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Discovery and Optimization of Pyrrolopyrimidines as Highly Potent, Selective and Brain-Penetrant LRRK2 Inhibitors

Jeffrey M. Axten[a,*], Xiao Ding[b], Luigi Piero Stasi[b], Baowei Zhao[b], Yingxia Sang[b], Ming-Hsun Ho[a], Lizhen Wang[b], Minhua Zhang[b], Xianjun Guo[b], Chengfang Tan[b], Xu Feng[b], Colin Edge[c], Klara Valko[c], Yi Li[b], Kelly Dong[b], Xiaoming Guan[b], Nico Zinn[d], F. David Tattersall[a], Feng Ren[b], Dai-Shi Su[a], Alastair D. Reith[c]

GSK Research and Development, [a]1250 S. Collegeville Road, Collegeville, Pennsylvania, 19426, USA, [b]898 Halei Road, Zhangjiang Hi-Tech Park, Pudong, Shanghai 201203, PR China, [c]Medicines Research Centre, Gunnels Wood Road, Stevenage, SG1 2NY, UK, [d] Omics Technologies, Cellzome a GSK company, Meyerhofstrasse 1, D-69117 Heidelberg, Germany

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Synthetic procedures and characterization data for compounds 3-42

General methods

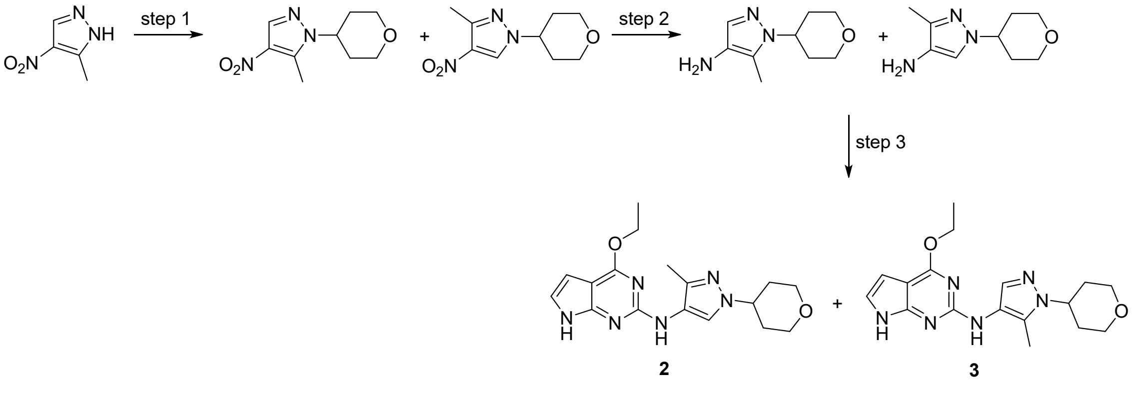
All reagents used were purchased from commercial suppliers and used without further purification. ^{1}H NMR, ^{19}F NMR and ^{13}C NMR spectra were recorded on a Bruker 400 or 600 NMR spectrometer using $CDCl_3$ (deuterochloroform), CD_3OD (deuterated methanol), or DMSO-d_6 (hexadeuterio-dimethyl sulfoxide) as the solvents and TMS (tetramethylsilane) as the internal standard. Chemical shifts (d) are given in parts per million (ppm) downfield from the TMS signal. The following abbreviations are used to indicate the multiplicity in NMR spectra: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets, app = apparent, br = broad. J indicates the NMR coupling constant measured in Hertz. High resolution mass (HRMS) was operated in a positive mode of electrospray ionization (ESI) at an orthogonal acceleration time-of-flight (oa-TOF) SYNAPT G2 HDMSTM (Waters, Manchester, UK). LCMS analysis was conducted for all compounds under the following conditions:1) Acidic conditions: mobile phase: water containing 0.05 % TFA / CH_3CN, column: Agilent SB-C18 4.6 x 30 mm-1.8 microns, detection: MS and photodiode array detector (PDA); 2) Basic conditions: mobile phase: water containing 10 mmol NH_4HCO_3 / CH_3CN, Column: XBridgeTM C18 4.6×50 mm-3.5 microns, detection: MS and photodiode array detector (PDA); 3) Basic conditions: mobile phase: water containing 0.02% NH_4OAc / CH_3CN, column: Welch Ultimate XB-C18 5µm 4.6×33mm, detection: MS and photodiode array detector (PDA). Column chromatography was performed on Isco or Biotage using a pre-packed silica gel column, a detector with UV wavelength at 254 nm and 280 nm. Prep-HPLC was performed on Waters instrument using Column: Xbridge Prep C18 column OBD (10 um, 19 x 250 mm) and water containing 0.08% ammonia/ acetonitrile as the mobile phase.

Abbreviations:

ACN: Acetonitrile
Boc_2O: Di-tert-butyl decarbonate
n-BuLi: *n*-Butyl lithium
CbzCl: Benzyl chloroformate
DAST: Diethylamino sulfur trifluoride
dba: dibenylidineacetone
DBU: 1,8-Diazabioyclo[5.4.0]undeo-7-ene
DCE: 1,2-Dichloroethane
DCM: Dichlormethane
DEA: Diethylamine

DIAD: Diisopropyl azodicarboxylate
DIPEA: *N,N*-Diisopropylethylamine
DMA: *N,N*-Dimethylacetamide
DMF: Dimethylformamide
DMAP: 4-Dimethylaminopyridine
DMSO: Dimethylsulfoxide
dppf: 1,1'-Bis(diphenylphosphino)ferrocene
EA: Ethyl acetate
Et: Ethyl
Et_2O: Diethyl ether
EtOAc: Ethyl acetate
LDA: Lithium diisopropylamide
LHMDS (or LiHMDS): Lithium bis(trimethylsilyl)amide
Me: Methyl
MsCl: Methanesulfonyl chloride
NIS: *N*-Iodosuccinimide
HOAc: Acetic acid
SEMCl: (2-(Chloromethoxy)ethyl)trimethylsilane
TBAF: Tetrabutylammonium fluoride
TEA: Triethylamine
TFA: Trifluoroacetic acid
PE: Petroleum ether
X-Phos: 4,5-Bis(diphenylphosphino)-9,9-dimethylxanthene

Synthesis of 4-ethoxy-*N*-(3-methyl-1-(tetrahydro-2*H*-pyran-4-yl)-1*H*-pyrazol-4-yl)-7*H*-pyrrolo-[2,3-*d*]-pyrimidin-2-amine (2) and 4-ethoxy-*N*-(5-methyl-1-(tetrahydro-2*H*-pyran-4-yl)-1*H*-pyrazol-4-yl)-7*H*-pyrrolo-[2,3-*d*]-pyrimidin-2-amine (3)



Step 1: *5-methyl-4-nitro-1-(tetrahydro-2H-pyran-4-yl)-1H-pyrazole and 3-methyl-4-nitro-1-(tetrahydro-2H-pyran-4-yl)-1H-pyrazole.* To a solution of 5-methyl-4-nitro-1H-pyrazole (800 mg, 6.29 mmol) and NaH (252 mg, 6.29 mmol) in DMF (5 mL) stirred at room temperature was added tetrahydro-2*H*-pyran-4-yl methanesulfonate (1475 mg, 8.18 mmol). The reaction was stirred overnight at 100 °C. The mixture was partitioned between ethyl acetate (10 mL) and water (25 mL). The organic phase was washed with satur. $NaHCO_3$ solution (10 mL), brine (10 mL), dried over Na_2SO_4 and concentrated to give the mixture of title compounds as brown oil (600 mg). LCMS: 212 $[M+H]^+$.

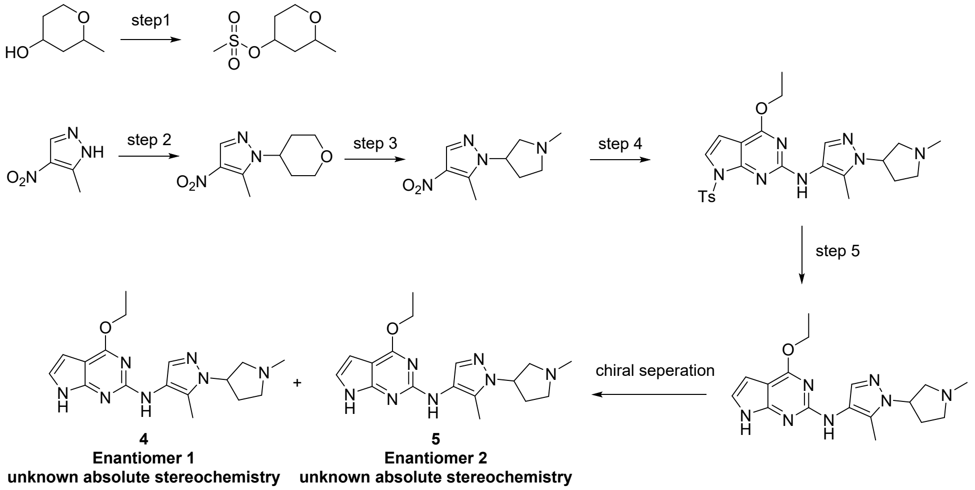
Step 2: *5-methyl-1-(tetrahydro-2H-pyran-4-yl)-1H-pyrazol-4-amine and 3-methyl-1-(tetrahydro-2H-pyran-4-yl)-1H-pyrazol-4-amine.* The mixture of 3-methyl-4-nitro-1-(tetrahydro-2H-pyran-4-yl)-1H-pyrazole, 5-methyl-4-nitro-1-(tetrahydro-2*H*-pyran-4-yl)-1H-pyrazole (470 mg, 2.225 mmol) and iron (1243 mg, 22.25 mmol) in ethanol (20 mL) and water (20.00 mL) was stirred overnight at 20 °C. The mixture was filtered and the filtrate concentrated. The crude was purified by column chromatography on silica gel (DCM: MeOH=10:1) to give the title compounds (180 mg, 44.6 % yield) as oil. LCMS: 182 $[M+H]^+$.

Step 3: *4-ethoxy-N-(3-methyl-1-(tetrahydro-2H-pyran-4-yl)-1H-pyrazol-4-yl)-7H-pyrrolo-[2,3-d]-pyrimidin-2-amine (**2**) and 4-ethoxy-N-(5-methyl-1-(tetrahydro-2H-pyran-4-yl)-1H-pyrazol-4-yl)-7H-pyrrolo-[2,3-d]-pyrimidin-2-amine (**3**).* A solution of **52** (90 mg, 0.455 mmol), a mixture of 5-methyl-1-(tetrahydro-2*H*-pyran-4-yl) -1*H*-pyrazol-4-amine and 3-methyl-1-(tetrahydro-2*H*-pyran-4-yl)-1*H*-pyrazol-4-amine (41.3 mg, 0.228 mmol), $Pd_2(dba)_3$ (41.7 mg, 0.046 mmol), (9,9-dimethyl-9*H*-xanthene-4,5-diyl)bis-(diphenylphosphine) and potassium carbonate (189 mg, 1.366 mmol) in 2-butanol (3.0 mL) was irradiated by microwave at 120 ^oC for 45 min. The reaction mixture was quenched with water, partitioned between ethyl acetate (25 mL) and water (5 mL). The organic layer was washed with water, and then saturated $NaHCO_3$ solution. The resulting organic layer was then dried over Na_2SO_4, filtered and concentrated. The crude was purified by prep-HPLC to give the title compounds **2** (40 mg, 0.117 mmol, 25.7 % yield) and **3** (16 mg, 0.047 mmol, 10.26 % yield) as brown solids.

2: LCMS: 343 $[M+H]^+$. t_R =1.121 mins. ^1H NMR (400MHz, DMSO-d_6): δ 11.23 (br. s., 1H), 8.09 (s, 1H), 7.93 (s, 1H), 6.74 - 7.02 (m, 1H), 6.22 (d, *J* = 1.2 Hz, 1H), 4.47 (q, *J* = 7.2 Hz, 2H), 4.12 - 4.32 (m, 1H), 3.84 - 4.05 (m, 2H), 3.45 (td, *J* = 11.2, 2.4 Hz, 2H), 2.12 (s, 3H), 1.81 - 2.02 (m, 4H), 1.37 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 162.8, 157.0, 155.1, 121.4, 120.9, 120.3, 98.6, 97.9, 66.5, 61.5, 57.3, 33.5, 15.2, 12.0. HRMS (ESI): m/z calcd for $C_{17}H_{22}N_6O_2$ $[M+H]^+$ 343.1882, found $[M+H]^+$ 343.1888.

3: LCMS: 343 $[M+H]^+$. t_R =1.478 mins. ^1H NMR (400MHz, DMSO-d_6) : δ 10.94 - 11.33 (m, 1H), 8.01 (s, 1H), 7.60 (s, 1H), 6.85 (d, *J* = 3.2 Hz, 1H), 6.20 (d, *J* = 3.2 Hz, 1H), 4.44 (q, *J* = 7.2 Hz, 2H), 4.20 - 4.39 (m, 1H), 3.96 (dd, *J* = 10.8, 3.6 Hz, 2H), 3.48 (t, *J* = 11.2 Hz, 2H), 2.20 (s, 3H), 2.03 (qd, *J* = 12.4, 4.4 Hz, 2H), 1.76 (dd, *J* = 12.4, 2.0 Hz, 2H), 1.35 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 162.8, 157.8, 154.5, 136.2, 120.5, 120.1, 98.5, 97.9, 66.6, 61.4, 54.0, 33.0, 15.1, 9.1. HRMS (ESI): m/z calcd for $C_{17}H_{22}N_6O_2$ $[M+H]^+$ 343.1882, found $[M+H]^+$ 343.1886.

Synthesis of cis-4-ethoxy-*N*-(5-methyl-1-(2-methyl-tetrahydro-2*H*-pyran-4-yl)-1*H*-pyrazol-4-yl)-7*H*-pyrrolo-[2,3-*d*]-pyrimidin-2-amine (4 and 5)



Step 1: *2-methyl-tetrahydro-2H-pyran-4-yl-methanesulfonate.* To a solution of 2-methyltetrahydro-2*H*-pyran-4-ol (1 g, 8.61 mmol) and DIPEA (2.255 mL, 12.91 mmol) in DCM (10 mL) stirred at 0 °C was added a solution of methanesulfonyl chloride (0.356 mL, 10.33 mmol) in DCM (2 mL) dropwise. The reaction mixture was stirred at room temperature for 1 hour. Satur. $NaHCO_3$ solution was added and the mixture was extracted with DCM (10 mL×3). The organic layer was dried over Na_2SO_4 and concentrated in vacuum to give the title compound (1.1 g, 5.66 mmol, 65.8 % yield) as colorless oil. ^{1}H NMR (400MHz, $CDCl_3$): δ 4.70 - 4.87 (m, 1H), 4.04 (ddd, *J* = 12.0, 4.9, 1.6 Hz, 1H), 3.31 - 3.52 (m, 2H), 3.03 (s, 3H), 2.01 - 2.20 (m, 2H), 1.73 - 1.87 (m, 1H), 1.44 - 1.57 (m, 1H), 1.20 - 1.26 (m, 3H).

Step 2: *5-methyl-1-(2-methyl-tetrahydro-2H-pyran-4-yl)-4-nitro-1H-pyrazole.* A solution of 5-methyl-4-nitro-1*H*-pyrazole (3.04 g, 23.89 mmol), 2-methyl-tetrahydro-2*H*-pyran-4-yl- methanesulfonate (5.8 g, 29.9 mmol) and Cs_2CO_3 (9.73 g, 29.9 mmol) in acetonitrile (50 mL) was stirred overnight at 80 °C. The mixture was filtered and the solution was evaporated. The crude was purified by prep-HPLC to give the title compound (870 mg, 3.86 mmol, 16.1% yield) as colorless oil. LCMS: 226 $[M+H]^+$.

Step 3: *5-methyl-1-(2-methyl-tetrahydro-2H-pyran-4-yl)-1H-pyrazol-4-amine.* A solution of 5-methyl-1-(2-methyl-tetrahydro-2*H*-pyran-4-yl)-4-nitro-1*H*-pyrazole (220 mg, 0.977 mmol) and iron (545 mg, 9.77 mmol) in ethanol (4 mL) and water (4.0 mL) was stirred overnight at room temperature. The mixture was filtered and the solution was concentrated. The crude was purified by chromatography on silica gel (DCM: MeOH=10:1) to give the title compound (200 mg, 0.727 mmol, 74.5 % yield) as black oil. LCMS: 196 $[M+H]^+$. ^{1}H NMR (400MHz, $CDCl_3$): δ 7.15 (s, 1H), 4.43 (m, *J* = 4.5 Hz, 1H), 4.20 - 4.30 (m, *J* = 9.3, 6.3, 6.3, 6.3, 3.0 Hz, 1H), 4.11 (td, *J* = 10.9, 3.0 Hz, 2H), 3.76 - 3.89 (m, 2H), 2.17 (s, 3H), 1.98 - 2.09 (m, 2H), 1.88 - 1.97 (m, 1H), 1.74 (dt, *J* = 9.2, 4.7 Hz, 1H), 1.20 (d, *J* = 6.3 Hz, 3H).

Step 4: *4-ethoxy-N-(5-methyl-1-(2-methyl-tetrahydro-2H-pyran-4-yl)-1H-pyrazol-4-yl)-7-tosyl-7H-pyrrolo [2,3-d] pyrimidin-2-amine.* To a solution of 2-chloro-4-ethoxy-7-tosyl-7*H*-pyrrolo-[2,3-*d*]-pyrimidine (541 mg, 1.536 mmol), 5-methyl-1-(2-methyl-tetrahydro-2*H*-pyran-4-yl)-1*H*-pyrazol-4-amine (200 mg, 1.024 mmol) and xtanphos (73.2 mg, 0.154 mmol) in 1,4-dioxane (2.0 mL) and water (0.2 mL) was added K_2CO_3 (283 mg, 2.049 mmol) and $PdCl_2$(pddf) (84 mg, 0.102 mmol). The reaction was stirred overnight at 90 ^oC. The mixture was cooled to room temperature and partitioned between EtOAc (100 mL) and water (80 mL). The organic layer was washed with water (80 mL), dried over Na_2SO_4 and evaporated in vacuum. The crude was purified by column chromatography on silica gel (PE: EA=3:1) to give the title compound (300 mg, 0.382 mmol, 37.3 % yield) as a white solid. LCMS: 511 $[M+H]^+$. ^1H NMR (400MHz, $CDCl_3$): δ 7.70 - 7.86 (m, 2H), 7.61 (s, 1H), 7.12 - 7.21 (m, 3H), 6.42 (d, *J* = 4.0 Hz, 1H), 4.62 (t, *J* = 4.4 Hz, 1H), 4.43 (q, *J* = 7.2 Hz, 2H), 4.27 - 4.37 (m, 1H), 4.16 - 4.26 (m, 1H), 3.88 (dt, *J* = 11.2, 4.4 Hz, 1H), 2.36 (s, 3H), 2.31 (s, 3H), 2.14 - 2.23 (m, 1H), 2.11 (dt, *J* = 8.8, 4.4 Hz, 2H), 1.82 (ddd, *J* = 13.9, 9.0, 5.0 Hz, 1H), 1.38 (t, *J* = 7.0 Hz, 3H), 1.19 (d, *J* = 6.4 Hz, 3H).

Step 5: *cis-4-ethoxy-N-(5-methyl-1-(2-methyl-tetrahydro-2H-pyran-4-yl)-1H- pyrazol-4-yl)-7H-pyrrolo-[2,3-d]-pyrimidin-2-amine (4 and 5).* A solution of 4-ethoxy-*N*-(5-methyl-1-(2-methyl-tetrahydro-2*H*-pyran-4-yl)-1*H*-pyrazol-4-yl)-7-tosyl-7*H*- pyrrolo-[2,3-*d*] pyrimidin-2-amine (300 mg, 0.588 mmol) and sodium hydroxide (0.588 mL, 1.175 mmol, 2M in water) in THF (2 mL) and methanol (0.500 mL) was stirred at 50 ^oC for 3 hours. After cooling, the reaction mixture was poured into water (50 mL) and extracted with EtOAc (40 mL× 3). The combined organic layer was dried and evaporated in vacuum. The crude was purified by prep-HPLC to give the product (150 mg, 0.391 mmol, 66.6 % yield), which was further purified by chiral-HPLC (Column AY-H (4.6*250mm, 5µm); Mobile Phase: n-Hexane (0.1%DEA): EtOH(0.1%DEA) =85:15) to give the title compounds **4** (44 mg, 0.123 mmol, 29.3 % yield) and **5** (50 mg, 0.140 mmol, 33.3 % yield) as white solids.

4: LCMS: 357 $[M+H]^+$. t_R =1.21 mins. (LCMS condition 2)

Chiral HPLC: t_R =6.61 mins. (Condition: Column AY-H (4.6×250mm, 5µm); Co-Solvent MeOH) unknown absolute stereochemistry

^1H NMR (400MHz, $CDCl_3$): δ 9.99 (br. s., 1H), 7.66 (s, 1H), 6.42 (br. s., 1H), 6.30 (d, *J* = 2.2 Hz, 1H), 6.10 (s, 1H), 4.49 (q, *J* = 7.3 Hz, 2H), 4.43 (t, *J* = 4.3 Hz, 1H), 4.21 (t, *J* = 6.1 Hz, 1H), 4.07 - 4.16 (m, 1H), 3.81 (dt, *J* = 11.4, 4.2 Hz, 1H), 2.22 (s, 3H), 1.95 - 2.05 (m, 2H), 1.85 - 1.95 (m, 1H), 1.64 - 1.75 (m, 3H), 1.43 (t, *J* = 7.1 Hz, 3H), 1.16 (d, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 162.8, 157.7, 155.2, 133.7, 120.8, 120.2, 98.5, 97.9, 68.3, 62.0, 61.4, 49.8, 37.4, 30.9, 20.9, 15.1, 9.3.

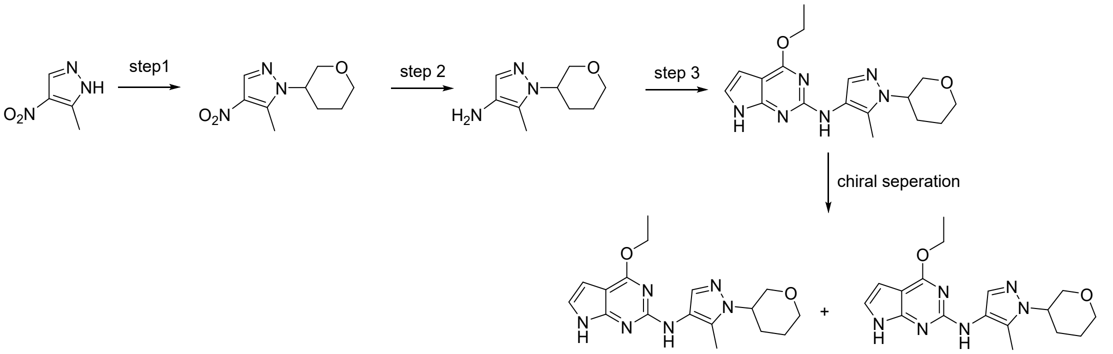
5: LCMS: 357 $[M+H]^+$. t_R =1.21 mins. (LCMS condition 2)

Chiral HPLC: t_R =7.99 mins. (Condition: Column AY-H (4.6*250mm, 5µm); Co-Solvent MeOH) unknown absolute stereochemistry

^1H NMR (400MHz, CDCl3): δ 9.66 (br. s., 1H), 7.68 (s, 1H), 6.49 (br. s., 1H), 6.32 (br. s., 1H), 6.08 (s, 1H), 4.41 - 4.54 (m, 3H), 4.18 - 4.28 (m, 1H), 4.13 (td, *J* = 11.0, 2.7 Hz, 1H), 3.82 (dt, *J* = 11.5, 4.2 Hz, 1H), 2.22 (s, 3H), 1.97 - 2.07 (m, 2H), 1.87 - 1.97 (m, 1H), 1.71 (ddd, *J* = 14.0, 9.2, 4.9 Hz, 3H), 1.43 (t, *J* = 7.1 Hz, 3H), 1.17 (d, 3H). ^{13}C NMR (101 MHz, DMSO-d_6):

δ 162.8, 157.7, 155.2, 133.7, 130.2, 120.8, 120.2, 98.5, 97.9, 68.3, 62.0, 61.4, 49.8, 37.4, 30.9, 20.9, 15.1, 9.3.

Synthesis of 4-ethoxy-*N*-(5-methyl-1-(tetrahydro-2*H*-pyran-3-yl)-1*H*-pyrazol-4-yl)-7*H*-pyrrolo-[2,3-*d*]pyrimidin-2-amine (6 and 7)



6
Enantiomer 1
unknown absolute stereochemistry

7
Enantiomer 2
unknown absolute stereochemistry

Step 1: *5-methyl-4-nitro-1-(tetrahydro-2H-pyran-3-yl)-1H-pyrazole***.** A solution of 5-methyl-4-nitro-1*H*-pyrazole (1.5 g, 11.80 mmol) and tetrahydro-2*H*-pyran-3-yl methanesulfonate (3.19 g, 17.70 mmol) in DMF (15 mL) was added potassium carbonate (2.447 g, 17.70 mmol) was stirred overnight at 90 °C. The mixture was diluted with water and extracted with EtOAc. The organic layer was concentrated in vacuum and purified by column chromatography on silica gel (PE: EA=2: 1) and further purified with pre-HPLC to get the title compounds 5-methyl-4-nitro-1-(tetrahydro-2*H*-pyran-3-yl)-1*H*-pyrazole (500 mg, 50% yield, mixture) as a white solid. LCMS: 212 $[M+H]^+$. t_R =1.266 mins. (LCMS condition 2)

Step 2: *5-methyl-1-(tetrahydro-2H-pyran-3-yl)-1H-pyrazol-4-amine***.** A solution of 5-methyl-4-nitro-1-(tetrahydro-2*H*-pyran-3-yl)-1*H*-pyrazole (450 mg, 2.131 mmol, not pure) and iron (595 mg, 10.65 mmol) in ethanol (2 mL) and water (2.000 mL) was stirred at 80 °C about 1 hours. The mixture was filtered and concentrated. The crude was purified by column chromatography on silica gel (PE: EA=1:1) to give the title compound 5-methyl-1-(tetrahydro-2*H*-pyran-3-yl)-1*H*-pyrazol-4-amine (150 mg, 0.828 mmol, 38.8 % yield, not pure) as a yellow solid. LCMS: 182 $[M+H]^+$. t_R =0.98 mins. (LCMS condition 2)

Step 3: *4-ethoxy-N-(5-methyl-1-(tetrahydro-2H-pyran-3-yl)-1H-pyrazol-4-yl)-7H- pyrrolo-[2,3-d]pyrimidin-2-amine* **(6 and 7).** A solution of **52** (126 mg, 0.638 mmol), 5-methyl-1-(tetrahydro-2*H*-pyran-3-yl)-1*H*-pyrazol-4-amine (139 mg, 0.765 mmol), $Pd_2(dba)_3$ (55.6 mg, 0.061 mmol, not pure), potassium carbonate (264 mg, 1.913 mmol) and xtanphos (30.4 mg, 0.064 mmol) in 2-butanol (4 mL) was irradiated by microwave at 120 °C for 45 min. After filtration, the filtrate was concentrated and the crude was purified by chiral-HPLC (Co-Solvent MeOH (0.1%DEA); Column AD-H (4.6*250mm, 5μm); Column Temperature 39.8; CO_2 Flow Rate 2.1; Co-Solvent Flow Rate 0.9; Co-Solvent % 30; Back Pressure 120; Total Flow 3; PDA Start Wavelength 214; PDA Stop Wavelength 359) to give the title compounds **6** (21 mg, 0.061 mmol, 9.62 % yield) and **7** (21 mg, 0.061 mmol, 9.62 % yield) as white solids.

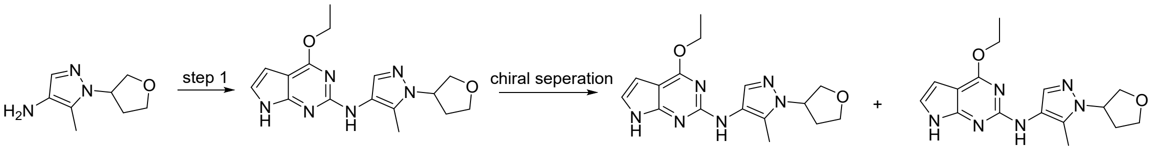
6: LCMS: 343 $[M+H]^+$. t_R =1.329 mins. (LCMS condition 2), Chiral HPLC: t_R =3.53 mins. Unknown absolute stereochemistry

^1H NMR (400MHz, DMSO-d_6) : δ 11.17 (br. s., 1H), 8.00 (s, 1H), 7.61 (s, 1H), 6.78 - 6.93 (m, 1H), 6.20 (dd, *J* = 3.3, 2.0 Hz, 1H), 4.37 - 4.51 (m, 2H), 4.09 - 4.26 (m, 1H), 3.87 (dd, *J* = 10.8, 2.3 Hz, 2H), 3.46 - 3.61 (m, 1H), 3.33 - 3.38 (m, 1H), 2.20 (s, 3H), 1.92 - 2.16 (m, 2H), 1.63 - 1.82 (m, 2H), 1.30 - 1.44 (m, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 162.8, 157.7, 155.2, 134.4, 130.4, 120.6, 120.2, 98.5, 98.0, 70.7, 67.5, 61.4, 53.7, 29.7, 25.6, 15.1, 9.0.

7: LCMS: 343 $[M+H]^+$. t_R =1.330 mins. (LCMS condition 2), Chiral HPLC: t_R =4.15 mins. Unknown absolute stereochemistry

^1H NMR (400MHz, DMSO-d_6) : δ 11.17 (br. s., 1H), 8.00 (s, 1H), 7.61 (s, 1H), 6.85 (br. s., 1H), 6.21 (br. s., 1H), 4.44 (q, *J* = 6.9 Hz, 2H), 4.05 - 4.29 (m, 1H), 3.87 (d, *J* = 10.3 Hz, 2H), 3.54 (t, *J* = 10.5 Hz, 1H), 3.41 (m, 1H), 2.20 (s, 3H), 1.94 - 2.14 (m, 2H), 1.65 - 1.83 (m, 2H), 1.35 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 162.8, 157.7, 155.2, 134.4, 130.3, 120.6, 120.2, 98.5, 98.0, 70.8, 67.5, 61.4, 53.7, 29.7, 25.6, 15.1, 9.0.

2.4 Synthesis of 4-ethoxy-*N*-(5-methyl-1-(tetrahydrofuran-3-yl)-1*H*-pyrazol-4-yl)-7*H*-pyrrolo-[2,3-*d*]-pyrimidin-2-amine (8 and 9)



8
Enantiomer 1
unknown absolute stereochemistry

9
Enantiomer 2
unknown absolute stereochemistry

A solution of 2-chloro-4-ethoxy-7*H*-pyrrolo[2,3-d]pyrimidine(which can be prepared according to D1) (49 mg, 0.248 mmol), 5-methyl-1-(tetrahydrofuran-3-yl)-1*H*- pyrazol-4-amine (49.8 mg, 0.298 mmol) (which may be prepared according to PCT Int. Appl., WO2012062783), $Pd_2(dba)_3$ (11.35 mg, 0.012 mmol), potassium carbonate (103 mg, 0.744 mmol) and (9,9-dimethyl-9*H*-xanthene-4,5-diyl)-bis(diphenylphosphine) (11.82 mg, 0.025 mmol) in 2-butanol (5 mL) was irradiated by microwave at 120 °C for 45 min. The reaction mixture was quenched with water, partitioned between ethyl acetate (25 mL) and water (5 mL). The organic layer was washed with water, and then saturated $NaHCO_3$. Then, the resulting organic phase was dried over Na_2SO_4, filtered and concentrated. The crude was purified by chiral-HPLC (Co-Solvent MeOH (0.1%DEA); Column OJ-H (4.6*250mm, 5µm); Column Temperature 39.8; CO_2 Flow Rate 2.4; Co-Solvent Flow Rate 0.6; Co-Solvent % 20; Back Pressure 120; Total Flow 3; PDA Start Wavelength 214; PDA Stop Wavelength 359) to give the title compounds **8** (18 mg, 24.56% yield) and **9** (10mg, 15.43% yield) as white solids.

8: LCMS: 329 $[M+H]^+$. t_R =1.267 mins. (LCMS condition 2)

Chiral HPLC: t_R =2.85 mins. (Conditions: Column OJ-H (4.6*250mm, 5µm); Co-Solvent MeOH (0.1%DEA)) unknown absolute stereochemistry

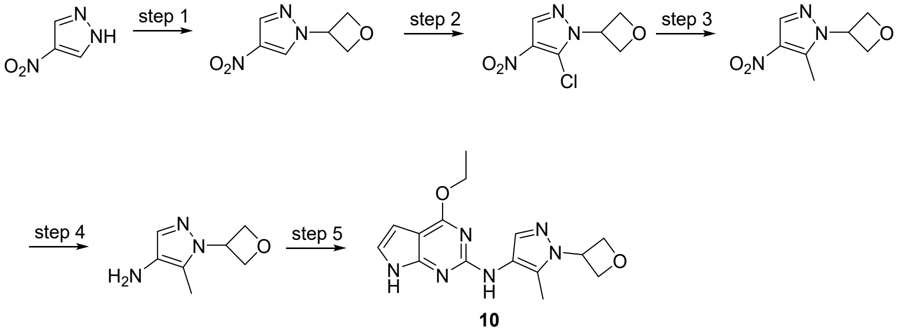
^{1}H NMR (400MHz, DMSO-*d*$_6$): *δ* 10.95 - 11.29 (m, 1H), 8.02 (s, 1H), 7.60 (s, 1H), 6.84 (d, *J* = 2.3 Hz, 1H), 6.20 (d, *J* = 2.8 Hz, 1H), 4.88 - 5.00 (m, 1H), 4.43 (d, *J* = 7.0 Hz, 2H), 3.90 - 4.13 (m, 2H), 3.68 - 3.90 (m, 2H), 2.23 - 2.32 (m, 2H), 2.18 (s, 3H), 1.34 (t, 3H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$): *δ* 162.8, 157.7, 155.2, 134.4, 121.0, 120.2, 98.5, 98.0, 72.0, 67.8, 61.4, 57.7, 32.4, 15.1, 9.3.

9: LCMS: 329 $[M+H]^+$. t_R =1.255 mins. (LCMS condition 2)

Chiral HPLC: t_R =4.28 mins. (Conditions: Column OJ-H (4.6*250mm, 5µm); Co-Solvent MeOH (0.1%DEA) unknown absolute stereochemistry

^{1}H NMR (400MHz, DMSO-*d*$_6$): *δ* 11.20 (br. s., 1H), 8.06 (s, 1H), 7.61 (s, 1H), 6.78 - 6.96 (m, 1H), 6.00 - 6.34 (m, 1H), 4.89 - 5.05 (m, 1H), 4.44 (q, *J* = 6.9 Hz, 2H), 3.92 - 4.10 (m, 2H), 3.66 - 3.89 (m, 2H), 2.29 (q, *J* = 6.4 Hz, 2H), 2.20 (s, 3H), 1.36 (t, 3H). HRMS (ESI): m/z calcd for $C_{16}H_{20}N_6O_2$ $[M+H]^+$ 329.1726, found $[M+H]^+$ 329.1723.

Synthesis of 4-ethoxy-*N*-(5-methyl-1-(oxetan-3-yl)-1*H*-pyrazol-4-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (10)



Step 1: *4-nitro-1-(oxetan-3-yl)-1H-pyrazole*. A solution of *4-nitro-1H-pyrazole* (4.46 g, 39.4 mmol), oxetan-3-yl methanesulfonate (5.0 g, 32.9 mmol) and K_2CO_3 (9.08 g, 65.7 mmol) in DMF (50 mL) was stirred at 80 °C for 60 hours. The mixture was added to water and extracted with ethyl acetate. The ethyl acetate layer was dried with Na_2SO_4 and evaporated to give the crude product which was added to a silica gel column and eluted with PE/DCM (5/1 to1/1, 3000 mL) to the title compound (3.5 g, 18.62 mmol, 56.7 % yield) as yellow oil. LCMS: rt = 1.339 mins, MH+ =170

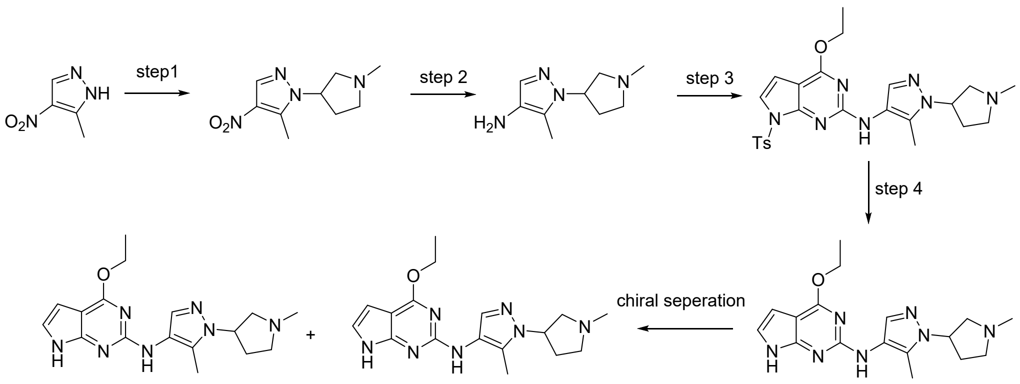
Step 2: *5-chloro-4-nitro-1-(oxetan-3-yl)-1H-pyrazole.* To a solution of *4-nitro-1-(oxetan-3-yl)-1H-pyrazole* (2 g, 11.82 mmol) in THF (5 mL) stirred under nitrogen at -78 °C was added a solution of lithium bis(trimethylsilyl)amide (6.66 mL, 35.5 mmol) in THF) (5 mL dropwise during 15 min. The reaction mixture was stirred at -78 °C for 30 min. A solution of perchloroethane (3.35 mL, 29.6 mmol) was added and the mixture was stirred for 3h at -78 °C under N2. The mixture was quenched with aq NH4Cl and evaporated to give the crude product. The crude product was added to a silica gel column and eluted with PE/ EtOAc (15/1)to afford (600 mg, 2.95 mmol, 24.92 % yield)(N28616-97-A1) as white solid. LCMS: rt = 1.363 mins, M+H = 204

Step 3: *5-methyl-4-nitro-1-(oxetan-3-yl)-1H-pyrazole*. To a solution of methylboronic acid (353 mg, 5.89 mmol), 5-chloro-4-nitro-1-(oxetan-3-yl)-1H-pyrazole (400 mg, 1.965 mmol) and sodium carbonate (625 mg, 5.89 mmol) in 1,4- Dioxane (4 mL) and Water (0.400 mL) was added PdCl2(dppf)-CH2Cl2 adduct (160 mg, 0.196 mmol). The reaction mixture was combined in a thick walled glass tube. The tube was sealed with a rubber septum, purged with nitrogen and stirred at 75 °C overnight. The mixture was cooled to room temperature, and evaporated in vacuo to give a black solid, which was added to a silica gel column and eluted with PE/ EtOAc (15/1 to 10/1, 1000 mL) to give (180 mg, 0.796 mmol, 40.5 % yield) as a white solid. LCMS: rt =1.072mins, MH+ = 184

Step 4: *5-methyl-1-(oxetan-3-yl)-1H-pyrazol-4-amine.* The mixture of *5-methyl-4-nitro-1-(oxetan-3-yl)-1H-pyrazole* (160 mg, 0.874 mmol) and iron (244 mg, 4.37 mmol) in Ethanol (2 mL) and Water (2.000 mL) was stirred at 80 °C about 1 h. The mixture was filtered and concentrated, the residue was purified by Pre-TLC (PE:EtOAc=1:1) to get 5-methyl-1-(oxetan-3-yl)-1H-pyrazol-4-amine (120 mg, 0.705 mmol, 81 % yield).

Step 5: *4-ethoxy-N-(5-methyl-1-(oxetan-3-yl)-1H-pyrazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidin-2-amine.* A solution of 50 (120mg, 0.607 mmol*), 5-methyl-1-(oxetan-3-yl)-1H-pyrazol-4-amine* (102 mg, 0.668 mmol), $Pd_2(dba)_3$ (55.6 mg, 0.061 mmol), (9,9-dimethyl-9*H*-xanthene-4,5-diyl)bis- (diphenylphosphine) (52.7 mg, 0.091 mmol) and potassium carbonate (252 mg, 1.822 mmol) in 2-butanol (1 mL) was irradiated by microwave at 130 ^{o}C for 90 min. The reaction mixture was partitioned between ethyl acetate (25 mL) and water (25 mL). The organic layer was washed with water, saturated $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated. The crude was purified by prep-HPLC to give the title compound (60 mg, 0.191 mmol, 31.4 % yield) as a white solid. LCMS: 315 $[M+H]^+$. t_R =1.445 mins. (LCMS condition 2) ^{1}H NMR (400MHz, DMSO-*d$_6$*): δ 11.18 (br. s., 1H), 8.07 (s, 1H), 7.75 (s, 1H), 6.80 - 6.92 (m, 1H), 6.21 (dd, *J* = 3.3, 1.8 Hz, 1H), 5.37 - 5.62 (m, 1H), 4.92 - 4.99 (m, 2H), 4.84 - 4.91 (m, 2H), 4.45 (q, *J* = 7.1 Hz, 2H), 2.13 (s, 3H), 1.36 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-*d$_6$*): δ 162.8, 157.6, 155.2, 135.3, 121.2, 120.2, 98.5, 98.0, 76.8, 61.5, 51.3, 15.1, 9.0. HRMS (ESI): m/z calcd for $C_{15}H_{18}N_6O_2$ $[M+H]^+$ 315.1569, found $[M+H]^+$ 315.1570.

Synthesis of 4-ethoxy-*N*-(5-methyl-1-(1-methylpyrrolidin-3-yl)-1*H*-pyrazol-4-yl)-7*H*-pyrrolo-[2,3-*d*]-pyrimidin-2-amine (11 and 12)



11
Enantiomer 1
unknown absolute stereochemistry

12
Enantiomer 2
unknown absolute stereochemistry

Step 1: *5-methyl-1-(1-methylpyrrolidin-3-yl)-4-nitro-1H-pyrazole.* A mixture of 3-methyl-4-nitro-1H-pyrazole (1.4 g, 11.01 mmol), 1-methylpyrrolidin-3-yl methanesulfonate (2.369 g, 13.22 mmol) and Cs_2CO_3 (10.77g, 33.0 mmol) was stirred at 80 °C for 12 h. After cooling the reaction and water (30 ml) was poured into the mixture, extracted with EtOAc (15 ml*3). The organic phase was dried over sodium sulphate and evaporated in vacuo to give residue. The residue was purified by silica gel column chromatography (PE: EA = 3:1) to the title compound (290 mg, 1.379 mmol, 12.52 % yield) as a yellow oil. MH+ = 211.0

Step 2: *(±)-5-methyl-1-(1-methylpyrrolidin-3-yl)-1Hpyrazol-4-amine.* To a solution of 5-*methyl-1-(1-methylpyrrolidin-3-yl)-4-nitro-1H-pyrazole* (290 mg, 1.379 mmol) in methanol (30 mL) stirred in Hydrogen at room temp was added solid Pd/C (44 mg, 0.413 mmol) in one charge. The reaction mixture was stirred at rt for 3 h. Filtered and concentrated to get the product (240 mg, 1.331 mmol, 97 % yield) as a yellow oil. LCMS: rt = 1.73 mins, MH+ = 181.0.

