK2	92
MKK2	101	CDK9-Cyclin T1	91
MARK2	101	Aurora B	90
CK1 $\gamma$ 2	100	TTBK2	90
IKKb	100	PDGFRA	89
JAK2	99	PINK	89
PKA	99	PDK1	88
DDR2	98	PIM1	88
MAP4K3	98	EPH-B4	86
MAPKAP-K3	97	YES1	85
Aurora B	97	MEKK1	85
NEK2a	97	ZAP70	85
Lck	97	MAPKAP-K3	84
TESK1	97	TrkA	84
PIM3	96	RIPK2	83
RSK1	96	NEK6	83

TAK1	96		MST2	82
IRAK4	95		IRAK4	82
MINK1	95		PIM2	82
HIPK1	95		MARK2	82
PIM2	95		SYK	81
PIM1	95		p38b MAPK	79
MEKK1	94		EIF2AK3	79
ERK2	94		MELK	79
HER4	94		MNK1	78
BRSK1	94		MARK1	77
CK2	94		SGK1	76
FGF-R1	94		PIM3	75
EPH-B3	92		MKK2	72
PDK1	91		ERK2	71
MKK1	91		ERK8	71
SYK	91		HER4	71
MST2	90		CK2	70
p38a MAPK	90		PAK5	70
MARK1	89		ERK1	68
CSK	89		MINK1	68
PAK5	89		TBK1	67
SRPK1	89		CDK2-Cyclin A	67
TBK1	88		RSK1	60
GCK	86		EPH-B2	58
SGK1	86		MKK1	56
MAPKAP-K2	86		ERK5	52
YES1	85		CK1 $\gamma$ 2	45
TrkA	85		HIPK1	44
HIPK2	84		HIPK2	43
GSK3b	82		TAK1	42
MLK1	81		MAP4K3	41
EPH-B4	80		GCK	41
CAMK1	80		MAPKAP-K2	40
MLK3	78		MLK1	38
ERK5	76		MLK3	35
PKCz	76		CAMK1	34
MNK2	75		CK1 $\delta$	33
NUAK1	75		SRPK1	31
CK1 $\delta$	74		DYRK3	30
DYRK3	74		MNK2	25
TTK	69		BRK	19
DYRK2	67		DYRK2	18

BRK	62		NUAK1	17
PLK1	62		CAMKKb	16
OSR1	61		STK33	15
EPH-B2	60		PLK1	13
ASK1	56		TTK	12
CAMKKb	50		OSR1	12
DYRK1A	49		MKK6	11
PKD1	47		IRR	10
PRAK	43		IR	10
JNK1	38		ASK1	10
MKK6	37		IGF-1R	9
IRR	31		JNK3	9
JNK3	29		DYRK1A	8
JNK2	27		PKD1	7
STK33	22		TSSK1	7
DAPK1	21		PRAK	6
IR	21		JNK1	6
TSSK1	14		DAPK1	5
CLK2	13		JNK2	4
PHK	13		CLK2	2
IGF-1R	13		PHK	1
SmMLCK	9		CHK2	1
CHK2	5		SmMLCK	1

**Supplementary Fig.1 IC50s of JH-II-127 against SmMLCK and CHK2**

### **KinomeScan analysis <sup>5</sup>**

Compound ID	<b>JH-II-127</b>
Compound Concentration (uM)	1

AAK1	88
ABL1(E255K)-phosphorylated	95
ABL1(F317I)-nonphosphorylated	96
ABL1(F317I)-phosphorylated	100
ABL1(F317L)-nonphosphorylated	93
ABL1(F317L)-phosphorylated	100
ABL1(H396P)-nonphosphorylated	100
ABL1(H396P)-phosphorylated	95
ABL1(M351T)-phosphorylated	80
ABL1(Q252H)-nonphosphorylated	88
ABL1(Q252H)-phosphorylated	94
ABL1(T315I)-nonphosphorylated	91
ABL1(T315I)-phosphorylated	96
ABL1(Y253F)-phosphorylated	97
ABL1-nonphosphorylated	100
ABL1-phosphorylated	100
ABL2	96
ACVR1	91
ACVR1B	82
ACVR2A	100
ACVR2B	100
ACVRL1	85
ADCK3	100
ADCK4	100
AKT1	97
AKT2	93
AKT3	93
ALK	70
ALK(C1156Y)	68
ALK(L1196M)	74
AMPK-alpha1	97
AMPK-alpha2	98
ANKK1	95
ARK5	89
ASK1	84