Step 3: *(±)-4-ethoxy-N-(5-methyl-1-(1-methyl-pyrrolidin-3-yl)-1H-pyrazol-4-yl)-7-tosyl-7H-pyrrolo-[2,3-d]-pyrimidin-2-amine***.** To a solution of (±)-5-methyl-1-(1-methylpyrrolidin-3-yl)-1*H*-pyrazol-4-amine (237 mg, 1.313 mmol), 2-chloro-4-ethoxy-7-tosyl-7*H*-pyrrolo-[2,3-*d*]-pyrimidine (420 mg, 1.194 mmol) and (9,9-dimethyl-9*H*-xanthene-4,5-diyl)bis-(diphenylphosphine) (104 mg, 0.179 mmol) in 1,4-dioxane (2.0 mL) and water (0.2 mL) was added potassium carbonate (330 mg, 2.388 mmol) and $PdCl_2$(dppf)-CH_2Cl_2 (97 mg, 0.119 mmol). The reaction was stirred overnight at 90 ^{o}C. The mixture was diluted with water (100 mL) and extracted with ethyl acetate (2 × 40 mL). The combined organic layer was washed with brine (20 mL), dried with Na_2SO_4, filtered and concentrated. The residue was purified by chromatography on silica gel (DCM: MeOH = 3:1) to give the title compound (±)-4-ethoxy-*N*-(5-methyl-1-(1-methyl-pyrrolidin-3-yl)-1*H*-pyrazol-4-yl)-7-tosyl-7*H*-pyrrolo-[2,3-*d*]-pyrimidin-2-amine (240 mg, 0.498 mmol, 41.7 % yield) as a black solid. LCMS: 495.7 $[M+H]^+$. t_R =1.58mins. (LCMS condition 2)

Step 4: *4-ethoxy-N-(5-methyl-1-(1-methylpyrrolidin-3-yl)-1H-pyrazol-4-yl)-7H- pyrrolo-[2,3-d]-pyrimidin-2-amine.* A solution of (±)-4-ethoxy-*N*-(5-methyl-1-(1-methyl-pyrrolidin-3-yl)-1*H*-pyrazol-4-yl)-7-tosyl-7*H*-pyrrolo-[2,3-*d*]-pyrimidin-2-amine (240 mg, 0.484 mmol) and sodium hydroxide (3 mL, 6.00 mmol) in methanol (15 mL) was stirred at 50 ^{o}C for 2 hours. The mixture was concentrated and saturated $NaHCO_3$ was added until pH=8.

The mixture was extracted with EtOAc (50 mL×3). The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude was purified by chromatography on silica gel (DCM: MeOH = 8:1) to give the racemic product, which was further purified by chiral-HPLC (Co-Solvent MeOH (0.1%DEA); Column AD-H (4.6*250mm, 5µm); Column Temperature 39.8; CO_2 Flow Rate 1.95; Co-Solvent Flow Rate 1.05; Co-Solvent % 35; Back Pressure 120; Total Flow 3; PDA Start Wavelength214; PDA Stop Wavelength 359) to give the title compounds **13**(30 mg, 0.088 mmol, 18.15 % yield) and **14** (30 mg, 0.088 mmol, 18.15 % yield) as white solids.

11: LCMS: 342 $[M+H]^+$. t_R =1.244 mins. (LCMS condition 2)

Chiral HPLC: t_R =3.2 mins. (Conditions: Column AD-H (4.6*250mm, 5µm); Co-Solvent MeOH (0.1%DEA)) unknown absolute stereochemistry

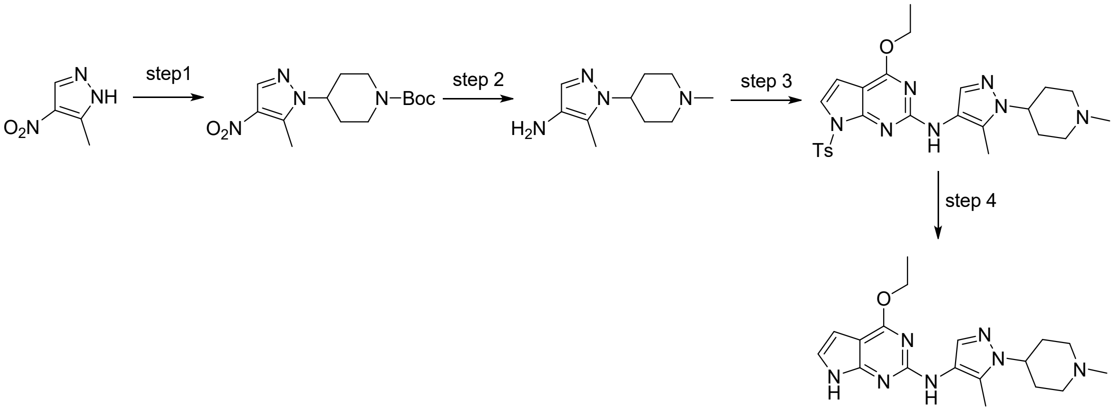
^1H NMR (400MHz, DMSO-*d6*): *δ* 8.01 (s, 1H), 7.58 (s, 1H), 6.63 - 6.96 (m, 1H), 6.20 (d, *J* = 1.3 Hz, 1H), 4.71 - 4.96 (m, 1H), 4.44 (q, *J* = 7.0 Hz, 2H), 3.00 (t, *J* = 8.3 Hz, 1H), 2.64 - 2.76 (m, 1H), 2.53 - 2.61 (m, 2H), 2.26 - 2.31 (m, 2H), 2.13 - 2.26 (m, 5H), 1.35 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*): *δ* 162.8, 157.4, 155.2, 134.2, 120.8, 120.1, 98.5, 97.9, 61.5, 61.4, 57.1, 55.6, 42.2, 31.4, 15.1, 9.3. HRMS (ESI): m/z calcd for $C_{17}H_{23}N_7O$ $[M+H]^+$ 342.2042, found $[M+H]^+$ 342.2042.

12: LCMS: 342 $[M+H]^+$. t_R =1.237 mins. (LCMS condition 2)

Chiral HPLC: t_R =4.9 mins. (Conditions: Column AD-H (4.6*250mm, 5µm); Co-Solvent MeOH (0.1%DEA)) unknown absolute stereochemistry

^1H NMR (400MHz, DMSO-*d6*): *δ* 11.18 (br. s., 1H), 8.00 (s, 1H), 7.58 (s, 1H), 6.85 (br. s., 1H), 6.21 (d, *J* = 1.0 Hz, 1H), 4.83 (m, 1H), 4.44 (q, *J* = 6.9 Hz, 2H), 3.00 (t, *J* = 8.3 Hz, 1H), 2.65 - 2.74 (m, 1H), 2.53 - 2.61 (m, 2H), 2.28 (s, 3H), 2.12 - 2.26 (m, 5H), 1.35 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*): *δ* 162.8, 157.7, 155.2, 134.3, 120.8, 120.1, 98.5, 97.9, 61.5, 61.4, 57.1, 55.6, 42.2, 31.4, 15.1, 8.8.

Synthesis of 4-ethoxy-*N*-(5-methyl-1-(1-methylpiperidin-4-yl)-1*H*-pyrazol-4-yl)-7*H*-pyrrolo-[2,3-*d*]-pyrimidin-2-amine (13)



13

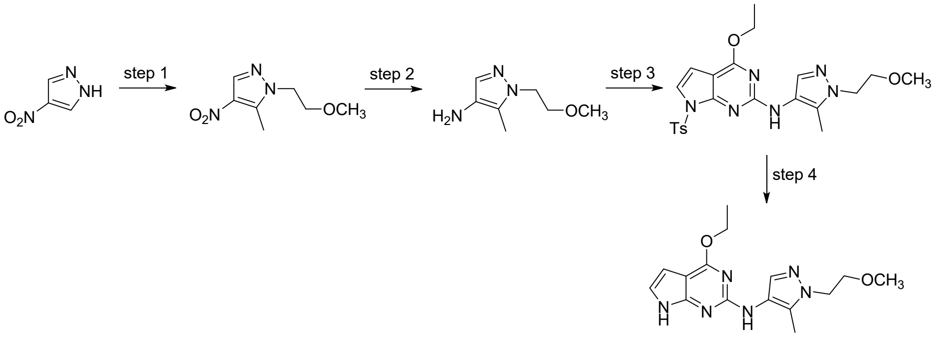
Step 1: To a solution of tert-butyl 4-((methylsulfonyl)oxy)piperidine-1-carboxylate (286 mg, 1.023 mmol)and potassium carbonate (109 mg, 0.787 mmol) in N,N-Dimethylformamide (DMF) (5 mL) was added 5-methyl-4-nitro-1H-pyrazole (100mg, 0.787 mmol). The reaction mixture was stirred at 80 °C for 2 h.(N28381-91-R1). The reaction mixture was poured into EtOAc(20 ml) and washed with water (3*30 ml). The combined organic layer was evaporated in vacuo to give the crude (N28381-91-A1) tertbutyl 4-(5-methyl-4-nitro-1H-pyrazol-1-yl)piperidine-1-carboxylate (200 mg, 0.374 mmol, 47.5 % yield) as yellow oil. LCMS: [M-Boc+1]=255 ,tR=1.87 min

Step 2: To a solution of tert-butyl 4-(5-methyl-4-nitro-1H-pyrazol-1-yl)piperidine-1-carboxylate (240 mg, 0.773 mmol) in Tetrahydrofuran (THF) (8 mL) was added LiAlH4 (176 mg, 4.64 mmol) at room temperature. The reaction mixture was refluxed at 80 °C for 2 h. LCMS: rt = 0.95 mins, MH+ =195.1

Step 3: *4-ethoxy-N-(3-methyl-1-(1-methylpiperidin-4-yl)-1H-pyrazol-4-yl)-7-tosyl-7H-pyrrolo-[2,3-d]pyrimidin-2-amine.* To a solution of 2-chloro-4-ethoxy-7-tosyl-7*H*-pyrrolo-[2,3-*d*]-pyrimidine (362 mg, 1.029 mmol), a mixture of 5-methyl-1-(1-methylpiperidin-4-yl)-1H-pyrazol-4-amine and 3-methyl-1-(1-methylpiperidin-4-yl)-1*H*- pyrazol-4-amine (200 mg, 1.029 mmol) (9,9-dimethyl-9*H*-xanthene-4,5-diyl)bis(diphenylphosphine) (89 mg, 0.154 mmol) in 1,4-dioxane (2.0 mL) and water (0.2 mL) was added potassium carbonate (285 mg, 2.059 mmol) and $PdCl_2$(pddf) (84 mg, 0.103 mmol). The reaction mixture was stirred at 90 ^oC overnight. The mixture was cooled to room temperature and partitioned between EtOAc (25 mL) and water (20 mL). The organic layer was washed with water (20 mL), dried over Na_2SO_4 and evaporated. The crude was purified by Biotage to give the mixture of the title compounds (130 mg, 0.140 mmol, 13.63 % yield) as yellow oil. LCMS: 510.1 $[M+H]^+$. t_R =1.45mins. (LCMS condition 2)

Step 4: *4-ethoxy-N-(5-methyl-1-(1-methylpiperidin-4-yl)-1H-pyrazol-4-yl)-7H-pyrrolo-[2,3-d]-pyrimidin-2-amine.* A solution of a mixture of 4-ethoxy-*N*-(5-methyl-1-(1-methylpiperidin-4-yl)-1*H*-pyrazol-4-yl)-7-tosyl-7*H*-pyrrolo-[2,3-*d*]pyrimidin-2-amine (260 mg, 0.510 mmol), sodium hydroxide (0.510 mL, 1.020 mmol, 2M in water) in THF (5 mL) was stirred at 60 ^oC for 1 hour. The reaction mixture was poured into EtOAc (20 mL) and extracted with water (3×30 mL). The organic layer was evaporated in vacuo and purified by prep-HPLC to give the title compound (16 mg, 0.045 mmol, 8.82 % yield) as a white solid. LCMS: 356.2 $[M+H]^+$. t_R =1.27 mins. (LCMS condition 2) ^{1}H NMR (400MHz, CDCl3): δ 9.16 (br. s., 1H), 7.69 (s, 1H), 6.60 (m, 1H), 6.35 (d, *J* = 1.8 Hz, 1H), 6.06 (s, 1H), 4.49 (q, *J* = 7.0 Hz, 2H), 4.00 (m, 1H), 3.05 (m, 2H), 2.10 - 2.44 (m, 10H), 1.90 (m, 2H), 1.43 (t, *J* = 7.0 Hz, 3H). HRMS (ESI): m/z calcd for $C_{18}H_{25}N_7O$ $[M+H]^+$ 356.2199, found $[M+H]^+$ 356.2196.

Synthesis of 4-ethoxy-*N*-(1-(2-methoxyethyl)-5-methyl-1*H*-pyrazol-4-yl)-7*H*-pyrrolo-[2,3-*d*]-pyrimidin-2-amine (14)



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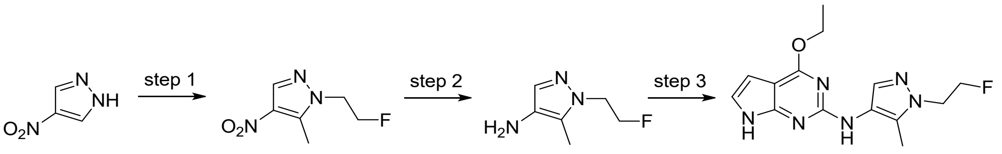
Step 1: *1-(2-methoxyethyl)-5-methyl-4-nitro-1H-pyrazole.* A mixture of 3-methyl-4-nitro-1*H*-pyrazole (1.50 g, 11.80 mmol), 1-bromo-2-methoxyethane (1.968 g, 14.16 mmol) and Cs_2CO_3 (7.69 g, 23.6 mmol) in acetonitrile (20 mL) was stirred overnight at 80 °C. The mixture was filtered and concentrated in vacuo to give the title compound (1.1 g, together with the isomer) as oil. LCMS: 186 $[M+H]^+$.

Step 2: *1-(2-methoxyethyl)-5-methyl-1H-pyrazol-4-amine.* A solution of 1-(2-methoxyethyl)-5-methyl-4-nitro-1H-pyrazole (1.1 g, 5.94 mmol, together with the isomer) and Pd/C (165 mg, 1.550 mmol) in methanol (10 mL) was stirred under H_2 at room temperature for 1 hour. The mixture was filtrated and the filtrate was concentrated to give the title compound (920 mg, together with the isomer) as a yellow oil. LCMS: 156 $[M+H]^+$.

Step 3: *4-ethoxy-N-(1-(2-methoxyethyl)-5-methyl-1H-pyrazol-4-yl)-7-tosyl-7H-pyrrolo-[2,3-d]-pyrimidin-2-amine.* A solution of 2-chloro-4-ethoxy-7-tosyl-7*H*-pyrrolo-[2,3-*d*]-pyrimidine (318 mg, 2.047 mmol), 1-(2-methoxyethyl)-5-methyl-1*H*-pyrazol-4-amine (318 mg, 2.047 mmol, together with the isomer), xphos (148 mg, 0.256 mmol), K_2CO_3 (471 mg, 3.41 mmol) and $PdCl_2(dppf)$-CH_2Cl_2 (139 mg, 0.171 mmol) in 1,4-dioxane (2.0 mL) and water (0.2 mL) was stirred overnight at 90 °C. Then mixture was diluted with water (100 mL) and extracted with ethyl acetate (2 × 40 mL). The combined organic layer was washed with brine (20 mL), dried with Na_2SO_4, filtered and concentrated. The crude was purified by column chromatography on silica gel (PE: EA=3:1) to give the title compound (650 mg, 58.3 % yield, together with the isomer) as oil. LCMS: 471 $[M+H]^+$.

Step 4: *4-ethoxy-N-(1-(2-methoxyethyl)-5-methyl-1H-pyrazol-4-yl)-7H-pyrrolo-[2,3-d]-pyrimidin-2-amine.* A solution of 4-ethoxy-*N*-(1-(2-methoxyethyl)-5-methyl-1*H*-pyrazol-4-yl)-7-tosyl-7*H*-pyrrolo-[2,3-*d*]-pyrimidin-2-amine (300 mg, 0.191 mmol, together with the isomer), sodium hydroxide (3 mL, 6.00 mmol, 2M in water) in methanol (15 mL) was stirred at 50 °C for 2 hours. The mixture was concentrated and saturated $NaHCO_3$ was added until pH= 8. The mixture was extracted with EtOAc (50 mL×3). The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude was purified by chromatography on silica gel (DCM: MeOH = 20:1) to give the title compound (25 mg, 0.079 mmol, 17.71 % yield) as a white solid. LCMS: 316.9 $[M+H]^+$. t_R =1.25 mins (LCMS condition 2). ^1H NMR (400MHz, $CDCl_3$): δ 10.05 - 10.33 (m, 1H), 7.64 (s, 1H), 6.41 (br. s., 1H), 6.25 - 6.34 (m, 1H), 6.12 (s, 1H), 4.49 (q, J = 7.2 Hz, 2H), 4.11 (t, J = 5.6 Hz, 2H), 3.66 (t, J = 5.6 Hz, 2H), 3.25 (s, 3H), 2.21 (s, 3H), 1.44 (t, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 162.8, 157.8, 155.2, 134.7, 120.5, 120.1, 98.5, 97.9, 71.4, 61.4, 58.6, 48.9, 15.1, 9.3. HRMS (ESI): m/z calcd for $C_{15}H_{20}N_6O_2$ [M+H] $^+$ 317.1726, found [M+H] $^+$ 317.1731.

Synthesis of 4-ethoxy-*N*-(1-(2-fluoroethyl)-5-methyl-1*H*-pyrazol-4-yl)-7*H*-pyrrolo-[2,3-*d*]-pyrimidin-2-amine (15)



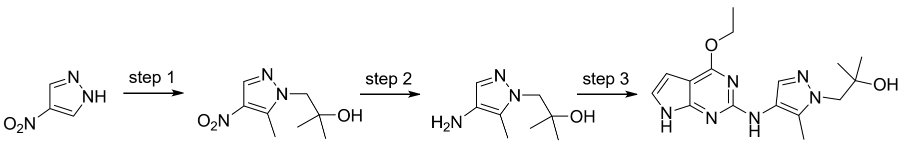
15

Step 1: *1-(2-fluoroethyl)-5-methyl-4-nitro-1H-pyrazole.* A solution of 5-methyl-4-nitro-1*H*-pyrazole (2.0 g, 15.74 mmol), 1-bromo-2-fluoroethane (2.197 g, 17.31 mmol) and Cs_2CO_3 (10.25 g, 31.5 mmol) in acetonitrile (100 mL) was stirred overnight at 60 °C. The mixture was filtered and the solution was concentrated in vacuo to give the mixture of the title compound (1.05 g, 38.2 % yield, not pure) as yellow oil. LCMS: 174 $[M+H]^+$.

Step 2: *1-(2-fluoroethyl)-5-methyl-1H-pyrazol-4-amine.* A solution of the mixture of 1-(2-fluoroethyl)-5-methyl-4-nitro-1*H*-pyrazole and Pd/C (100 mg, 0.940 mmol) in methanol (20 mL) was stirred under hydrogen at room temperature for 2 hours. The crude was then filtered and the solution was concentrated to give the mixture of the title compound (201 mg, 50 % yield, not pure) as yellow oil. LCMS: 144 $[M+H]^+$.

Step 3: *4-ethoxy-N-(1-(2-fluoroethyl)-5-methyl-1H-pyrazol-4-yl)-7H-pyrrolo-[2,3-d]-pyrimidin-2-amine.* A solution of 2-chloro-4-ethoxy-7H-pyrrolo[2,3-d]pyrimidine (200 mg, 1.012 mmol), 1-(2-fluoroethyl)-5-methyl-1*H*-pyrazol-4-amine (200 mg, 0.838 mmol, not pure), $Pd_2(dba)_3$ (46.3 mg, 0.051 mmol), Xtanphos and K_2CO_3 (280 mg, 2.024 mmol) in 2-butanol (10 mL) was irradiated by microwave at 100 °C for 2 hours. Then water (100 mL) was added and the aqueous phase was extracted with ethyl acetate (50 mL×3). The combined organic layer was washed with brine (20 mL), dried with Na_2SO_4, filtered and concentrated. The crude was purified by chromatography on silica gel (PE: EA=1:3) and further purified via Biotage (2M NH_4OH in Methanol; 40+M Biotage column) to give the title compound (35 mg, 11.36 % yield) as a white solid. LCMS: 305 $[M+H]^+$. t_R =1.263 mins. (LCMS condition 2) ^1H NMR (400MHz, CDCl3): δ 9.70 (br. s., 1H), 7.70 (s, 1H), 6.44 - 6.58 (m, 1H), 6.28 - 6.40 (m, 1H), 6.09 (s, 1H), 4.76 (t, J = 4.8 Hz, 1H), 4.64 (t, J = 4.8 Hz, 1H), 4.49 (q, J = 7.2 Hz, 2H), 4.26 - 4.34 (m, 1H), 4.14 - 4.25 (m, 1H), 2.18 - 2.28 (m, 3H), 1.43 (t, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 162.8, 157.8, 155.2, 135.1, 131.7, 120.8, 120.2, 98.5, 98.0, 83.0, 61.4, 49.6, 15.1, 9.3. HRMS (ESI): m/z calcd for $C_{14}H_{17}FN_6O$ [M+H] $^+$ 305.1526, found [M+H] $^+$ 305.1520.

Synthesis of 1-(4-((4-ethoxy-7*H*-pyrrolo-[2,3-*d*]pyrimidin-2-yl)amino)-5-methyl-1*H*-pyrazol-1-yl)-2-methylpropan-2-ol (16)



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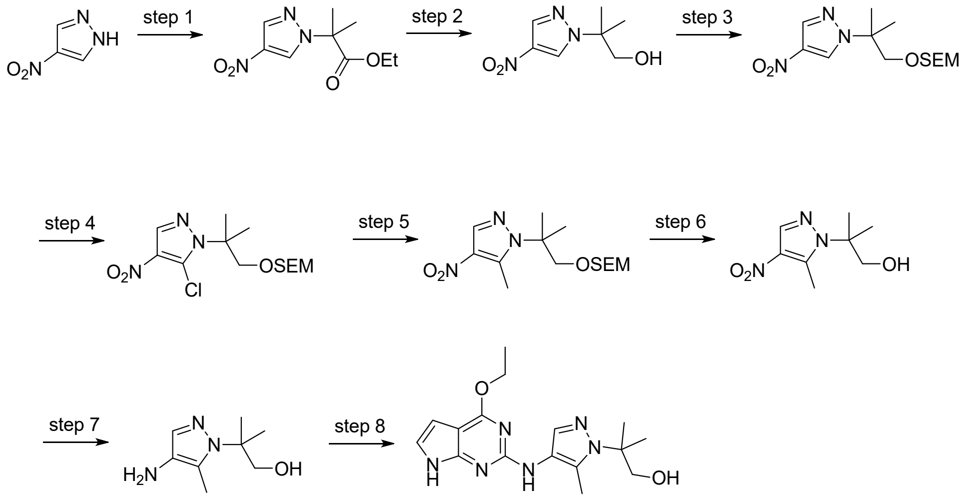
Step 1: *2-methyl-1-(5-methyl-4-nitro-1H-pyrazol-1-yl)propan-2-ol.* To a solution of 3-methyl-4-nitro-1*H*-pyrazole (1.0 g, 7.87 mmol) in acetonitrile (10 mL) was added 2,2-dimethyloxirane (1.702 g, 23.60 mmol) and DBU (2.372 mL, 15.74 mmol). The reaction was stirred at 60 °C for 20 hrs. The mixture was concentrated and the residue dissolved in ethyl acetate and washed with 1 N HCl, water and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude was purified by column chromatography on silica gel (DCM: MeOH=20:1) to give the title compound (800 mg, 51.0 % yield, together with the isomer). LCMS: 200.1 $[M+H]^+$.

Step 2: *1-(4-amino-3-methyl-1H-pyrazol-1-yl)-2-methylpropan-2-ol.* To a solution of 2-methyl-1-(3-methyl-4-nitro-1H-pyrazol-1-yl)propan-2-ol (800 mg, 4.02 mmol, together with the isomer) in ethanol (50 mL) was added Pd/C (85 mg, 0.080 mmol) under N_2. The mixture was stirred overnight under H_2 at room temperature. The suspension was filtered through a pad of Celite and the pad was washed with EtOH (10 mL×3). The combined filtrates were concentrated. The crude was purified by column chromatography on silica gel (DCM: MeOH=20:1) to give the title compound (650 mg, 96 % yield, together with the isomer). LCMS: 170.2 $[M+H]^+$.

Step 3: *1-(4-((4-ethoxy-7H-pyrrolo-[2,3-d]pyrimidin-2-yl)amino)-5-methyl-1H-pyrazol-1-yl)-2-methylpropan-2-ol.* A solution of 2-chloro-4-ethoxy-7H-pyrrolo[2,3-d]pyrimidine (200 mg, 1.012 mmol), 1-(4-amino-5-methyl-1*H*-pyrazol-1-yl)-2-methylpropan-2-ol (206 mg, 1.214 mmol, together with the isomer), $Pd_2(dba)_3$ (46.3 mg, 0.051 mmol), Xtanphos (58.6 mg, 0.101 mmol) and K_2CO_3 (420 mg, 3.04 mmol) in 2-butanol (2.0 mL) was irradiated by microwave at 120 ^oC for 45 min. The reaction mixture was quenched with water and extracted with EtOAc (20 mL×3). The combined organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. The crude was purified by column chromatography on silica gel (DCM: MeOH=20:1) to get a mixture, which was further purified by chiral-HPLC (Co-Solvent MeOH (0.1%DEA); Column AY-H (4.6*250mm, 5µm); Column Temperature 40; CO_2 Flow Rate 2.4; Co-Solvent Flow Rate 0.6; Co-Solvent % 20; Back Pressure 120; Total Flow 31; PDA Start Wavelength 214; PDA Stop Wavelength 359) to give the title compound (20 mg, 5.76 % yield) as grey solids. The structure was determined by NOE effect between methyl group on position 5 of pyrazole (CH_3, 2.23 ppm) and methylene group on position N1 of pyrazole (CH_2, 3.96 ppm). LCMS: 331.1 $[M+H]^+$, t_R =1.31 min (LCMS condition 2). ^{1}H NMR(400MHz, CDCl3): δ 9.04 (s, 1H), 7.75 (s, 1H), 6.71 (d, J = 4.0 Hz, 1H), 6.41 (d, J= 4.4 Hz, 1H), 6.15 (s, 1H), 4.50 (dd, J = 9.2 Hz, 9.2 Hz, 2H), 3.96, (s, 2H), 2.23 (s, 3H), 1.89 (s, 1H), 1.45 (t, J = 9.2 Hz, 3H), 1.20 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 162.8, 157.8, 155.2, 134.3, 132.2, 120.5, 120.1, 98.5, 97.9, 70.8, 61.4, 59.2, 28.0, 15.1, 10.0. HRMS (ESI): m/z calcd for $C_{16}H_{22}N_6O_2$ $[M+H]^+$ 331.1882, found $[M+H]^+$ 331.1890.

Synthesis of 2-(4-((4-ethoxy-7*H*-pyrrolo-[2,3-*d*]pyrimidin-2-yl)amino)-5-methyl-1*H*-pyrazol-1-yl)-2-methylpropan-1-ol (17)



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Step 1: *ethyl 2-methyl-2-(4-nitro-1H-pyrazol-1-yl)propanoate.* A mixture of 4-nitro-1*H*-pyrazole (10.0 g, 8.85 mmol), ethyl 2-bromo-2-methylpropanoate (20.7 g, 10.6 mmol) and K_2CO_3 (24.4 g, 177 mmol) in DMF (100 mL) was stirred at 80 ^oC for 2 hours. The mixture was filtered through a Celite pad and the filtrate was concentrated. The residue was diluted with EtOAc (300 mL), then washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated in vacuo. The crude was purified by column chromatography on silica gel (PE: EA = 15:1 to 8:1) to give the title compound (16.7 g, 83% yield) as yellow oil. ^{1}H NMR (400 MHz, CDCl3): δ 8.31 (s, 1H), 8.06 (s, 1H), 4.18 (q, *J* = 7.2 Hz, 2H), 1.86 (s, 6H), 1.20 (t, *J* = 7.2 Hz, 3H).

Step 2: *2-methyl-2-(4-nitro-1H-pyrazol-1-yl)propan-1-ol.* To a solution of ethyl 2-methyl-2-(4-nitro-1*H*-pyrazol-1-yl)propanoate (17.0 g, 74.8 mmol) in THF (50 mL) and water (3 mL) was added $NaBH_4$ (5.66 g, 150 mmol) at 0 ºC. The reaction was stirred at room temperature for 2 hours. The mixture was quenched with aq. $NaHCO_3$ and extracted with DCM (20 mL×3). The combined extracts were dried over Na_2SO_4, filtered and concentrated in vacuum. The crude was purified by column chromatography on silica gel (DCM: MeOH=20:1) to give the title compound **(**10.0 g, 72.2 % yield). LCMS: 186 [M+H]$^+$. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.79 (s, 1H), 8.26 (s, 1H), 5.09 (t, *J*= 9.2 Hz, 1H), 3.57 (d, *J*= 9.2 Hz, 2H), 1.48 (s, 6H).

Step 3: *1-(2-methyl-1-((2-(trimethylsilyl)ethoxy)methoxy)propan-2-yl)-4-nitro-1H-pyrazole.* A solution of 2-methyl-2-(4-nitro-1*H*-pyrazol-1-yl)propan-1-ol (2 g, 10.80 mmol) and NaH (0.864 g, 21.60 mmol) in DMF (30 mL) was stirred in an ice-bath for 30 min. SEMCl (2.299 mL, 12.96 mmol) was added and then the mixture was stirred at room temperature for 3 hours. The reaction was then quenched with water (100 mL) and extracted with diethyl ether (50 mL×3). The organic layer was dried and concentrated. The crude was purified by column chromatography on silica gel (PE: EA=10:1) to give the title compound (1.2 g, 33.5 % yield) as yellow oil. LCMS: 314 [M+H]$^+$. ^{1}H NMR (400MHz, DMSO-d_6): δ 8.91 (s, 1H), 8.31 (s, 1H), 4.56 (s, 2H), 3.30-3.45 (m, 3H), 2.55 (br. s., 1H), 1.60 (s, 6H), 0.79-0.94 (m, 2H), 0.00 (s, 9H).

Step 4: *5-chloro-1-(2-methyl-1-((2-(trimethylsilyl)ethoxy)methoxy)propan-2-yl)-4-nitro-1H-pyrazole.* To a solution of 1-(2-methyl-1-((2-(trimethylsilyl)ethoxy) methoxy)propan-2-yl)-4-nitro-1*H*-pyrazole (1.2 g, 3.80 mmol) in dry THF (30 mL) stirred under nitrogen at -70 ^oC was added a solution of LiHMDS (11.41 mL, 11.41 mmol, 1M in THF) dropwise during 20 min. The reaction mixture was stirred at -78 ^oC for 30 min. A solution of perchloroethane (1.351 g, 5.71 mmol) was added and the mixture was stirred for 1 hour at -78 ^oC under nitrogen. The mixture was quenched with aq NH_4Cl. Then the mixture was extracted with EtOAc (2 ×100 mL), washed with brine, dried with Na_2SO_4, filtered and concentrated. The crude was purified by column chromatography on silica gel (PE: EA=30:1) to give the title compound (1.2 g, 90 % yield) as a yellow solid. LCMS: 322 $[M+H]^+$. ^1H NMR (400MHz, CDCl3): δ 8.13 (s, 1H), 4.58-4.69 (m, 2H), 3.89-3.99 (m, 2H), 3.41-3.59 (m, 2H), 1.70-1.84 (m, 6H), 0.79-0.97 (m, 2H), 0.00 (s, 9H).

Step 5: *5-methyl-1-(2-methyl-1-((2-(trimethylsilyl)-ethoxy)-methoxy)propan-2-yl)-4-nitro-1H-pyrazole.* A solution of 2,4,6-trimethyl-1,3,5,2,4,6-trioxatriborinane (0.897 g, 7.15 mmol), 5-chloro-1-(2-methyl-1-((2-(trimethylsilyl)ethoxy)methoxy)propan-2-yl)-4-nitro-1*H*-pyrazole (1.0 g, 2.86 mmol), Na_2CO_3 (0.909 g, 8.57 mmol) and $PdCl_2(dppf)-CH_2Cl_2$ adduct (0.467 g, 0.572 mmol) in 1,4-dioxane (2 mL) and water (0.4 mL) was combined in a thick-walled glass tube and stirred at 90 ^oC for 40 hours. Then the mixture was diluted with water (100 mL) and extracted with ethyl acetate (100 mL×2). The combined organic phase was washed with brine (20 mL), dried with Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (PE: EA=30:1) to give the title compound (530 mg, 56.3 % yield) as yellow oil. LCMS: 330 $[M+H]^+$. ^1H NMR (400MHz, CDCl3): δ 8.06 (s, 1H), 4.62 (s, 2H), 3.82 (s, 2H), 3.34-3.56 (m, 2H), 2.71-2.95 (m, 3H), 1.72 (s, 6H), 0.79-0.95 (m, 2H), 0.00 (s, 9H).

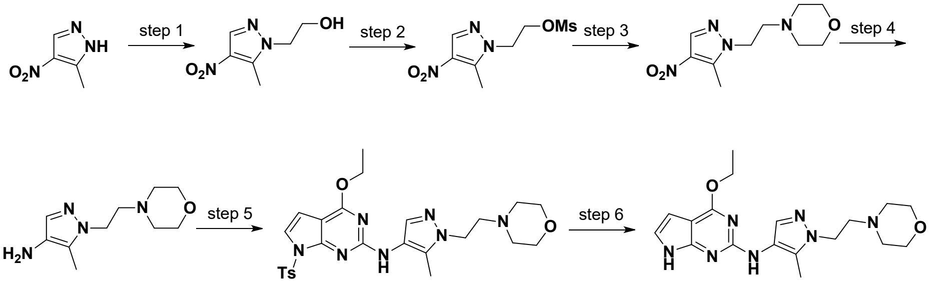
Step 6: *2-methyl-2-(5-methyl-4-nitro-1H-pyrazol-1-yl)propan-1-ol.* A solution of 5-methyl-1-(2-methyl-1-((2-(trimethylsilyl)-ethoxy)-methoxy)propan-2-yl)-4-nitro-1*H*-pyrazole (500 mg, 1.518 mmol) and hydrogen chloride (15 mL, 60.0 mmol, 4M in water) was stirred at room temperature for 5 hours. The mixture was treated with saturated $NaHCO_3$ solution until the pH = 8. The mixture was then extracted with EtOAc (50 mL×2). The organic layer was dried with Na_2SO_4, filtered and concentrated to give the title compound (270 mg, 89 % yield) as brown oil. LCMS: 200 $[M+H]^+$. ^1H NMR (400MHz, CDCl3): δ 8.06 (s, 1H), 7.26 (s, 1H), 3.94 (s, 2H), 2.83 (s, 3H), 1.46-1.75 (m, 6H).

Step 7: *2-(4-amino-5-methyl-1H-pyrazol-1-yl)-2-methylpropan-1-ol.* A solution of 2-methyl-2-(5-methyl-4-nitro-1*H*-pyrazol-1-yl)propan-1-ol (260 mg, 1.305 mmol) and Pd/C (290 mg, 2.73 mmol) in methanol (30 mL) was stirred under hydrogen at room temperature for 4 hours. The mixture was then filtered and the solution was concentrated to give the title compound (200 mg, 81 % yield) as brown oil. LCMS: 170 $[M+H]^+$. ^1H NMR (400MHz, CDCl3): δ 7.27 (s, 1H), 7.09 (s, 1H), 3.88 (s, 2H), 2.31 (s, 3H), 1.49 (s, 6H).

Step 8: *2-(4-((4-ethoxy-7H-pyrrolo-[2,3-d]pyrimidin-2-yl)amino)-5-methyl-1H-pyrazol-1-yl)-2-methylpropan-1-ol.* A solution of 2-chloro-4-ethoxy-7H-pyrrolo[2,3-d]pyrimidine (60 mg, 0.304 mmol), 2-(4-amino-5-methyl-1H-pyrazol-1-yl)-2-methylpropan-1-ol (46.2 mg, 0.273 mmol), xantphos (26.4 mg, 0.046 mmol), K_2CO_3 (84 mg, 0.607 mmol) and $Pd_2(dba)_3$

(27.8 mg, 0.030 mmol) in 2-butanol (2 mL) was irradiated by microwave at 120 ^oC for 1 hour. The mixture was filtered and concentrated. The crude was then purified by prep-HPLC to give the title compound (30 mg, 0.091 mmol, 29.9 % yield) as a white solid. LCMS: 331 $[M+H]^+$. t_R =1.16 mins.(LCMS conditions 2) ^1H NMR (400MHz, $CDCl_3$): δ 9.45 (br. s., 1H), 7.61 (s, 1H), 6.48-6.64 (m, 1H), 6.35 (dd, *J*=3.2, 2.0 Hz, 1H), 6.04 (s, 1H), 4.42 - 4.59 (m, 3H), 3.89 (br. s., 2H), 2.35 (s, 3H), 1.41 - 1.49 (m, 9H). ^{13}C NMR (101 MHz, DMSO-*d$_6$*): δ 162.8, 158.0, 155.2, 133.4, 131.7, 121.9, 120.1, 98.5, 97.9, 69.0, 63.6, 61.4, 25.2, 15.1, 12.0.

Synthesis of 4-ethoxy-*N*-(5-methyl-1-(2-morpholinoethyl)-1H-pyrazol-4-yl)-7*H*-pyrrolo-[2,3-*d*]pyrimidin-2-amine (18)



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Step 1: *2-(5-methyl-4-nitro-1H-pyrazol-1-yl)-ethanol.* To a solution of 3-methyl-4-nitro-1*H*-pyrazole (2.0g, 15.74 mmol) and 1, 3-dioxolan-2-one (6.93 g, 79 mmol) in acetonitrile (5 mL) was added sodium hydroxide (1.888 g, 47.2 mmol). The reaction mixture was stirred at 80 ^oC for 15 hours. The mixture was then diluted with water (100 ml) and extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude was purified by pre-HPLC to give the title compound (400 mg, 14.85 % yield) as a white solid. LCMS: 172 $[M+H]^+$. ^1H NMR (400MHz, CDCl3): δ 8.11 (s, 1H), 4.17 - 4.26 (m, 2H), 3.99 - 4.13 (m, 2H), 2.77 (br., 1H), 2.68 (s, 3H).

Step 2: *2-(5-methyl-4-nitro-1H-pyrazol-1-yl) ethyl-methanesulfonate.* To a solution of 2-(5-methyl-4-nitro-1*H*-pyrazol-1-yl)-ethanol (200 mg, 1.169 mmol) and DIPEA (0.204 mL, 1.169 mmol) in THF (5 mL) was added hypochlorous methanesulfonic anhydride (0.210 mL, 1.169 mmol). The reaction was then stirred at 0 ^oC for 30 min. The mixture was diluted with aq. $NaHCO_3$ (20 mL), extracted with EtOAc. The organic layer was dried and concentrated to give the title compound (300 mg, 81 % yield) as oil. LCMS: 250 $[M+H]^+$.

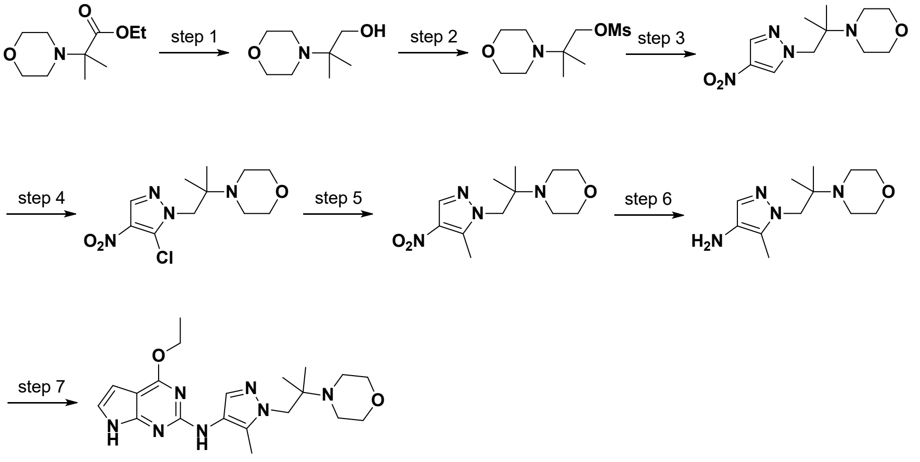
Step 3: *4-(2-(5-methyl-4-nitro-1H-pyrazol-1-yl)ethyl)-morpholine.* A solution of 2-(5-methyl-4-nitro-1*H*-pyrazol-1-yl)ethyl-methanesulfonate (200 mg, 0.802 mmol), morpholine (80 mg, 0.918 mmol) and K_2CO_3 (381 mg, 2.75 mmol) in acetonitrile (10 mL) was stirred overnight at 80 ^oC. The mixture was concentrated and purified by column chromatography on silica gel (PE: EA =1:1) to give the title compound (150 mg, 34.0 % yield) as oil. LCMS: 241 $[M+H]^+$. ^1H NMR (400MHz, CDCl3): δ 8.10 (s, 1H), 4.16 - 4.24 (m, 2H), 3.62 - 3.74 (m, 4H), 2.80 (t, *J* = 6.4 Hz, 2H), 2.69 (s, 3H), 2.44 - 2.52 (m, 4H).

Step 4: *5-methyl-1-(2-morpholinoethyl)-1H-pyrazol-4-amine.* A solution of 4-(2-(5-methyl-4-nitro-1*H*-pyrazol-1-yl)ethyl)-morpholine (150 mg, 0.624 mmol) and Pd/C (33.2 mg, 0.031 mmol) in methanol (5 mL) was stirred overnight at 20 ^oC under hydrogen. The mixture was filtered and the solution was concentrated to give the title compound (100 mg, 76 % yield) as oil. LCMS: 211 [M+H]$^+$.