ASK2	100
AURKA	100
AURKB	80
AURKC	100
AXL	97
BIKE	97
BLK	100
BMPR1A	91
BMPR1B	98
BMPR2	100
BMX	100
BRAF	100
BRAF(V600E)	100
BRK	97
BRSK1	100
BRSK2	100
BTK	100
BUB1	100
CAMK1	100
CAMK1D	100
CAMK1G	85
CAMK2A	92
CAMK2B	86
CAMK2D	99
CAMK2G	95
CAMK4	100
CAMKK1	96
CAMKK2	100
CASK	88
CDC2L1	85
CDC2L2	100
CDC2L5	100
CDK11	63
CDK2	95
CDK3	96

CDK4-cyclinD1	95
CDK4-cyclinD3	92
CDK5	100
CDK7	95
CDK8	74
CDK9	92
CDKL1	87
CDKL2	86
CDKL3	86
CDKL5	100
CHEK1	100
CHEK2	31
CIT	92
CLK1	87
CLK2	56
CLK3	99
CLK4	63
CSF1R	79
CSF1R-autoinhibited	47
CSK	100
CSNK1A1	80
CSNK1A1L	97
CSNK1D	82
CSNK1E	100
CSNK1G1	97
CSNK1G2	100
CSNK1G3	100
CSNK2A1	93
CSNK2A2	70
CTK	96
DAPK1	54
DAPK2	40
DAPK3	52
DCAMKL1	74
DCAMKL2	100

DCAMKL3	77
DDR1	100
DDR2	93
DLK	89
DMPK	98
DMPK2	84
DRAK1	89
DRAK2	86
DYRK1A	95
DYRK1B	66
DYRK2	89
EGFR	93
EGFR(E746-A750del)	100
EGFR(G719C)	91
EGFR(G719S)	88
EGFR(L747-E749del, A750P)	81
EGFR(L747-S752del, P753S)	94
EGFR(L747-T751del,Sins)	84
EGFR(L858R)	83
EGFR(L858R,T790M)	100
EGFR(L861Q)	57
EGFR(S752-I759del)	97
EGFR(T790M)	97
EIF2AK1	100
EPHA1	100
EPHA2	88
EPHA3	88
EPHA4	94
EPHA5	98
EPHA6	88
EPHA7	98
EPHA8	99
EPHB1	100
EPHB2	98
EPHB3	89

EPHB4	83
EPHB6	100
ERBB2	88
ERBB3	84
ERBB4	100
ERK1	100
ERK2	96
ERK3	62
ERK4	100
ERK5	85
ERK8	100
ERN1	92
FAK	93
FER	95
FES	99
FGFR1	99
FGFR2	87
FGFR3	83
FGFR3(G697C)	100
FGFR4	98
FGR	81
FLT1	97
FLT3	100
FLT3(D835H)	98
FLT3(D835Y)	100
FLT3(ITD)	94
FLT3(K663Q)	100
FLT3(N841I)	100
FLT3(R834Q)	100
FLT3-autoinhibited	100
FLT4	93
FRK	100
FYN	90
GAK	65
GCN2(Kin.Dom.2,S808G)	91

GRK1	85
GRK4	90
GRK7	99
GSK3A	100
GSK3B	84
HASPIN	76
HCK	99
HIPK1	90
HIPK2	100
HIPK3	91
HIPK4	85
HPK1	100
HUNK	91
ICK	81
IGF1R	95
IKK-alpha	100
IKK-beta	98
IKK-epsilon	100
INSR	83
INSRR	100
IRAK1	100
IRAK3	98
IRAK4	100
ITK	88
JAK1(JH1domain-catalytic)	77
JAK1(JH2domain-pseudokinase)	99
JAK2(JH1domain-catalytic)	100
JAK3(JH1domain-catalytic)	100
JNK1	60
JNK2	78
JNK3	67
KIT	79
KIT(A829P)	100
KIT(D816H)	99
KIT(D816V)	76