Step 5: *4-ethoxy-N-(5-methyl-1-(2-morpholinoethyl)-1H-pyrazol-4-yl)-7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-2-amine.* To a solution of 2-chloro-4-ethoxy-7-tosyl-7*H*-pyrrolo-[2,3-*d*]-pyrimidine (150 mg, 0.426mmol), 5-methyl-1-(2-morpholinoethyl)-1*H*-pyrazol-4-amine (100 mg, 0.476 mmol) and Xanphos (37.0 mg, 0.064 mmol) in 1,4- dioxane (2.0 mL) and water (0.2 mL) was added K_2CO_3 (118 mg, 0.853 mmol) and Pd(dppf)Cl_2 (34.8 mg, 0.043 mmol). The reaction mixture was stirred overnight at 90 ^{o}C. The mixture was cooled to room temperature and partitioned between EtOAc (25 mL) and water (20 mL). The organic layer was washed with water (20 mL), dried over Na_2SO_4 and concentrated. The crude was purified by column chromatography on silica gel (PE: EA=1:1) to give the title compound (70 mg, 25.6 % yield) as a yellow solid. LCMS: 525 [M+H]$^+$.

Step 6: *4-ethoxy-N-(5-methyl-1-(2-morpholinoethyl)-1H-pyrazol-4-yl)-7H-pyrrolo-[2,3-d]pyrimidin-2-amine.* A solution of 4-ethoxy-*N*-(5-methyl-1-(2-morpholinoethyl)-1*H*-pyrazol-4-yl)-7-tosyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (70 mg, 0.133 mmol) and sodium hydroxide (0.2 mL, 0.4 mmol) in isopropanol (2 mL) was stirred overnight at 60 ^{o}C. The mixture was concentrated and 2N HCl was added until pH=7. The mixture was then extracted with EtOAc twice. The combined organic layer was dried and evaporated. The crude was purified by prep-HPLC to give the title compound (25 mg, 49.0 % yield) as a white solid. LCMS: 372 [M+H]$^+$. t_R =1.223 mins. (LCMS condition 2) ^{1}H NMR (400MHz, DMSO-d_6): δ 11.17 (br. s., 1H), 7.98 (s, 1H), 7.55 (s, 1H), 6.66 - 6.94 (m, 1H), 6.19 (dd, *J* = 3.3, 1.8 Hz, 1H), 4.42 (q, *J* = 7.0 Hz, 2H), 4.09 (t, *J* = 6.7 Hz, 2H), 3.55 (t, *J* = 4.5 Hz, 4H), 2.56 - 2.73 (m, 2H), 2.41 (br. s., 4H), 2.18 (s, 3H), 1.34 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 162.8, 157.8, 155.2, 134.4, 131.0, 120.6, 120.1, 98.5, 97.9, 66.6, 61.4, 58.4, 53.9, 46.8, 15.1, 9.3.

Synthesis of 4-ethoxy-*N*-(5-methyl-1-(2-methyl-2-morpholinopropyl)-1*H*-pyrazol-4-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (19)



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Step 1: *2-methyl-2-morpholinopropan-1-ol.* To a solution of ethyl 2-methyl-2-morpholinopropanoate (3.8 g, 18.88 mmol) in THF (30 mL) was added $LiAlH_4$ (2.87 g, 76 mmol) at 0ºC. The reaction mixture was stirred overnight at 25 °C. The reaction was quenched with water and 10% NaOH solution. The mixture was filtered through a pad of Celite and the pad was washed with THF (10 mL). The combined filtrates were concentrated and purified by column chromatgraphy on silica gel (PE: EA =1:2) to give the title compound (2.5 g, 83 % yield). LCMS: 160 $[M+H]^+$. ^1H NMR (400 MHz, CDCl3): δ 3.72 (m, 4H), 3.33 (s, 2H), 2.55 (m, 4H), 1.03 (s, 6H).

Step 2: *2-methyl-2-morpholinopropyl methanesulfonate.* To a solution of 2-methyl-2-morpholinopropan-1-ol (2.2 g, 13.82 mmol) and DIPEA (4.83 mL, 27.6 mmol) in DCM (10 mL) was added methanesulfonyl chloride (1.283 mL, 16.58 mmol) at 0 °C. The reaction was stirred at 0 °C for 2 hours. The mixture was quenched with aqueous $NaHCO_3$ and extracted with DCM (20 mL×3). The combined extracts were dried over Na_2SO_4, filtered and concentrated to give the title compound (3.28 g, 100 % yield).

Step 3: *4-(2-methyl-1-(4-nitro-1H-pyrazol-1-yl)propan-2-yl)morpholine.* To a solution of 4-nitro-1*H*-pyrazole (1.560 g, 13.80 mmol) and 2-methyl-2-morpholinopropyl methanesulfonate (3.27 g, 13.80 mmol) in DMF (10 mL) was added K_2CO_3 (5.72 g, 41.4 mmol). The reaction was stirred overnight at 90 °C. The mixture was quenched with water and extracted with DCM (20 mL×3). The combined extracts were dried over Na_2SO_4, filtered and concentrated. The crude was purified by column chromatography on silica gel (PE: EA=4:1) to give the title compound (1.50 g, 42.7 % yield). LCMS: 255 $[M+H]^+$.

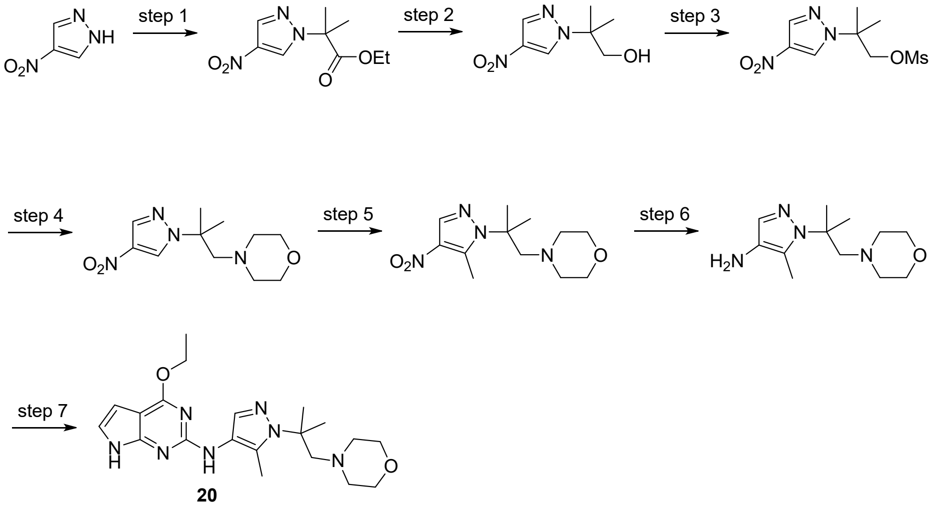
Step 4: *4-(1-(5-chloro-4-nitro-1H-pyrazol-1-yl)-2-methylpropan-2-yl)morpholine.* To a solution of 4-(2-methyl-1-(4-nitro-1*H*-pyrazol-1-yl)propan-2-yl)morpholine (1.50 g, 5.90 mmol) in THF (100 mL) was added LiHMDS (1M in THF, 23.60 mL, 23.60 mmol) at -78 °C under nitrogen. After stirring at -78 °C for 30min, perchloroethane (4.19 g, 17.70 mmol) in THF (100 mL) was added and the mixture was stirred for another 2 hours at -78 °C under nitrogen. The reaction was quenched with aq. NH_4Cl. The mixture was extracted with EA (100 mL×2), washed with brine, dried with Na_2SO_4, filtered and concentrated. The crude was purified by column chromatography on silica gel (PE: EA =4:1) to give the title compound (1.1 g, 53.4 % yield) as yellow oil. LCMS: 289 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 8.18 (s, 1H), 4.17 (s, 2H), 3.70 (m, 4H), 2.66 (m, 4H), 1.11 (s, 6H).

Step 5: *4-(2-methyl-1-(5-methyl-4-nitro-1H-pyrazol-1-yl)propan-2-yl)morpholine.* A solution of 4-(1-(5-chloro-4-nitro-1*H*-pyrazol-1-yl)-2-methylpropan-2-yl)morpholine (1.1 g, 3.81 mmol), methylboronic acid (0.684 g, 11.43 mmol), Na_2CO_3 (1.211 g, 11.43 mmol) and $PdCl_2(dppf)-CH_2Cl_2$ adduct (0.311 g, 0.381 mmol) in 1,4-dioxane (3 mL) and water (0.3 mL) was stirred under at 80 °C for 12 hours. Solvent was evaporated and the crude was directly purified via column chromatography on silica gel (PE: EA=4: 1) to give the title compound (800 mg, 74.3 % yield). LCMS: 269 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 8.07 (s, 1H), 4.06 (s, 2H), 3.69 (m, 4H), 2.68 (s, 3H), 2.63 (m, 4H), 1.06 (s, 6H).

Step 6: *5-methyl-1-(2-methyl-2-morpholinopropyl)-1H-pyrazol-4-amine.* A solution of 4-(2-methyl-1-(5-methyl-4-nitro-1*H*-pyrazol-1-yl)propan-2-yl)morpholine (800 mg, 2.98 mmol) and Pd/C (79 mg, 0.075 mmol) in methanol (10 mL) was stirred overnight under hydrogen. The mixture was filtered and the solution was evaporated to give the title compound (600 mg, 82 % yield) as yellow oil. LCMS: 239 $[M+H]^+$. ^1H NMR (400 MHz, CDCl3): δ 7.15 (s, 1H), 3.94 (s, 2H), 3.71 (m, 4H), 2.63 (m, 6H), 2.19 (s, 3H), 1.03 (s, 6H).

Step 7: *4-ethoxy-N-(5-methyl-1-(2-methyl-2-morpholinopropyl)-1H-pyrazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidin-2-amine.* A solution of 2-chloro-4-ethoxy-7H-pyrrolo[2,3-d]pyrimidine (100 mg, 0.506 mmol), 5-methyl-1-(2-methyl-2-morpholinopropyl)-1H-pyrazol-4-amine (145 mg, 0.607 mmol), X-phos (18.09 mg, 0.038 mmol), K_2CO_3 (210 mg, 1.518 mmol) and $Pd_2(dba)_3$ (24.12 mg, 0.051 mmol) in 2-butanol (2 mL) was irradiated by microwave at 120 °C for 45 min. The mixture was filtered and concentrated. The crude was then purified by prep-HPLC to give the title compound (105 mg, 51.9 % yield) as a white solid. LCMS: 400 $[M+H]^+$. t_R =1.28 mins (LCMS conditions 2). ^1H NMR (300 MHz, $CDCl_3$): δ 11.20 (s, 1H), 8.00 (s, 1H), 7.57 (s, 1H), 6.86 (d, *J*=3.5 Hz, 1H), 6.21 (d, *J*=3.5 Hz, 1H), 4.42 (dd, *J*=9.0 Hz, 2H), 3.97 (s, 2H), 3.57 (m, 4H), 2.58 (m, 4H), 2.19 (s, 3H), 1.34 (t, J=9.0 Hz, 3H), 0.97 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 162.8, 157.8, 155.2, 134.4, 132.1, 120.5, 120.1, 98.5, 97.9, 67.5, 61.3, 58.3, 55.5, 46.6, 22.3, 15.1, 10.2.

Synthesis of 4-ethoxy-*N*-(5-methyl-1-(2-methyl-1-morpholinopropan-2-yl)-1*H*-pyrazol-4-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (20)



20

Step 1: ethyl 2-methyl-2-(4-nitro-1*H*-pyrazol-1-yl)propanoate. A mixture of 4-nitro-1*H*-pyrazole (10.0 g, 8.85 mmol), ethyl 2-bromo-2-methylpropanoate (20.7 g, 10.6 mmol) and K_2CO_3 (24.4 g, 177 mmol) in DMF (100 mL) was stirred at 80 °C for 2 hours. The mixture was filtered through a Celite pad and the filtrate was concentrated. The residue was diluted with EtOAc (300 mL), then washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated in vacuo. The crude was purified by column chromatography on silica gel (PE: EA = 15:1 to 8:1) to give the title compound (16.7 g, 83% yield) as yellow oil. ^1H NMR (400 MHz, CDCl3): δ 8.31 (s, 1H), 8.06 (s, 1H), 4.18 (q, *J* = 7.2 Hz, 2H), 1.86 (s, 6H), 1.20 (t, *J* = 7.2 Hz, 3H).

Step 2: *2-methyl-2-(4-nitro-1H-pyrazol-1-yl)propan-1-ol.* To a solution of ethyl 2-methyl-2-(4-nitro-1*H*-pyrazol-1-yl)propanoate (17.0 g, 74.8 mmol) in THF (50 mL) and water (3 mL) was added $NaBH_4$ (5.66 g, 150 mmol) at 0 ºC. The reaction was stirred at room temperature for 2 hours. The mixture was quenched with aq. $NaHCO_3$ and extracted with DCM (20 mL×3). The combined extracts were dried over Na_2SO_4, filtered and concentrated in vacuum. The crude was purified by column chromatography on silica gel (DCM: MeOH=20:1) to give the title compound **(**10.0 g, 72.2 % yield). LCMS: 186 [M+H]$^+$. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.79 (s, 1H), 8.26 (s, 1H), 5.09 (t, *J*= 9.2 Hz, 1H), 3.57 (d, *J*= 9.2 Hz, 2H), 1.48 (s, 6H).

Step 3: *2-methyl-2-(4-nitro-1H-pyrazol-1-yl)propyl methanesulfonate.* To a solution of 2-methyl-2-(4-nitro-1*H*-pyrazol-1-yl)propan-1-ol (5 g, 27.0 mmol) and DIPEA (9.43 mL, 54.0 mmol) in DCM (100 mL) at 0°C was added a solution of MsCl (2.95 mL, 37.8 mmol) in DCM (10 mL) dropwise. The reaction was stirred at room temperature for 1 hour. Satur. $NaHCO_3$ solution was added and the mixture was extracted with DCM (100 mL×3). The combined organic layers were dried over Na_2SO_4 and concentrated to give the title compound (6.2 g, 81 % yield) as a yellow solid. LCMS: 264 [M+H]$^+$.

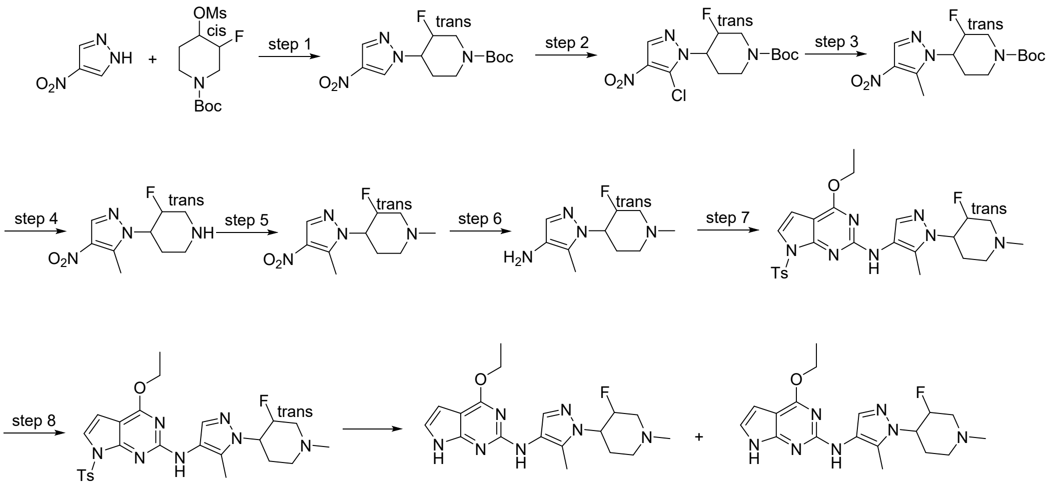
Step 4: *4-(2-methyl-2-(4-nitro-1H-pyrazol-1-yl)propyl)morpholine.* A mixture of 2-methyl-2-(4-nitro-1*H*-pyrazol-1-yl)propyl methanesulfonate (6.2 g, 23.55 mmol) and morpholine (30 mL, 23.55 mmol) was stirred at 135 °C for 7 days. Then water (150 mL) was added and the aqueous phase was extracted with EA (100 mL×2). The combined organic phase was washed with brine (50 mL), dried over Na_2SO_4 and concentrated. The crude was purified by column chromatography on silica gel (PE: EA=10:1) to give the title compound (3.7 g, 61.8 % yield) as a yellow solid. LCMS: 255 [M+H]$^+$.

Step 5: *4-(2-methyl-2-(5-methyl-4-nitro-1H-pyrazol-1-yl)propyl)morpholine.* To a solution of 4-(2-methyl-2-(4-nitro-1*H*-pyrazol-1-yl)propyl)morpholine (1g, 3.93 mmol) in THF(100 mL) was added LiHMDS (1M in THF) (1.974 g, 11.80 mmol) at -70 °C under nitrogen. After stirring at -70 °C for 30min, iodomethane (1.675 g, 11.80 mmol) was added and the mixture was stirred for 30 min at -78 °C under nitrogen. The reaction was quenched with aq. NH_4Cl. Then the mixture was extracted with EA, washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude was purified by prep-HPLC to give the title compound (100 mg, 0.373 mmol, 9.48 % yield) as a white solid. LCMS: 269 [M+H]$^+$.

Step 6: *5-methyl-1-(2-methyl-1-morpholinopropan-2-yl)-1H-pyrazol-4-amine.* A solution of 4-(2-methyl-2-(5-methyl-4-nitro-1*H*-pyrazol-1-yl)propyl)morpholine (100 mg, 0.373 mmol) and Pd/C (70 mg, 0.658 mmol) in methanol (30 mL) was stirred overnight under hydrogen. The mixture was filtered with diatomite and the solution was evaporated to give the title compound (70 mg, 79 % yield) as oil. LCMS: 239 [M+H]$^+$.

Step 7: *4-ethoxy-N-(5-methyl-1-(2-methyl-1-morpholinopropan-2-yl)-1H-pyrazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidin-2-amine.* A solution of 2-chloro-4-ethoxy-7H-pyrrolo[2,3-d]pyrimidine (50 mg, 0.253 mmol), 5-methyl-1-(2-methyl-1-morpholinopropan-2-yl)-1*H*-pyrazol-4-amine (70 mg, 0.294 mmol), X-phos (14.64 mg, 0.025 mmol), K_2CO_3 (69.9 mg, 0.506 mmol) and $Pd_2(dba)_3$ (23.17 mg, 0.025 mmol) in 2-butanol (1.5 mL) was irradiated by microwave at 120 ^{o}C for 45 min. The mixture was filtered and concentrated. The crude was then purified by prep-HPLC to give the title compound (45 mg, 44.5 % yield) as a white solid. LCMS: 400 [M+H]$^+$. t_R =1.75 mins.(LCMS conditions 2) ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.63 (s, 1H), 6.56 (br. s., 1H), 6.34 (dd, *J*= 2.0, 3.2 Hz, 1H), 6.02 (s, 1H), 4.48 (q, *J*=7.2 Hz, 2H), 3.51-3.67 (m, 4H), 2.60 (s, 2H), 2.43 (s, 3H), 2.18-2.30 (m, 4H), 1.61 (s, 6H), 1.43 (t, *J*=7.2 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 162.8, 158.1, 155.2, 133.7, 132.5, 121.9, 120.1, 98.5, 97.9, 68.0, 67.0, 63.7, 61.3, 55.1, 26.8, 15.1, 12.5.

Synthesis of *trans*-4-ethoxy-N-(1-(3-fluoro-1-methylpiperidin-4-yl)-5-methyl-1H-pyrazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidin-2-amine (21 and 22)



21
***trans*-Enantiomer 1**
unknown absolute stereochemistry

22
***trans*-Enantiomer 2**
unknown absolute stereochemistry

Step 1: *tert-butyl 3-fluoro-4-(4-nitro-1H-pyrazol-1-yl)piperidine-1-carboxylate*. To a solution of *tert*-butyl 3-fluoro-4-((methylsulfonyl)oxy)piperidine-1-carboxylate (14.93 g,50.2 mmol) (which may be prepared according to PCT Int. Appl., 2012062783) in DMF (25.0 mL) was added K_2CO_3 (13.88 g, 100mmol) and 4-nitro-1*H*-pyrazole (5.68 g, 50.2 mmol). The reaction mixture was stirred at 90 ^oC for overnight. The reaction mixture was quenched with water and extracted with DCM (20 mL × 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated in vacuo. The crude was purified by column chromatography on silica gel (PE: EA=4: 1) to give the title compound (10.0 g, 31.2 mmol, 62.1 % yield) as yellow oil. LCMS: 259.1 [M-56+H]$^+$. t_R =1.45 mins. (LCMS condition 2)

Step 2: *tert-butyl 4-(5-chloro-4-nitro-1H-pyrazol-1-yl)-3-fluoropiperidine-1-carboxylate.* To a solution of *tert*-butyl-3-fluoro-4-(4-nitro-1*H*-pyrazol-1-yl)piperidine-1-carboxylate (which may be prepared according to D103) (10.0 g, 31.8 mmol) in dry THF (50.0 mL) under nitrogen at -70 ^oC was added lithium bis(trimethylsilyl)amide (127 mL, 127 mmol, 1M in THF) dropwise during 15 min. The reaction mixture was stirred at -78 ^oC for 30 min. A solution of perchloroethane (22.60 g, 95 mmol) in dry THF (50.0 mL) was added and the mixture was stirred for 2 hours at -78 ^oC under nitrogen. The mixture was quenched with aq NH_4Cl and extracted with EtOAc (2 ×100 mL). The organic layer was dried with Na_2SO_4, filtered and concentrated. The crude was purified by chromatography on silica gel (PE: EA=4: 1) to give the title compound (6.0 g, 15.31 mmol, 48.1 % yield) as yellow oil.
LCMS: 293 [M-56+H]$^+$. t_R =1.55 mins. (LCMS condition 2)

Step 3: *tert-butyl 3-fluoro-4-(5-methyl-4-nitro-1H-pyrazol-1-yl)piperidine-1-carboxylate.* A solution of methylboronic acid (3.09 g, 51.6 mmol), *tert*-butyl 4-(5-chloro-4-nitro-1*H*-pyrazol-1-yl)-3-fluoropiperidine-1-carboxylate (6.0 g), $PdCl_2$(dppf)-CH_2Cl_2 adduct (1.405 g, 1.720 mmol) and sodium carbonate (5.47 g, 51.6 mmol) in 1,4-dioxane (30 mL) and water (3.0 mL) was combined in a thick walled glass tube and stirred overnight at 75 ^oC. The mixture was poured into water and extracted with DCM (20 mL × 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated in vacuo. The crude was purified by column chromatography on silica (PE: EA=4: 1) to give the title compound (2.0 g, 6.09 mmol, 35.4 % yield) as a yellow solid. LCMS: 273.1 $[M-56+H]^+$. t_R =1.53 mins. (LCMS condition 2)

Step 4: *3-fluoro-4-(5-methyl-4-nitro-1H-pyrazol-1-yl)piperidine* A solution of *tert*-butyl 3-fluoro-4-(5-methyl-4-nitro-1*H*-pyrazol-1-yl)piperidine-1-carboxylate (6.0 g, 18.27 mmol) in DCM (50 mL) was added TFA (14.08 mL, 183 mmol) was stirred at room temperature for 2 hours. The reaction mixture was quenched with aq. $NaHCO_3$ and extracted with DCM (20 mL ×3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated in vacuum. The crude was purified by column chromatography on silica gel (DCM: MeOH =20:1) to give the title compound (4.0 g, 17.53mmol, 96 % yield) as a yellow solid. LCMS: 229.1 $[M+H]^+$. t_R =1.11 mins. (LCMS condition 2)

Step 5: *3-fluoro-1-methyl-4-(5-methyl-4-nitro-1H-pyrazol-1-yl)piperidine***.** To a solution of 3-fluoro-4-(5-methyl-4-nitro-1*H*-pyrazol-1-yl)piperidine (4.0 g, 17.53 mmol) and formaldehyde (1.579 g, 52.6 mmol) in methanol (5.0 mL) was added AcOH (0.100 mL, 1.753 mmol). The reaction was stirred at 65 °C for 2 hours. The reaction mixture was then cooled to 0 °C, sodium triacetoxyborohydride (3.71 g, 17.53 mmol) was added. The reaction was stirred at room temperature for 2 hours. The reaction mixture was quenched with water and extracted with mix solvent of DCM and MeOH (10:1, 20 mL × 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated in vacuo. The crude was purified by column chromatography on silica gel (PE: EA=2: 1) to give the title compound (3.0 g, 12.38 mmol, 70.7 % yield) as a yellow solid. LCMS: 243.1 $[M+H]^+$. t_R =1.47 mins. (LCMS condition 2)

Step 6: *1-(3-fluoro-1-methylpiperidin-4-yl)-5-methyl-1H-pyrazol-4-amine* To a solution of 3-fluoro-1-methyl-4-(5-methyl-4-nitro-1*H*-pyrazol-1-yl)piperidine (3.0 g, 12.38 mmol) in ethanol (10.0 mL) and water (10.0 mL) was added iron (1.383 g, 24.77 mmol) and ammonium chloride (0.331 g, 6.19 mmol). The reaction was stirred overnight at room temperature. The mixture was filtered through a pad of Celite and washed with EtOH (10 mL x3). The filtrate was concentrated and the crude was purified by column chromatography on silica gel (DCM: MeOH=20:1) to give the title compound (1.5 g, 7.07 mmol, 57.1 % yield) as yellow oil. LCMS: 213.1 $[M+H]^+$. t_R =0.94 mins. (LCMS condition 2)

Step 7: *4-ethoxy-N-(1-(3-fluoro-1-methylpiperidin-4-yl)-5-methyl-1H-pyrazol-4-yl)-7-tosyl-7H-pyrrolo-[2,3-d]pyrimidin-2-amine*. A solution of 2-chloro-4-ethoxy-7-tosyl-7*H*-pyrrolo-[2,3-*d*]-pyrimidine (180 mg, 0.512 mmol), 1-(3-fluoro-1-methylpiperidin-4-yl)-5-methyl-1*H*-pyrazol-4-amine (108 mg, 0.512 mmol), K_2CO_3 (212 mg, 1.535 mmol), X-Phos (73.2 mg, 0.153 mmol) and $PdCl_2(dppf)-CH_2Cl_2$ adduct (84 mg, 0.102 mmol) in 1,4-dioxane (1.50 mL) and water (0.20 mL) under nitrogen was stirred overnight at 90 °C. The reaction mixture was poured into water and extracted with DCM (20 mL × 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated in vacuum. The crude was purified by column chromatography on silica gel (PE: EA=2: 1) to give the title compound (250 mg, 0.256 mmol, 50.1 % yield) as a yellow solid. LCMS: 528.3 $[M+H]^+$. t_R =1.57 mins. (LCMS condition 2)

Step 8: *trans-4-ethoxy-N-(1-(3-fluoro-1-methylpiperidin-4-yl)-5-methyl-1H-pyrazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidin-2-amine.* A solution of 4-ethoxy-N-(1-(3-fluoro-1-methylpiperidin-4-yl)-5-methyl-1H-pyrazol-4-yl)-7-tosyl-7H-pyrrolo-[2,3-d]pyrimidin-2-amine (750 mg, 1.421 mmol) and TBAF (1858 mg, 7.11 mmol, 1M in THF) in THF (10.0 mL) was stirred at 80 °C for 2 hours. The mixture was quenched with aqueous NH_4Cl and extracted with DCM (20 mL × 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated in vacuum The residue was purified by column chromatography on silica gel (DCM: MeOH=20:1) to give the mixture (220 mg, 0.554 mmol, 39.0 % yield), which was further purified by chiral-HPLC (Co-Solvent MeOH(0.1%DEA); Column OJ-H (4.6*250mm, 5µm); Column Temperature 40; CO_2 Flow Rate 2.25; Co-Solvent Flow Rate 0.45; Co-Solvent % 15; Back Pressure 120; Total Flow 3; PDA Start Wavelength 214; PDA Stop Wavelength 359) to give the title compounds **21**(19.0 mg, 0.051 mmol, 8.64 % yield) and **22** (13.6 mg, 0.036 mmol, 6.18 % yield) as white solids.

21: LCMS: 374.2 $[M+H]^+$. t_R =1.21mins. (LCMS condition 2)

Chiral HPLC: t_R =3.27 mins. (Condition: Column OJ-H (4.6*250mm, 5µm); (Co-Solvent MeOH) unknown absolute stereochemistry

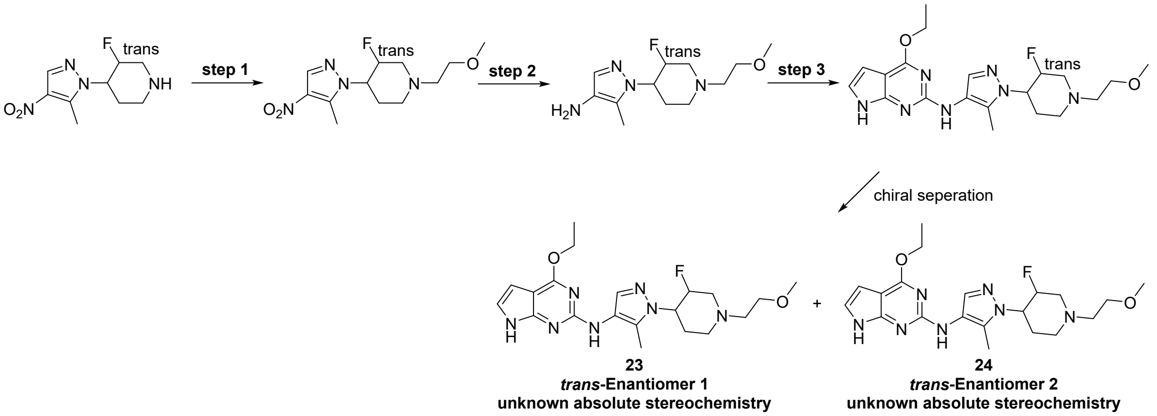
^{1}H NMR (400MHz, CDCl3): δ 9.10 (br. s., 1H), 7.77 (s, 1H), 6.54-6.74 (m, 1H), 6.35 (dd, *J*=3.4, 1.9 Hz, 1H), 6.06 (s, 1H), 4.82-5.07 (m, 1H), 4.42-4.54 (m, *J*=7.2, 7.2, 7.2 Hz, 2H), 3.85-4.07 (m, 1H), 3.18-3.37 (m, 1H), 2.79-3.01 (m, 1H), 2.41-2.52 (m, 1H), 2.37 (s, 3H), 2.23 (s, 3H), 2.05-2.19 (m, 2H), 1.91 (dd, *J*=7.5, 5.0 Hz, 1H), 1.42 (t, *J*=7.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 162.8, 157.7, 155.2, 135.1, 131.7, 120.5, 120.2, 98.5, 98.0, 90.2, 61.4, 59.0, 58.5, 53.9, 45.7, 30.1, 15.1, 9.1.

22: LCMS: 374.2 $[M+H]^+$. t_R =1.21mins. (LCMS condition 2)

Chiral HPLC: t_R =3.85 mins. (Condition: Column OJ-H (4.6*250mm, 5µm); (Co-Solvent MeOH) unknown absolute stereochemistry

^{1}H NMR (400MHz, $CDCl_3$): δ 7.77 (s, 1H), 6.63 (d, *J*=3.0 Hz, 1H), 6.36 (d, *J*=1.8 Hz, 1H), 6.06 (s, 1H), 4.83-5.08 (m, 1H), 4.48 (q, *J*=7.2 Hz, 2H), 3.87-4.05 (m, 1H), 3.21-3.35 (m, 1H), 2.92 (d, *J*=9.8 Hz, 1H), 2.40-2.52 (m, 1H), 2.38 (s, 3H), 2.23 (s, 3H), 2.04-2.20 (m, 2H), 1.92 (dd, *J*=7.8, 5.3 Hz, 1H), 1.42 (t, *J*=7.0 Hz, 3H).

Synthesis of *trans*-4-ethoxy-*N*-(1-(3-fluoro-1-(2-methoxyethyl) piperidin-4-yl)-5-methyl-1*H*-pyrazol-4-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (23 and 24)



23
***trans*-Enantiomer 1**
unknown absolute stereochemistry

24
***trans*-Enantiomer 2**
unknown absolute stereochemistry

Step 1: *trans-3-fluoro-1-(2-methoxyethyl)-4-(5-methyl-4-nitro-1H-pyrazol-1-yl)piperidine***.** To a solution of 3-fluoro-4-(5-methyl-4-nitro-1H-pyrazol-1-yl)piperidine (450 mg, 1.97 mmol) (according to synthesis of **21** and **22**), 1-bromo-2-methoxyethane (820 mg, 6.00 mmol) in DMF (15 mL) was added TEA (800 mg, 8.00 mmol). After stirring at 60 ^oC overnight, the mixture was poured into water (200 mL) and extracted with EtOAc (150 mL×2). The extracts were dried over Na_2SO_4 and concentrated. The crude was purified by column on C18 (30~60% CH_3CN in H_2O) to give the title compound (500 mg, 83 %) as yellow oil. LC-MS: 287 $[M+H]^+$. ^1H NMR (300MHz, $CDCl_3$): δ 8.13 (s, 1H), 4.82 - 5.07 (m, 1H), 4.01 - 4.16 (m, 1H), 3.52 (t, *J* = 5.4 Hz, 2H), 3.41 - 3.48 (m, 1H), 3.67 (s, 3H), 3.04 - 3.11 (m, 1H), 2.68 - 2.73 (m, 2H), 2.67 (s, 3H), 2.42 - 2.56 (m, 1H), 2.19 - 2.30 (m, 2H), 1.86 - 1.96 (m, 1H).

Step 2: *trans-1-(3-fluoro-1-(2-methoxyethyl)piperidin-4-yl)-5-methyl-1H-pyrazol-4-amine***.** To a solution of *trans*-3-fluoro-1-(2-methoxyethyl)-4-(5-methyl-4-nitro-1*H*-pyrazol-1-yl)piperidine (500 mg, 1.75 mmol) in MeOH (25 mL) was added Pd/C (10 %, 100 mg). The reaction was stirred at 40 ^oC for 30 min. The mixture was filtered. The filtrate was concentrated to give the title compound (430 mg, 90%) as colorless oil. LC-MS: 257 $[M+H]^+$. ^1H NMR (300 MHz, CDCl3): δ 7.21 (s, 1H), 4.82 - 5.06 (m, 1H), 3.87 - 3.98 (m, 1H), 3.52 (t, *J* = 5.4 Hz, 2H), 3.35 - 3.42 (m, 4H), 3.00 - 3.04 (m, 1H), 2.61 - 2.71 (m, 2H), 2.33 - 2.46 (m, 1H), 2.19 - 2.27 (m, 2H), 2.17 (s, 3H), 1.84 - 1.93 (m, 1H).

Step 3: *trans-4-ethoxy-N-(1-(3-fluoro-1-(2-methoxyethyl) piperidin-4-yl)-5-methyl-1H-pyrazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidin-2-amine.* To a solution of 1-(3-fluoro-1-(2-methoxyethyl)piperidin-4-yl)-5-methyl-1*H*-pyrazol-4-amine (430 mg, 1.68 mmol), **52** (365 mg, 1.85 mmol), xphos (146 mg, 0.310 mmol), K_2CO_3 (927 mg, 6.72 mmol) in dioxane (15 mL) was added $Pd_2(dba)_3$ (154 mg, 0.168 mmol) under N_2 atmosphere. Then the reaction was stirred overnight at reflux. The mixture was filtered and the filtrate was concentrated. The crude was purified by column C18 (30~55% CH_3CN in H_2O) to afford desired racemate (350 mg) as yellow solid which was separated by SFC (Instrument Method: 70-30-CO_2-MeOH, Co-solvent MeOH (0.2dea); column ID, Sample Well 11B,Col.Temp 39.8, CO_2 Flow Rate 2.1 , Co-solvent Flow Rate 0.899 Back pressure 100) to give the title compound **23** (53 mg, t_R= 6.819 min) as a yellow solid and **24** (62 mg, t_R= 8.406 min) as a white solid.

23: LCMS: 418 $[M+H]^+$. t_R= 1.90 min. (LCMS condition 2)

Chiral HPLC: t_R =6.82 mins. (Condition: column ID, Col.Temp 39.8, CO_2 Flow Rate 2.1) unknown absolute stereochemistry

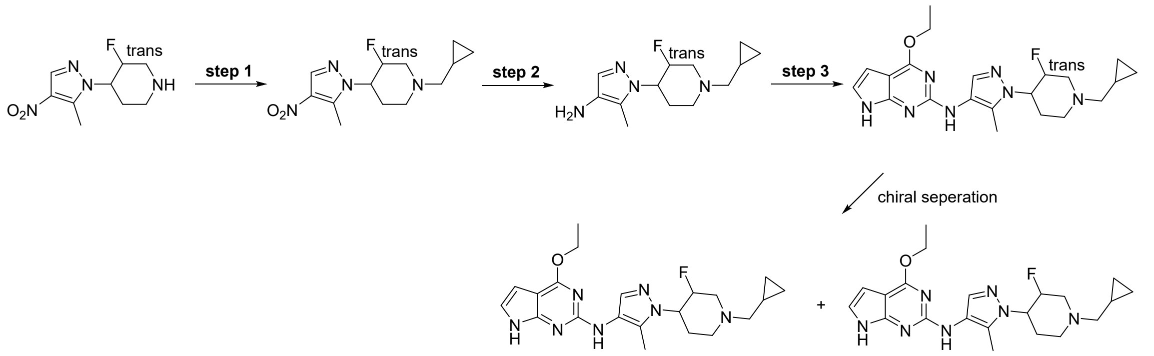
^1H NMR (400 MHz, DMSO-d_6): δ 11.22 (br. s., 1H), 8.03 (s, 1H), 7.66 (s, 1H), 6.86 (dd, *J*= 2.0, 3.2 Hz, 1H), 6.21 (dd, *J*= 2.0, 3.6 Hz, 1H), 4.70-5.02 (m, 1H), 4.44 (q, *J*= 7.2 Hz, 2H), 4.22 (dd, *J*= 4.0, 9.2 Hz, 1H), 3.47 (t, *J*= 5.6 Hz, 2H), 3.26 (s, 3H), 2.92 (d, *J*= 9.2 Hz, 1H), 2.62 (t, *J*= 4.8 Hz, 2H), 2.21-2.34 (m, 2H), 2.18 (s, 3H), 2.01-2.14 (m, 1H), 1.79-1.93 (m, 1H), 1.36 (t, *J*= 7.2 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 162.8, 157.8, 155.2, 135.2, 131.7, 120.4, 120.2, 98.5, 98.0, 90.5, 70.5, 61.4, 59.3, 58.5, 56.8, 56.7, 52.0, 30.3, 15.1, 9.1. HRMS (ESI): m/z calcd for $C_{20}H_{28}FN_7O_2$ $[M+H]^+$ 418.2367, found $[M+H]^+$ 418.2361.

24: LCMS: 418 $[M+H]^+$. t_R= 1.90 min. (LCMS condition 2)

Chiral HPLC: t_R= 8.41 min. (Condition: column ID, Col.Temp 39.8, CO_2 Flow Rate 2.1) unknown absolute stereochemistry

^{1}H NMR (400 MHz, DMSO-d_6): δ 11.22 (br. s., 1H), 8.03 (s, 1H), 7.66 (s, 1H), 6.86 (dd, *J*=2.4, 3.2 Hz, 1H), 6.21 (dd, *J*= 2.0, 3.2 Hz, 1H), 4.66-5.00 (m, 1H), 4.44 (q, *J*= 7.2 Hz, 2H), 4.08-4.31 (m, 1H), 3.48 (t, *J*= 5.6 Hz, 2H), 3.26 (s, 3H), 2.93 (d, *J*= 9.2 Hz, 1H), 2.63 (br. s., 2H), 2.21-2.34 (m, 2H), 2.00-2.14 (m, 1H), 1.78-1.94 (m, 1H), 1.36 (t, *J*=7.2 Hz, 3H). DMSO-d_6): δ 162.8, 157.8, 155.2, 120.4, 120.2, 98.5, 98.0, 70.5, 61.4, 59.2, 58.5, 56.7, 52.0, 30.2, 15.1, 9.1. HRMS (ESI): m/z calcd for $C_{20}H_{28}FN_7O_2$ [M+H]$^+$ 418.2367, found [M+H]$^+$ 418.2360.

2.16. (*trans*)-*N*-(1-(1-(cyclopropylmethyl)-3-fluoropiperidin-4-yl)-5-methyl-1H-pyrazol-4-yl)-4-ethoxy-7H-pyrrolo[2,3-d]pyrimidin-2-amine (25 and 26)



25
***trans*-Enantiomer 1**
unknown absolute stereochemistry

26
***trans*-Enantiomer 2**
unknown absolute stereochemistry

Step 1: *(±)-(trans)-1-(cyclopropylmethyl)-3-fluoro-4-(5-methyl-4-nitro-1H-pyrazol-1-yl)piperidine.* To a solution of *(±)-(trans)-3-fluoro-4-(5-methyl-4-nitro-1H-pyrazol-1-yl)piperidine* (300 mg, 1.32 mmol), cyclopropanecarbaldehyde (230 mg, 3.30 mmol) in 1,2-dichloroethane (15 mL) was added NaBH(OAc)$_3$ (840 mg, 3.96 mmol) at room temperature. Then the reaction mixture was stirred at room temperature for 2 hrs. The reaction mixture was poured into 100 mL of saturated Na_2CO_3 aqueous and extracted with DCM (100 mL × 2). The combined extracts were dried over Na_2SO_4 and concentrated. The residue was purified by column C18 (ACN/H_2O = 40-60%) to afford title product (310 mg, 83%) as white solid. ^{1}H NMR (300MHz, $CDCl_3$): δ 8.14 (s ,1H), 4.83-5.08 (m, 1H), 4.03-4.15 (m, 1H), 3.51-3.57 (m, 1H), 3.14-3.17 (m, 1H), 2.67 (s, 3H), 2.04-2.50 (m, 5H), 1.91-1.95 (m, 1H), 0.83-0.90 (m, 1H), 0.54-0.57 (m, 2H), 0.12-0.14 (m, 2H). LC-MS: 283 [M+H]$^+$.