KIT(L576P)	51
KIT(V559D)	82
KIT(V559D,T670I)	92
KIT(V559D,V654A)	94
KIT-autoinhibited	87
LATS1	78
LATS2	93
LCK	99
LIMK1	100
LIMK2	82
LKB1	100
LOK	100
LRRK2	11
LRRK2(G2019S)	6.5
LTK	59
LYN	100
LZK	92
MAK	98
MAP3K1	90
MAP3K15	100
MAP3K2	98
MAP3K3	100
MAP3K4	100
MAP4K2	97
MAP4K3	100
MAP4K4	100
MAP4K5	100
MAPKAPK2	95
MAPKAPK5	99
MARK1	97
MARK2	100
MARK3	84
MARK4	82
MAST1	100
MEK1	98

MEK2	100
MEK3	79
MEK4	72
MEK5	92
MEK6	99
MELK	91
MERTK	66
MET	97
MET(M1250T)	80
MET(Y1235D)	100
MINK	94
MKK7	100
MKNK1	85
MKNK2	91
MLCK	88
MLK1	100
MLK2	100
MLK3	100
MRCKA	99
MRCKB	100
MST1	100
MST1R	100
MST2	80
MST3	91
MST4	77
MTOR	100
MUSK	100
MYLK	61
MYLK2	82
MYLK4	81
MYO3A	100
MYO3B	85
NDR1	92
NDR2	100
NEK1	96

NEK10	100
NEK11	100
NEK2	96
NEK3	100
NEK4	90
NEK5	83
NEK6	100
NEK7	100
NEK9	100
NIK	62
NIM1	100
NLK	100
OSR1	88
p38-alpha	96
p38-beta	82
p38-delta	100
p38-gamma	95
PAK1	100
PAK2	100
PAK3	100
PAK4	100
PAK6	100
PAK7	83
PCTK1	93
PCTK2	88
PCTK3	100
PDGFRA	80
PDGFRB	78
PDPK1	86
PFCDPK1(P.falciparum)	96
PFPK5(P.falciparum)	100
PFTAIRE2	72
PFTK1	82
PHKG1	88
PHKG2	66

PIK3C2B	98
PIK3C2G	100
PIK3CA	87
PIK3CA(C420R)	95
PIK3CA(E542K)	100
PIK3CA(E545A)	99
PIK3CA(E545K)	100
PIK3CA(H1047L)	90
PIK3CA(H1047Y)	100
PIK3CA(I800L)	97
PIK3CA(M1043I)	91
PIK3CA(Q546K)	82
PIK3CB	86
PIK3CD	83
PIK3CG	100
PIK4CB	90
PIM1	98
PIM2	98
PIM3	99
PIP5K1A	84
PIP5K1C	100
PIP5K2B	62
PIP5K2C	100
PKAC-alpha	98
PKAC-beta	100
PKMYT1	100
PKN1	100
PKN2	100
PKNB(M.tuberculosis)	100
PLK1	89
PLK2	100
PLK3	100
PLK4	97
PRKCD	100
PRKCE	100

PRKCH	100
PRKCI	100
PRKCQ	93
PRKD1	85
PRKD2	98
PRKD3	100
PRKG1	72
PRKG2	99
PRKR	96
PRKX	100
PRP4	93
PYK2	100
QSK	86
RAF1	100
RET	96
RET(M918T)	96
RET(V804L)	92
RET(V804M)	100
RIOK1	91
RIOK2	100
RIOK3	67
RIPK1	76
RIPK2	96
RIPK4	82
RIPK5	100
ROCK1	88
ROCK2	93
ROS1	93
RPS6KA4(Kin.Dom.1-N-terminal)	97
RPS6KA4(Kin.Dom.2-C-terminal)	14
RPS6KA5(Kin.Dom.1-N-terminal)	100
RPS6KA5(Kin.Dom.2-C-terminal)	78
RSK1(Kin.Dom.1-N-terminal)	69
RSK1(Kin.Dom.2-C-terminal)	88
RSK2(Kin.Dom.1-N-terminal)	85