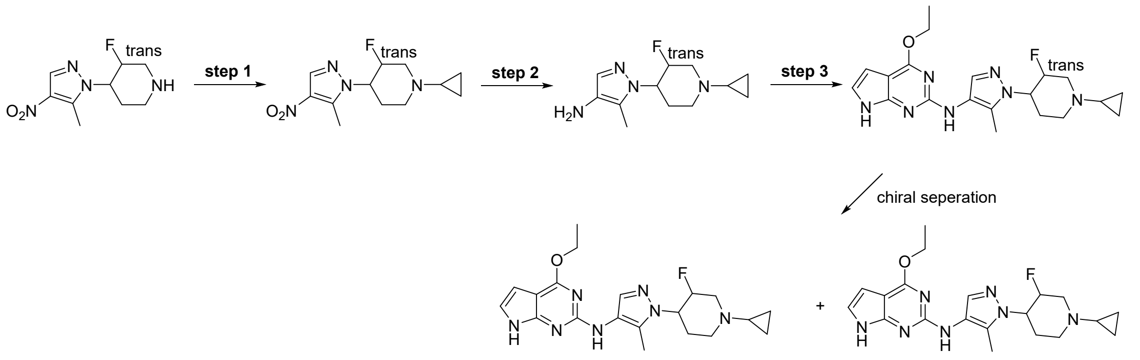
Step 2: *(±)-(trans)-1-(1-(cyclopropylmethyl)-3-fluoropiperidin-4-yl)-5-methyl-1H-pyrazol-4-amine.* To a solution of *(±)-(trans)-1-(cyclopropylmethyl)-3-fluoro-4-(5-methyl-4-nitro-1H-pyrazol-1-yl)piperidine* (310 mg, 1.09 mmol) in 15 mL of MeOH was added Pd/C (10%, 100 mg) and then the mixture was stirred under H_2 atmosphere for 2 hrs. The reaction mixture was filtered and the filtrate was concentrated to afford the title product (230 mg, 84%) as colorless oil. ^{1}H NMR (300MHz, $CDCl_3$): δ 7.22 (s ,1H), 4.81-5.06 (m, 1H), 3.86-3.99 (m, 1H), 3.45-3.53 (m, 1H), 3.08-3.11 (m, 1H), 2.37(br s, 2H), 2.27-2.43 (m, 3H), 2.10-2.27 (m, 5H), 1.80-1.94 (m, 1H) 0.83-0.92 (m, 1H), 0.52-0.54 (m, 2H) ,0.11-0.12 (m, 2H). LC-MS: 253 [M+H]$^+$.

Step 2: *(trans)-N-(1-(1-(cyclopropylmethyl)-3-fluoropiperidin-4-yl)-5-methyl-1H-pyrazol-4-yl)-4-ethoxy-7H-pyrrolo[2,3-d]pyrimidin-2-amine.* To a solution of *(±)-(trans)-1-(1-(cyclopropylmethyl)-3-fluoropiperidin-4-yl)-5-methyl-1H-pyrazol-4-amine* (230 mg, 0.913 mmol), 2-chloro-4-ethoxy-7H-pyrrolo[2,3-d]pyrimidine (198 mg, 1.00 mmol), Xphos (79 mg, 0.14 mmol) and K_2CO_3 (504 mg, 3.65 mmol) in 20 ml of dioxane was added $Pd_2(dba)_3$ (83 mg , 0.090 mmol) under N_2 atmosphere. Then the reaction mixture was stirred at 120 ^oC for overnight. The reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated and the residue was purified by column C18 (CAN/H_2O = 30-50%) to afford racemate (150 mg) as yellow solid, which was separated by SFC (Instrument Method: 70-30-CO_2/MeOH, Co-solvent: MeOH (0.2% DEA); column ID: Sample Well 11B; Col.Temp: 39.8; CO_2 Flow Rate: 2.1, Co-solvent Flow Rate: 0.899, Back pressure: 100) to give **25** (13.5 mg, Rt= 4.55 min) as white solid and **26** (15.0 mg, Rt = 5.43 min) as white solids. LC-MS: Rt = 3.23 min; 414 $[M+H]^+$.

25: (Rt= 4.55 min) 95.35% ee. ^1H NMR (400 MHz, DMSO-*d₆*): *δ* 11.22 (br. s., 1H), 8.03 (s, 1H), 7.66 (s, 1H), 6.86 (dd, *J*= 2.0, 3.2 Hz, 1H), 6.21 (dd, *J*=2.0, 3.2 Hz, 1H), 4.73-5.01 (m, 1H), 4.44 (d, *J*= 7.2 Hz, 2H), 4.09-4.28 (m, 1H), 3.01 (d, *J*= 10.0 Hz, 1H), 2.27-2.38 (m, 2H), 2.04-2.26 (m, 6H), 1.81-1.95 (m, 1H), 1.36 (t, *J*= 7.2 Hz, 3H), 0.73-0.98 (m, 1H), 0.36-0.58 (m, 2H), 0.11 (d, *J*= 4.0 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-*d₆*): *δ* 162.8, 157.8, 155.2, 135.1, 120.4, 120.2, 98.5, 98.0, 91.4, 89.7, 62.4, 61.4, 59.5, 56.2, 51.5, 30.2, 15.1, 9.1, 8.6, 4.2, 4.1. HRMS (ESI): m/z calcd for $C_{21}H_{28}FN_7O$ $[M+H]^+$ 414.2417, found $[M+H]^+$ 414.2424.

26: (Rt = 5.43 min) 100% ee. ^1H NMR (400 MHz, DMSO-*d₆*): *δ* 11.22 (br. s., 1H), 8.03 (s, 1H), 7.66 (s, 1H), 6.86 (dd, *J*= 2.0, 3.2 Hz, 1H), 6.21 (dd, *J*= 2.0, 3.2 Hz, 1H), 4.72-5.02 (m, 1H), 4.44 (q, *J*= 7.2 Hz, 2H), 4.05-4.32 (m, 1H), 3.01 (d, *J*=10.0 Hz, 1H), 2.28-2.40 (m, 2H), 2.02-2.26 (m, 6H), 1.80-1.97 (m, 1H), 1.36 (t, *J*=7.2 Hz, 3H), 1.08-1.28 (m, 1H), 0.78-0.94 (m, 1H), 0.42-0.57 (m, 2H), 0.11 (d, *J*= 4.0 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-*d₆*): *δ* 162.8, 157.8, 155.2, 120.4, 120.1, 98.5, 98.0, 91.4, 89.7, 62.4, 61.4, 59.5, 56.2, 51.5, 30.2, 15.1, 9.1, 8.6, 4.2, 4.1. HRMS (ESI): m/z calcd for $C_{21}H_{28}FN_7O$ $[M+H]^+$ 414.2417, found $[M+H]^+$ 414.2411.

Synthesis of (*trans*)-N-(1-(1-cyclopropyl-3-fluoropiperidin-4-yl)-5-methyl-1H-pyrazol-4-yl)-4-ethoxy-7H-pyrrolo[2,3-d]pyrimidin-2-amine (27 and 28)



27
***trans*-Enantiomer 1**
unknown absolute stereochemistry

28
***trans*-Enantiomer 2**
unknown absolute stereochemistry

Step 1: *(±)-(trans)-1-cyclopropyl-3-fluoro-4-(5-methyl-4-nitro-1H-pyrazol-1-yl)piperidine.* To a solution of (±)-(*trans*)-3-fluoro-4-(5-methyl-4-nitro-1*H*-pyrazol-1-yl)piperidine hydrochloride (230 mg, 0.903 mmol) and (1-ethoxycyclopropoxy)trimethylsilane (250 mg, 1.43 mmol) in MeOH (5 mL) and acetic acid (1 mL) was added $NaBH_3CN$ (90 mg, 1.43 mmol) slowly. The reaction was stirred at 65^{o}C overnight. MeOH was removed and the residue was portioned between sat. $NaHCO_3$ solution (10mL) and EtOAc (10 mL). The $NaHCO_3$ solution was extracted with EtOAc (10 mL×2). The combined EtOAc layers were dried over Na_2SO_4 and concentrated. The residue was purified by Prep-TLC (PE/EtOAc= 3: 1) to give the title product (130 mg, yield 57%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 8.11 (s, 1 H), 4.95~4.70 (m, 1 H), 4.15~4.03 (m, 1 H), 3.51~3.44 (m, 1 H), 3.13~3.09 (m, 1 H), 2.66 (s, 3 H), 2.44~2.31 (m, 3 H), 1.94~1.87 (m, 1H), 1.81-1.74 (m, 2 H), 0.53~0.0.39 (m, 4 H).

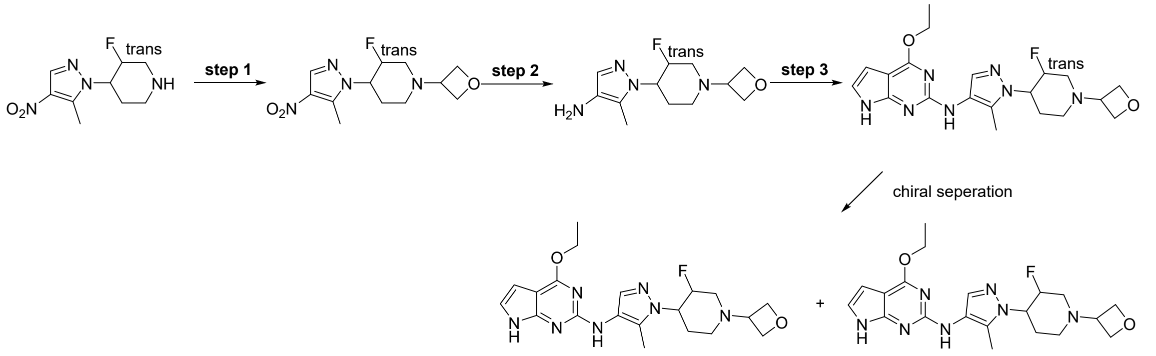
Step 2: *(±)-(trans)-1-(1-cyclopropyl-3-fluoropiperidin-4-yl)-5-methyl-1H-pyrazol-4-amine***.** To a solution of (±)-(*trans*)-1-cyclopropyl-3-fluoro-4-(5-methyl-4-nitro-1*H*-pyrazol-1-yl)piperidine (120 mg, 0.447 mmol) in MeOH (10 mL) was added Pd/C (30 mg, 10%) at room temperature. The reaction was stirred at room temperature under H_2 atmosphere for 3 hours. The Pd/C was filtered and washed with MeOH (5 mL). The filtrate was concentrated to give the title product (80 mg, yield 75%) as yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* (s, 1 H), 4.94~4.69 (m, 1 H), 3.99~3.86 (m, 1 H), 3.43~3.40 (m, 1 H), 3.07~3.04 (m, 1 H), 2.66 (br s, 2H), 2.40~2.23 (m, 3 H), 2.15 (s, 3 H), 1.87~1.71 (m, 2 H), 0.49~0.37 (m, 4 H).

Step 3: *(trans)-N-(1-(1-cyclopropyl-3-fluoropiperidin-4-yl)-5-methyl-1H-pyrazol-4-yl)-4-ethoxy-7H-pyrrolo[2,3-d]pyrimidin-2-amine.* To a solution of ((±)-(*trans*)-1-(1-cyclopropyl-3-fluoropiperidin-4-yl)-5-methyl-1*H*-pyrazol-4-amine (80 mg, 0.33 mmol) in dioxane (15 mL) was added 2-chloro-4-ethoxy-7H-pyrrolo[2,3-d]pyrimidine (132 mg, 0.672 mmol), $Pd_2(dba)_3$ (30 mg, 0.033 mmol), Xphos (32 mg, 0.067 mmol), and K_2CO_3 (138 mg,1.00 mmol) at room temperature under N_2 atmosphere. The mixture was stirred at 100 ^{o}C overnight. The mixture was filtered and the filtrate was concentrated. The residue was purified by column (PE/EtOAc = 1/1~1/2) to give racemate as a white solid (70 mg, yield 52%), which was separated by SFC (Chiral condition: chiralpak IE, 60-40-CO_2-MeOH, Flow: 1.799, T = 43 ^{o}C) to give **27** (11.1 mg, Rt = 4.4 min, 100% ee) and **28** (13.0 mg, Rt = 5.6 min, 98.12% ee). LC-MS: Rt= 4.24 min; 400 $[M+H]^+$.

27: ^{1}H NMR (400 MHz, DMSO-*d*$_6$): *δ* 11.22 (br. s., 1H), 8.03 (br. s., 1H), 7.65 (s, 1H), 6.86 (br. s., 1H), 6.21 (br. s., 1H), 4.61-4.96 (m, 1H), 4.44 (q, *J*= 6.8 Hz, 2H), 4.13-4.32 (m, 1H), 2.97 (d, *J*=10.0 Hz, 1H), 2.33-2.47 (m, 2H), 2.19 (s, 3H), 1.94-2.10 (m, 1H), 1.73-1.92 (m, 2H), 1.35 (t, *J*= 6.8 Hz, 3H), 0.18-0.58 (m, 4H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$): *δ* 162.8, 157.8, 155.2, 135.2, 120.4, 120.2, 98.5, 98.0, 91.1, 89.4, 61.4, 59.5, 59.3, 56.6, 56.3, 51.7, 38.0, 30.2, 15.1, 9.1, 6.9, 6.6. ^{19}F NMR (376MHz, CD_3OD): -189.5. HRMS (ESI): m/z calcd for $C_{20}H_{26}FN_7O$ $[M+H]^+$ 400.2261, found $[M+H]^+$ 400.2446.

28: ^{1}H NMR (400 MHz, DMSO-*d*$_6$): *δ* 10.95-11.42 (m, 1H), 8.03 (br., 1H), 7.65 (s, 1H), 6.86 (br., 1H), 6.21 (br., 1H), 4.63-4.96 (m, 1H), 4.44 (q, *J*= 6.8 Hz, 2H), 4.25 (d, *J*= 5.6 Hz, 1H), 2.97 (d, *J*= 10.4 Hz, 1H), 2.31-2.45 (m, 2H), 2.19 (s, 3H), 1.93-2.11 (m, 1H), 1.73-1.90 (m, 2H), 1.35 (t, *J*= 6.8 Hz, 3H), 0.16-0.60 (m, 4H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$): *δ* 162.8, 157.8, 155.2, 135.2, 131.4, 120.4, 120.2, 98.5, 98.0, 91.1, 89.4, 61.4, 59.5, 56.5, 51.7, 38.0, 29.9, 15.1, 9.1, 6.9, 6.6. HRMS (ESI): m/z calcd for $C_{20}H_{26}FN_7O$ $[M+H]^+$ 400.2261, found $[M+H]^+$ 400.2458.

Synthesis of 4-ethoxy-*N*-(1-((3*S*,4*S*)-3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-5-methyl-1*H*-pyrazol-4-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (29 and 30)



29
trans-Enantiomer 1

30
trans-Enantiomer 2

Step 1: *(±)-(trans)-3-fluoro-4-(5-methyl-4-nitro-1H-pyrazol-1-yl)-1-(oxetan-3-yl)piperidine.* To a solution of *tert*-butyl 3-fluoro-4-(5-methyl-4-nitro-1*H*-pyrazol-1-yl)piperidine-1-carboxylate (1.00 g, 4.38 mmol), oxetan-3-one (785 mg, 10.9 mmol) in DCE (40 mL) was added portions $NaBH(OAc)_3$ (2.78 g, 13.1 mmol) at room temperature. The reaction was stirred overnight at room temperature. Aq. Na_2CO_3 solution (30 mL) was added and the mixture was then extracted with DCM (50 mL*2). The combined organic layers were dried and concentrated. The crude was purified by column chromatography on silica gel (PE: EA=1:1) to give the title compound (1.00 g, 80% yield) as a yellow solid. LCMS: 285 [M+H ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.14 (s, 1H), 5.07-4.83 (m, 1H), 4.71-4.58 (m, 4H), 4.17-4.04 (m, 1H), 3.70-3.61 (m, 1H), 3.26-3.18 (m, 1H), 2.90-2.84 (m, 1H), 2.67 (s, 3H), 2.52-2.44 (m, 1H), 2.14-1.93 (m, 3H).

Step 2: *(±)-(trans)-1-(3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-5-methyl-1H-pyrazol-4-amine.* A solution of (±)-(*trans*)-3-fluoro-4-(5-methyl-4-nitro-1H-pyrazol-1-yl)-1-(oxetan-3-yl)piperidine (500 mg, 1.76 mmol) and Pd/C (160 mg, 10%) in MeOH (20 mL) was stirred at 30 °C under H_2 for 2 hrs. The mixture was filtered and the filtrate was concentrated. The crude was purified by column (DCM: MeOH=15: 1) to give the title compound (420 mg, 94 % yield) as a white solid. LCMS: 255 $[M+H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.23 (s, 1H), 5.07-4.83 (m, 1H), 4.69-4.61 (m, 4H), 4.01-3.89 (m, 1H), 3.67-3.60 (m, 1H), 3.49 (s, 1H), 3.21-3.13 (m, 1H), 2.85-2.79 (m, 1H), 2.48-2.34 (m, 1H), 2.18 (s, 3H), 2.07-1.90 (m, 3H).

Step 3: *4-ethoxy-N-(1-((3S,4S)-3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-5-methyl-1H-pyrazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidin-2-amine.* A solution of 2-chloro-4-ethoxy-7H-pyrrolo[2,3-d]pyrimidine (390 mg, 1.98 mmol), (±)-(*trans*)-1-(3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-5-methyl-1*H*-pyrazol-4-amine (420 mg, 1.65 mmol), X-phos (157 mg, 0.33 mmol), $Pd_2(dba)_3$ (144 mg, 0.16 mmol) and K_2CO_3 (683 mg, 4.95 mmol) in dioxane (20 mL) was stirred overnight at 100 °C under N_2. The mixture was filtered and the filtrate was concentrated. The crude was purified by column chromatography on silica gel (DCM: MeOH= 40:1) to give the racemate (320 mg, 49 % yield) as a light yellow solid, which was further separated by chiral-HPLC and purified by prep-HPLC [Waters xbridge^{TM} C18, 5 um, 19*150 mm;Flowing phase: H_2O (0.1%NH_4HCO_3)/MeCN: MeCN form 10% to 95%, 15 ml/min,T= 6 min] to give the title compounds **29** and **30**.

29: LCMS: 416 $[M+H]^+$. t_R =3.50 mins. (LCMS condition 3)

Chiral HPLC: t_R =6.688 mins. (Chiralpak OD-H 5μm 4.6*250mm, Hex:EtOH:DEA = 70:30:0.2, Flow: 1.0ml/min, 230 nm, T = 30^{o}C)

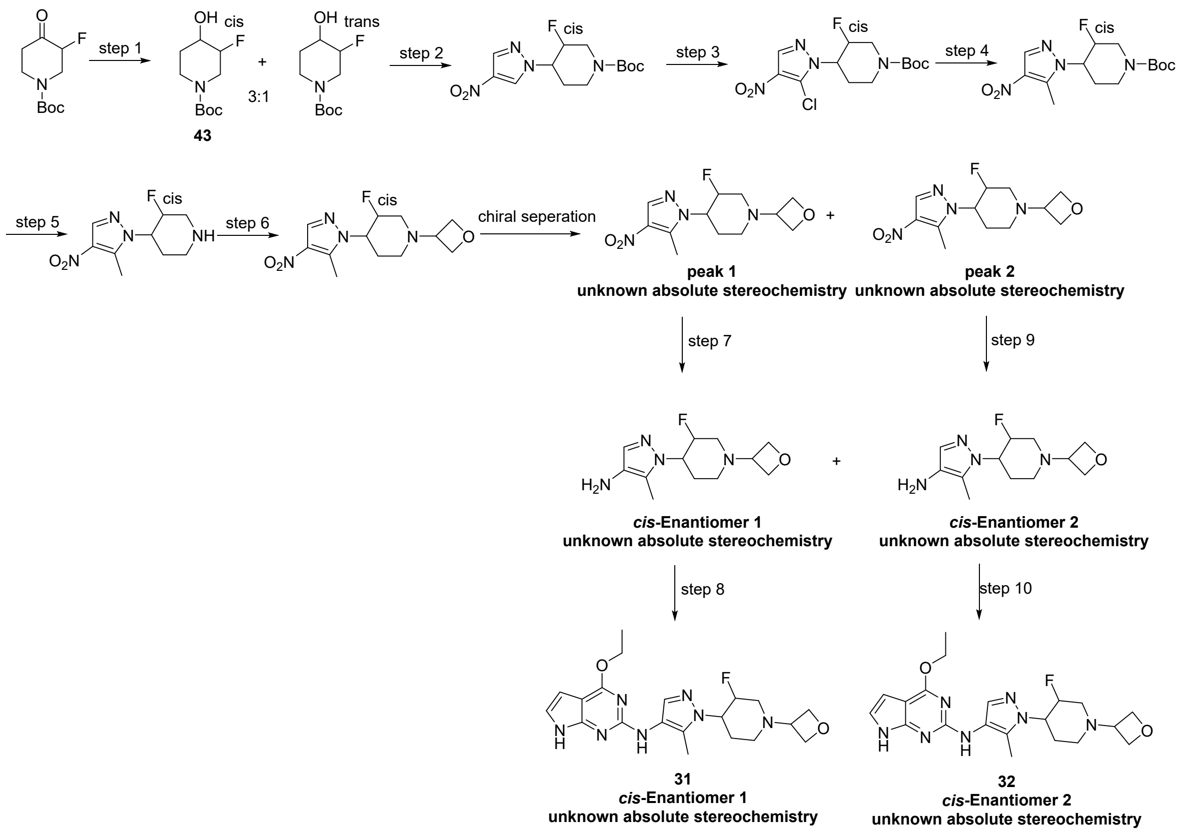
^{1}H NMR (300 MHz, DMSO-d_6): δ 11.20 (s, 1H), 8.03 (s, 1H), 7.67 (s, 1H), 6.85 (s, 1H), 6.20 (s, 1H), 5.03-4.77 (m, 1H), 4.58-4.55 (m, 2H), 4.50-4.40 (m, 4H), 4.30-4.24 (m, 1H), 3.59-3.55 (m, 1H), 3.20-3.15 (m, 1H), 2.78-2.75 (m, 1H), 2.18 (s, 3H), 2.12-2.04 (m, 3H), 1.96-1.87 (m, 1H), 1.35 (t, *J* = 6.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 162.7, 157.6, 155.1, 120.5, 120.2, 98.5, 98.0, 91.1, 89.4, 74.9, 61.4, 59.2, 58.4, 48.1, 29.9, 15.1, 9.1. ^{19}F NMR (376 MHz, DMSO-d_6): δ -186.1. HRMS (ESI): m/z calcd for $C_{20}H_{26}FN_7O_2$ $[M+H]^+$ 416.2210, found $[M+H]^+$ 416.2217.

30: LCMS: 416 $[M+H]^+$. t_R =2.98 mins. (LCMS condition 3)

Chiral HPLC: t_R =5.96 mins. (Chiralpak OD-H 5μm 4.6*250mm, Hex:EtOH:DEA = 70:30:0.2, Flow: 1.0ml/min, 230 nm, T = 30 ^{o}C)

^{1}H NMR (300 MHz, DMSO-d_6): δ 11.20 (s, 1H), 8.03 (s, 1H), 7.67 (s, 1H), 6.85 (s, 1H), 6.20 (s, 1H), 5.03-4.77 (m, 1H), 4.58-4.55 (m, 2H), 4.50-4.40 (m, 4H), 4.30-4.24 (m, 1H), 3.59-3.55 (m, 1H), 3.20-3.15 (m, 1H), 2.78-2.75 (m, 1H), 2.18 (s, 3H), 2.12-2.04 (m, 3H), 1.96-1.87 (m, 1H), 1.35 (t, *J* = 6.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 162.8, 157.8, 155.2, 135.2, 132.0, 120.5, 120.2, 98.5, 98.0, 91.1, 89.4, 75.0, 74.9, 61.4, 59.2, 58.4, 52.7, 48.1, 30.0, 15.1, 9.1. ^{19}F NMR (376 MHz, DMSO-d_6): δ -186.1. HRMS (ESI): m/z calcd for $C_{20}H_{26}FN_7O_2$ $[M+H]^+$ 416.2210, found $[M+H]^+$ 416.2213.

Synthesis of *cis*-4-ethoxy-*N*-(1-(3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-5-methyl-1*H*-pyrazol-4-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (enantiomers 31 and 32)



Step 1: *(±)-trans-tert-butyl 3-fluoro-4-hydroxypiperidine-1-carboxylate.* To a solution of *tert-butyl 3-fluoro-4-oxopiperidine-1-carboxylate* (10 g, 23 mmol) in MeOH (100 mL) was added $NaBH_4$ (2.27 g, 60.0 mmol) under ice cooling. The reaction mixture was stirred at this temperature for 2 hours. Aq. NH_4Cl (100 mL) was added and the solution was extracted with EtOAc (80 mL×3). The organic layer was dried and concentrated. The crude was purified by flash chromatography on C18 to give the title compound (2 g, 20 % yield) as a white solid. LCMS: 220[M+H]$^+$. ^{1}H NMR (300 MHz, $CDCl_3$): 4.16 - 4.39 (m, 2H), 3.74 - 3.90 (m, 2H), 2.89 - 2.98 (m, 2H), 1.93 - 2.01 (m, 1H), 1.45 - 1.54 (m, 1H), 1.45 (s, 9H).
The major cis-product **43** was also isolated as a white solid (6g, 60% yield). ^{1}H NMR (300 MHz, CDCl3): δ 4.50-4.68 (m, 1H), 3.89-3.93 (m, 2H), 3.65-3.71 (m, 1H), 3.35-3.44 (m, 1H), 3.17-3.21 (m, 1H), 2.07-2.09 (m, 1H), 1.76-1.86 (m, 2H), 1.45 (s, 9H). LCMS:220 [M+H] +.
Step 2: *(±)-cis-tert-butyl 3-fluoro-4-(4-nitro-1H-pyrazol-1-yl)piperidine-1-carboxylate.* To a solution *(±)-trans-tert-butyl 3-fluoro-4-hydroxypiperidine-1-carboxylate.* (2.0 g, 9.1 mmol), 4-nitro-1*H*-pyrazole (1.03 g, 9.11 mmol), PPh_3 (3.57 g, 13.6 mmol) in THF (50 mL) was added slowly DIAD (2.75 g, 13.6 mmol) at 0 °C. The mixture was stirred overnight at room temperature. Solvent was evaporated and the residue was dissolved in DCM (30 mL) and *n*-hexane (60 mL) was added. The suspension was stirred vigorously for 1 hour and then filtered. The filtrate was concentrated and the crude was purified by column chromatography on C18 (CH_3CN: H_2O=4:1~1:1) to give the title compound as yellow oil (2.4 g, 86 % yield). LCMS: 314 [M+H]$^+$. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 8.25 (s, 1H), 8.10 (s,

1H), 4.86 - 5.03 (m, 1H), 4.39 - 4.55 (m, 3H), 2.85 - 3.13 (m, 2H), 2.24 - 2.38 (m, 1H), 2.00 - 2.08 (m, 1H), 1.48 (s, 9H).

Step 3: *(±)-cis-tert-butyl 4-(5-chloro-4-nitro-1H-pyrazol-1-yl)-3-fluoropiperidine-1-carboxylate.* To a solution of DIPEA (0.98 g, 9.7 mmol) in THF (15 mL) was added *n*-BuLi (3.9 mL, 9.7 mmol) at -78 °C under N_2 atmosphere. The reaction mixture was stirred at this temperature for 30 min and then at 0 °C for 30 min. To a solution of *(±)-cis-tert-butyl 3-fluoro-4-(4-nitro-1H-pyrazol-1-yl)piperidine-1-carboxylate* (1.7 g, 5.40 mmol) in THF (20 mL) was added above LDA solution at -78 °C. The reaction mixture was stirred at this temperature for 1.5 h and perchloroethane (2.30 g, 9.72 mmol) was added. The reaction mixture was stirred at -78 °C for 30 min and then allowed to warm to room temperature for 1 hour. NH_4Cl aq. (40 mL) was added and the solution was extracted with EtOAc (50 mL×2). The organic layer was dried and concentrated. The crude was purified by chromatography on silica gel (PE: EA = 8:1) to give the title compound (640 mg, 36 % yield) as a white solid. ^1H NMR (300 MHz, $CDCl_3$): δ 8.22 (s, 1H), 4.94-4.74 (m, 1H), 4.68-4.33 (m, 3H), 3.26-2.97 (m, 2H), 2.84-2.71 (m, 1H), 1.96-1.88 (m, 1H), 1.48 (s, 9H).

Step 4: *(±)-cis-tert-butyl 3-fluoro-4-(5-methyl-4-nitro-1H-pyrazol-1-yl)piperidine-1-carboxylate.* A solution of *(±)-cis-tert-butyl 4-(5-chloro-4-nitro-1H-pyrazol-1-yl)-3-fluoropiperidine-1-carboxylate* (640 mg, 1.86 mmol), 2,4,6-trimethyl-cyclotriboroxane (224 mg, 1.77 mmol), Pd(dppf)Cl_2 (310 mg, 0.37 mmol), Na_2CO_3 (297 mg, 2.80 mmol) and KOAc (224 mg, 2.80 mmol) in H_2O (2.8 mL) and acetonitrile (15 mL) was irradiated under microwave at 130 °C for 1.5 hours. The mixture was cooled to room temperature and filtered through a celite pad. The solution was concentrated and the crude was purified by column chromatography on silica gel (PE: EA = 5:1) to give the title compound (710 mg, 60% yield) as a yellow solid. LCMS: 229 $[M+H-100]^+$.^1H NMR (300 MHz, $CDCl_3$): δ 8.11 (s, 1H), 4.84-4.69 (m, 1H), 4.52-4.26 (m, 3H), 3.33-3.10 (m, 2H), 2.77-2.64 (m, 1H), 2.71 (s, 3H), 1.99-1.92 (m, 1H), 1.47 (s, 9H).

Step 5: *(±)-cis-3-fluoro-4-(5-methyl-4-nitro-1H-pyrazol-1-yl)piperidine.* To a solution of *(±)-cis-tert-butyl 3-fluoro-4-(5-methyl-4-nitro-1H-pyrazol-1-yl)piperidine-1-carboxylate* (710 mg, 2.16 mmol) in MeOH (7 mL) was added HCl/dioxane (5.7 M, 10 mL). The reaction was stirred at room temperature for 2 hrs. Solvent was removed, and the residue was dissolved in Na_2CO_3 aqueous solution (20 mL) and extracted with DCM/MeOH (10:1, 20 mL*5). The combined organic layers were dried and concentrated to give the title compound (500 mg, 100% yield) as a yellow solid. LCMS: 229 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$): δ 8.12 (s, 1H), 4.78-4.61 (m, 1H), 4.44-4.32 (m, 1H), 3.47-3.35 (m, 3H), 2.87-2.63 (m, 2H), 2.71 (s, 3H), 1.91-1.85 (m, 1H), 1.64-1.57 (m, 1H).

Step 6: *cis-3-fluoro-4-(5-methyl-4-nitro-1H-pyrazol-1-yl)-1-(oxetan-3-yl)piperidine.* To a solution of *(±)-cis-3-fluoro-4-(5-methyl-4-nitro-1H-pyrazol-1-yl)piperidine* (500 mg, 2.20 mmol), oxetan-3-one (395 mg, 5.48 mmol) in DCE (20 mL) was added portions $NaBH(OAc)_3$ (1.4 g, 6.6 mmol) at room temperature. The reaction mixture was stirred overnight at room temperature. Na_2CO_3 aqueous solution (30 mL) was added and extracted with DCM (530 mL*5). The combined organic layer was dried and concentrated. The crude was purified by column chromatography on C18 (25-60% ACN in water) to afford desired product (470 mg, 75% yield) as a white solid, which was separated by chiral HPLC (chiralpak IA 5 um 4.6*250 mm, Hex/EtOH: 50/50, 1.0 mL/min) to give the title compounds **peak 1** (200 mg, t$_R$=7.939 min) and **peak 2** (180 mg, t$_R$=10.224 min) was prepared as white solids. LCMS: 285 [M+H]$^+$. ^{1}H NMR (300 MHz, CDCl$_3$): δ 8.09 (s, 1H), 4.93-4.76 (m, 1H), 4.70-4.60 (m, 4H), 4.50-4.37 (m, 1H), 3.74-3.68 (m, 1H), 3.09-2.96 (m, 2H), 2.77-2.75 (m, 1H), 2.70 (s, 3H), 2.50-2.27 (m, 2H), 2.09-2.00 (m, 1H).

Step 7: *cis-1-(3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-5-methyl-1H-pyrazol-4-amine (enantiomer 1).* A solution of *cis-3-fluoro-4-(5-methyl-4-nitro-1H-pyrazol-1-yl)-1-(oxetan-3-yl)piperidine* (**peak 1**, 200 mg, 0.70 mmol) and Pd/C (60 mg, 10%) in MeOH (8 mL) was stirred at 30 ^{o}C under hydrogen for 1 hour. The mixture was filtered and the filtrate was concentrated to give the title compound (150 mg, 85 % yield) as a white solid. LCMS: 255 [M+H]$^+$. ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.18 (s, 1H), 4.89-4.61 (m, 5H), 4.32-4.18 (m, 1H), 3.73-3.64 (m, 1H), 3.10-3.02 (m, 2H), 2.78-2.74 (m, 1H), 2.66-2.61 (br. s., 2H), 2.40-2.17 (m, 2H), 2.21 (s, 3H), 2.01-1.93 (m, 1H).

Step 8: *cis-4-ethoxy-N-(1-(3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-5-methyl-1H-pyrazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidin-2-amine Enantiomer 1 (**31**).* A solution of 2-chloro-4-ethoxy-7H-pyrrolo[2,3-d]pyrimidine (128 mg, 0.648 mmol), *cis-1-(3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-5-methyl-1H-pyrazol-4-amine (entiomer 1)* (150 mg, 0.590 mmol), X-phos (60 mg, 0.11 mmol), Pd$_2$(dba)$_3$ (53 mg, 0.058 mmol), K$_2$CO$_3$ (244 mg, 1.77 mmol) in dioxane (10 mL) was stirred at 110 ^{o}C under N$_2$ for 8 hrs. The mixture was filtered and the filtrate was concentrated. The crude was purified by column chromatography on C18 (20-50% ACN in water) and further purified by prep-HPLC(Instrument: Column: Boston C18, 5 um, 21*150 mm; Mobile phase: H$_2$O (0.1%NH$_4$HCO$_3$)/MeCN: MeCN form 20% to 70%, 20 ml/min, T = 15 min, rt= 7.2 min) to give the title compound (30 mg, 100% ee) as a light yellow solid.

LCMS: 416 [M+H]$^+$. t$_R$ =3.29 mins. (LCMS condition 3)

Chiral HPLC: t$_R$ =6.73 mins. (Chiral condition: OD-H; 5µm 4.6*250mm, Hex:EtOH:DEA = 70:30:0.2, Flow: 1.0ml/min, 230 nm, T = 30^{o}C.) unknown absolute stereochemistry

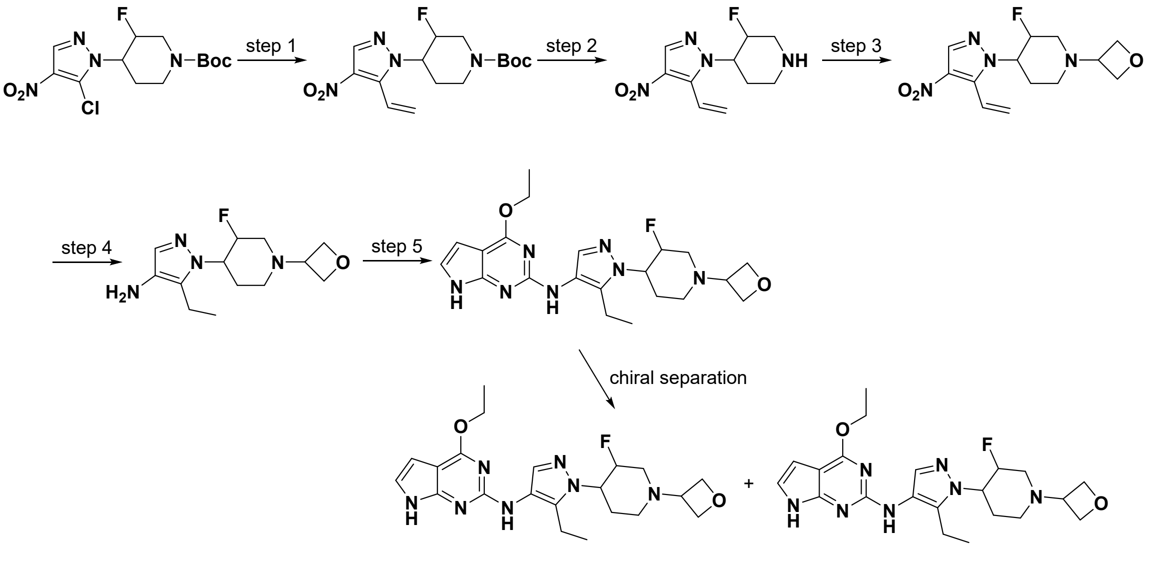
^{1}H NMR (300 MHz, DMSO-d_6): δ 11.20 (s, 1H), 8.03 (s, 1H), 7.59 (s, 1H), 6.85 (m, 1H), 6.20 (m 1H), 4.89-4.77 (m, 1H), 4.57-4.36 (m, 7H), 3.57-3.52 (m, 1H), 2.99-2.89 (m, 2H), 2.67-2.58 (m, 1H), 2.21 (s, 3H), 2.35-2.07 (m, 2H), 1.82-1.78 (m, 1H), 1.35 (t, J = 6.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 162.8, 157.7, 155.2, 134.3, 130.4, 124.8, 121.1, 120.2, 98.5, 98.0, 88.7, 86.9, 74.9, 61.4, 58.5, 57.0, 52.0, 48.1, 25.1, 15.1, 9.3. ^{19}F NMR (376 MHz, DMSO-d_6): δ -198.1. HRMS (ESI): m/z calcd for C$_{20}$H$_{26}$FN$_7$O$_2$ [M+H]$^+$ 416.2210, found [M+H]$^+$ 416.2213.

Step 9: *cis-1-(3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-5-methyl-1H-pyrazol-4-amine (enantiomer 2).* A solution of *cis-3-fluoro-4-(5-methyl-4-nitro-1H-pyrazol-1-yl)-1-(oxetan-3-yl)piperidine* (**peak 2**, 180 mg, 0.63 mmol) and Pd/C (30 mg, 10%) in MeOH (8 mL) was stirred at 30 ^{o}C under H$_2$ balloon for 1 hour. The mixture was filtered and the filtrate was concentrated to give the title compound (140 mg, 87 % yield) as a white solid. LCMS: 255 [M+H]$^+$. ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.18 (s, 1H), 4.89-4.61 (m, 5H), 4.32-4.18 (m, 1H), 3.73-3.64 (m, 1H), 3.10-3.02 (m, 2H), 2.78-2.74 (m, 1H), 2.66-2.61 (br. s., 2H), 2.40-2.17 (m, 2H), 2.21 (s, 3H), 2.01-1.93 (m, 1H).

Step 10: *cis-4-ethoxy-N-(1-(3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-5-methyl-1H-pyrazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidin-2-amine Enantiomer 2 (**32**).* A solution of 2-chloro-4-ethoxy-7H-pyrrolo[2,3-d]pyrimidine (128 mg, 0.648 mmol), *cis-1-(3-fluoro-1-(oxetan-3-*

yl)piperidin-4-yl)-5-methyl-1H-pyrazol-4-amine (enantiomer 2) (150 mg, 0.590 mmol), X-phos (60 mg, 0.11 mmol), $Pd_2(dba)_3$ (53 mg, 0.058 mmol), K_2CO_3 (244 mg, 1.77 mmol) in dioxane (10 mL) was stirred at 110 °C under N_2 for 8 hrs. The mixture was filtered and the filtrate was concentrated. The crude was purified by column chromatography on C18 (20-50% ACN in water) and further purified by prep-HPLC(Instrument: Column: Boston C18, 5 um, 21*150 mm; Mobile phase: H_2O (0.1%NH_4HCO_3)/MeCN: MeCN form 20% to 70%, 20 ml/min, T = 15 min, rt= 7.2 min) to give the title compound (30 mg, 100% ee) as a light yellow solid.
LCMS: 416 $[M+H]^+$. t_R =3.29 mins. (LCMS condition 3)
Chiral HPLC: t_R =6.73 mins. (Chiral condition: OD-H; 5µm 4.6*250mm, Hex:EtOH:DEA = 70:30:0.2, Flow: 1.0ml/min, 230 nm, T = 30°C.) unknown absolute stereochemistry
^1H NMR (300 MHz,DMSO-d_6): δ 11.20 (s, 1H), 8.03 (s, 1H), 7.59 (s, 1H), 6.85 (m, 1H), 6.20 (m 1H), 4.89-4.77 (m, 1H), 4.57-4.36 (m, 7H), 3.57-3.52 (m, 1H), 2.99-2.89 (m, 2H), 2.67-2.58 (m, 1H), 2.21 (s, 3H), 2.35-2.07 (m, 2H), 1.82-1.78 (m, 1H), 1.35 (t, *J* = 6.6 Hz, 3H). ^{19}F NMR (376 MHz, DMSO-d_6): δ -198.1.

Synthesis of (*trans*)-4-ethoxy-*N*-(5-ethyl-1-(3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-1*H*-pyrazol-4-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (33 and 34)



33
***trans*-Enantiomer 1**
unknown absolute stereochemistry

34
***trans*-Enantiomer 2**
unknown absolute stereochemistry

Step 1: *(±)-(trans)-tert-butyl 3-fluoro-4-(4-nitro-5-vinyl-1H-pyrazol-1-yl)piperidine-1-carboxylate.* A solution of *(±)-(trans)-tert-butyl 4-(5-chloro-4-nitro-1H-pyrazol-1-yl)-3-fluoropiperidine-1-carboxylate* (700 mg, 2.01 mmol), 4,4,5,5-tetramethyl-2-vinyl-1,3,2-dioxaborolane (775 mg, 5.03 mmol), Na_2CO_3 (640 mg, 6.03 mmol) and $PdCl_2(dppf)$ (180 mg, 0.22 mmol) in dioxane (10 mL) and water (2 mL) was stirred at 120 °C under nitrogen for 2 days. The mixture was cooled to room temperature and filtered. The filtrate was concentrated. The residue was poured into water (50 mL) and extracted with EtOAc (50 mL×3). The extracts were dried over Na_2SO_4 and concentrated. The crude was purified by column chromatography on silica gel (PE: EA = 5:1) to give the title compound (400 mg, 58%) as red oil. LCMS: 241 [M+H-100]$^+$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.16 (s, 1H), 6.85 - 6.95 (m, 1H), 5.80 - 5.97 (m, 2H), 4.82 - 5.06 (m, 1H), 4.42 - 4.65 (m, 2H), 4.19 - 4.26 (m, 1H), 2.72 - 2.93 (m, 2H), 2.23 - 2.37 (m, 1H), 1.90 - 1.95 (m, 1H), 1.48 (s, 9H).

Step 2: *(±)-(trans)-3-fluoro-4-(4-nitro-5-vinyl-1H-pyrazol-1-yl)piperidine.* To a solution of *(±)-(trans)-tert-butyl 3-fluoro-4-(4-nitro-5-vinyl-1H-pyrazol-1-yl)piperidine-1-carboxylate* (400 mg, 1.18 mmol) in MeOH (5 mL) added HCl/dioxane (4 M, 5mL). The reaction was stirred overnight at room temperature. The mixture was concentrated and the residue was poured into water (40 mL) and extracted with EtOAc (40 mL×2). The aqueous layer was basified with NaOH (aq., 2N, 10 mL) to pH=9 and extracted with EtOAc (40 mL×3). The extracts were dried over Na_2SO_4 and concentrated to give the title compound (200 mg, yield 70%) as brown oil. LCMS: 241 [M+H]$^+$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.17 (s, 1H), 6.85 - 6.95 (m, 1H), 5.81 - 5.96 (m, 2H), 4.81 - 5.07 (m, 1H), 4.38 - 4.52 (m, 1H), 3.51 - 3.56 (m, 1H), 3.15 - 3.19 (m, 1H), 2.62 - 2.76 (m, 2H), 2.04 - 2.30 (m, 1H), 1.93 - 1.98 (m, 1H).

Step 3: *(±)-(trans)-3-fluoro-4-(4-nitro-5-vinyl-1H-pyrazol-1-yl)-1-(oxetan-3-yl)piperidine.* To a solution of *(±)-(trans)-3-fluoro-4-(4-nitro-5-vinyl-1H-pyrazol-1-yl)piperidine* (200 mg, 0.83 mmol) and oxetan-3-one (150 mg, 2.08 mmol) in 1,2-dichloroethane (10 mL) was added $NaBH(OAc)_3$ at room temperature as portions. Then the reaction was stirred at room temperature for 2 hour. The mixture was poured into satur. Na_2CO_3 aqueous solution (40 mL) and extracted with DCM (40 mL×3). The extracts were dried over Na_2SO_4 and concentrated. The crude was purified by column C18 (30 ~ 60% CH_3CN in water) to give the title compound (150 mg, 61% yield) as colorless oil. LCMS: 297 [M+H]$^+$. ^{1}H NMR (300 MHz, CDCl3): δ 8.18 (s, 1H), 6.84 - 6.94 (m, 1H), 5.79 - 5.95 (m, 2H), 4.98 - 5.22 (m, 1H), 4.60 - 4.69 (m, 5H), 4.31 - 4.44 (m, 1H), 3.61 - 3.69 (m, 1H), 3.21 - 3.27 (m, 1H), 2.83 - 2.87 (m, 1H), 2.36 - 2.50 (m, 1H), 1.90 - 2.12 (m, 2H).

Step 4: *(±)-(trans)-5-ethyl-1-(3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-1H-pyrazol-4-amine.* A solution of *(±)-(trans)-3-fluoro-4-(4-nitro-5-vinyl-1H-pyrazol-1-yl)-1-(oxetan-3-yl)piperidine* (150 mg, 0.51 mmol) and Pd/C (10%, 50 mg) in MeOH (5 mL) was stirred under H_2 at room temperature for 2 hours. The reaction mixture was filtered and the filtrate was concentrated to give the title compound (125 mg, 91% yield) as colorless oil. LCMS: 269 [M+H]$^+$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.23 (s, 1H), 4.88 - 5.13 (m, 1H), 4.61 - 4.67 (m, 4H), 3.88 - 3.98 (m, 1H), 3.60 - 3.69 (m, 1H), 3.15 - 3.21 (m, 1H), 2.79 - 2.84 (m, 1H), 2.61 - 2.64 (m, 2H), 2.32 - 2.46 (m, 1H), 1.98 - 2.11 (m, 2H), 1.89 - 1.95 (m, 1H), 1.16 (t, *J* = 7.6 Hz, 3H).

Step 5: *(trans)-4-ethoxy-N-(5-ethyl-1-(3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-1H-pyrazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidin-2-amine.* To a solution of *(±)-(trans)-5-ethyl-1-(3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-1H-pyrazol-4-amine* (125 mg, 0.47 mmol), 2-chloro-4-ethoxy-7H-pyrrolo[2,3-d]pyrimidine (101 mg, 0.51 mmol), K_2CO_3 (259 mg, 1.88 mmol) and X-phos (41 mg, 0.071 mmol) in dioxane (15 mL) was added $Pd_2(dba)_3$ (42 mg, 0.047 mmol) at room temperature under N_2 atmosphere. The reaction was stirred at 120^{o}C for 16 hours. The mixture was filtered and the filtrate was concentrated. The crude was purified by column chromatography on silica gel (DCM: MeOH= 50: 1) to afford the desired racemate (80 mg, 75%), which was separated by chiral HPLC (OJ-H 5µm 4.6*250mm phase: Hex/EtOH = 70/30, F: 1ml/min w: 230nm T: 30) and column on C18 (35 ~ 55%

CH_3CN in water) to give the title compounds **33** (14 mg, t_R = 8.735 min, 100% ee) and **34** (10 mg, t_R = 11.262 min, 97.5% ee) as white solids.

33: LCMS: 430 $[M+H]^+$. t_R =3.298 mins. (LCMS condition 3)

Chiral HPLC: t_R =8.735 mins. (Chiralcel OJ-H 5µm 4.6×250 mm, phase: Hex: EtOH = 70: 30, F: 1 mL/min, w: 230 nm T: 30 °C) unknown absolute stereochemistry

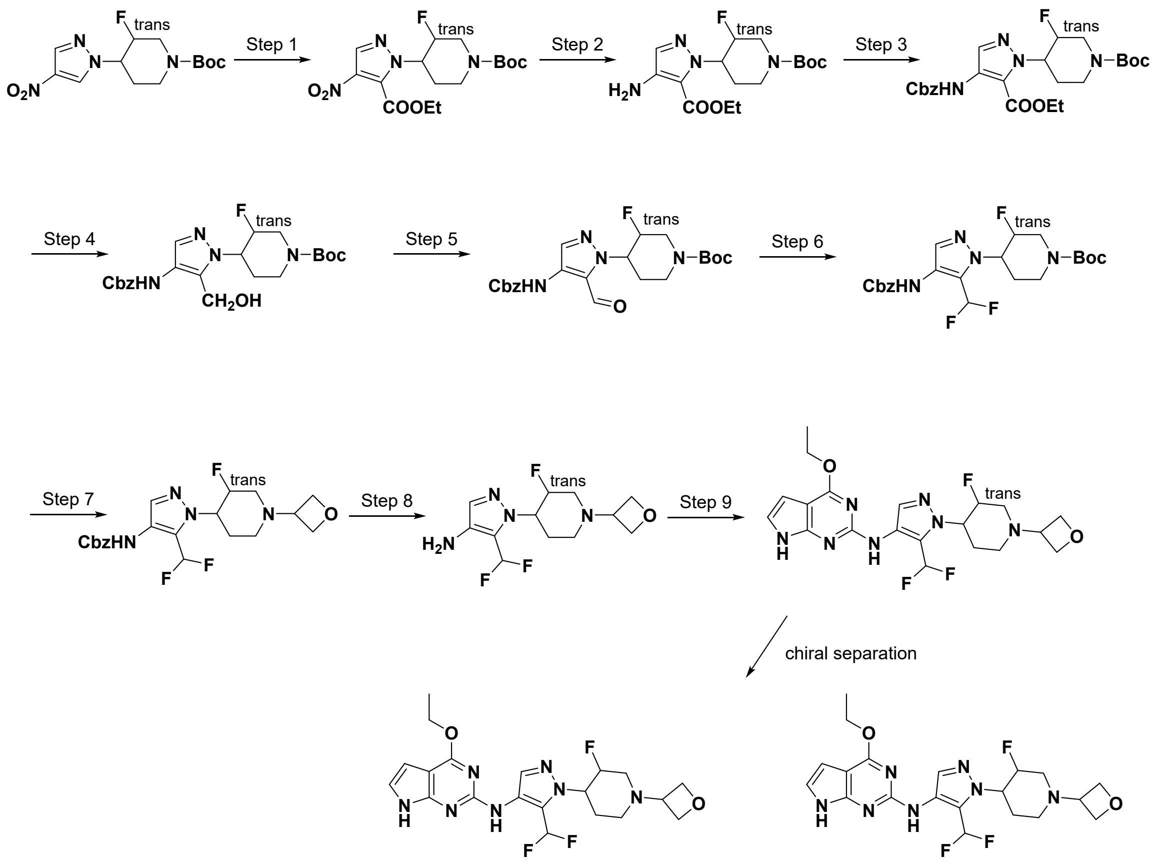
^{1}H NMR (400 MHz, CD_3OD): δ 7.74 (s, 1H), 6.80 (d, *J*= 3.6 Hz, 1H), 6.28 (d, *J*= 3.6 Hz, 1H), 4.09 - 5.09 (m, 1H), 4.71 (t, *J*= 6.4 Hz, 2H), 4.63 (q, *J*= 6.4 Hz, 2H), 4.47 (q, *J*= 7.2 Hz, 2H), 4.20 - 4.30 (m, 1H), 3.65 - 3.72 (m, 1H), 3.23 - 3.25 (m, 1H), 2.86 - 2.89 (m, 1H), 2.73 (q, *J*= 7.6 Hz, 2H), 2.28 - 2.38 (m, 1H), 2.10 - 2.18 (m, 2H), 1.97 - 2.03 (m, 1H), 1.40 (t, *J*= 7.2 Hz, 3H), 1.17 (t, *J*= 7.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 162.8, 158.1, 155.2, 135.9, 120.1, 119.6, 98.5, 97.9, 90.9, 89.1, 75.0, 74.9, 61.4, 59.3, 59.1, 58.4, 52.8, 52.6, 48.1, 30.6, 16.2, 15.1, 14.2. ^{19}F NMR (CD_3OD, 376 MHz): δ -189.1.

34: LCMS: 430 $[M+H]^+$. t_R =3.298 mins. (LCMS condition 3)

Chiral HPLC: t_R =11.262 mins. (Chiralcel OJ-H 5µm 4.6×250 mm, phase: Hex: EtOH = 70: 30, F: 1 mL/min, w: 230 nm T: 30 °C) unknown absolute stereochemistry

^{1}H NMR (400 MHz, CD_3OD): δ 7.74 (s, 1H), 6.80 (d, *J*= 3.6 Hz, 1H), 6.28 (d, *J*= 3.6 Hz, 1H), 4.90 - 5.08 (m, 1H), 4.71 (t, *J*= 6.4 Hz, 2H), 4.63 (q, *J*= 6.4 Hz, 2H), 4.47 (q, *J*= 7.2 Hz, 2H), 4.20 - 4.30 (m, 1H), 3.65 - 3.72 (m, 1H), 3.23 - 3.25 (m, 1H), 2.86 - 2.89 (m, 1H), 2.73 (q, *J*= 7.6 Hz, 2H), 2.28 - 2.38 (m, 1H), 2.10 - 2.18 (m, 2H), 1.96 - 2.03 (m, 1H), 1.40 (t, *J*= 7.2 Hz, 3H), 1.17 (t, *J*= 7.6 Hz, 3H). ^{19}F NMR (CD_3OD, 376 MHz): δ -189.1.

Synthesis of (*trans*)-*N*-(5-(difluoromethyl)-1-(3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-1*H*-pyrazol-4-yl)-4-ethoxy-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (35 and 36)



35
***trans*-Enantiomer 1**
unknown absolute stereochemistry

36
***trans*-Enantiomer 2**
unknown absolute stereochemistry

Step 1: *(±)-(trans)-tert-butyl 4-(5-(ethoxycarbonyl)-4-nitro-1H-pyrazol-1-yl)-3-fluoro piperidine-1-carboxylate.* To a solution of *(±)-(trans)-tert-butyl 3-fluoro-4-(4-nitro-1H-pyrazol-1-yl)piperidine-1-carboxylate* (2.34 g, 7.45 mmol)(according to the synthesis of **21** and **22**) in dry THF (50 mL) was added dropwise LiHMDS (14.9 mL, 1 M in THF) at -78 ^{o}C under N_2. After stirred at -78 ^{o}C for 30 min, ethyl chloroformate (1.43 mL, 14.9 mmol) was added at -78^{o}C. The mixture was then warmed to room temperature and stirred for additional 2 hours. The mixture was quenched with satur. NH_4Cl and then was extracted with EtOAc (20 mL×3). The combined organic layers were washed with brine and concentrated. The crude was purified by column chromatography on silica gel (PE: EA = 20:1) to give the title compound (2.20 g, 76%) as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 8.09 (s, 1H), 4.62 - 4.91 (m, 2H), 4.44 - 4.54 (m, 2H), 4.23 (q, *J* = 7.2 Hz, 2H), 2.80 - 2.89 (m, 2H), 2.22 - 2.36 (m, 1H), 2.04 - 2.16 (m, 1H), 1.48 (s, 9H), 1.40 (t, *J* = 7.2 Hz, 3H).

Step 2: *(±)-(trans)-tert-butyl 4-(4-amino-5-(ethoxycarbonyl)-1H-pyrazol-1-yl)-3-fluoropiperidine-1-carboxyl-ate.* A mixture of *(±)-(trans)-tert-butyl 4-(5-(ethoxycarbonyl)-4-nitro-1H-pyrazol-1-yl)-3-fluoro piperidine-1-carboxylate* (4.25 g, 11.0 mmol) and Pd/C (10%, 500 mg) in MeOH (40 mL) was stirred overnight at room temperature under H_2. The suspension was filtrated and the filtrate was concentrated to give the title compound (3.74 g, 96%) as colorless oil. LC-MS: 301 [M+H-56]$^{+}$. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 7.19 (s, 1H), 5.28 - 5.39 (m, 1H), 4.80 - 5.07 (m, 1H),4.44 - 4.56 (m, 1H), 4.38 (q, *J* = 7.2 Hz, 2H), 4.04 - 4.18 (m, 3H), 2.80 - 2.97 (m, 2H), 1.98 - 2.07 (m, 2H), 1.46 (s, 9H), 1.40 (t, *J* = 7.2 Hz, 3H).

Step 3: *(±)-(trans)-tert-butyl 4-(4-(((benzyloxy)carbonyl)amino)-5-(ethoxycarbonyl)-1H-pyrazol-1-yl)-3-fluoro -piperidine-1-carboxylate.* To a solution of *(±)-(trans)-tert-butyl 4-(4-amino-5-(ethoxycarbonyl)-1H-pyrazol-1-yl)-3-fluoropiperidine-1-carboxyl-ate* (3.74 g, 10.5 mmol) in THF (100 mL) was added aq. Na_2CO_3 (3.34 g, 31.5 mmol, in 30 mL water). The mixture was cooled to 0 ^oC, CbzCl (2.69 g, 15.8 mmol) was added dropwise. After addition, the mixture was warmed to room temperature and stirred overnight. The mixture was quenched with Satur. NH_4Cl (20 mL) and water (20 mL), and then extracted with EtOAc (30 mL×3). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated. The crude was purified by column chromatography on silica gel (PE: EA = 10:1) to give the title compound (4.62 g, 90% yield) as colorless oil. LC-MS: 435 $[M+H-56]^+$. ^1H NMR (300 MHz, $CDCl_3$): δ 8.21 (s, 1H), 8.05 (s, 1H), 7.36 - 7.40 (m, 5H), 5.28 - 5.39 (m, 1H), 5.22 (s, 2H), 4.81 - 5.06 (m, 1H), 4.48 - 4.58 (m, 1H), 4.42 (q, *J* = 7.2 Hz, 2H), 4.10 - 4.28 (m, 1H), 2.79 - 2.98 (m, 2H), 1.99 - 2.21 (m, 2H), 1.47 (s, 9H), 1.42 (t, *J* = 7.2 Hz, 3H).

Step 4: *(±)-(trans)-tert-butyl 4-(4-(((benzyloxy)carbonyl)amino)-5-(hydroxymethyl)-1H-pyrazol-1-yl)-3-fluoro -piperidine-1-carboxylate.* To a solution of *(±)-(trans)-tert-butyl 4-(4-(((benzyloxy)carbonyl)amino)-5-(ethoxycarbonyl)-1H-pyrazol-1-yl)-3-fluoro-piperidine-1-carboxylate* (4.62 g, 9.43 mmol) in THF/MeOH (100 mL/10 mL) was added $NaBH_4$ (2.87 g, 75.4 mmol) in portions at 0 ^oC. After addition, the mixture was warmed to room temperature and stirred for 1 hour. The mixture was poured into Satur. NH_4Cl (100 mL) and extracted with EtOAc (50 mL×3). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated to give the title compound (4.29 g, 100% yield) as a white solid. LC-MS: 331 $[M+H-118]^+$. ^1H NMR (300 MHz, $CDCl_3$): δ 7.42 (s, 1H), 7.36 - 7.38 (m, 5H), 6.36 (s, 1H), 5.14 - 5.23 (m, 2H), 4.74 - 4.98 (m, 1H), 4.55 - 4.70 (m, 1H), 4.33 - 4.45 (m, 1H), 4.11 - 4.29 (m, 1H), 3.48 (s, 2H), 2.79 - 2.96 (m, 2H), 2.20 - 2.34 (m, 1H), 1.98 - 2.10 (m, 1H), 1.47 (s, 9H).

Step 5: *(±)-(trans)-tert-butyl 4-(4-(((benzyloxy)carbonyl)amino)-5-formyl-1H-pyrazol-1-yl)-3-fluoropiperidine-1-carboxylate.* A mixture of *(±)-(trans)-tert-butyl 4-(4-(((benzyloxy)carbonyl)amino)-5-(hydroxymethyl)-1H-pyrazol-1-yl)-3-fluoro-piperidine-1-carboxylate* (1.66 g, 3.71 mmol) and MnO_2 (2.90 g, 33.3 mmol) in $CHCl_3$ was stirred at 70 ^oC for 5 hours. The mixture was cooled to room temperature and then filtered. The filtrate was concentrated and the crude was purified by column chromatography on silica gel (PE: EA= 10: 1~ 0: 1) to give the title compound (1.49 g, 90% yield) as colorless oil. LC-MS: 391 $[M+H-56]^+$. ^1H NMR (300 MHz, $CDCl_3$): δ 10.01 (s, 1H), 8.55 (s, 1H), 8.18 (s, 1H), 7.35 - 7.39 (m, 5H), 5.22 (s, 2H), 4.61 - 4.84 (m, 1H), 4.55 - 4.58 (m, 2H), 4.20 - 4.35 (m, 1H), 2.82 - 2.93 (m, 2H), 2.38 - 2.51 (m, 1H), 2.07 - 2.11 (m, 1H), 1.48 (s, 9H).

Step 6: *(±)-(trans)-tert-butyl 4-(4-(((benzyloxy)carbonyl)amino)-5-(difluoromethyl)-1H-pyrazol-1-yl)-3-fluoro -piperidine-1-carboxylate.* To a solution of *(±)-(trans)-tert-butyl 4-(4-(((benzyloxy)carbonyl)amino)-5-formyl-1H-pyrazol-1-yl)-3-fluoropiperidine-1-carboxylate* (1.49 g, 3.34 mmol) in dry DCM (40 mL) was added dropwise DAST (3.23 g, 20.0 mmol) at -78^oC under N_2. After addition, the mixture was warmed to room temperature and stirred for 2 hour. The mixture was poured into Satur. $NaHCO_3$ (100 mL) in an ice-bath and stirred for 10 min, then extracted with DCM (20 mL ×3). The combined organic layers were dried over Na_2SO_4, concentrated and purified by column chromatography on silica gel (PE: EA = 10: 1) to give the title compound (676 mg, 43% yield) as a yellow solid. LC-MS: 413 $[M+H-56]^+$. ^1H NMR (300 MHz, $CDCl_3$): δ 7.89 (s, 1H), 7.35 - 7.38 (m, 5H), 6.88 (t, J = 56.8 Hz, 1H), 6.63 (s, 1H), 5.19 (s, 2H), 4.74 - 4.99 (m, 1H), 4.47 - 4.62 (m, 1H), 4.17 - 4.33 (m, 2H), 2.78 - 2.90 (m, 2H), 2.20 - 2.33 (m, 1H), 1.99 - 2.06 (m, 1H), 1.47 (s, 9H).

Step 7: *(±)-(trans)-benzyl (5-(difluoromethyl)-1-(3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-1H-pyrazol-4-yl) carbamate.* To a solution of *(±)-(trans)-tert-butyl 4-(4-(((benzyloxy)carbonyl)amino)-5-(difluoromethyl)-1H-pyrazol-1-yl)-3-fluoro-piperidine-1-carboxylate* (676 mg, 1.44 mmol) in DCM (20 mL) was added HCl/dioxane (4 mL, 6 N). The mixture was stirred at room temperature for 30 min and then concentrated. The residue was diluted with 1,2-dichloroethane (20 mL), oxetan-3-one (624 mg, 8.67 mmol) was added, followed by NaBH $(OAc)_3$ (1.84 g, 8.67 mmol). The reaction was stirred at room temperature for 1 hour. The mixture was quenched with Satur. $NaHCO_3$ (50 mL) and extracted with DCM (20 mL×3). The combined organic layers were concentrated and purified by column chromatography on silica gel (DCM: MeOH= 100: 1) to give the title compound (588 mg, 96% yield) as a yellow solid. LC-MS: 425 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$): δ 7.90 (s, 1H), 7.34 - 7.39 (m, 5H), 6.87 (t, J = 40.8 Hz, 1H), 6.60 (s, 1H), 5.20 (s, 2H), 4.91 - 5.09 (m, 1H), 4.58 - 4.69 (m, 4H), 4.10 - 4.19 (m, 1H), 3.61 - 3.67 (m, 1H), 3.18 - 3.22 (m, 1H), 2.81 - 2.84 (m, 1H), 2.36 - 2.45 (m, 1H), 2.01 - 2.11 (m, 3H).

Step 8: *(±)-(trans)-5-(difluoromethyl)-1-(3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-1H-pyrazol-4-amine.* A mixture of *(±)-(trans)-benzyl (5-(difluoromethyl)-1-(3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-1H-pyrazol-4-yl)* carbamate (588 mg, 1.39 mmol) and Pd/C (10%, 100 mg) in MeOH/THF (10 mL/10 mL) was stirred at room temperature under H_2 for 1 hour. The suspension was filtered and the filtrate was concentrated. The crude was purified by column chromatography on silica gel (DCM: MeOH= 100: 1~50:1) to give the title compound (275 mg, 68% yield) as a yellow solid. ^1H NMR (300 MHz, $CDCl_3$): δ 7.21 (s, 1H), 6.83 (dd, J = 56.4, 52.8 Hz, 1H), 4.86 - 5.11 (m, 1H), 4.57 - 4.69 (m, 4H), 4.00 - 4.15 (m, 1H), 3.58 - 3.67 (m, 1H), 3.13 - 3.19 (m, 2H), 2.79 - 2.82 (m, 1H), 2.27 - 2.41 (m, 1H), 1.98 - 2.01 (m, 3H).

Step 9: *(trans)-N-(5-(difluoromethyl)-1-(3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-1H-pyrazol-4-yl)-4-ethoxy-7H-pyrrolo[2,3-d]pyrimidin-2-amine.* A mixture of *(±)-(trans)-5-(difluoromethyl)-1-(3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-1H-pyrazol-4-amine* (275 mg, 0.95 mmol), 2-chloro-4-ethoxy-7H-pyrrolo[2,3-d]pyrimidine (374 mg, 1.90 mmol), $Pd_2(dba)_3$ (174 mg, 0.19 mmol), X-phos (181 mg, 0.38 mmol) and K_2CO_3 (393 mg, 2.85 mmol) in dioxane (20 mL) was stirred at 110 ^oC under N_2 for 4 hours. The mixture was concentrated and the crude purified by column chromatography on silica gel (DCM: MeOH= 100: 1 to 50: 1) and C18 column (15~60 % CH_3CN in water) to give the racemic product (141 mg, 33% yield) as a white solid, which was further separated by chiral column (chiralpak: Column ID (4.6 × 250 mm, 5 um), phase: Hex: EtOH = 70: 30). to give the title compounds **35** (t_R = 13.035 min, 63 mg) and **36** (t_R = 14.930 min, 59 mg).

35: LC-MS: 452 $[M+H]^+$. t_R= 4.209 min (LCMS condition 3).

Chiral HPLC: t_R=13.035mins. (chiralpak: Column ID (4.6 × 250 mm, 5 um), phase: Hex: EtOH = 70: 30) unknown absolute stereochemistry

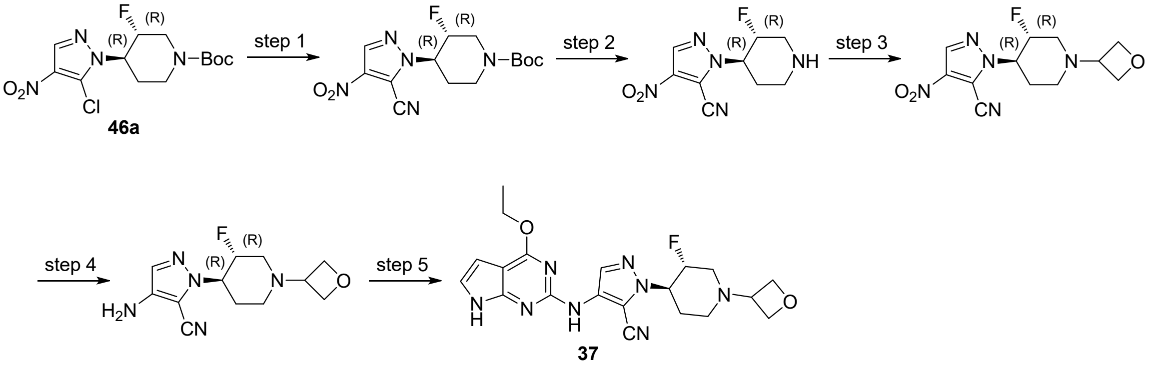
^{1}H NMR (400 MHz, DMSO-d_6): δ 11.36 (br., 1H), 8.71 (s, 1H), 8.09 (s, 1H), 7.13 - 7.56 (m, 1H), 6.96 (dd, *J*= 2.0, 3.2 Hz, 1H), 6.27 (dd, *J*= 2.0, 3.2 Hz, 1H), 4.87 - 5.34 (m, 1H), 4.54 - 4.62 (m, 2H), 4.32 - 4.52 (m, 5H), 3.60 (t, *J*= 6.4 Hz, 1H), 3.16 - 3.27 (m, 1H), 2.79 (d, *J*= 4.8 Hz, 1H), 1.86 - 2.20 (m, 4H), 1.38 (t, *J*=7.2 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 162.9, 156.3, 154.7, 134.3, 124.9, 120.9, 109.0, 98.7, 90.1, 88.3, 74.9, 74.8, 62.1, 61.8, 58.2, 52.7, 48.0, 30.9, 15.1. ^{19}F NMR (376 MHz, DMSO-d_6): δ -111.3 (s, 2F), -187.1 (s, 1F).

36: LC-MS: 452 $[M+H]^+$. t_R= 4.209 min (LCMS condition 3).

Chiral HPLC: t_R=14.93 mins. (chiralpak: Column ID (4.6 × 250 mm, 5 um), phase: Hex: EtOH = 70: 30) unknown absolute stereochemistry

^{1}H NMR (400 MHz, DMSO-d_6): δ 11.36 (br. s., 1H), 8.71 (s, 1H), 8.09 (s, 1H), 7.18 - 7.59 (m, 1H), 6.96 (dd, *J*= 2.0, 3.2 Hz, 1H), 6.27 (dd, *J*= 2.0, 3.2 Hz, 1H), 4.90 - 5.26 (m, 1H), 4.54 - 4.60 (m, 2H), 4.32 - 4.52 (m, 5H), 3.60 (t, *J*= 6.4 Hz, 1H), 3.20 - 3.27 (m, 1H), 2.79 (d, *J*= 5.2 Hz, 1H), 1.97 - 2.15 (m, 4H), 1.38 (t, *J*= 7.2 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 162.9, 156.3, 154.7, 134.3, 124.9, 120.9, 108.9, 98.7, 98.7, 90.1, 88.3, 74.9, 74.8, 62.1, 61.8, 58.2, 52.7, 30.9, 15.1. ^{19}F NMR (376 MHz, DMSO-d_6): δ -111.3 (s, 2F), -187.1 (s, 1F).

Synthesis of 4-((4-ethoxy-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)amino)-1-((3*R*,4*R*)-3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-1*H*-pyrazole-5-carbonitrile (37)



Step 1: *(3R,4R)-tert-butyl 4-(5-cyano-4-nitro-1H-pyrazol-1-yl)-3-fluoropiperidine-1-carboxylate.* To a solution of **46a** (590 mg, 1.69 mmol) in DMF (50 mL) was added NaCN (150 mg, 3.06 mmol). The reaction was stirred at 70 °C for 2 hours. The mixture was quenched by poured into H_2O (200 mL). The water phase was extracted with EtOAc (50 mL×3). The combined organic layer was washed with water (40 mL×3), brine (20 mL×3), dried over Na_2SO_4 and concentrated to give the title compound (570 mg, 99 % yield) as yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.25 (s, 1H), 4.51 - 4.92 (m, 3H), 4.25 - 4.38 (m, 1H), 2.83 - 2.99 (m, 2H), 2.25 - 2.41 (m, 1H), 2.08 - 2.16 (m, 1H), 1.48 (s, 9H).

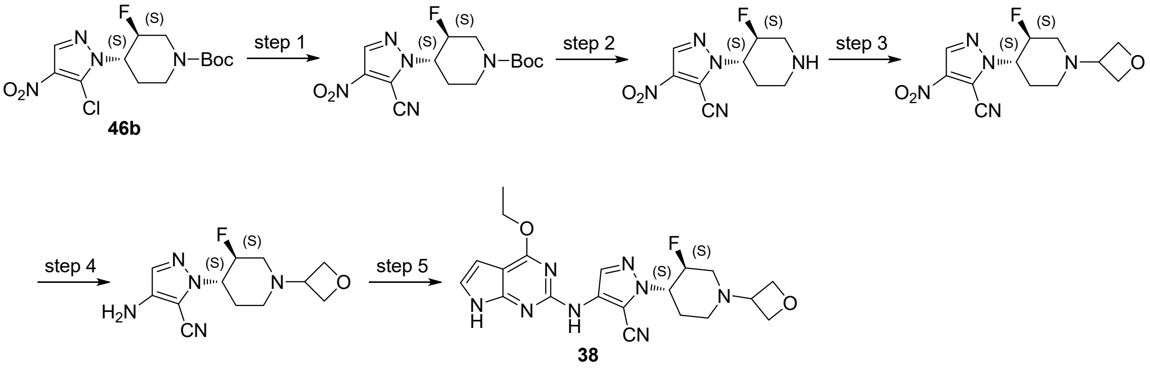
Step 2: *1-((3R,4R)-3-fluoropiperidin-4-yl)-4-nitro-1H-pyrazole-5-carbonitrile hydrochloride.* To a solution of 5N HCl/dioxane (10 mL) was added *(3R,4R)-tert-butyl 4-(5-cyano-4-nitro-1H-pyrazol-1-yl)-3-fluoropiperidine-1-carboxylate* (450 mg, 1.33 mmol) at room temperature. The reaction was stirred for 1 hour at room temperature and then concentrated to give the title compound (453 mg, 100% yield) as yellow oil. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.55 - 9.73 (m, 2H), 8.76 (s, 1H), 5.05 - 5.24 (m, 2H), 3.62 - 3.71 (m, 3H), 3.17 - 3.30 (m, 1H), 2.40 - 2.48 (m, 2H).

Step 3: *1-((3R,4R)-3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-4-nitro-1H-pyrazole-5-carbonitrile.* To a solution of *1-((3R,4R)-3-fluoropiperidin-4-yl)-4-nitro-1H-pyrazole-5-carbonitrile hydrochloride* (453 mg, 1.64 mmol) in MeOH (10 mL) was added oxetan-3-one (300 mg, 4.16 mmol). After stirred for 10 minutes, $NaBH_3CN$ (200 mg, 2.90 mmol) and AcOH (20 mg, 0.33 mmol) was added. The reaction was stirred at room temperature for 2 hours. The mixture was concentrated and the crude was purified by Prep-TLC (PE: EA = 1:2) to give the title compound (134 mg, 31% yield) as yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 8.25 (s, 1 H), 4.83 - 5.07 (m, 1H), 4.69 - 4.74 (m, 2H), 4.53 - 4.62 (m, 1H), 4.41 - 4.53 (m, 1H), 3.64 - 3.72 (m, 1H), 3.22 - 3.27 (m, 1H), 2.88 - 2.93 (m, 1H), 2.38 - 2.53 (m, 1H), 2.01 - 2.22 (m, 3H).

Step 4: *4-amino-1-((3R,4R)-3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-1H-pyrazole-5-carbonitrile*. To a solution of *1-((3R,4R)-3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-4-nitro -1H-pyrazole-5-carbonitrile* (110 mg, 0.372 mmol) in MeOH (8 mL) was added Pd/C (80 mg, 10% wet). The mixture was stirred at room temperature with 1 atm H_2 for 2 hours. The suspension was filtered and washed with MeOH (5 mL). The filtrate was concentrated to give the title compound (67 mg, 68% yield) as yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.20 (s, 1 H), 4.77 - 5.01 (m, 1H), 4.55 - 4.68 (m, 4H), 3.99 - 4.22 (m, 1H), 3.14 - 3.16 (m, 1H), 2.79 - 2.82 (m, 1H), 2.23 - 2.45 (m, 1H), 1.99 - 2.33 (m, 3H).

Step 5: *4-((4-ethoxy-7H-pyrrolo[2,3-d]pyrimidin-2-yl)amino)-1-((3R,4R)-3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-1H-pyrazole-5-carbonitrile.* To a solution of 2-chloro-4-ethoxy-7H-pyrrolo[2,3-d]pyrimidine (65 mg, 0.33 mmol), *4-amino-1-((3R,4R)-3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-1H-pyrazole-5-carbonitrile* (65 mg, 0.22 mmol), X-phos (21 mg, 0.044 mmol), K_2CO_3 (91 mg, 0.66 mmol) in dioxane (40 mL) was added $Pd_2(dba)_3$ (20 mg, 0.022 mmol). The mixture was stirred overnight at 100 °C under N_2. The mixture was filtered and the filtrate was concentrated. The crude was purified by prep-HPLC to give the title compound as a white solid (6.2 mg, 7% yield). LC-MS: 427 $[M+H]^+$. t_R= 3.43 min (LCMS condition 3). ^{1}H NMR (400 MHz, CD_3OD): *δ* 8.24 (s, 1H), 6.90 (d, *J* = 3.6 Hz 1H), 6.37 (d, *J*= 3.6 Hz, 1H), 4.89 - 5.05 (m, 1H), 4.74 (t, *J*= 6.8 Hz, 2H), 4.65 (q, *J*= 6.0 Hz, 2H), 4.58 (q, *J*= 7.2 Hz, 2H), 4.40 - 4.49 (m, 1H), 3.69 - 3.75 (m, 1H), 3.26 - 3.31 (m, 1H), 2.92 - 2.95 (m, 1H), 2.34 - 2.41 (m, 1H), 2.14 - 2.34 (m, 3H), 1.47 (t, *J*= 7.2 Hz, 3H).

Synthesis of 4-((4-ethoxy-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)amino)-1-((3*S*,4*S*)-3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-1H-pyrazole-5-carbonitrile (38)



Step 1: *(3S,4S)-tert-butyl 4-(5-cyano-4-nitro-1H-pyrazol-1-yl)-3-fluoropiperidine-1-carboxylate.* To a solution of **46b** (1.10 g, 3.17 mmol) in DMF (50 mL) was added NaCN (310 mg, 6.34 mmol). The mixture was stirred at 70°C for 2 hours. The mixture was quenched by poured in H_2O (200 mL). The water phase was extracted with EtOAc (80 mL×3). The combined organic layer was washed with water (50 mL×3), brine (50 mL×2), dried over Na_2SO_4 and concentrated to give the title compound (1.0g, 94% yield) as yellow oil.

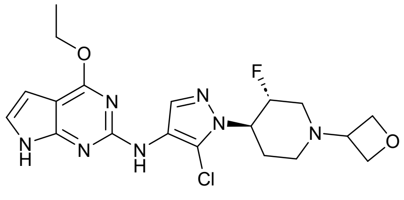
Step 2: *1-((3S,4S)-3-fluoropiperidin-4-yl)-4-nitro-1H-pyrazole-5-carbonitrile hydrochloride.* To a solution of 4N HCl/dioxane (10 mL) was added (3S,4S)-tert-butyl 4-(5-cyano-4-nitro-1H-pyrazol-1-yl)-3-fluoropiperidine-1-carboxylate (1.1 g, 3.26 mmol) at room temperature. The reaction was stirred for 2 hour at room temperature and then concentrated to give the title compound (1.3 g 100% yield) as yellow oil. ^{1}H NMR: (300 MHz, DMSO-d_6): δ 9.84 (br., 2H), 8.77 (s, 1H), 5.08 - 5.29 (m, 2H), 3.39 - 3.50 (m, 2H), 3.27 - 3.37 (m, 1H), 3.15 - 3.27 (m, 1H), 2.37 - 2.44 (m, 2H).

Step 3: *1-((3S,4S)-3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-4-nitro-1H-pyrazole-5-carbonitrile.* To a solution of 1-((3S,4S)-3-fluoropiperidin-4-yl)-4-nitro-1H-pyrazole-5-carbonitrile hydrochloride (1.10 g, 3.99 mmol) in MeOH (50 mL) was added oxetan-3-one (1.0 g, 13.9 mmol). After stirring at room temperature for 10 minutes, $NaBH_3CN$ (200 mg, 2.90 mmol) in portions was added followed by AcOH (1 mL). The reaction was stirred at room temperature for 1 hour. The mixture was concentrated and the crude was purified by column chromatography on silica gel (PE: EA= 2:1) to give a white solid. The solid was triturated in a mixed solution (PE: EA= 4: 1, 5 mL) to give the title compound (300 mg, 25% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.26 (s, 1 H), 4.84 - 5.09 (m, 4H), 4.70 (t, J = 6.4 Hz, 2H), 4.60 (td, J= 6.4, 2.0 Hz, 1H), 4.41 - 4.54 (m, 1H), 3.64 - 3.72 (m, 1H), 3.23 - 3.30 (m, 1H), 2.88 - 2.94 (m, 1H), 2.39 - 2.53 (m, 1H), 2.10 - 2.22 (m, 3H).

Step 4: *4-amino-1-((3S,4S)-3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-1H-pyrazole-5-carbonitrile.* To a solution of *1-((3S,4S)-3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-4-nitro-1H-pyrazole -5-carbonitrile* (300 mg, 1.02 mmol) in MeOH (20 mL) was added Pd/C (100 mg, 10% wet). The mixture was stirred at room temperature with 1 atm H_2 for 2 hours. The suspension was filtered and washed with MeOH (5 mL), EtOAc (5 mL). The filtrate was concentrated to give the title compound (200 mg, 74% yield) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.23 (s, 1 H), 4.79 - 5.04 (m, 1H), 4.57 - 4.70 (m, 4H), 4.13 - 4.27 (m, 1H), 3.62 - 3.68 (m, 1H), 3.15 - 3.24 (m, 1H), 2.80 - 2.88 (m, 1H), 2.27 - 2.38 (m, 1H), 2.02 - 2.16 (m, 3H).

Step 5: *4-((4-ethoxy-7H-pyrrolo[2,3-d]pyrimidin-2-yl)amino)-1-((3S,4S)-3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-1H-pyrazole-5-carbonitrile.* To a solution of 2-chloro-4-ethoxy-7H-pyrrolo[2,3-d]pyrimidine (200 mg, 1.02 mmol), *4-amino-1-((3S,4S)-3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-1H-pyrazole-5-carbonitrile* (200 mg, 0.755 mmol), X-phos (83 mg, 0.174 mmol), K_2CO_3 (300 mg, 2.17 mmol) in dioxane (20 mL) was added $Pd_2(dba)_3$ (78 mg, 0.085 mmol). The mixture was stirred overnight at 100 ^oC under N_2. The mixture was filtered and the filtrate was concentrated. The crude was purified by prep-HPLC to give the title compound as a white solid (21.4 mg, 7% yield). LC-MS: 427 $[M+H]^+$. t_R= 3.43 min (LCMS condition 3). ^1H NMR (300 MHz, CD_3OD): δ 8.23 (s, 1H), 6.93 (d, *J*= 3.6 Hz, 1H), 6.37 (d, *J*= 3.6 Hz, 1H), 4.98 - 5.20 (m, 1H), 4.71 - 4.88 (m, 5H), 4.55 (q, *J*= 7.2 Hz, 1H), 4.14 - 4.22 (m, 1H), 3.60 - 3.69 (m, 1H), 3.33 - 3.38 (m, 1H), 2.77 - 2.97 (m, 2H), 2.33 - 2.53 (m, 2H), 1.44 (t, *J*= 7.2 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 162.9, 155.2, 154.4, 133.8, 131.3, 121.4, 111.4, 106.5, 99.1, 98.7, 90.9, 89.1, 74.9, 74.8, 62.7, 61.9, 58.2, 52.3, 47.7, 29.4, 15.1. ^{19}F NMR (376 MHz, CD_3OD): -185.0.

Synthesis of N-(5-chloro-1-((3R,4R)-3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-1H-pyrazol-4-yl)-4-ethoxy-7H-pyrrolo[2,3-d]pyrimidin-2-amine (39)



GSK3357679

(cis)-tert-butyl 3-fluoro-4-hydroxypiperidine-1-carboxylate (**43**). To a solution of tert-butyl 3-fluoro-4-oxopiperidine-1-carboxylate (5.0 g, 23 mmol) in MeOH (50 mL) was added $NaBH_4$ (1.13 g, 29.9 mmol) under ice cooling. The reaction mixture was stirred at this temperature for 2 hrs. The *cis*- and *trans*- isomers were detected by LCMS in a ratio of 3:1. NH_4Cl aq. (50 mL) was added and the solution was extracted with EtOAc (80 mL×3). The organic layer was dried and concentrated. The major *cis*- product was purified by flash chromatography on C18 to afford desired product (2.2 g, 44 % yield) as a white solid. ^1H NMR (300 MHz, CDCl3): δ 4.50-4.68 (m, 1H), 3.89-3.93 (m, 2H), 3.65-3.71 (m, 1H), 3.35-3.44 (m, 1H), 3.17-3.21 (m, 1H), 2.07-2.09 (m, 1H), 1.76-1.86 (m, 2H), 1.45 (s, 9H). LCMS:220 [M+H] +.