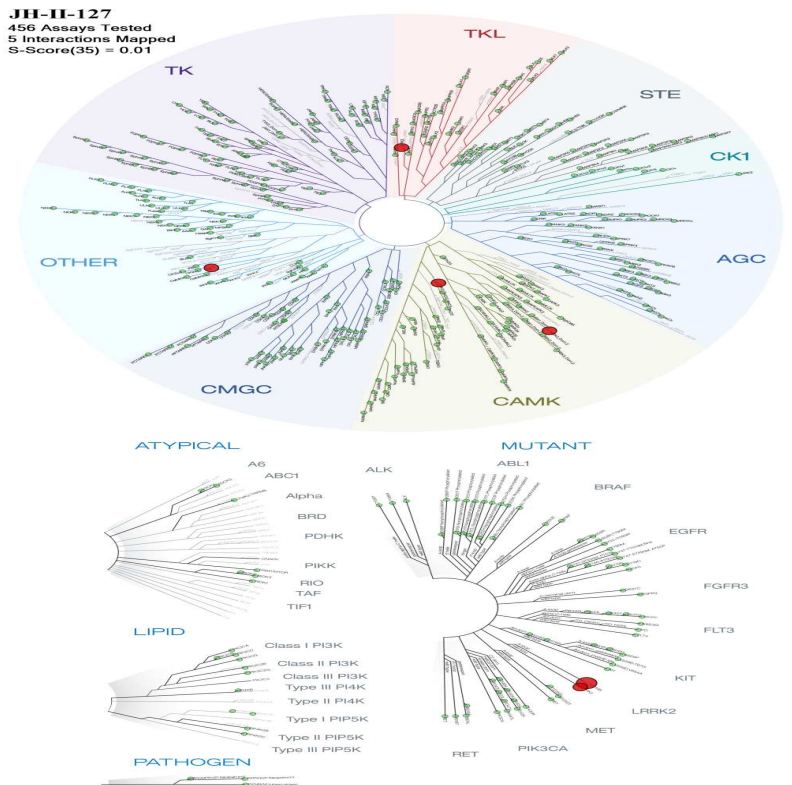
RSK2(Kin.Dom.2-C-terminal)	97
RSK3(Kin.Dom.1-N-terminal)	100
RSK3(Kin.Dom.2-C-terminal)	96
RSK4(Kin.Dom.1-N-terminal)	93
RSK4(Kin.Dom.2-C-terminal)	90
S6K1	99
SBK1	96
SGK	100
SgK110	74
SGK2	100
SGK3	100
SIK	94
SIK2	97
SLK	96
SNARK	100
SNRK	99
SRC	99
SRMS	98
SRPK1	76
SRPK2	100
SRPK3	100
STK16	100
STK33	42
STK35	100
STK36	94
STK39	81
SYK	80
TAK1	91
TAOK1	100
TAOK2	85
TAOK3	100
TBK1	100
TEC	100
TESK1	100
TGFBR1	99

TGFBR2	80
TIE1	94
TIE2	97
TLK1	92
TLK2	98
TNIK	100
TNK1	100
TNK2	100
TNNI3K	97
TRKA	99
TRKB	100
TRKC	88
TRPM6	100
TSSK1B	100
TTK	13
TXK	100
TYK2(JH1domain-catalytic)	99
TYK2(JH2domain-pseudokinase)	100
TYRO3	68
ULK1	100
ULK2	100
ULK3	96
VEGFR2	91
VRK2	99
WEE1	100
WEE2	97
WNK1	95
WNK3	96
YANK1	92
YANK2	100
YANK3	100
YES	92
YSK1	89
YSK4	81
ZAK	100