(3R,4S)-tert-butyl 3-fluoro-4-hydroxypiperidine-1-carboxylate (**44a**) and (3S,4R)-tert-butyl 3-fluoro-4-hydroxypiperidine-1-carboxylate (**44b**). (cis)-tert-butyl 3-fluoro-4-hydroxypiperidine-1-carboxylate **43** (6 g) was separated by chiral-HPLC to give **44a** (enantiomer 1 (3R,4S), t_R= 11.77 min, 100% ee, 2.2g, 73% recovery) and **44b** (enantiomer 2 (3S,4R), t_R= 9.55 min, 100% ee, 2.4 g, 80% recovery). Chiral condition: Chiralpak IC 5µm 4.6×250 mm, Hex: EtOH = 90:10, Flow: 1.0 mL/min, 230 nm, T = 30 ^oC. Absolute stereochemistry was assigned by aligning chiral HPLC retention times with those previously reported.[1]

(3R,4R)-tert-butyl 3-fluoro-4-(4-nitro-1H-pyrazol-1-yl)piperidine-1-carboxylate (**45a**). To a solution of **44a** (2.2 g, 10.04 mmol), 4-nitro-1H-pyrazole (1.19 g, 10.5 mmol), PPh_3 (3.94 g, 15.06 mmol) in THF (50 mL) was added slowly DIAD (3.04 g, 15.06 mmol) under ice cooling. The mixture was stirred overnight at room temperature. Solvent was removed and the residue was dissolved in Et_2O (50 mL). The suspension was stirred vigorously for 30 min and then filtered. The filtrate was concentrated, and the crude was purified by flash column chromatography on C18 (30~60% CH_3CN in water) to give the title compound (2.7 g, 85% yield) as yellow oil. LCMS: 313 [M-H] +. ^1H NMR (300 MHz, CDCl3): δ 8.23 (s, 1H), 8.13 (s, 1H), 4.62-4.87 (m, 2H), 4.17-4.29 (m, 2H), 2.80-2.93 (m, 2H), 2.12-2.33 (m, 2H), 1.47 (s, 9H).

(3R,4R)-tert-butyl 4-(5-chloro-4-nitro-1H-pyrazol-1-yl)-3-fluoropiperidine-1-carboxylate (**46a**). To a solution of **45a** (2.7 g, 8.6 mmol) in THF (50 mL) was added dropwise LiHMDS (17 mL, 17.2 mmol, 1M in THF) at -78 ^oC under N2. The reaction mixture was stirred at this temperature for 1 hour. A solution of hexachloroethane (4.07 g, 17.2 mmol) in THF (20 mL) was added at -78 oC. The reaction mixture was stirred at -78 ^oC for 20 min under N_2. NH_4Cl aq. solution (40 mL) was added and the solution was extracted with EA (50 mL×2). The organic layers were dried and concentrated. The crude was purified by column chromatography on silica gel (PE: EA = 5:1) to give the title compound (2.7 g, 90 % yield) as a yellow solid. ^1H NMR (300 MHz, CDCl3): δ 8.24 (s, 1H), 5.00-4.76 (m, 1H), 4.61-4.54 (m, 2H), 4.29-4.24 (m, 1H), 2.91-2.87 (m, 2H), 2.26-2.20 (m, 1H), 2.03-2.01 (m, 1H), 1.48 (s, 9H).

(3R,4R)-4-(5-chloro-4-nitro-1H-pyrazol-1-yl)-3-fluoropiperidine (**47a**). To a solution of **46a** (2.7 g, 7.75 mmol) in MeOH (20 mL) was added HCl/dioxane (8 M, 20 mL). The reaction mixture was stirred at room temperature for 2 hrs. Solvent was removed and the residue was dissolved in Na_2CO_3 aqueous solution (30 mL) and extracted with DCM/ MeOH (10:1, 50 mL×3). The combined organic layer was dried and concentrated to give the title compound (1.83 g, 95 % yield) as a yellow solid. ^1H NMR (300 MHz, CDCl3): δ 8.23 (s, 1H), 4.74-5.00 (m, 1H), 4.44-4.56 (m, 1H), 3.51-3.56 (m, 1H), 3.16-3.20 (m, 1H), 2.66-2.81 (m, 2H), 2.12-2.27 (m, 1H), 1.97-2.05 (m, 2H).

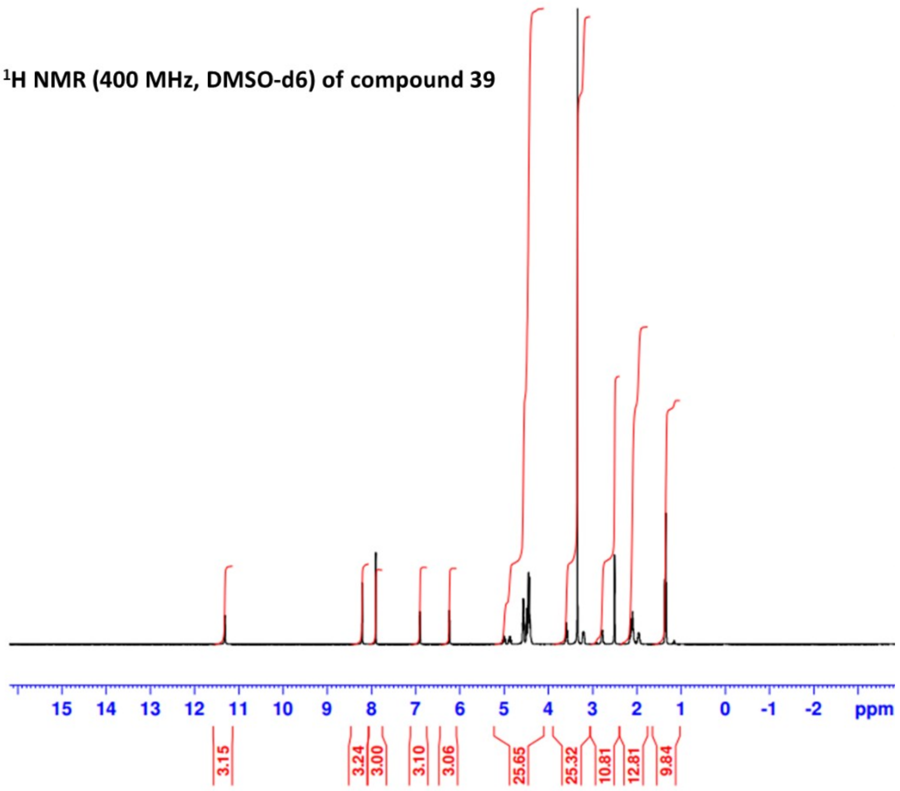
(3R,4R)-4-(5-chloro-4-nitro-1H-pyrazol-1-yl)-3-fluoro-1-(oxetan-3-yl)piperidine (**48a**). To a solution of **47a** (500 mg, 2.01 mmol), oxetan-3-one (363 mg, 5.04 mmol) in DCE (16 mL) was added in portions $NaBH(OAc)_3$ (1.27 g, 6.03 mmol) at room temperature. The reaction was stirred overnight at room temperature. Na_2CO_3 aqueous solution (60 mL) was added and the mixture was extracted with DCM (80 mL×4). The combined organic layers were dried and concentrated. The crude was purified by column chromatography on silica gel (PE: EA=2:1) to give the title compound (520 mg, 85% yield) as colorless oil. LCMS: 305 $[M+H]^+$. ^1H NMR (300 MHz, CDCl3): δ 8.24 (s, 1H), 4.94-5.12 (m, 1H), 4.58-4.71 (m, 4H), 4.38-4.48 (m, 1H), 3.63-3.69 (m, 1H), 3.22-3.25 (m, 1H), 2.85-2.89 (m, 1H), 2.30-2.41 (m, 1H), 2.03-2.16 (m, 2H), 1.99-2.01 (m, 1H).

5-chloro-1-((3R,4R)-3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-1H-pyrazol-4-amine (**49a**). To a solution of **48a** (5.90 g, 19.4 mmol) in EtOH/H_2O (50 mL/ 50 mL) was added iron powder (5.4 g, 97 mmol) and NH_4Cl (5.2 g, 97 mmol). The reaction mixture was stirred overnight at 50 oC. The solution was filtered through a Celite pad and washed with MeOH (50 mL×3). The combined organic layers were concentrated, dissolved in EtOAc (50 mL) and filtered. The solution was concentrated and purified by flash column chromatography on C18 (10~40% CH_3CN in water, tR= 20 min) to give the title compound (3.5 g, 66% yield) as a white solid. LCMS: 275 [M+H] +. ^{1}H NMR (300 MHz, CDCl3): δ 7.29 (s, 1H), 4.88-5.13 (m, 1H), 4.57-4.69 (m, 4H), 4.15-4.28 (m, 1H), 3.59-3.68 (m, 1H), 3.14-3.21 (m, 1H), 2.90 (br., 2H), 2.79-2.83 (m, 1H), 2.20-2.34 (m, 1H), 1.92-2.13 (m, 3H).

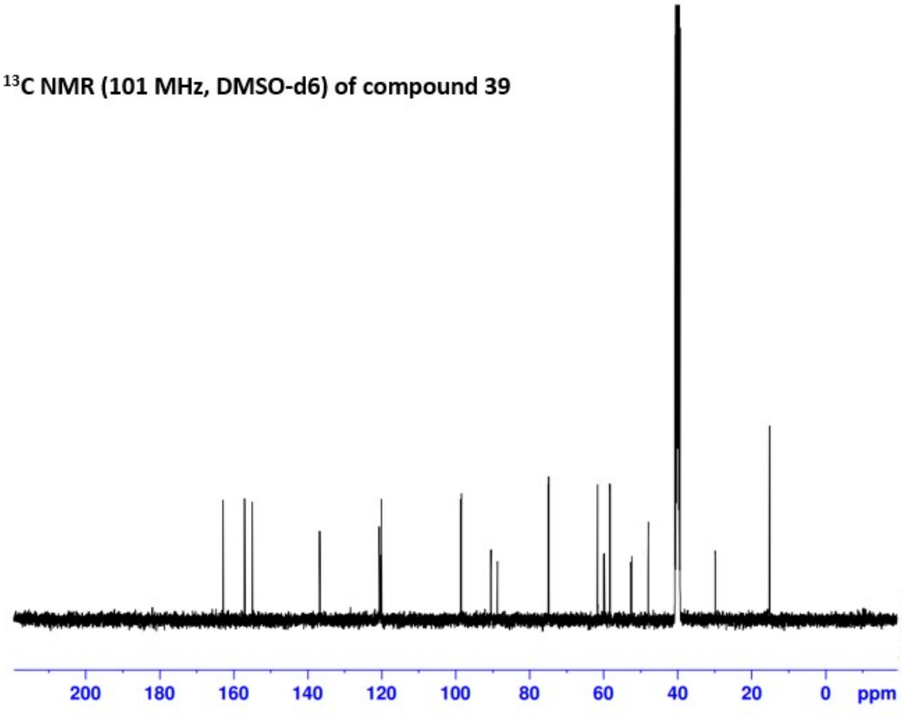
2-chloro-4-ethoxy-7H-pyrrolo[2,3-d]pyrimidine. A solution of 2,4-dichloro-7H-pyrrolo-[2,3-d]-pyrimidine (500 mg, 2.66 mmol) and sodium ethoxide (181 mg, 2.66 mmol) in ethanol (8 mL) and THF (8.00 mL) was stirred at 90 ^{o}C overnight. The mixture was cooled to room temperature and evaporated. The crude was purified via chromatography on silica gel (PE: EA=25:1) to give the title compound (300 mg, 1.214 mmol, 45.7 % yield) as a white solid. LCMS:198 [M+H] +. ^{1}H NMR (400MHz, DMSO-d6): δ 12.23 (br. s., 1H), 7.38 (d, J = 3.6 Hz, 1H), 6.50 (d, J = 3.6 Hz, 1H), 4.51 (q, J = 7.2 Hz, 2H), 1.39 (t, J = 7.2 Hz, 3H).

N-(5-chloro-1-((3R,4R)-3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-1H-pyrazol-4-yl)-4-ethoxy-7H-pyrrolo[2,3-d]pyrimidin-2-amine (**39**). A mixture of 2-chloro-4-ethoxy-7H-pyrrolo[2,3-d]pyrimidine (258 mg, 1.31 mmol), **49a** (300 mg, 1.09 mmol), X-phos (99 mg, 0.21 mmol), $Pd_2(dba)_3$ (90 mg, 0.10 mmol) and K_2CO_3 (451 mg, 3.27 mmol) in dioxane (30 mL) was stirred overnight at 110 ^{o}C under N_2. The mixture was cooled to room temperature and filtered. The filter was concentrated and the crude was purified by flash chromatography on C18 (20~50% CH_3CN in water) to give crude product (200 mg, 50 % yield) as a light yellow solid, which was further purified by prep-HPLC to give the title compound **39** as a white solid (100 mg, 99.5 % ee). LCMS: 436 [M+H] +. t_R =3.85 mins (LCMS condition 3). Chiral HPLC: t_R =6.87 mins (ID, CO2: MEOH = 70:30, Flow: CO2 Flow rate: 2.1, Co-solvent: 0.899, back pressure: 100, T = 39.9 ^{o}C). ^{1}H NMR (400 MHz, DMSO-d6): δ 11.31 (s, 1H), 8.21 (s, 1H), 7.90 (s, 1H), 6.90 (d, J = 2.8 Hz, 1H), 6.23 (d, J = 2.8 Hz, 1H), 4.86-5.00 (m, 1H), 4.55-4.58 (m, 2H), 4.41-4.51(m, 5H), 3.61-3.57 (m, 1H), 3.17-3.23 (m, 1H), 2.77-2.79 (m, 1H), 2.09-2.16 (m, 3H), 1.94-1.97 (m, 1H), 1.34 (t, J = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d6): δ 162.9, 157.0, 155.0, 136.7, 120.6, 120.4, 120.0, 98.6, 98.4, 90.4, 88.7, 74.9, 74.8, 61.6, 59.8, 58.3, 52.5, 47.9, 29.7, 15.1. ^{19}F NMR (376 MHz, DMSO-d6): δ -186.6. HRMS (ESI): m/z calcd for C19H23ClFN7O2 [M+H] + 436.1664, found [M+H] + 436.1665.

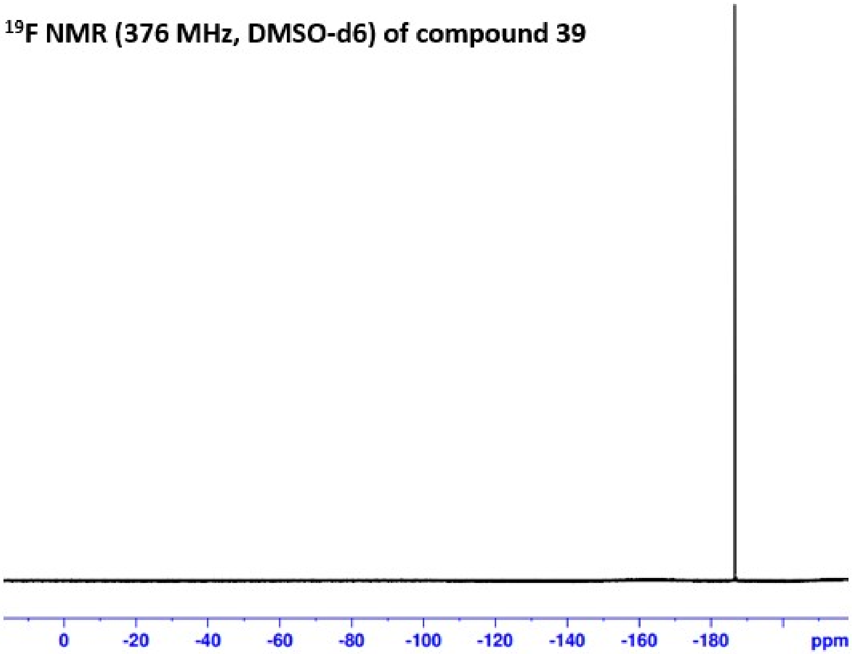
^{1}H NMR (400 MHz, DMSO-d6) of compound 39



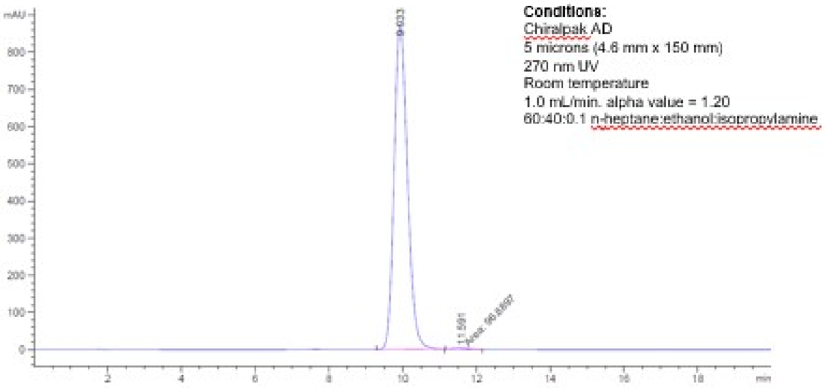
^{13}C NMR (101 MHz, DMSO-d6) of compound 39



^{19}F NMR (376 MHz, DMSO-d6) of compound 39



Analytical Chiral HPLC of compound 39



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270 nm UV
Room temperature
1.0 mL/min. alpha value = 1.20
60:40:0.1 n-heptane:ethanol:isopropylamine

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=====================================================================
                         Area Percent Report
=====================================================================

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Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


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----|-------|----|-------|----------|----------|--------|
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   2  11.591 MM    0.4821   96.88973    3.34985   0.4408

Totals :                  2.19794e4  875.84790
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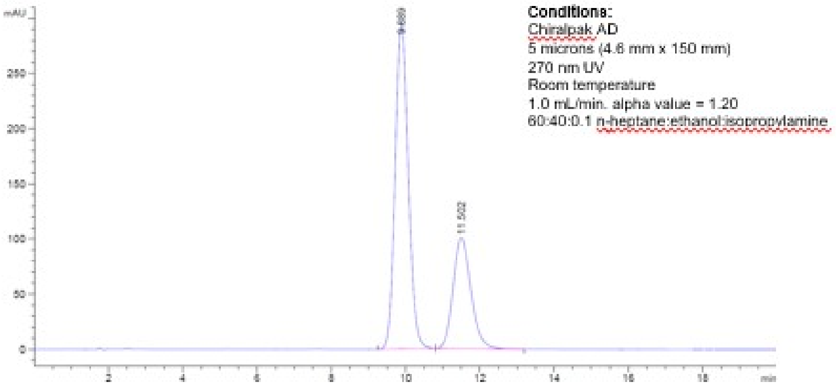
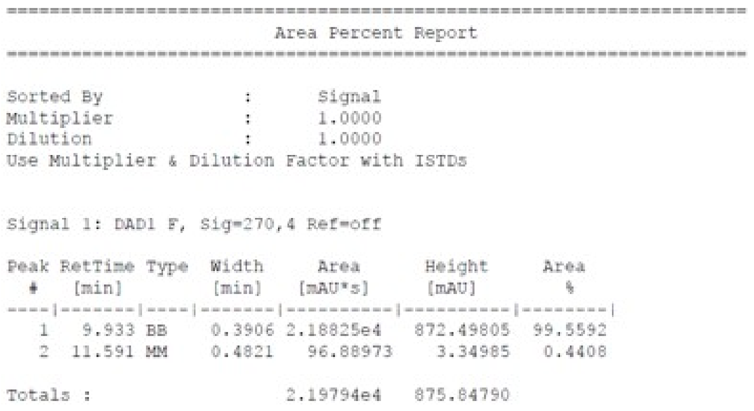
Analytical Chiral HPLC of compound 39 and 40 racemic mixture





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Use Multiplier & Dilution Factor with ISTDs


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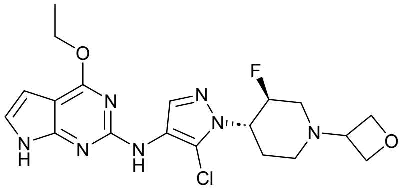
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   9.933 BB    0.3906 2.18825e4  872.49805  99.5592
   2  11.591 MM    0.4821  96.88973    3.34985   0.4408

Totals :                  2.19794e4  875.84790
```

Synthesis of N-(5-chloro-1-((3S,4S)-3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-1H-pyrazol-4-yl)-4-ethoxy-7H-pyrrolo[2,3-d]pyrimidin-2-amine (40)



(3S,4S)-tert-butyl 3-fluoro-4-(4-nitro-1H-pyrazol-1-yl)piperidine-1-carboxylate (**45b**). To a solution of **44b** (3.40 g, 15.5 mmol), 4-nitro-1H-pyrazole (1.75 g, 15.5 mmol), PPh_3 (6.10 g, 23.3 mmol) in THF (100 mL) was added slowly DIAD (4.71 g, 23.3 mmol) at 0°C. The mixture was stirred overnight at room temperature. The mixture was concentrated, the residue was dissolved in EtOAc (50 mL) and then n-hexane (100 mL) was added. The suspension was stirred vigorously for 1 hour and then filtered. The filtrate was concentrated, and the crude was purified by column chromatography on C18 (20~80% CH_3CN in water) to give the title compound (4.05 g, 83% yield) as yellow oil. 1H NMR (300

MHz, CDCl3): δ 8.22 (s, 1H), 8.12 (s, 1H), 4.57-4.86 (m, 2H), 4.18-4.29 (m, 2H), 2.85 (br., 2H), 2.12-2.28 (m, 2H), 1.48 (s, 9H).

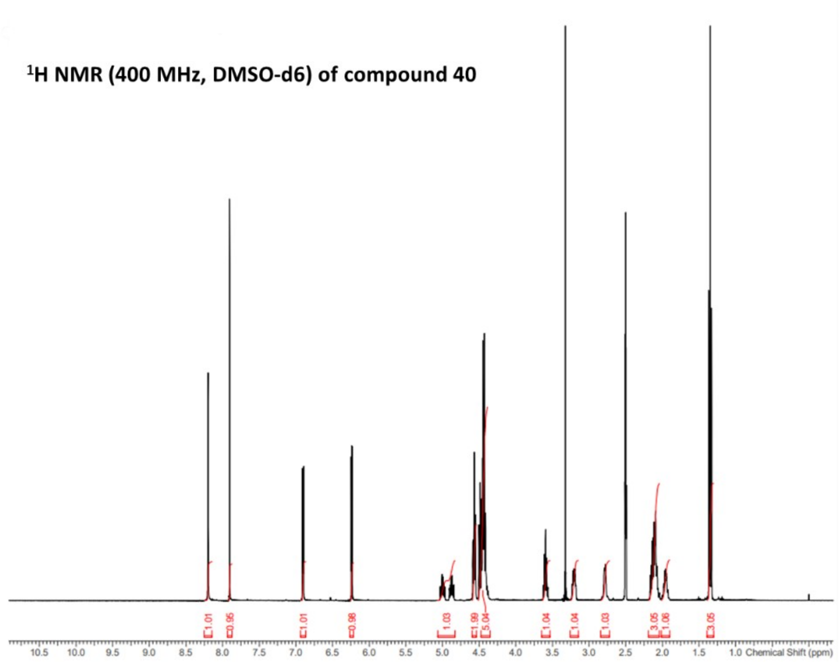
(3S,4S)-tert-butyl 4-(5-chloro-4-nitro-1H-pyrazol-1-yl)-3-fluoropiperidine-1-carboxylate (**46b**). To a solution of **45b** (2.7 g, 8.6 mmol) in THF (50 mL) was added LiHMDS (26 mL, 25.8 mmol, 1M in THF) at -78 oC under N2. The reaction mixture was stirred at -78 ^{o}C for 40 min. A solution of hexachloroethane (4.07 g, 17.2 mmol) in THF (20 mL) was added and the reaction was stirred at -78 ^{o}C for 2 hrs under N_2. Aq. NH_4Cl solution (40 mL) was added and the solution was extracted with EtOAc (50 mL×2). The organic layers were dried and concentrated. The crude was purified by column chromatography on silica gel (PE: EA = 5:1) to give the title compound (1.2 g, 40 % yield) as a yellow solid. ^{1}H NMR (300 MHz, CDCl3): δ 8.23 (s, 1H), 4.75-4.99 (m, 1H), 4.48-4.61(m, 2H), 4.22-4.32 (m, 1H), 2.83-2.99 (m, 2H), 2.16-2.31 (m, 1H), 1.96-2.03 (m, 1H), 1.48 (s, 9H).

(3S,4S)-4-(5-chloro-4-nitro-1H-pyrazol-1-yl)-3-fluoropiperidine (**47b**). To a solution of **46b** (1.2 g, 3.44 mmol) in MeOH (6 mL) was added HCl/dioxane (8 M, 6 mL). The reaction was stirred at room temperature for 2 hrs. Solvent was evaporated, and the residue was dissolved in Na_2CO_3 aqueous solution (20 mL) and extracted with DCM/MeOH (10:1, 50 mL×3). The combined organic layers were dried and concentrated to give the title compound (820 mg, 96 % yield). ^{1}H NMR (300 MHz, CDCl3): δ 8.23 (s, 1H), 4.75-4.99 (m, 1H), 4.44-4.56 (m, 1H), 3.51-3.58 (m, 1H), 3.15-3.23(m, 1H), 2.68-2.81 (m, 2H), 2.13-2.27 (m, 1H), 1.97-2.06 (m, 1H).

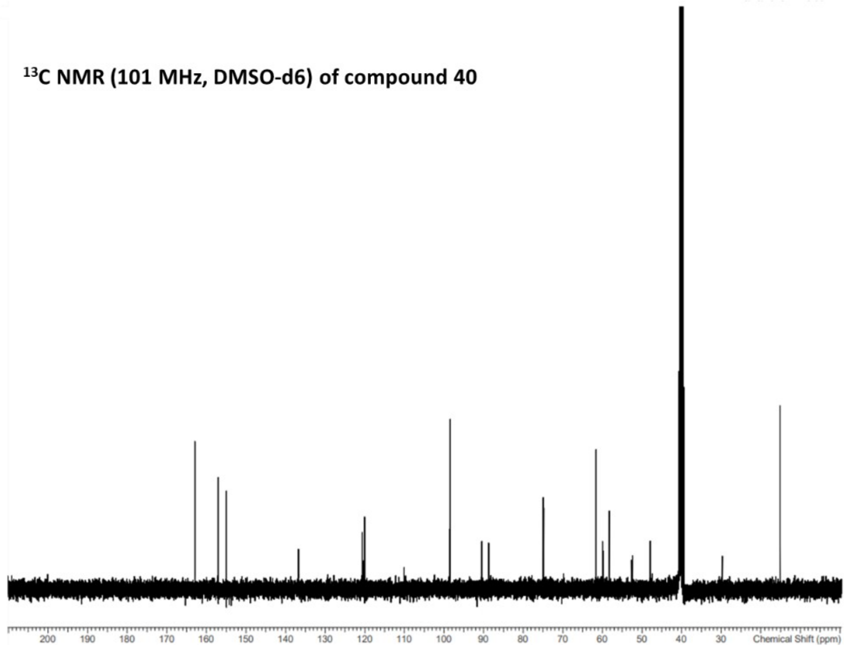
(3S,4S)-4-(5-chloro-4-nitro-1H-pyrazol-1-yl)-3-fluoro-1-(oxetan-3-yl)piperidine (**48b**). To a solution of **47a** (400 mg, 1.61 mmol), oxetan-3-one (290 mg, 4.03 mmol) in DCE (15 mL) was added in portions $NaBH(OAc)_3$ (1.02 g, 4.83 mmol) at room temperature. The reaction mixture was stirred overnight at room temperature. Na_2CO_3 aqueous solution (30 mL) was added and extracted with DCM (30 mL×5). The combined organic layers were dried and concentrated. The crude was purified by column chromatography on silica gel (PE: EA = 1:1) to give the title compound (410 mg, 83% yield) as a light yellow solid. LCMS: 305 [M+H] +. ^{1}H NMR (300 MHz, CDCl3): δ 8.24 (s, 1H), 4.91-5.16 (m, 1H), 4.69 (t, J = 6.6 Hz, 2H), 4.58-4.63 (m, 2H), 4.37-4.49 (m, 1H), 3.62-3.70 (m, 1H), 3.20-3.27 (m, 1H), 2.85-2.90 (m, 1H), 2.28-2.43 (m, 1H), 2.08-2.18 (m, 2H), 1.96-2.02 (m, 1H).

5-chloro-1-((3S,4S)-3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-1H-pyrazol-4-amine (**49b**). To a solution of 48b (410 mg, 1.35 mmol) in $EtOH/H_2O$ (4 mL/ 4mL) was added iron powder (151 mg, 2.70 mmol) and NH_4Cl (150 mg, 2.70 mmol). The reaction was stirred overnight at room temperature. The solution was filtered through a Celite pad and washed with MeOH (10 mL×3). The combined organic layers were concentrated, and the crude was purified by column chromatography on C18 (20~50% CH_3CN in water) to give the title compound (260 mg, 70% yield) as red oil. LCMS: 275 [M+H] +. ^{1}H NMR (300 MHz, CDCl3): δ 7.29 (s, 1H), 4.88-5.13 (m, 1H), 4.58-4.69 (m, 4H), 4.15-4.28 (m, 1H), 3.59-3.68 (m, 1H), 3.15-3.21 (m, 1H), 2.76-2.97 (m, 3H), 2.20-2.34 (m, 1H), 1.92-2.13 (m, 3H).

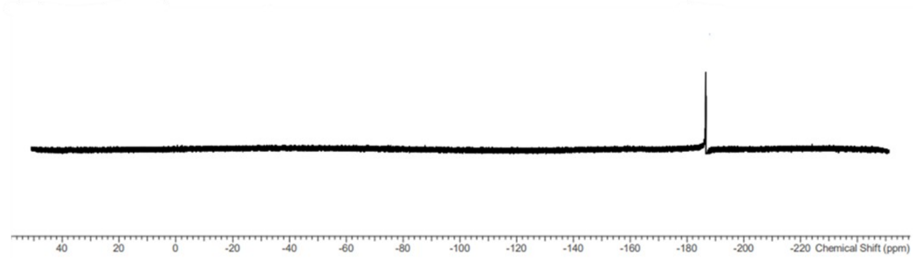
N-(5-chloro-1-((3S,4S)-3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-1H-pyrazol-4-yl)-4-ethoxy-7H-pyrrolo[2,3-d]pyrimidin-2-amine (**40**). A mixture of 2-chloro-4-ethoxy-7H-pyrrolo[2,3-d]pyrimidine (181 mg, 0.92 mmol), **49b** (210 mg, 0.76 mmol), X-phos (71 mg, 0.15 mmol), $Pd2(dba)_3$ (70 mg, 0.07 mmol) and K_2CO_3 (314 mg, 2.28 mmol) in dioxane (20 mL) was stirred at 110 oC under N_2 for 8 hrs. The mixture was cooled to room temperature and filtered. The filtrate was concentrated and the crude was purified by flash chromatography on C18 (20~50% CH_3CN in water) to give crude product (100 mg, 30 % yield) as a light yellow solid, which was further purified by prep-HPLC to give the title compound (60 mg, 99.7 % ee) as a white solid. LCMS: 436 [M+H] +. t_R =3.85 mins (LCMS condition 3). Chiral HPLC: t_R =8.92 mins (ID, CO2: MEOH = 70:30, Flow: CO2 Flow rate: 2.1, Co-solvent: 0.899, back pressure: 100, T = 39.9 oC). ^{1}H NMR (400 MHz, DMSO-d6): δ 11.31 (s, 1H), 8.20 (s, 1H), 7.90 (s, 1H), 6.90 (d, J = 2.8 Hz, 1H), 6.23(d, J = 2.8 Hz, 1H), 4.86-5.00 (m, 1H), 4.54-4.58 (m, 2H), 4.39-4.51 (m, 5H), 3.57-3.60 (m, 1H), 3.18-3.21 (m, 1H), 2.77-2.79 (m, 1H), 2.07-2.16 (m, 3H), 1.94-1.96 (m, 1H), 1.35 (t, J = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d6): δ 162.9, 157.0, 155.0, 136.7, 120.6, 120.4, 120.0, 98.6, 98.4, 90.4, 88.7, 74.9, 74.8, 61.6, 59.8, 58.3, 52.5, 47.9, 29.7, 15.1. ^{19}F NMR (376 MHz, DMSO-d6): δ -186.6.



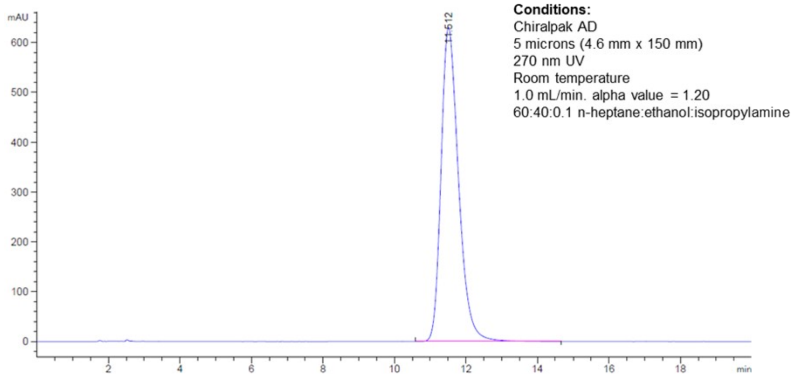
^{13}C NMR (101 MHz, DMSO-d6) of compound 40



^{19}F NMR (376 MHz, DMSO-d6) of compound 40



Chiral HPLC of compound 40



Conditions:
Chiralpak AD
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270 nm UV
Room temperature
1.0 mL/min. alpha value = 1.20
60:40:0.1 n-heptane:ethanol:isopropylamine

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=====================================================================
                         Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 F, Sig=270,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  11.512 BB    0.5212 2.13470e4   630.17877 100.0000

Totals :                  2.13470e4   630.17877
```
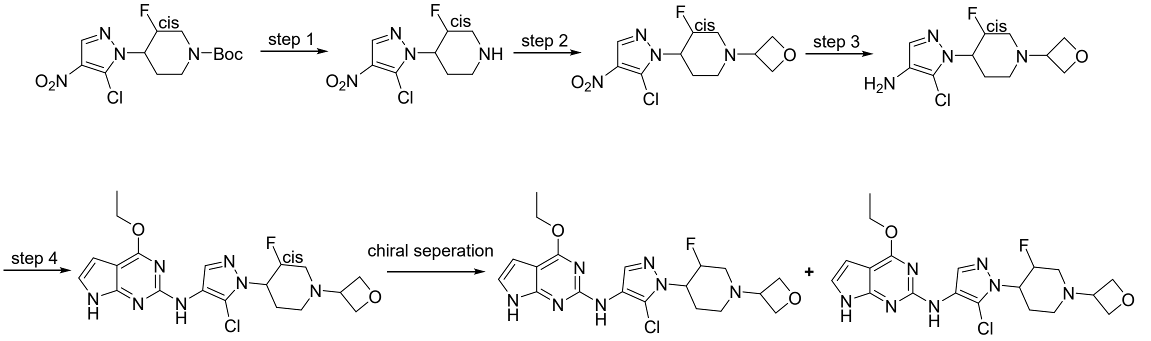

Synthesis of *cis-N*-(5-chloro-1-(3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-1*H*-pyrazol-4-yl)-4-ethoxy-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (41 and 42)



41
***cis*-Enantiomer 1**
unknown absolute stereochemistry

42
***cis*-Enantiomer 2**
unknown absolute stereochemistry

Step 1: *(±)-(cis)-4-(5-chloro-4-nitro-1H-pyrazol-1-yl)-3-fluoropiperidine.* To a solution of (±)-cis-tert-butyl 4-(5-chloro-4-nitro-1H-pyrazol-1-yl)-3-fluoropiperidine-1-carboxylate (1.0 g, 2.87 mmol) (according to the synthesis of **33** and **34**) in MeOH (10 mL) was added HCl/dioxane (5.7 M, 10 mL). The reaction mixture was stirred at room temperature for 1 hour. Solvent was removed and the residue was dissolved in aq. Na_2CO_3 solution (40 mL) and extracted with EtOAc (50 mL×3). The organic layers were dried and concentrated to give the title compound as a yellow solid (650 mg, 90% yield). LCMS: 249 $[M+H]^+$.

Step 2: *(±)-(cis)-4-(5-chloro-4-nitro-1H-pyrazol-1-yl)-3-fluoro-1-(oxetan-3-yl)piperidine.* To a solution of *(±)-(cis)-4-(5-chloro-4-nitro-1H-pyrazol-1-yl)-3-fluoropiperidine* (650 mg, 2.62 mmol), oxetan-3-one (472 mg, 6.55 mmol) in DCE (30 mL) was added in portions $NaBH(OAc)_3$ (1.66 g, 7.86 mmol) at room temperature. The reaction mixture was stirred at room temperature for 2 hour. Aq. Na_2CO_3 solution (40 mL) was added and the mixture was extracted with DCM (50 mL×3). The organic layers were dried and concentrated. The crude was purified by column chromatography on silica gel (PE: EA=1:2) to give the title compound (640 mg, 84% yield) as a white solid. LCMS: 305 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$): δ 8.21 (s, 1H), 4.83 - 4.99 (m, 1H), 4.55 - 4.70 (m, 5H), 3.67 - 3.75 (m, 1H), 3.02 - 3.16 (m, 2H), 2.80 - 2.93 (m, 1H), 2.24 - 2.42 (m, 2H), 1.98 - 2.08 (m, 1H).

Step 3: *(±)-(cis)-5-chloro-1-(3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-1H-pyrazol-4-amine.* To a solution of *(±)-(cis)-4-(5-chloro-4-nitro-1H-pyrazol-1-yl)-3-fluoro-1-(oxetan-3-yl)piperidine* (640 mg, 2.10 mmol) in $EtOH/H_2O$ (10 mL/10 mL) was added iron powder (707 mg, 12.6 mmol) and NH_4Cl (670 mg, 12.6 mmol). The reaction was stirred overnight at 50 °C. The mixture was filtered through a Celite pad. The solution was concentrated and purified by flash column chromatography on C18 (0~20% CH_3CN in water) to give the title compound (400 mg, 80 % yield) as red oil. LCMS: 275 $[M+H]^+$. ^1H NMR (300 MHz, CDCl3): δ 7.27 (s, 1H), 4.77 - 4.93 (m, 1H), 4.64 - 4.72 (m, 4H), 4.25 - 4.38 (m, 1H), 3.65 - 3.74 (m, 1H), 2.78 - 3.15 (m, 5H), 2.18 - 2.41 (m, 2H), 1.94 - 1.99 (m, 1H).

Step 4: *cis-N-(5-chloro-1-(3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-1H-pyrazol-4-yl)-4-ethoxy-7H-pyrrolo[2,3-d]pyrimidin-2-amine.* A mixture of 2-chloro-4-ethoxy-7H-pyrrolo[2,3-d]pyrimidine (345 mg, 1.75 mmol), *(±)-(cis)-5-chloro-1-(3-fluoro-1-(oxetan-3-yl)piperidin-4-yl)-1H-pyrazol-4-amine* (400 mg, 1.46 mmol), X-phos (139 mg, 0.29 mmol), $Pd_2(dba)_3$ (132 mg, 0.14 mmol) and K_2CO_3 (604 mg, 4.38 mmol) in dioxane (30 mL) was stirred overnight at 105 °C under N_2. The mixture was cooled to room temperature and filtered. The filtrate was concentrated and the crude was purified by flash chromatography on C18 (20~50% CH_3CN in water) to give the racemic product (150 mg, 24 % yield) as a light yellow solid, which was further separated by SFC to give the title compounds **41** (40 mg, t_R = 5.5 min, 100% ee) and **42** (40 mg, t_R = 6.5 min, 99% ee)

41: LCMS: 436 $[M+H]^+$. t_R =3.61 mins. (LCMS condition 3)

Chiral HPLC: t_R =5.5 mins. (Chiralpak: column OD-H (250 mm×4.6 mm, 5 um), CO_2: MeOH (0.2% DEA) = 70:30) unknown absolute stereochemistry

^1H NMR (400 MHz, DMSO-d_6): δ 11.30 (s, 1H), 8.20 (s, 1H), 7.82 (s, 1H), 6.90 (t, J = 2.8 Hz, 1H), 6.23 (dd, J = 3.2, 1.6 Hz, 1H), 4.83 - 4.95 (m, 1H), 4.40 - 4.61 (m, 7H), 3.52 - 3.58 (m, 1H), 2.90 - 3.04 (m, 2H), 2.57 - 2.67 (m, 1H), 2.18 - 2.38 (m, 2H), 1.87 - 1.90 (m, 1H), 1.35 (t, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 162.9, 156.9, 155.0, 135.6, 120.6, 120.6, 119.1, 98.6, 98.4, 88.2, 86.4, 74.8, 61.6, 58.4, 57.4, 51.8, 47.7, 24.6, 15.1. ^{19}F NMR (376 MHz, DMSO-d_6): δ -198.6.

42: LCMS: 436 $[M+H]^+$. t_R =3.62 mins. (LCMS condition 3)

Chiral HPLC: t_R =6.5 mins. (Chiralpak: column OD-H (250 mm×4.6 mm, 5 um), CO_2: MeOH (0.2% DEA) = 70:30) unknown absolute stereochemistry

^{1}H NMR (400 MHz, DMSO-d_6): δ 11.30 (s, 1H), 8.20 (s, 1H), 7.82 (s, 1H), 6.90 (t, J = 2.8 Hz, 1H), 6.23 (dd, J = 3.2, 1.6 Hz, 1H), 4.83 - 4.95 (m, 1H), 4.40 - 4.61 (m, 7H), 3.52 - 3.58 (m, 1H), 2.90 - 3.04 (m, 2H), 2.58 - 2.67 (m, 1H), 2.16 - 2.38 (m, 2H), 1.87 - 1.90 (m, 1H), 1.35 (t, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 162.9, 157.0, 155.0, 135.6, 120.6, 120.6, 119.1, 98.6, 98.4, 88.2, 86.4, 74.9, 61.6, 58.4, 57.4, 51.8, 47.7, 24.6, 15.1. ^{19}F NMR (376 MHz, DMSO-d_6): δ -198.6.

Cellular LRRK2 pS935 AlphaScreen assay: The assay measures the activity of compounds against LRRK2 kinase activity in cells. The observed LRRK2 kinase-dependent modulation of LRRK2 Ser 935 phosphorylation was utilized to develop a quantitative 384 well plate-based immunoassay of LRRK2 Ser935 phosphorylation in the human neuroblastoma cell line SH-SY5Y, engineered to over-express recombinant full length LRRK2 protein. SH-SY5Y cells were grown in DMEM/F12 medium with 10% dialyzed fetal bovine serum and harvested by treatment with 0.5 % trypsin-EDTA for 5 minutes at 37^{o}C followed by centrifugation at 1000rpm for 4 minutes. The cell pellet was suspended in Opti-MEM reduced serum media (Invitrogen) at 200,000 cells/ml and mixed with the BacMam LRRK2 virus at MOI=50. 50 µl cell solutions were then dispensed to each well of a 384-well plate and incubated at 37 °C, 5 % CO_2 for 24 hours. Serial dilutions of test compounds were prepared in Opti-MEM reduced serum media (Invitrogen) and 5.6ul transferred from compound plate to cell assay plate to achieve a top final assay concentration of 10uM. DMSO was used in certain wells as controls. Cells were incubated at 37 °C, 5% CO2 for 60 minutes. The medium was then removed, and cells lysed by addition of 20ul cell lysis buffer (Cell Signaling Technology) and incubation at 4 ^{o}C for 20 minutes. 10ul of antibody/acceptor bead mix [(1/1000 biotinylated-pS935 LRRK2 antibody, 1/1000 total-LRRK2 antibody, 1/100 Acceptor beads in AlphaScreen detection buffer (25mM Hepes (pH 7.4), 0.5% Triton X-100, 1mg/ml Dextran 500 and 0.1% BSA)] was then added to each well and plates incubated for 2 hours at ambient temperature in the dark. 10 µl of donor beads solution (1/33.3 donor beads in AlphaScreen detection buffer) was then added to each well. Following incubation for a further 2 hours at ambient temperature in the dark, plates were read on an EnVision™ plate reader at emission 520-620nm with excitation 680nm. Dose response curve data was based on sigmoidal dose-response model.

P-gp/Bcrp Permeability Ratio (ER) and Cell Permeability Assay. Polarized Madin-Darby canine kidney (MDCKII) cells stably transfected with human P-gp (MDCKII-MDR1 cell line) were obtained from The Netherlands Cancer Institute (Amsterdam, The Netherlands). MDCKII-MDR1 cells transduced with BacMam2-BCRP according to experimental protocols reported previously were used for the in vitro transport studies in the Transwell system. The cell culture and transport studies were described previously. The transport of test compounds (3 µM) was measured in duplicates in one direction [apical to basolateral (A→B)] for 90 min in the absence and presence of GF120918 (5 µM, a dual P-gp and BCRP inhibitor). The exact permeability (P_{exact}, nm/s) for test compounds across MDCKII monolayers were calculated using the equation reported previously. The permeability ratio is defined as the ratio of permeability of apical-to-basolateral direction in the presence to absence of GF120918. The functionality of human P-gp and Bcrp in this cell line was evidenced by typical ERs of amprenavir (a P-gp specific substrate) and dantrolene (a P-gp specific substrate) greater than 12 and 5, respectively.

Animal Studies

All studies were conducted in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals and were reviewed by the Institutional Animal Care and Use Committee either at GSK or by the ethical review process at the institution where the work was performed.

Pharmacokinetics and tissue distribution. Pharmacokinetics and tissue distribution of compounds was evaluated in the male Han-Wistar rats following oral gavage administration at 2 mg/kg, respectively. Test compound was formulated as homogenous suspension in a dosing vehicle containing DMSO: 1% methylcellulose (w/v) =1:99. At each time point post dose (15 min, 0.5h, 1h, 2h, 4h, 7h), blood samples (>60µL/time point/each site) were collected via cardiac puncture, into separate EDTA-K2(0.5M) containers following anaesthesia with isofluorane. 50 uL of blood samples were diluted with 150 µL of deionized H_2O (1:3), snap frozen on dry ice and further stored at -80°C. Immediately after blood collection, the brains, lungs, and kidneys from all the animals were excised, transferred suitable containers and immediately stored frozen. Brain and kidney were homogenized with 3-fold PBS (w/v) and further stored at -80°C (dilution factor=3.9). Separated calibration curves were prepared in blank blood/water mixture, kidney, brain homogenate respectively. Study samples and their corresponding calibration curves were extracted by protein precipitation. Exact 50 uL sample was aliquot into a clean extract plate and then 150uL of acetonitrile containing analytical internal standard was added to each sample. After mixing and centrifugation, approximately 100uL of supernatant was removed into an injection plate and sample was further diluted with same volume of deionized water. Quantitation of test compound was carried out by UPLC/MS/MS. Brain, lung, and kidney exposure were estimated by area under curve (AUC) up to last time point at 7 hrs. Kp (tissue to blood ratio) of test compounds was evaluated from the ratio of AUC_{tissue}/AUC_{blood}.

Dog pharmacokinetic studies. Oral bioavailability was determined in male Beagle dogs (n = 3) using a crossover study design with a 7-day washout period. Compounds **39** and **40** were administered by 1 hr i.v. infusion (1%DMSO:10% hydroxypropyl-betacyclodextrin, 4 mL/kg) or oral gavage (1% MC, 10 mL/kg). Blood samples (50 µL) were collected at 0.25, 0.5, 1, 1.08, 1.25, 1.5, 2, 3, 5, 8, 24, 32 h (i.v.) or 0, 0.25, 0.5, 1, 2, 4, 7, 24, 32 h (oral gavage) timepoints into separate EDTA-K2(0.5M) containers, diluted with 150 µL of deionized H2O (1:3), and snap frozen on dry ice and further stored at -80°C prior to analysis. Blood samples were assayed using protein precipitation followed by UPLC/MS/MS analysis, and the resulting concentration–time data were analyzed by noncompartmental methods.

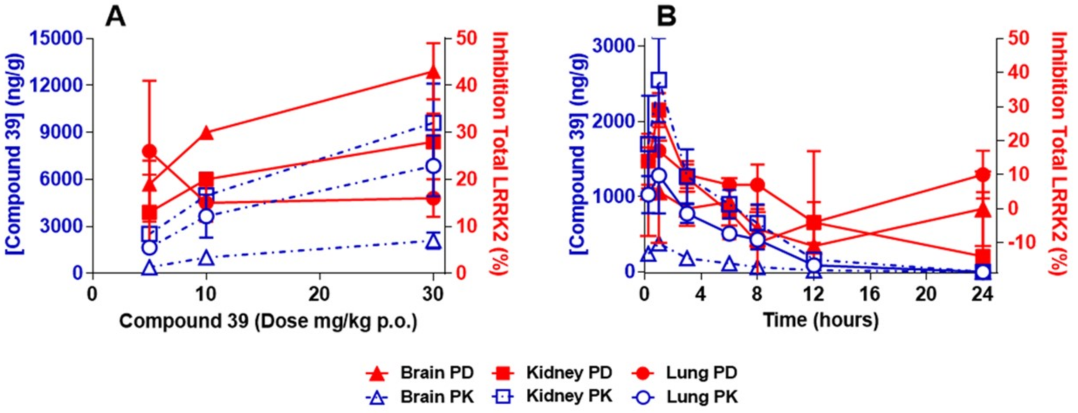
Rat pharmacodynamic studies. Compounds **39** and **40** were dissolved in 1% MC and 8-week-old naïve male Han-rats were assigned to treatment groups. For time course of pharmacodynamic study, rats received oral gavage administration of compound **39** at dose of 3 mg/kg for 0.25, 1, 3, 6, 8, 12 and 24 h (3 rats per time point) or vehicle (1% MC, 3 rats) for 6 h; rats received oral gavage administration of compound **40** at dose of 3 mg/kg for 0.25, 1, 3, 6, 8 and 12h (3 rats per time point) or vehicle (1% MC, 3 rats) for 6 h. For pharmacodynamic assessment, mice were dosed by oral gavage administration of **39** at dose of 5 mg/kg, 10 mg/kg and 30 mg/kg for 4 h (3 rats for each dose) or vehicle (1% MC, 3 rats) for 4h. At the end of the study animals were euthanized to collect 100 µL blood (immediately dilute by 300 µL distilled deionized H_2O and completely mix, followed by snap-frozen on dry ice and then exsanguinated by transcranial perfusion of PBS. Tissue samples (lung, kidney, brain) were quickly removed, weighed and snap-frozen on dry ice.

Mouse pharmacodynamic studies. Male C57BL/6 mice (~8 weeks old; Charles River (China)) were dosed with **39** at 3, 10 and 15mg/kg p.o. suspended in methycellulose (1% w/v; 10 ml/kg dosing volume). Blood samples (20 µL) were collected at 0.25, 1, 2, 4, 6, 8 and 12h post dose from a tail vein into sample tubes containing 15% EDTA-K2, diluted 5-fold with water and mixed by vortexing. At 0.25, 1, 2, 4, 6, 8 and 12h post dose animals were anaesthetised with isoflurane and a blood sample taken and the animals were exsanguinated by transcardial perfusion of chilled PBS or cold saline. Samples of brain, lung and kidney tissues were quickly dissected out, weighed and snap-frozen on dry ice and stored at -80^{o}C until further analysed for either compound concentrations by Mass spectrometry or levels pS935 by an ELISA method.

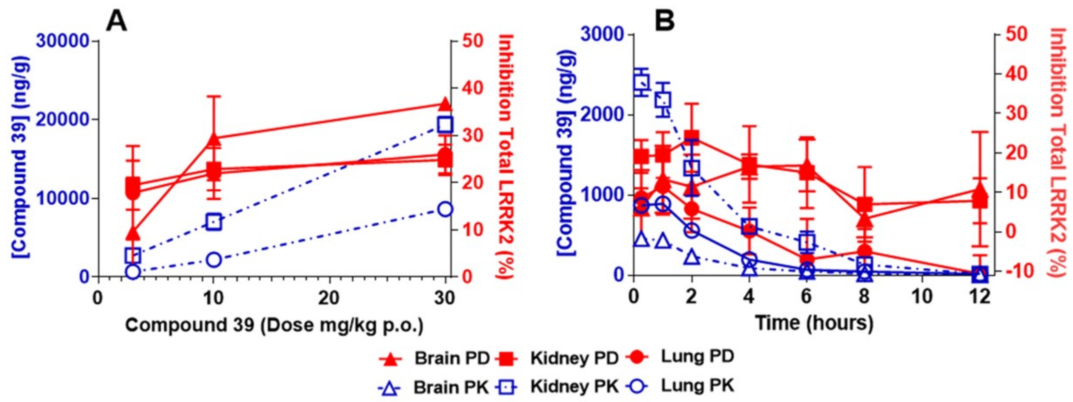
Tissue lysis. For measurement of pS935 tissues were thawed and homogenized in homogenization buffer (10 v/w) This buffer was comprised of RIPA buffer (Sigma-R0278; 150 mM NaCl, 1.0% IGEPAL® CA-630, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris, pH 8.0.); 1X Halt Protease Inhibitor Cocktail, EDTA-Free ((100X; Pierce, #78437); 1X Halt Phosphatase Inhibitor Cocktail, (Pierce, #78426); 1mM DTT (MW 154.25, stock 50 mg/ml in water, Sigma, #D9779); 1mM Benzamidine (Sigma 12072); 1mM PMSF (Sigma P7626); 1:1000 Okadaic acid (stock 100ng/µl in ethanol, Sigma, # O4511) 1:1000 Calyculin A (stock 100ng/µl in ethanol, Invitrogen, # PHZ1044). The homogenization was carried out using a Tissuelyser II (Qiagen; Cat No. 85300) at 30Hz (brain 3 minutes; kidney and lung 5 minutes; precooling adapter set at 4^{o}C) followed by further homogenization using a Bio-Gen Pro 200 homogenizer (Proscientific Cat No. 01-02200), power level 3) on ice. Tissues were further ultra-sonicated on ice using a Sonics Vibra cell (Misonix Cat No. XL-2000) power set at 3 using 3 pulses of 3 seconds. The tissue lysates were kept on ice for 30 minutes and centrifuged at 14,000 rpm for 30 minutes at 4^{o}C. The supernatant was removed and protein concentration was determined by the BCA method.

Protein concentration determination in tissue samples. The BCA Working Reagent (WR) was prepared by mixing 50 parts of Reagent A with 1 part of Reagent B (Thermo Cat No. 23225). Tissue lysates were diluted 1:20 with distilled water, (Invitrogen) 10 µl of each standard or sample was pipetted into a Microplate (NUNC Cat no. 442404). Buffer diluted 1:20 with distilled H_2O was used for background measurements for the tissue samples and distilled water as for the standards. WR (200 µl) was added to each well and the plate contents were thoroughly mixed on a plate shaker for 30 seconds followed by incubating at 37°C for 30 minutes. The plate was then cooled to room temperature and the absorbance at or near 562 nm was measured on a multifunctional microplate reader (BioTek, SYNERGY 4).

Determination of pS935 LRRK2 levels in tissue samples. To measure pS935 levels in tissue samples, 50 µl/well of capture antibody was pipetted into a 96-well Microplate (5 µg/ml LRRK2 antibody; Covance (SIG-39840); in coating buffer: 10mM Tris-HCl,10mM NaCl, pH 8.5) and the plate was incubated overnight at 4°C. Following washing the plate twice with washing buffer (PBST), the plate was blocked with blocking buffer (1% BSA in PBST) and incubated at 37°C for 2 hours after which the buffer was thoroughly removed. Brain, lung and kidney samples were diluted with RIPA buffer (with 1X phosphatase inhibitor) to 5, 0.5, 0.5 mg/ml respectively. Dilution buffer (1% BSA, 1x Phosphatase Inhibitor and 1x Protease Inhibitor in PBST) was added (37.5 µl/well) to the plate and 12.5 µl of diluted brain, lung or kidney samples were added to the plate which was incubated at 4 °C for 2 h. Detection antibody (50 µl/well) was added (1000 -fold diluted pS935-LRRK2 antibody (Epitomics (5099-1) in dilution buffer) was added to the plate which was incubated overnight at 4°C with slow shaking. The plate was washed 3 times with washing buffer and following thorough removal 100 µl/well of HRP labeled anti-rabbit antibody (Perox-AffiniPure Dnk Anti-Rabbit IgG (H+L), Jackson Immuno Research, Cat No 711-035-152; 0. 16 µg/ml; secondary antibody) in dilution buffer was added to each well of the plate and incubated at RT for 30 min with slow shaking. The plate was washed 5 times with washing buffer and 200 µl of the ELISA mixed substrates (equal volumes of A solution and B solution (DY999 R&D Systems)) was added to each well and incubated at RT with slow shaking for 15 min following which 50 µl of stop buffer (ELISA stop solution (DY994); R&D Systems) was added to each well followed by gentle mixing. Absorbance was measured on a Spectrum Max M5 plate reader.

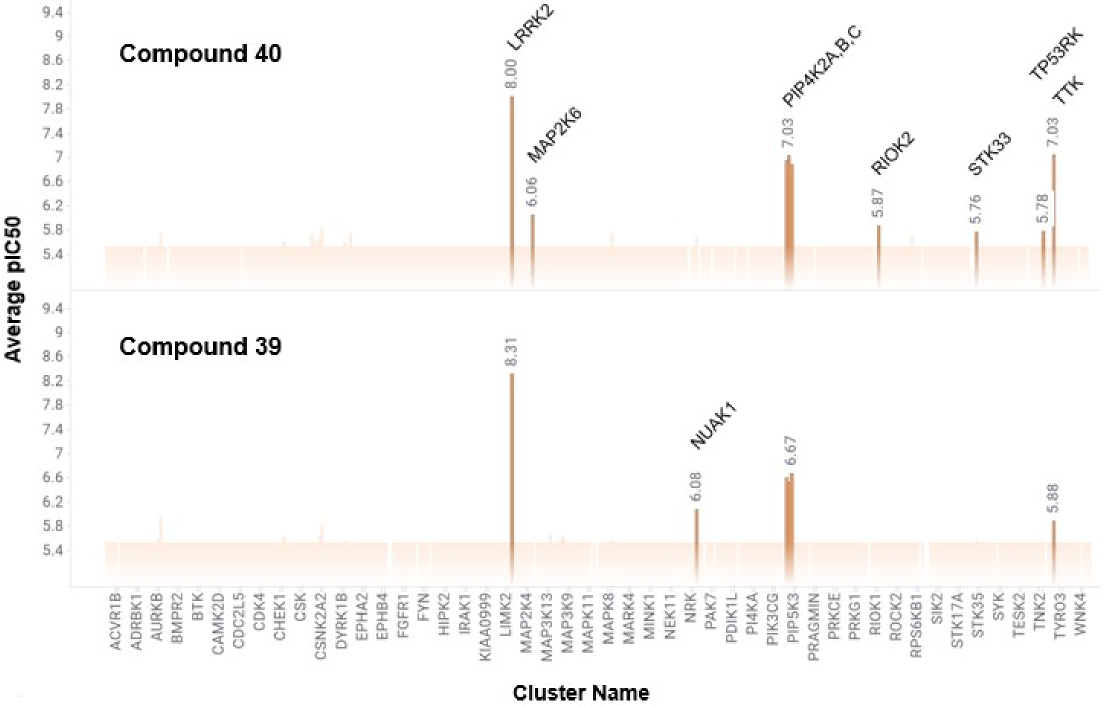


Supplementary Figure S1. Dose-response (A) and time course (B) of the pharmacodynamic effects of compounds **39** in rats. Total LRRK2 levels (red solid symbols and lines) were measured by an ELISA assay in rat brain, kidney and lung after oral administration of a single dose of 5 – 30 mg/kg of compound **39** (4 hr pretreatment) (A: Dose-response) or 3 mg/kg p.o. of Compound **39** (B: Time course)). Compound levels in the tissues at each time point (blue dashed lines and open symbols) correlated with the pharmacodynamic effect. N=3 rats per time point.



Supplementary Figure S2. Dose-response (A) and time course (B) of the pharmacodynamic effects of compounds **39** in mice. Total LRRK2 levels (red solid symbols and lines) were measured by an ELISA assay in mouse brain, kidney and lung after oral administration of a single dose of 3 – 30 mg/kg of compound **39** (4 hr pretreatment) (A: Dose-response) or 3 mg/kg p.o. of Compound **39** (B: Time course))). Compound levels in the tissues at each time point (blue dashed lines and open symbols) correlated with the pharmacodynamic effect. N= 3 rats per time point for (A) and 2 rats per timepoint for (B).

Kinase selectivity determination of compounds 39 and 40. Competition binding assays were conducted as previously described using Kinobeads and Lipid-Kinobeads.[2-3] In brief, one milliliter (1 mL) of cell extract, with a protein concentration of 5 mg/mL, was incubated with the test compound at seven different concentrations ranging from 0.73 nM to 3.0 µM (using ¼ dilution increments) and a vehicle control, for 45 minutes at 4 °C. This was followed by a 1-hour incubation with either Kinobeads or Lipid-Kinobeads. The samples were then processed according to the protocol described.[3]



Supplementary Figure S3. Kinase selectivity profiles of compounds **39** and **40** determined using Kinobeads and Lipid-Kinobeads.

Supplementary Table S1. Individual kinase data of compound **39** determined using Kinobeads and Lipid-Kinobeads (qupm = quantified unique peptides measured).

Clustername	Focusname	Focusgroup	qupm	IC_{50} [uM]	pIC50
AAK1	AAK1	KINASE	35	>3	<5.52
AAK1	AAK1	KINASE	37	>3	<5.52
ABL1	ABL	KINASE	61	>3	<5.52
ABL1	ABL	KINASE	60	>3	<5.52
ABL2	ARG	KINASE	42	>3	<5.52
ABL2	ARG	KINASE	49	>3	<5.52
ACVR1	ALK2	KINASE	13	>3	<5.52
ACVR1	ALK2	KINASE	16	>3	<5.52
ACVR1B	Afg3-like protein 1A, ALK4	PROTEASE, KINASE	12	>3	<5.52
ACVR1B	Afg3-like protein 1A, ALK4	KINASE, PROTEASE	14	>3	<5.52
ACVR2A	ACTR2	KINASE	14	>3	<5.52
ACVR2A	ACTR2	KINASE	10	>3	<5.52
ACVR2B	ACTR2B	KINASE	16	>3	<5.52
ACVR2B	ACTR2B	KINASE	14	>3	<5.52
ACVRL1	ALK1	KINASE	5	>3	<5.52
ADCK1	ADCK1	KINASE	4	>3	<5.52
ADCK1	ADCK1	KINASE	25	>3	<5.52
ADCK1	ADCK1	KINASE	6	>3	<5.52
ADCK1	ADCK1	KINASE	24	>3	<5.52
ADCK4	ADCK4	KINASE	4	>3	<5.52
ADCK5	ADCK5	KINASE	5	>3	<5.52
ADCK5	ADCK5	KINASE	19	>3	<5.52
ADRBK1	BARK1	KINASE	6	>3	<5.52
AKT1	AKT1	KINASE	14	>3	<5.52
AKT1	AKT1	KINASE	13	>3	<5.52
AKT2	AKT2	KINASE	11	>3	<5.52
AKT2	AKT2	KINASE	11	>3	<5.52
ALK	ALK	KINASE	8	>3	<5.52
ARAF	ARAF	KINASE	2	>3	<5.52
ARAF	ARAF	KINASE	17	>3	<5.52
ARAF	ARAF	KINASE	19	>3	<5.52
ATM	ATM	KINASE	51	>3	<5.52
ATM	ATM	KINASE	12	>3	<5.52
ATM	ATM	KINASE	64	>3	<5.52
ATM	ATM	KINASE	52	>3	<5.52
ATR	ATR	KINASE	15	>3	<5.52
ATR	ATR	KINASE	13	>3	<5.52
ATR	ATR	KINASE	6	>3	<5.52
ATR	ATR	KINASE	32	>3	<5.52

AURKA	AurA	KINASE	2	>3	<5.52
AURKA	AurA	KINASE	34	>3	<5.52
AURKA	AurA	KINASE	34	>3	<5.52
AURKB	AurB	KINASE	21	2.3	5.64
AURKB	AurB	KINASE	28	>3	<5.52
AURKC	AurC	KINASE	2	1.1	5.96
AXL	AXL	KINASE	29	>3	<5.52
AXL	AXL	KINASE	15	>3	<5.52
BCR	BCR, complement component C2a	KINASE, PROTEASE	59	>3	<5.52
BCR	BCR	KINASE	41	>3	<5.52
BLK	BLK	KINASE	39	>3	<5.52
BMP2K	BIKE	KINASE	46	>3	<5.52
BMP2K	BIKE	KINASE	48	>3	<5.52
BMPR1A	BMPR1A	KINASE	15	>3	<5.52
BMPR1A	BMPR1A	KINASE	22	>3	<5.52
BMPR1B	BMPR1B	KINASE	13	>3	<5.52
BMPR1B	BMPR1B	KINASE	19	>3	<5.52
BMPR2	BMPR2	KINASE	18	>3	<5.52
BMPR2	BMPR2	KINASE	30	>3	<5.52
BMX	BMX	KINASE	22	>3	<5.52
BMX	BMX	KINASE	9	>3	<5.52
BRAF	BRAF	KINASE	19	>3	<5.52
BRAF	BRAF	KINASE	28	>3	<5.52
BRD2	BRD2	KINASE, BROMO	12	>3	<5.52
BRD2	BRD2	KINASE, BROMO	17	>3	<5.52
BRD3	BRD3	BROMO, KINASE	22	>3	<5.52
BRD3	BRD3	KINASE, BROMO	21	>3	<5.52
BRD4	BRD4	KINASE, BROMO	44	>3	<5.52
BRD4	BRD4	KINASE, BROMO	10	>3	<5.52
BRD4	BRD4	KINASE, BROMO	31	>3	<5.52
BRD4	BRD4	KINASE, BROMO	8	>3	<5.52
BRSK1	BRSK1	KINASE	5	>3	<5.52
BRSK1	BRSK1	KINASE	8	>3	<5.52
BRSK2	BRSK2	KINASE	14	>3	<5.52
BRSK2	BRSK2	KINASE	19	>3	<5.52
BTK	BTK	KINASE	51	>3	<5.52
BTK	BTK	KINASE	55	>3	<5.52
BUB1	BUB1	KINASE	32	>3	<5.52
BUB1	BUB1	KINASE	44	>3	<5.52
BUB1B	BUBR1	KINASE	4	>3	<5.52
CABC1	ADCK3	KINASE	2	>3	<5.52
CABC1	ADCK3	KINASE	28	>3	<5.52
CABC1	ADCK3	KINASE	31	>3	<5.52

CAMK1	CaMK1a	KINASE	4	>3	<5.52
CAMK1D	CaMK1d	KINASE	4	>3	<5.52
CAMK1D	CaMK1d	KINASE	17	>3	<5.52
CAMK2A	CaMK2a	KINASE	2	>3	<5.52
CAMK2A	CaMK2a	KINASE	2	>3	<5.52
CAMK2B	CaMK2b	KINASE	11	>3	<5.52
CAMK2B	CaMK2b	KINASE	8	>3	<5.52
CAMK2D	CaMK2d	KINASE	24	>3	<5.52
CAMK2D	CaMK2d	KINASE	28	>3	<5.52
CAMK2G	CaMK2g	KINASE	22	>3	<5.52
CAMK2G	CaMK2g	KINASE	26	>3	<5.52
CAMK4	CaMK4	KINASE	5	>3	<5.52
CAMK4	CaMK4	KINASE	24	>3	<5.52
CAMKK1	CaMKK1	KINASE	20	>3	<5.52
CAMKK1	CaMKK1	KINASE	12	>3	<5.52
CAMKK2	CaMKK2	KINASE	29	>3	<5.52
CAMKK2	CaMKK2	KINASE	40	>3	<5.52
CASK	CASK	KINASE	6	>3	<5.52
CASK	CASK	KINASE	4	>3	<5.52
CDC2	CDC2	KINASE	14	>3	<5.52
CDC2	CDC2	KINASE	23	>3	<5.52
CDC2L1	PITSLRE	KINASE	6	>3	<5.52
CDC2L5	CHED	KINASE	4	>3	<5.52
CDC2L5	CHED	KINASE	15	>3	<5.52
CDC2L6	CDK11	KINASE	3	>3	<5.52
CDC42BPA	MRCKa	KINASE	34	>3	<5.52
CDC42BPA	MRCKa	KINASE	21	>3	<5.52
CDC42BPB	MRCKb	KINASE	37	>3	<5.52
CDC42BPB	MRCKb	KINASE	41	>3	<5.52
CDC42BPG	DMPK2	KINASE	16	>3	<5.52
CDC42BPG	DMPK2	KINASE	8	>3	<5.52
CDC7	CDC7	KINASE	7	>3	<5.52
CDC7	CDC7	KINASE	13	>3	<5.52
CDK10	CDK10	KINASE	11	>3	<5.52
CDK10	CDK10	KINASE	14	>3	<5.52
CDK2	CDK2	KINASE	2	>3	<5.52
CDK2	CDK2	KINASE	21	>3	<5.52
CDK2	CDK2	KINASE	22	>3	<5.52
CDK4	CDK4	KINASE	16	>3	<5.52
CDK5	CDK5	KINASE	26	>3	<5.52
CDK5	CDK5	KINASE	27	>3	<5.52
CDK6	CDK6	KINASE	4	>3	<5.52
CDK6	CDK6	KINASE	27	>3	<5.52

CDK7	CDK7	KINASE	15	>3	<5.52
CDK7	CDK7	KINASE	16	>3	<5.52
CDK8	CDK8	KINASE	8	>3	<5.52
CDK8	CDK8	KINASE	6	>3	<5.52
CDK9	CDK9	KINASE	22	>3	<5.52
CDK9	CDK9	KINASE	24	>3	<5.52
CDKL2	CDKL2	KINASE	4	>3	<5.52
CDKL5	CDKL5	KINASE	16	>3	<5.52
CDKL5	CDKL5	KINASE	15	>3	<5.52
CHEK1	CHK1	KINASE	22	>3	<5.52
CHEK1	CHK1	KINASE	38	>3	<5.52
CHEK2	CHK2	KINASE	28	2	5.70
CHEK2	CHK2	KINASE	29	2.8	5.55
CHUK	IKKa	KINASE	16	>3	<5.52
CHUK	IKKa	KINASE	22	>3	<5.52
CIT	CRIK	KINASE	82	>3	<5.52
CIT	CRIK	KINASE	141	>3	<5.52
CLK1	CLK1	KINASE	8	>3	<5.52
CLK1	CLK1	KINASE	13	>3	<5.52
CLK2	CLK2	KINASE	2	>3	<5.52
CLK2	CLK2	KINASE	5	>3	<5.52
CRKRS	CRK7	KINASE	16	>3	<5.52
CRKRS	CRK7	KINASE	29	>3	<5.52
CSF1R	FMS	KINASE	15	>3	<5.52
CSK	CSK	KINASE	36	>3	<5.52
CSK	CSK	KINASE	40	>3	<5.52
CSNK1A1	CK1a	KINASE	17	>3	<5.52
CSNK1A1	CK1a	KINASE	24	>3	<5.52
CSNK1D	CK1d	KINASE	6	>3	<5.52
CSNK1D	CK1d	KINASE	10	>3	<5.52
CSNK1E	CK1e	KINASE	12	>3	<5.52
CSNK1E	CK1e	KINASE	12	>3	<5.52
CSNK1G1	CK1g1	KINASE	2	>3	<5.52
CSNK1G1	CK1g1	KINASE	4	>3	<5.52
CSNK1G2	CK1g2	KINASE	2	>3	<5.52
CSNK1G3	CK1g3	KINASE	2	>3	<5.52
CSNK1G3	CK1g3	KINASE	2	>3	<5.52
CSNK2A1	CK2a1	KINASE	13	1.2	5.92
CSNK2A1	CK2a1	KINASE	11	1.4	5.85
CSNK2A1	CK2a1	KINASE	2	>3	<5.52
CSNK2A1	CK2a1	KINASE	6	>3	<5.52
CSNK2A1	CK2a1	KINASE	5	>3	<5.52
CSNK2A1	CK2a1	KINASE	3	>3	<5.52

CSNK2A2	CK2a2	KINASE	22	0.6	6.22
CSNK2A2	CK2a2	KINASE	24	0.98	6.01
CSNK2A2	CK2a2	KINASE	24	>3	<5.52
CSNK2A2	CK2a2	KINASE	27	>3	<5.52
DAPK1	DAPK1	KINASE	35	>3	<5.52
DAPK1	DAPK1	KINASE	12	>3	<5.52
DAPK2	DAPK2	KINASE	2	>3	<5.52
DAPK3	DAPK3	KINASE	4	>3	<5.52
DAPK3	DAPK3	KINASE	7	>3	<5.52
DDR1	DDR1	KINASE	23	>3	<5.52
DDR1	DDR1	KINASE	21	>3	<5.52
DDR2	DDR2	KINASE	9	>3	<5.52
DDR2	DDR2	KINASE	29	>3	<5.52
DSTYK	SgK496	KINASE	18	>3	<5.52
DSTYK	SgK496	KINASE	24	>3	<5.52
DYRK1A	DYRK1A	KINASE	22	>3	<5.52
DYRK1A	DYRK1A	KINASE	23	>3	<5.52
DYRK1B	DYRK1B	KINASE	3	>3	<5.52
DYRK1B	DYRK1B	KINASE	7	>3	<5.52
DYRK2	DYRK2	KINASE	6	2.5	5.60
DYRK2	DYRK2	KINASE	14	>3	<5.52
EGFR	EGFR	KINASE	67	>3	<5.52
EGFR	EGFR	KINASE	56	>3	<5.52
EIF2AK1	HRI	KINASE	18	>3	<5.52
EIF2AK1	HRI	KINASE	20	>3	<5.52
EIF2AK2	PKR	KINASE	9	>3	<5.52
EIF2AK2	PKR	KINASE	15	>3	<5.52
EIF2AK3	PEK	KINASE	13	>3	<5.52
EIF2AK3	PEK	KINASE	21	>3	<5.52
EIF2AK4	GCN2	KINASE	72	>3	<5.52
EIF2AK4	GCN2	KINASE	85	>3	<5.52
EPHA1	EphA1	KINASE	21	>3	<5.52
EPHA2	EphA2	KINASE	37	>3	<5.52
EPHA2	EphA2	KINASE	57	>3	<5.52
EPHA3	EphA3, EphA6	KINASE	37	>3	<5.52
EPHA3	EphA3, EphA6	KINASE	35	>3	<5.52
EPHA4	EphA4	KINASE	33	>3	<5.52
EPHA4	EphA4	KINASE	11	>3	<5.52
EPHA5	EphA5	KINASE	10	>3	<5.52
EPHA5	EphA5	KINASE	6	>3	<5.52
EPHA7	EphA7	KINASE	29	>3	<5.52
EPHA7	EphA7	KINASE	20	>3	<5.52
EPHB1	EphB1	KINASE	20	>3	<5.52

EPHB2	EphB2	KINASE	31	>3	<5.52
EPHB2	EphB2	KINASE	34	>3	<5.52
EPHB3	EphB3	KINASE	28	>3	<5.52
EPHB3	EphB3	KINASE	30	>3	<5.52
EPHB4	EphB4	KINASE	44	>3	<5.52
EPHB4	EphB4	KINASE	49	>3	<5.52
EPHB6	EphB6	KINASE	28	>3	<5.52
EPHB6	EphB6	KINASE	26	>3	<5.52
ERBB4	HER4/ErbB4	KINASE	12	>3	<5.52
ERN1	IRE1	KINASE	24	>3	<5.52
ERN1	IRE1	KINASE	23	>3	<5.52
FER	FER	KINASE	55	>3	<5.52
FER	FER	KINASE	65	>3	<5.52
FES	FES	KINASE	37	>3	<5.52
FGFR1	FGFR1	KINASE	15	>3	<5.52
FGFR1	FGFR1	KINASE	23	>3	<5.52
FGFR2	FGFR2	KINASE	15	>3	<5.52
FGFR2	FGFR2	KINASE	10	>3	<5.52
FGFR3	FGFR3	KINASE	8	>3	<5.52
FGFR3	FGFR3	KINASE	5	>3	<5.52
FGFR4	FGFR4	KINASE	4	>3	<5.52
FGFR4	FGFR4	KINASE	4	>3	<5.52
FGR	FGR	KINASE	14	>3	<5.52
FGR	FGR	KINASE	15	>3	<5.52
FLT4	FLT4	KINASE	32	>3	<5.52
FRK	FRK	KINASE	16	>3	<5.52
FRK	FRK	KINASE	3	>3	<5.52
FYN	FYN	KINASE	30	>3	<5.52
FYN	FYN	KINASE	33	>3	<5.52
GAK	GAK	KINASE	76	>3	<5.52
GAK	GAK	KINASE	83	>3	<5.52
GSG2	Haspin	KINASE	2	>3	<5.52
GSG2	Haspin	KINASE	2	>3	<5.52
GSK3A	GSK3A	KINASE	3	>3	<5.52
GSK3A	GSK3A	KINASE	15	>3	<5.52
GSK3A	GSK3A	KINASE	2	>3	<5.52
GSK3A	GSK3A	KINASE	17	>3	<5.52
GSK3B	GSK3B	KINASE	5	>3	<5.52
GSK3B	GSK3B	KINASE	22	>3	<5.52
GSK3B	GSK3B	KINASE	8	>3	<5.52
GSK3B	GSK3B	KINASE	24	>3	<5.52
HCK	HCK	KINASE	24	>3	<5.52
HIPK1	HIPK1	KINASE	11	>3	<5.52

HIPK1	HIPK1	KINASE	14	>3	<5.52
HIPK2	HIPK2	KINASE	9	>3	<5.52
HIPK2	HIPK2	KINASE	11	>3	<5.52
HIPK3	HIPK3	KINASE	6	>3	<5.52
HIPK3	HIPK3	KINASE	9	>3	<5.52
ICK	ICK	KINASE	8	>3	<5.52
ICK	ICK	KINASE	14	>3	<5.52
IGF1R	IGF1R	KINASE	40	>3	<5.52
IGF1R	IGF1R	KINASE	39	>3	<5.52
IKBKB	IKKb	KINASE	17	>3	<5.52
IKBKB	IKKb	KINASE	19	>3	<5.52
IKBKE	IKKe	KINASE	26	>3	<5.52
IKBKE	IKKe	KINASE	46	>3	<5.52
ILK	ILK	KINASE	13	>3	<5.52
ILK	ILK	KINASE	17	>3	<5.52
INSR	INSR	KINASE	51	>3	<5.52
INSR	INSR	KINASE	42	>3	<5.52
IRAK1	IRAK1	KINASE	19	>3	<5.52
IRAK1	IRAK1	KINASE	21	>3	<5.52
IRAK3	IRAK3	KINASE	13	>3	<5.52
IRAK4	IRAK4	KINASE	35	>3	<5.52
IRAK4	IRAK4	KINASE	39	>3	<5.52
ITK	ITK	KINASE	23	>3	<5.52
ITK	ITK	KINASE	45	>3	<5.52
JAK1	JAK1	KINASE	75	>3	<5.52
JAK1	JAK1	KINASE	94	>3	<5.52
JAK2	JAK2	KINASE	30	>3	<5.52
JAK2	JAK2	KINASE	55	>3	<5.52
JAK3	JAK3	KINASE	13	>3	<5.52
JAK3	JAK3	KINASE	55	>3	<5.52
KDR	KDR	KINASE	20	>3	<5.52
KIAA0999	QSK	KINASE	31	>3	<5.52
KIAA0999	QSK	KINASE	47	>3	<5.52
KIAA1804	MLK4	KINASE	25	>3	<5.52
KIAA1804	MLK4	KINASE	18	>3	<5.52
KIT	KIT	KINASE	17	>3	<5.52
KIT	KIT	KINASE	23	>3	<5.52
KSR1	KSR1	KINASE	11	>3	<5.52
KSR1	KSR1	KINASE	4	>3	<5.52
LATS1	LATS1	KINASE	41	>3	<5.52
LATS1	LATS1	KINASE	41	>3	<5.52
LATS2	LATS2	KINASE	7	>3	<5.52
LATS2	LATS2	KINASE	7	>3	<5.52

LCK	LCK	KINASE	11	>3	<5.52
LCK	LCK	KINASE	32	>3	<5.52
LIMK1	LIMK1	KINASE	26	>3	<5.52
LIMK1	LIMK1	KINASE	39	>3	<5.52
LIMK2	LIMK2	KINASE	23	>3	<5.52
LIMK2	LIMK2	KINASE	24	>3	<5.52
LOC100128443	MAST4	KINASE	14	>3	<5.52
LOC100128443	MAST4	KINASE	5	>3	<5.52
LRRK2	LRRK2	KINASE	7	0.0049	8.31
LTK	LTK	KINASE	3	>3	<5.52
LYN	LYN	KINASE	37	>3	<5.52
LYN	LYN	KINASE	50	>3	<5.52
MAP2K1	MAP2K1	KINASE	15	>3	<5.52
MAP2K1	MAP2K1	KINASE	13	>3	<5.52
MAP2K2	MAP2K2	KINASE	15	>3	<5.52
MAP2K2	MAP2K2	KINASE	22	>3	<5.52
MAP2K3	MAP2K3	KINASE	12	>3	<5.52
MAP2K3	MAP2K3	KINASE	15	>3	<5.52
MAP2K4	MAP2K4	KINASE	22	>3	<5.52
MAP2K4	MAP2K4	KINASE	17	>3	<5.52
MAP2K5	MAP2K5	KINASE	21	>3	<5.52
MAP2K5	MAP2K5	KINASE	24	>3	<5.52
MAP2K6	MAP2K6	KINASE	17	>3	<5.52
MAP2K6	MAP2K6	KINASE	23	>3	<5.52
MAP3K1	MAP3K1	KINASE	36	>3	<5.52
MAP3K1	MAP3K1	KINASE	47	>3	<5.52
MAP3K10	MLK2	KINASE	3	>3	<5.52
MAP3K11	MLK3	KINASE	23	>3	<5.52
MAP3K11	MLK3	KINASE	31	>3	<5.52
MAP3K12	DLK	KINASE	20	>3	<5.52
MAP3K13	LZK	KINASE	4	>3	<5.52
MAP3K13	LZK	KINASE	5	>3	<5.52
MAP3K15	MAP3K7	KINASE	14	1.6	5.80
MAP3K15	MAP3K7	KINASE	2	>3	<5.52
MAP3K2	MAP3K2	KINASE	18	>3	<5.52
MAP3K2	MAP3K2	KINASE	25	>3	<5.52
MAP3K3	MAP3K3	KINASE	22	>3	<5.52
MAP3K3	MAP3K3	KINASE	25	>3	<5.52
MAP3K4	MAP3K4	KINASE	70	>3	<5.52
MAP3K4	MAP3K4	KINASE	66	>3	<5.52
MAP3K5	MAP3K5	KINASE	50	2.4	5.62
MAP3K5	MAP3K5	KINASE	50	>3	<5.52
MAP3K6	MAP3K6	KINASE	28	1.8	5.74

MAP3K6	MAP3K6	KINASE	17	>3	<5.52
MAP3K7	TAK1	KINASE	22	>3	<5.52
MAP3K7	TAK1	KINASE	25	>3	<5.52
MAP3K9	MLK1	KINASE	5	>3	<5.52
MAP3K9	MLK1	KINASE	18	>3	<5.52
MAP4K1	HPK1	KINASE	5	>3	<5.52
MAP4K1	HPK1	KINASE	30	>3	<5.52
MAP4K2	GCK	KINASE	23	>3	<5.52
MAP4K2	GCK	KINASE	31	>3	<5.52
MAP4K3	KHS2	KINASE	18	>3	<5.52
MAP4K3	KHS2	KINASE	20	>3	<5.52
MAP4K4	ZC1/HGK	KINASE	36	>3	<5.52
MAP4K4	ZC1/HGK	KINASE	68	>3	<5.52
MAP4K5	KHS1	KINASE	30	>3	<5.52
MAP4K5	KHS1	KINASE	31	>3	<5.52
MAPK1	Erk2	KINASE	3	>3	<5.52
MAPK1	Erk2	KINASE	21	>3	<5.52
MAPK1	Erk2	KINASE	5	>3	<5.52
MAPK1	Erk2	KINASE	25	>3	<5.52
MAPK10	JNK3	KINASE	3	>3	<5.52
MAPK10	JNK3	KINASE	10	>3	<5.52
MAPK11	p38b	KINASE	7	>3	<5.52
MAPK11	p38b	KINASE	9	>3	<5.52
MAPK14	p38a	KINASE	25	>3	<5.52
MAPK14	p38a	KINASE	27	>3	<5.52
MAPK3	Erk1	KINASE	14	>3	<5.52
MAPK3	Erk1	KINASE	18	>3	<5.52
MAPK4	Erk4	KINASE	3	>3	<5.52
MAPK6	Erk3	KINASE	9	>3	<5.52
MAPK6	Erk3	KINASE	10	>3	<5.52
MAPK7	Erk5	KINASE	22	>3	<5.52
MAPK7	Erk5	KINASE	23	>3	<5.52
MAPK8	JNK1	KINASE	17	>3	<5.52
MAPK8	JNK1	KINASE	20	>3	<5.52
MAPK9	JNK2	KINASE	4	1.8	5.74
MAPK9	JNK2	KINASE	23	>3	<5.52
MAPK9	JNK2	KINASE	3	>3	<5.52
MAPK9	JNK2	KINASE	24	>3	<5.52
MAPKAPK2	MAPKAPK2	KINASE	7	>3	<5.52
MAPKAPK3	MAPKAPK3	KINASE	8	>3	<5.52
MAPKAPK3	MAPKAPK3	KINASE	8	>3	<5.52
MAPKAPK5	MAPKAPK5	KINASE	6	>3	<5.52
MAPKAPK5	MAPKAPK5	KINASE	9	>3	<5.52