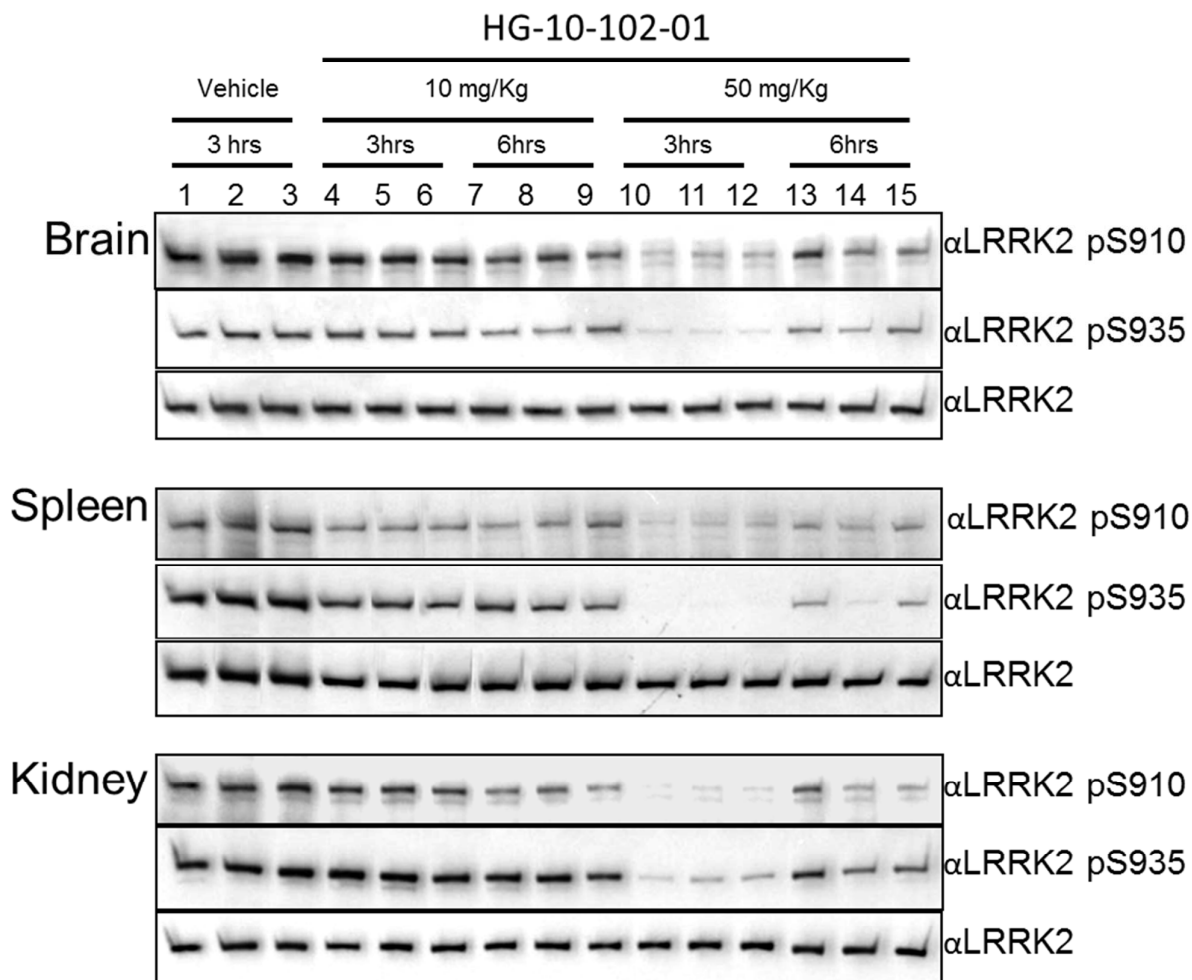
ZAP70	97
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# S-Score Table JH-II-127

**JH-II-127**  
 456 Assays Tested  
 5 Interactions Mapped  
 S-Score(35) = 0.01



Compound Name	Selectivity Score Type	Number of Hits	Number of Non-Mutant Kinases	Screening Concentration (nM)	Selectivity Score
JH-II-127	S(35)	4	392	1000	0.01
JH-II-127	S(10)	0	392	1000	0
JH-II-127	S(1)	0	392	1000	0



**Formulation:** 10% 1-methyl-2-pyrrolidinone (NMP) /90% PEG;

**IP doses:** 0, 10 (3 hrs), 10 (6hrs), 50 (3 hrs) and 50 (6hrs) mg/kg of HG-10-102-01 ;

**IP injection volume:** 200 µL/20g.

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