MARK1	MARK1	KINASE	23	>3	<5.52
MARK1	MARK1	KINASE	26	>3	<5.52
MARK2	MARK2	KINASE	41	>3	<5.52
MARK2	MARK2	KINASE	58	>3	<5.52
MARK3	MARK3	KINASE	31	>3	<5.52
MARK3	MARK3	KINASE	42	>3	<5.52
MARK4	MARK4	KINASE	19	>3	<5.52
MARK4	MARK4	KINASE	31	>3	<5.52
MAST1	MAST1	KINASE	8	>3	<5.52
MAST2	MAST2	KINASE	12	>3	<5.52
MAST2	MAST2	KINASE	19	>3	<5.52
MAST3	MAST3	KINASE	22	>3	<5.52
MAST3	MAST3	KINASE	35	>3	<5.52
MASTL	MASTL	KINASE	13	>3	<5.52
MASTL	MASTL	KINASE	21	>3	<5.52
MELK	MELK	KINASE	31	>3	<5.52
MELK	MELK	KINASE	41	>3	<5.52
MERTK	MER	KINASE	20	>3	<5.52
MERTK	MER	KINASE	25	>3	<5.52
MET	MET	KINASE	42	>3	<5.52
MINK1	ZC3/MINK	KINASE	30	>3	<5.52
MINK1	ZC3/MINK	KINASE	44	>3	<5.52
MKNK1	MNK1	KINASE	8	>3	<5.52
MKNK1	MNK1	KINASE	6	>3	<5.52
MKNK2	MNK2	KINASE	8	>3	<5.52
MKNK2	MNK2	KINASE	9	>3	<5.52
MST1R	RON	KINASE	13	>3	<5.52
mTOR	FRAP	KINASE	111	>3	<5.52
mTOR	FRAP	KINASE	51	>3	<5.52
mTOR	FRAP	KINASE	139	>3	<5.52
mTOR	FRAP	KINASE	50	>3	<5.52
MYLK	smMLCK	KINASE	37	>3	<5.52
MYLK	smMLCK	KINASE	13	>3	<5.52
MYLK3	CaMLCK	KINASE	14	>3	<5.52
MYLK3	CaMLCK	KINASE	2	>3	<5.52
NEK1	NEK1	KINASE	46	>3	<5.52
NEK1	NEK1	KINASE	46	>3	<5.52
NEK11	NEK11	KINASE	3	>3	<5.52
NEK2	NEK2	KINASE	24	>3	<5.52
NEK2	NEK2	KINASE	30	>3	<5.52
NEK3	NEK3	KINASE	8	>3	<5.52
NEK3	NEK3	KINASE	18	>3	<5.52
NEK6	NEK6	KINASE	10	>3	<5.52

NEK6	NEK6	KINASE	16	>3	<5.52
NEK7	NEK7	KINASE	11	>3	<5.52
NEK7	NEK7	KINASE	12	>3	<5.52
NEK9	NEK9	KINASE	56	>3	<5.52
NEK9	NEK9	KINASE	65	>3	<5.52
NLK	NLK	KINASE	13	>3	<5.52
NLK	NLK	KINASE	15	>3	<5.52
NPR2	ANPb	KINASE	2	>3	<5.52
NRK	ZC4/NRK	KINASE	8	>3	<5.52
NTRK1	TRKA	KINASE	18	>3	<5.52
NTRK1	TRKA	KINASE	9	>3	<5.52
NUAK1	NuaK1	KINASE	10	0.8	6.10
NUAK1	NuaK1	KINASE	9	0.85	6.07
NUAK2	NuaK2	KINASE	5	>3	<5.52
OXSR1	OSR1	KINASE	17	>3	<5.52
OXSR1	OSR1	KINASE	18	>3	<5.52
PAK4	PAK4	KINASE	32	>3	<5.52
PAK4	PAK4	KINASE	33	>3	<5.52
PAK6	PAK6	KINASE	5	>3	<5.52
PAK6	PAK6	KINASE	6	>3	<5.52
PAK7	PAK5	KINASE	12	>3	<5.52
PBK	PBK	KINASE	2	>3	<5.52
PBK	PBK	KINASE	2	>3	<5.52
PCTK1	PCTAIRE1	KINASE	23	>3	<5.52
PCTK1	PCTAIRE1	KINASE	19	>3	<5.52
PCTK2	PCTAIRE2	KINASE	15	>3	<5.52
PCTK2	PCTAIRE2	KINASE	18	>3	<5.52
PCTK3	PCTAIRE3	KINASE	13	>3	<5.52
PCTK3	PCTAIRE3	KINASE	16	>3	<5.52
PDGFRA	PDGFRa	KINASE	10	>3	<5.52
PDGFRA	PDGFRa	KINASE	34	>3	<5.52
PDGFRB	PDGFRb	KINASE	19	>3	<5.52
PDGFRB	PDGFRb	KINASE	23	>3	<5.52
PDIK1L	CLIK1L	KINASE	9	>3	<5.52
PDIK1L	CLIK1L	KINASE	6	>3	<5.52
PDK1	PDHK1	KINASE	2	>3	<5.52
PDPK1	PDK1	KINASE	23	>3	<5.52
PDPK1	PDK1	KINASE	25	>3	<5.52
PFTK1	PFTAIRE1	KINASE	7	>3	<5.52
PFTK1	PFTAIRE1	KINASE	14	>3	<5.52
PHKG2	PHKg2	KINASE	14	>3	<5.52
PHKG2	PHKg2	KINASE	20	>3	<5.52
PI4K2A	PIK4C2A	KINASE	5	>3	<5.52

PI4K2B	PIK4C2B	KINASE	4	>3	<5.52
PI4K2B	PIK4C2B	KINASE	7	>3	<5.52
PI4K2B	PIK4C2B	KINASE	7	>3	<5.52
PI4KA	PIK4Ca	KINASE	47	>3	<5.52
PI4KA	PIK4Ca	KINASE	4	>3	<5.52
PI4KA	PIK4Ca	KINASE	12	>3	<5.52
PI4KA	PIK4Ca	KINASE	51	>3	<5.52
PI4KA	PIK4Ca	KINASE	4	>3	<5.52
PI4KA	PIK4Ca	KINASE	10	>3	<5.52
PI4KB	PIK4Cb	KINASE	23	>3	<5.52
PI4KB	PIK4Cb	KINASE	22	>3	<5.52
PIK3C2A	PIK3C2a	KINASE	30	>3	<5.52
PIK3C2A	PIK3C2a	KINASE	40	>3	<5.52
PIK3C2A	PIK3C2a	KINASE	2	>3	<5.52
PIK3C2B	PIK3C2b	KINASE	68	>3	<5.52
PIK3C2B	complement component C2a, PIK3C2b	PROTEASE, KINASE	15	>3	<5.52
PIK3C2B	PIK3C2b	KINASE	71	>3	<5.52
PIK3C2B	PIK3C2b	KINASE	6	>3	<5.52
PIK3C3	PIK3C3	KINASE	50	>3	<5.52
PIK3C3	complement component C2a, PIK3C3	PROTEASE, KINASE	21	>3	<5.52
PIK3C3	PIK3C3	KINASE	55	>3	<5.52
PIK3C3	PIK3C3	KINASE	19	>3	<5.52
PIK3CA	PIK3Ca	KINASE	67	>3	<5.52
PIK3CA	PIK3Ca	KINASE	67	>3	<5.52
PIK3CA	PIK3Ca	KINASE	2	>3	<5.52
PIK3CB	PIK3Cb	KINASE	48	>3	<5.52
PIK3CB	PIK3Cb	KINASE	63	>3	<5.52
PIK3CB	PIK3Cb	KINASE	12	>3	<5.52
PIK3CD	PIK3Cd	KINASE	55	>3	<5.52
PIK3CD	PIK3Cd	KINASE	65	>3	<5.52
PIK3CD	PIK3Cd	KINASE	4	>3	<5.52
PIK3CG	PIK3Cg	KINASE	53	>3	<5.52
PIK3CG	PIK3Cg	KINASE	57	>3	<5.52
PIK3CG	PIK3Cg	KINASE	2	>3	<5.52
PIK3R4	PIK3R4	KINASE	65	>3	<5.52
PIK3R4	PIK3R4	KINASE	16	>3	<5.52
PIK3R4	PIK3R4	KINASE	64	>3	<5.52
PIK3R4	PIK3R4	KINASE	23	>3	<5.52
PIM1	PIM1	KINASE	2	>3	<5.52
PIM1	PIM1	KINASE	14	>3	<5.52
PIM1	PIM1	KINASE	7	>3	<5.52
PIM1	PIM1	KINASE	10	>3	<5.52

PIM2	PIM2	KINASE	9	>3	<5.52
PIM2	PIM2	KINASE	12	>3	<5.52
PIM3	PIM3	KINASE	6	>3	<5.52
PIM3	PIM3	KINASE	6	>3	<5.52
PIP4K2A	PIP5K2A	KINASE	11	0.23	6.64
PIP4K2A	PIP5K2A	KINASE	14	0.27	6.57
PIP4K2B	PIP5K2B	KINASE	11	0.28	6.55
PIP4K2B	PIP5K2B	KINASE	17	0.32	6.49
PIP4K2C	PIP5K2C	KINASE	18	0.2	6.70
PIP4K2C	PIP5K2C	KINASE	25	0.23	6.64
PIP5K3	PIP5K3	KINASE	40	>3	<5.52
PIP5K3	PIP5K3	KINASE	76	>3	<5.52
PIP5K3	PIP5K3	KINASE	52	>3	<5.52
PIP5K3	PIP5K3	KINASE	96	>3	<5.52
PKMYT1	MYT1	KINASE	17	>3	<5.52
PKMYT1	MYT1	KINASE	21	>3	<5.52
PKN1	complement component C2a, PKN1	PROTEASE, KINASE	15	>3	<5.52
PKN1	PKN1	KINASE	27	>3	<5.52
PKN2	complement component C2a, PKN2	PROTEASE, KINASE	30	>3	<5.52
PKN2	PKN2	KINASE	34	>3	<5.52
PKN3	complement component C2a, PKN3	PROTEASE, KINASE	19	>3	<5.52
PKN3	PKN3	KINASE	7	>3	<5.52
PLK1	PLK1	KINASE	20	>3	<5.52
PLK1	PLK1	KINASE	23	>3	<5.52
PLK4	PLK4	KINASE	23	>3	<5.52
PLK4	PLK4	KINASE	40	>3	<5.52
PRAGMIN	SgK223	KINASE	13	>3	<5.52
PRKAA1	AMPKa1	KINASE	36	>3	<5.52
PRKAA1	AMPKa1	KINASE	43	>3	<5.52
PRKAA2	AMPKa2	KINASE	17	>3	<5.52
PRKAA2	AMPKa2	KINASE	15	>3	<5.52
PRKACA	PKACa	KINASE	5	>3	<5.52
PRKACA	PKACa	KINASE	4	>3	<5.52
PRKACB	PKACb, SgK494	KINASE	3	>3	<5.52
PRKACB	PKACb, SgK494	KINASE	4	>3	<5.52
PRKCA	complement component C2a, PKCa	PROTEASE, KINASE	24	>3	<5.52
PRKCA	PKCa	KINASE	26	>3	<5.52
PRKCB	complement component C2a, PKCb	PROTEASE, KINASE	24	>3	<5.52
PRKCB	PKCb	KINASE	18	>3	<5.52
PRKCD	complement component C2a, PKCd	PROTEASE, KINASE	28	>3	<5.52
PRKCD	PKCd	KINASE	28	>3	<5.52
PRKCE	complement component C2a, PKCe	PROTEASE, KINASE	2	>3	<5.52
PRKCE	PKCe	KINASE	3	>3	<5.52

PRKCI	PKCi	KINASE	4	>3	<5.52
PRKCI	PKCi	KINASE	16	>3	<5.52
PRKCQ	PKCt	KINASE	35	>3	<5.52
PRKCQ	PKCt	KINASE	39	>3	<5.52
PRKCZ	PKCz	KINASE	4	>3	<5.52
PRKCZ	PKCz	KINASE	6	>3	<5.52
PRKD1	PKD1	KINASE	18	>3	<5.52
PRKD2	PKD2	KINASE	27	>3	<5.52
PRKD2	PKD2	KINASE	35	>3	<5.52
PRKD3	PKD3	KINASE	17	>3	<5.52
PRKD3	PKD3	KINASE	33	>3	<5.52
PRKDC	DNAPK	KINASE	319	>3	<5.52
PRKDC	DNAPK	KINASE	212	>3	<5.52
PRKDC	DNAPK	KINASE	330	>3	<5.52
PRKDC	DNAPK	KINASE	218	>3	<5.52
PRKG1	PKG1	KINASE	45	>3	<5.52
PRKG1	PKG1	KINASE	50	>3	<5.52
PRKX	PRKX	KINASE	7	>3	<5.52
PRKX	PRKX	KINASE	10	>3	<5.52
PTK2	FAK	KINASE	71	>3	<5.52
PTK2	FAK	KINASE	85	>3	<5.52
PTK2B	PYK2	KINASE	77	>3	<5.52
PTK2B	PYK2	KINASE	95	>3	<5.52
PTK6	BRK	KINASE	3	>3	<5.52
RAF1	RAF1	KINASE	3	>3	<5.52
RAF1	RAF1	KINASE	7	>3	<5.52
RET	RET	KINASE	39	>3	<5.52
RIOK1	RIOK1	KINASE	3	>3	<5.52
RIOK2	RIOK2	KINASE	3	>3	<5.52
RIOK2	RIOK2	KINASE	28	>3	<5.52
RIOK2	RIOK2	KINASE	5	>3	<5.52
RIOK2	RIOK2	KINASE	33	>3	<5.52
RIPK1	RIPK1	KINASE	3	>3	<5.52
RIPK1	RIPK1	KINASE	6	>3	<5.52
RIPK2	RIPK2	KINASE	9	>3	<5.52
RIPK2	RIPK2	KINASE	27	>3	<5.52
RIPK2	RIPK2	KINASE	13	>3	<5.52
RIPK2	RIPK2	KINASE	32	>3	<5.52
RIPK3	RIPK3	KINASE	3	>3	<5.52
RIPK3	RIPK3	KINASE	24	>3	<5.52
RIPK4	ANKRD3	KINASE	11	>3	<5.52
RIPK4	ANKRD3	KINASE	12	>3	<5.52
RNASEL	RNAseL	KINASE	3	>3	<5.52

RNASEL	RNAseL	KINASE	5	>3	<5.52
ROCK1	ROCK1	KINASE	7	>3	<5.52
ROCK1	ROCK1	KINASE	5	>3	<5.52
ROCK2	ROCK2	KINASE	17	>3	<5.52
ROCK2	ROCK2	KINASE	22	>3	<5.52
RP6-213H19 1	MST4	KINASE	5	>3	<5.52
RP6-213H19 1	MST4	KINASE	3	>3	<5.52
RPS6KA1	RSK3	KINASE	33	>3	<5.52
RPS6KA1	RSK3	KINASE	37	>3	<5.52
RPS6KA2	RSK1	KINASE	10	>3	<5.52
RPS6KA2	RSK1	KINASE	14	>3	<5.52
RPS6KA3	RSK2	KINASE	34	>3	<5.52
RPS6KA3	RSK2	KINASE	39	>3	<5.52
RPS6KA4	MSK2	KINASE	36	>3	<5.52
RPS6KA4	MSK2	KINASE	48	>3	<5.52
RPS6KA5	MSK1	KINASE	36	>3	<5.52
RPS6KA5	MSK1	KINASE	26	>3	<5.52
RPS6KA6	RSK4	KINASE	13	>3	<5.52
RPS6KA6	RSK4	KINASE	37	>3	<5.52
RPS6KB1	p70S6K	KINASE	12	>3	<5.52
RPS6KB1	p70S6K	KINASE	14	>3	<5.52
SBK1	SBK	KINASE	8	>3	<5.52
SGK1	SGK1	KINASE	2	>3	<5.52
SGK1	SGK1	KINASE	5	>3	<5.52
SGK3	SGK3	KINASE	9	>3	<5.52
SGK3	SGK3	KINASE	12	>3	<5.52
SIK1	SIK	KINASE	5	>3	<5.52
SIK1	SIK	KINASE	7	>3	<5.52
SIK2	QIK	KINASE	32	>3	<5.52
SIK2	QIK	KINASE	38	>3	<5.52
SLK	SLK	KINASE	82	>3	<5.52
SLK	SLK	KINASE	86	>3	<5.52
SMG1	SMG1	KINASE	4	>3	<5.52
SNRK	SNRK	KINASE	13	>3	<5.52
SNRK	SNRK	KINASE	10	>3	<5.52
SRC	SRC	KINASE	20	>3	<5.52
SRC	SRC	KINASE	22	>3	<5.52
STK10	LOK	KINASE	58	>3	<5.52
STK10	LOK	KINASE	80	>3	<5.52
STK11	LKB1	KINASE	8	>3	<5.52
STK11	LKB1	KINASE	8	>3	<5.52
STK16	MPSK1	KINASE	14	>3	<5.52
STK16	MPSK1	KINASE	15	>3	<5.52

STK17A	DRAK1, SgK085	KINASE	6	>3	<5.52
STK17A	DRAK1, SgK085	KINASE	14	>3	<5.52
STK17B	DRAK2	KINASE	8	>3	<5.52
STK17B	DRAK2	KINASE	9	>3	<5.52
STK24	MST3	KINASE	4	>3	<5.52
STK25	YSK1	KINASE	2	>3	<5.52
STK25	YSK1	KINASE	2	>3	<5.52
STK3	MST2	KINASE	18	>3	<5.52
STK3	MST2	KINASE	15	>3	<5.52
STK32B	YANK1, YANK2	KINASE	8	>3	<5.52
STK32C	YANK3	KINASE	2	>3	<5.52
STK33	STK33	KINASE	24	2.3	5.64
STK33	STK33	KINASE	21	>3	<5.52
STK35	CLIK1	KINASE	18	>3	<5.52
STK35	CLIK1	KINASE	24	>3	<5.52
STK36	Fused	KINASE	5	>3	<5.52
STK38	NDR1	KINASE	17	>3	<5.52
STK38	NDR1	KINASE	25	>3	<5.52
STK38L	NDR2	KINASE	11	>3	<5.52
STK38L	NDR2	KINASE	13	>3	<5.52
STK39	STLK3	KINASE	13	>3	<5.52
STK39	STLK3	KINASE	13	>3	<5.52
STK4	MST1	KINASE	25	>3	<5.52
STK4	MST1	KINASE	28	>3	<5.52
STRADA	STLK5	KINASE	9	>3	<5.52
STRADA	STLK5	KINASE	14	>3	<5.52
SYK	SYK	KINASE	28	>3	<5.52
SYK	SYK	KINASE	35	>3	<5.52
TAOK1	TAO1	KINASE	6	>3	<5.52
TAOK1	TAO1	KINASE	6	>3	<5.52
TAOK2	TAO2	KINASE	9	>3	<5.52
TAOK2	TAO2	KINASE	19	>3	<5.52
TAOK3	TAO3	KINASE	16	>3	<5.52
TAOK3	TAO3	KINASE	33	>3	<5.52
TBK1	TBK1	KINASE	45	>3	<5.52
TBK1	TBK1	KINASE	56	>3	<5.52
TEC	TEC	KINASE	52	>3	<5.52
TEC	TEC	KINASE	53	>3	<5.52
TEK	TIE2	KINASE	24	>3	<5.52
TESK1	TESK1	KINASE	11	>3	<5.52
TESK1	TESK1	KINASE	18	>3	<5.52
TESK2	TESK2	KINASE	16	>3	<5.52
TESK2	TESK2	KINASE	14	>3	<5.52

TGFBR1	TGFbR1	KINASE	25	>3	<5.52
TGFBR1	TGFbR1	KINASE	22	>3	<5.52
TGFBR2	TGFbR2	KINASE	22	>3	<5.52
TGFBR2	TGFbR2	KINASE	19	>3	<5.52
TIE1	TIE1	KINASE	2	>3	<5.52
TLK1	TLK1	KINASE	2	>3	<5.52
TLK1	TLK1	KINASE	3	>3	<5.52
TLK2	TLK2	KINASE	3	>3	<5.52
TLK2	TLK2	KINASE	3	>3	<5.52
TNIK	ZC2/TNIK	KINASE	44	>3	<5.52
TNIK	ZC2/TNIK	KINASE	41	>3	<5.52
TNK1	TNK1	KINASE	21	>3	<5.52
TNK1	TNK1	KINASE	4	>3	<5.52
TNK2	ACK	KINASE	27	>3	<5.52
TNK2	ACK	KINASE	32	>3	<5.52
TP53RK	PRPK	KINASE	12	>3	<5.52
TP53RK	PRPK	KINASE	13	>3	<5.52
TRIO	Trio	KINASE	5	>3	<5.52
TRRAP	TRRAP	KINASE	3	>3	<5.52
TRRAP	TRRAP	KINASE	20	>3	<5.52
TRRAP	TRRAP	KINASE	3	>3	<5.52
TRRAP	TRRAP	KINASE	26	>3	<5.52
TTK	TTK	KINASE	32	1.1	5.96
TTK	TTK	KINASE	37	1.6	5.80
TTN	TTN	KINASE	3	>3	<5.52
TYK2	TYK2	KINASE	57	>3	<5.52
TYK2	TYK2	KINASE	55	>3	<5.52
TYRO3	TYRO3	KINASE	10	>3	<5.52
TYRO3	TYRO3	KINASE	9	>3	<5.52
UHMK1	KIS	KINASE	3	>3	<5.52
UHMK1	KIS	KINASE	7	>3	<5.52
ULK1	ULK1	KINASE	23	>3	<5.52
ULK1	ULK1	KINASE	27	>3	<5.52
ULK2	ULK2	KINASE	2	>3	<5.52
ULK2	ULK2	KINASE	5	>3	<5.52
ULK3	ULK3	KINASE	26	>3	<5.52
ULK3	ULK3	KINASE	29	>3	<5.52
WEE1	Wee1, Wee1B	KINASE	39	>3	<5.52
WEE1	Wee1, Wee1B	KINASE	46	>3	<5.52
WNK1	Wnk1	KINASE	9	>3	<5.52
WNK1	Wnk1	KINASE	27	>3	<5.52
WNK2	Wnk2	KINASE	7	>3	<5.52
YES1	YES	KINASE	19	>3	<5.52

YES1	YES	KINASE	17	>3	<5.52
ZAK	ZAK	KINASE	20	>3	<5.52
ZAK	ZAK	KINASE	23	>3	<5.52
ZAP70	ZAP70	KINASE	5	>3	<5.52

Supplementary Table S2. Individual kinase data of compound **40** determined using Kinobeads and Lipid-Kinobeads (qupm = quantified unique peptides measured).

Clustername	Focusname	Focusgroup	qupm	IC_{50} [uM]	pIC50
AAK1	AAK1	KINASE	38	>3	<5.52
AAK1	AAK1	KINASE	38	>3	<5.52
ABL1	ABL	KINASE	69	>3	<5.52
ABL1	ABL	KINASE	78	>3	<5.52
ABL2	ARG	KINASE	49	>3	<5.52
ABL2	ARG	KINASE	59	>3	<5.52
ACVR1	ALK2	KINASE	18	>3	<5.52
ACVR1	ALK2	KINASE	20	>3	<5.52
ACVR1B	Afg3-like protein 1A, ALK4	PROTEASE, KINASE	17	>3	<5.52
ACVR1B	Afg3-like protein 1A, ALK4	KINASE, PROTEASE	16	>3	<5.52
ACVR1C	ALK7	KINASE	2	>3	<5.52
ACVR2A	ACTR2	KINASE	16	>3	<5.52
ACVR2A	ACTR2	KINASE	17	>3	<5.52
ACVR2B	ACTR2B	KINASE	20	>3	<5.52
ACVR2B	ACTR2B	KINASE	16	>3	<5.52
ACVRL1	ALK1	KINASE	7	>3	<5.52
ACVRL1	ALK1	KINASE	10	>3	<5.52
ADCK1	ADCK1	KINASE	26	>3	<5.52
ADCK1	ADCK1	KINASE	4	>3	<5.52
ADCK1	ADCK1	KINASE	24	>3	<5.52
ADCK1	ADCK1	KINASE	5	>3	<5.52
ADCK4	ADCK4	KINASE	7	>3	<5.52
ADCK4	ADCK4	KINASE	4	>3	<5.52
ADCK5	ADCK5	KINASE	9	>3	<5.52
ADCK5	ADCK5	KINASE	9	>3	<5.52
ADRBK1	BARK1	KINASE	2	>3	<5.52
AKT1	AKT1	KINASE	15	>3	<5.52
AKT1	AKT1	KINASE	18	>3	<5.52
AKT2	AKT2	KINASE	14	>3	<5.52
AKT2	AKT2	KINASE	16	>3	<5.52
ARAF	ARAF	KINASE	15	>3	<5.52
ARAF	ARAF	KINASE	17	>3	<5.52
ARAF	ARAF	KINASE	2	>3	<5.52
ATM	ATM	KINASE	38	>3	<5.52
ATM	ATM	KINASE	65	>3	<5.52
ATM	ATM	KINASE	28	>3	<5.52
ATM	ATM	KINASE	51	>3	<5.52
ATR	ATR	KINASE	25	>3	<5.52
ATR	ATR	KINASE	4	>3	<5.52

ATR	ATR	KINASE	12	>3	<5.52
ATR	ATR	KINASE	13	>3	<5.52
AURKA	AurA	KINASE	34	>3	<5.52
AURKA	AurA	KINASE	34	>3	<5.52
AURKB	AurB	KINASE	27	>3	<5.52
AURKB	AurB	KINASE	27	>3	<5.52
AURKC	AurC	KINASE	4	1.5	5.82
AURKC	AurC	KINASE	5	2.1	5.68
AXL	AXL	KINASE	30	>3	<5.52
AXL	AXL	KINASE	32	>3	<5.52
BCR	BCR, complement component C2a	KINASE, PROTEASE	67	>3	<5.52
BCR	BCR	KINASE	68	>3	<5.52
BMP2K	BIKE	KINASE	51	>3	<5.52
BMP2K	BIKE	KINASE	51	>3	<5.52
BMP2K	BIKE	KINASE	2	>3	<5.52
BMPR1A	BMPR1A	KINASE	17	>3	<5.52
BMPR1A	BMPR1A	KINASE	20	>3	<5.52
BMPR1B	BMPR1B	KINASE	14	>3	<5.52
BMPR1B	BMPR1B	KINASE	19	>3	<5.52
BMPR2	BMPR2	KINASE	24	>3	<5.52
BMPR2	BMPR2	KINASE	23	>3	<5.52
BMX	BMX	KINASE	27	>3	<5.52
BMX	BMX	KINASE	26	>3	<5.52
BRAF	BRAF	KINASE	25	>3	<5.52
BRAF	BRAF	KINASE	30	>3	<5.52
BRD2	BRD2	KINASE, BROMO	16	>3	<5.52
BRD2	BRD2	KINASE, BROMO	12	>3	<5.52
BRD3	BRD3	KINASE, BROMO	21	>3	<5.52
BRD3	BRD3	BROMO, KINASE	26	>3	<5.52
BRD4	BRD4	KINASE, BROMO	4	>3	<5.52
BRD4	BRD4	KINASE, BROMO	35	>3	<5.52
BRD4	BRD4	KINASE, BROMO	5	>3	<5.52
BRD4	BRD4	KINASE, BROMO	38	>3	<5.52
BRSK1	BRSK1	KINASE	6	>3	<5.52
BRSK1	BRSK1	KINASE	6	>3	<5.52
BRSK2	BRSK2	KINASE	23	>3	<5.52
BRSK2	BRSK2	KINASE	23	>3	<5.52
BTK	BTK	KINASE	57	>3	<5.52
BTK	BTK	KINASE	60	>3	<5.52
BUB1	BUB1	KINASE	42	>3	<5.52
BUB1	BUB1	KINASE	44	>3	<5.52
BUB1B	BUBR1	KINASE	2	>3	<5.52
CABC1	ADCK3	KINASE	32	>3	<5.52

CABC1	ADCK3	KINASE	3	>3	<5.52
CABC1	ADCK3	KINASE	38	>3	<5.52
CABC1	ADCK3	KINASE	2	>3	<5.52
CAMK1	CaMK1a	KINASE	4	>3	<5.52
CAMK1	CaMK1a	KINASE	5	>3	<5.52
CAMK1D	CaMK1d	KINASE	4	>3	<5.52
CAMK1D	CaMK1d	KINASE	4	>3	<5.52
CAMK2A	CaMK2a	KINASE	3	>3	<5.52
CAMK2A	CaMK2a	KINASE	2	>3	<5.52
CAMK2B	CaMK2b	KINASE	13	>3	<5.52
CAMK2B	CaMK2b	KINASE	12	>3	<5.52
CAMK2D	CaMK2d	KINASE	27	>3	<5.52
CAMK2D	CaMK2d	KINASE	29	>3	<5.52
CAMK2G	CaMK2g	KINASE	26	>3	<5.52
CAMK2G	CaMK2g	KINASE	27	>3	<5.52
CAMK4	CaMK4	KINASE	8	>3	<5.52
CAMK4	CaMK4	KINASE	8	>3	<5.52
CAMKK1	CaMKK1	KINASE	23	>3	<5.52
CAMKK1	CaMKK1	KINASE	25	>3	<5.52
CAMKK2	CaMKK2	KINASE	33	>3	<5.52
CAMKK2	CaMKK2	KINASE	36	>3	<5.52
CASK	CASK	KINASE	14	>3	<5.52
CASK	CASK	KINASE	7	>3	<5.52
CDC2	CDC2	KINASE	20	>3	<5.52
CDC2	CDC2	KINASE	23	>3	<5.52
CDC2L1	PITSLRE	KINASE	5	>3	<5.52
CDC2L1	PITSLRE	KINASE	3	>3	<5.52
CDC2L5	CHED	KINASE	10	>3	<5.52
CDC2L5	CHED	KINASE	16	>3	<5.52
CDC42BPA	MRCKa	KINASE	38	>3	<5.52
CDC42BPA	MRCKa	KINASE	47	>3	<5.52
CDC42BPB	MRCKb	KINASE	49	>3	<5.52
CDC42BPB	MRCKb	KINASE	50	>3	<5.52
CDC42BPG	DMPK2	KINASE	25	>3	<5.52
CDC42BPG	DMPK2	KINASE	25	>3	<5.52
CDC7	CDC7	KINASE	14	>3	<5.52
CDC7	CDC7	KINASE	10	>3	<5.52
CDK10	CDK10	KINASE	15	>3	<5.52
CDK10	CDK10	KINASE	14	>3	<5.52
CDK2	CDK2	KINASE	26	>3	<5.52
CDK2	CDK2	KINASE	25	>3	<5.52
CDK4	CDK4	KINASE	8	>3	<5.52
CDK4	CDK4	KINASE	8	>3	<5.52

CDK5	CDK5	KINASE	27	>3	<5.52
CDK5	CDK5	KINASE	27	>3	<5.52
CDK6	CDK6	KINASE	3	>3	<5.52
CDK6	CDK6	KINASE	2	>3	<5.52
CDK7	CDK7	KINASE	16	>3	<5.52
CDK7	CDK7	KINASE	18	>3	<5.52
CDK8	CDK8	KINASE	6	>3	<5.52
CDK8	CDK8	KINASE	6	>3	<5.52
CDK9	CDK9	KINASE	25	>3	<5.52
CDK9	CDK9	KINASE	24	>3	<5.52
CDKL2	CDKL2	KINASE	3	>3	<5.52
CDKL2	CDKL2	KINASE	2	>3	<5.52
CDKL5	CDKL5	KINASE	14	>3	<5.52
CDKL5	CDKL5	KINASE	13	>3	<5.52
CHEK1	CHK1	KINASE	30	>3	<5.52
CHEK1	CHK1	KINASE	29	>3	<5.52
CHEK2	CHK2	KINASE	27	1.9	5.72
CHEK2	CHK2	KINASE	27	>3	<5.52
CHUK	IKKa	KINASE	27	>3	<5.52
CHUK	IKKa	KINASE	23	>3	<5.52
CIT	CRIK	KINASE	108	>3	<5.52
CIT	CRIK	KINASE	107	>3	<5.52
CLK1	CLK1	KINASE	12	>3	<5.52
CLK1	CLK1	KINASE	14	>3	<5.52
CLK2	CLK2	KINASE	6	>3	<5.52
CLK2	CLK2	KINASE	4	>3	<5.52
CRKRS	CRK7	KINASE	32	>3	<5.52
CRKRS	CRK7	KINASE	33	>3	<5.52
CSF1R	FMS	KINASE	11	>3	<5.52
CSF1R	FMS	KINASE	14	>3	<5.52
CSK	CSK	KINASE	38	>3	<5.52
CSK	CSK	KINASE	43	>3	<5.52
CSNK1A1	CK1a	KINASE	20	>3	<5.52
CSNK1A1	CK1a	KINASE	21	>3	<5.52
CSNK1D	CK1d	KINASE	8	>3	<5.52
CSNK1D	CK1d	KINASE	10	>3	<5.52
CSNK1E	CK1e	KINASE	14	>3	<5.52
CSNK1E	CK1e	KINASE	14	>3	<5.52
CSNK1G1	CK1g1	KINASE	4	1.1	5.96
CSNK1G1	CK1g1	KINASE	4	>3	<5.52
CSNK1G2	CK1g2	KINASE	3	2.1	5.68
CSNK1G2	CK1g2	KINASE	3	2.7	5.57
CSNK1G3	CK1g3	KINASE	5	2.3	5.64

CSNK1G3	CK1g3	KINASE	5	2.7	5.57
CSNK2A1	CK2a1	KINASE	11	1.5	5.82
CSNK2A1	CK2a1	KINASE	13	1.7	5.77
CSNK2A1	CK2a1	KINASE	6	>3	<5.52
CSNK2A1	CK2a1	KINASE	25	>3	<5.52
CSNK2A2	CK2a2	KINASE	24	0.58	6.24
CSNK2A2	CK2a2	KINASE	23	0.89	6.05
CSNK2A2	CK2a2	KINASE	27	>3	<5.52
CSNK2A2	CK2a2	KINASE	30	>3	<5.52
DAPK1	DAPK1	KINASE	43	>3	<5.52
DAPK1	DAPK1	KINASE	44	>3	<5.52
DAPK2	DAPK2	KINASE	4	>3	<5.52
DAPK2	DAPK2	KINASE	6	>3	<5.52
DAPK3	DAPK3	KINASE	11	>3	<5.52
DAPK3	DAPK3	KINASE	12	>3	<5.52
DDR1	DDR1	KINASE	30	>3	<5.52
DDR1	DDR1	KINASE	28	>3	<5.52
DDR2	DDR2	KINASE	15	>3	<5.52
DDR2	DDR2	KINASE	14	>3	<5.52
DSTYK	SgK496	KINASE	20	>3	<5.52
DSTYK	SgK496	KINASE	24	>3	<5.52
DYRK1A	DYRK1A	KINASE	24	>3	<5.52
DYRK1A	DYRK1A	KINASE	29	>3	<5.52
DYRK1B	DYRK1B	KINASE	10	>3	<5.52
DYRK1B	DYRK1B	KINASE	11	>3	<5.52
DYRK2	DYRK2	KINASE	6	2	5.70
DYRK2	DYRK2	KINASE	9	>3	<5.52
EGFR	EGFR	KINASE	72	>3	<5.52
EGFR	EGFR	KINASE	75	>3	<5.52
EIF2AK1	HRI	KINASE	21	1.7	5.77
EIF2AK1	HRI	KINASE	20	1.9	5.72
EIF2AK2	PKR	KINASE	15	>3	<5.52
EIF2AK2	PKR	KINASE	13	>3	<5.52
EIF2AK3	PEK	KINASE	21	>3	<5.52
EIF2AK3	PEK	KINASE	15	>3	<5.52
EIF2AK4	GCN2	KINASE	82	>3	<5.52
EIF2AK4	GCN2	KINASE	81	>3	<5.52
EPHA1	EphA1	KINASE	28	>3	<5.52
EPHA1	EphA1	KINASE	21	>3	<5.52
EPHA2	EphA2	KINASE	43	>3	<5.52
EPHA2	EphA2	KINASE	45	>3	<5.52
EPHA3	EphA3, EphA6	KINASE	47	>3	<5.52
EPHA3	EphA3, EphA6	KINASE	45	>3	<5.52

EPHA4	EphA4	KINASE	41	>3	<5.52
EPHA4	EphA4	KINASE	38	>3	<5.52
EPHA5	EphA5	KINASE	18	>3	<5.52
EPHA5	EphA5	KINASE	14	>3	<5.52
EPHA7	EphA7	KINASE	36	>3	<5.52
EPHA7	EphA7	KINASE	35	>3	<5.52
EPHB1	EphB1	KINASE	28	>3	<5.52
EPHB1	EphB1	KINASE	26	>3	<5.52
EPHB2	EphB2	KINASE	31	>3	<5.52
EPHB2	EphB2	KINASE	34	>3	<5.52
EPHB3	EphB3	KINASE	31	>3	<5.52
EPHB3	EphB3	KINASE	22	>3	<5.52
EPHB4	EphB4	KINASE	48	>3	<5.52
EPHB4	EphB4	KINASE	52	>3	<5.52
EPHB6	EphB6	KINASE	29	>3	<5.52
EPHB6	EphB6	KINASE	35	>3	<5.52
ERBB2	HER2/ErbB2	KINASE	2	>3	<5.52
ERBB3	HER3/ErbB3	KINASE	2	>3	<5.52
ERBB4	HER4/ErbB4	KINASE	19	>3	<5.52
ERBB4	HER4/ErbB4	KINASE	19	>3	<5.52
ERN1	IRE1	KINASE	27	>3	<5.52
ERN1	IRE1	KINASE	31	>3	<5.52
FER	FER	KINASE	64	>3	<5.52
FER	FER	KINASE	64	>3	<5.52
FES	FES	KINASE	46	>3	<5.52
FES	FES	KINASE	41	>3	<5.52
FGFR1	FGFR1	KINASE	21	>3	<5.52
FGFR1	FGFR1	KINASE	26	>3	<5.52
FGFR2	FGFR2	KINASE	14	>3	<5.52
FGFR2	FGFR2	KINASE	17	>3	<5.52
FGFR3	FGFR3	KINASE	11	>3	<5.52
FGFR3	FGFR3	KINASE	12	>3	<5.52
FGFR4	FGFR4	KINASE	3	>3	<5.52
FGFR4	FGFR4	KINASE	6	>3	<5.52
FGR	FGR	KINASE	18	>3	<5.52
FGR	FGR	KINASE	12	>3	<5.52
FLT1	FLT1	KINASE	9	>3	<5.52
FLT1	FLT1	KINASE	3	>3	<5.52
FLT4	FLT4	KINASE	38	>3	<5.52
FLT4	FLT4	KINASE	35	>3	<5.52
FRK	FRK	KINASE	17	>3	<5.52
FRK	FRK	KINASE	20	>3	<5.52
FYN	FYN	KINASE	35	>3	<5.52

FYN	FYN	KINASE	38	>3	<5.52
GAK	GAK	KINASE	81	>3	<5.52
GAK	GAK	KINASE	82	>3	<5.52
GRK6	GPRK6	KINASE	2	>3	<5.52
GSG2	Haspin	KINASE	3	>3	<5.52
GSK3A	GSK3A	KINASE	16	>3	<5.52
GSK3A	GSK3A	KINASE	3	>3	<5.52
GSK3A	GSK3A	KINASE	18	>3	<5.52
GSK3A	GSK3A	KINASE	3	>3	<5.52
GSK3B	GSK3B	KINASE	25	>3	<5.52
GSK3B	GSK3B	KINASE	6	>3	<5.52
GSK3B	GSK3B	KINASE	23	>3	<5.52
GSK3B	GSK3B	KINASE	6	>3	<5.52
HCK	HCK	KINASE	27	>3	<5.52
HCK	HCK	KINASE	30	>3	<5.52
HIPK1	HIPK1	KINASE	12	>3	<5.52
HIPK1	HIPK1	KINASE	10	>3	<5.52
HIPK2	HIPK2	KINASE	13	>3	<5.52
HIPK2	HIPK2	KINASE	13	>3	<5.52
HIPK3	HIPK3	KINASE	14	>3	<5.52
HIPK3	HIPK3	KINASE	13	>3	<5.52
ICK	ICK	KINASE	19	>3	<5.52
ICK	ICK	KINASE	15	>3	<5.52
IGF1R	IGF1R	KINASE	53	>3	<5.52
IGF1R	IGF1R	KINASE	54	>3	<5.52
IKBKB	IKKb	KINASE	24	>3	<5.52
IKBKB	IKKb	KINASE	19	>3	<5.52
IKBKE	IKKe	KINASE	31	>3	<5.52
IKBKE	IKKe	KINASE	25	>3	<5.52
ILK	ILK	KINASE	15	>3	<5.52
ILK	ILK	KINASE	15	>3	<5.52
INSR	INSR	KINASE	60	>3	<5.52
INSR	INSR	KINASE	54	>3	<5.52
IRAK1	IRAK1	KINASE	19	>3	<5.52
IRAK1	IRAK1	KINASE	20	>3	<5.52
IRAK3	IRAK3	KINASE	20	>3	<5.52
IRAK3	IRAK3	KINASE	20	>3	<5.52
IRAK4	IRAK4	KINASE	40	>3	<5.52
IRAK4	IRAK4	KINASE	42	>3	<5.52
ITK	ITK	KINASE	23	>3	<5.52
ITK	ITK	KINASE	20	>3	<5.52
JAK1	JAK1	KINASE	83	>3	<5.52
JAK1	JAK1	KINASE	88	>3	<5.52

JAK2	JAK2	KINASE	42	>3	<5.52
JAK2	JAK2	KINASE	46	>3	<5.52
JAK3	JAK3	KINASE	21	>3	<5.52
JAK3	JAK3	KINASE	19	>3	<5.52
KDR	KDR	KINASE	20	>3	<5.52
KDR	KDR	KINASE	22	>3	<5.52
KIAA0999	QSK	KINASE	45	>3	<5.52
KIAA0999	QSK	KINASE	42	>3	<5.52
KIAA1804	MLK4	KINASE	34	>3	<5.52
KIAA1804	MLK4	KINASE	29	>3	<5.52
KIT	KIT	KINASE	27	>3	<5.52
KIT	KIT	KINASE	27	>3	<5.52
KSR1	KSR1	KINASE	15	>3	<5.52
KSR1	KSR1	KINASE	18	>3	<5.52
LATS1	LATS1	KINASE	44	>3	<5.52
LATS1	LATS1	KINASE	45	>3	<5.52
LATS2	LATS2	KINASE	17	>3	<5.52
LATS2	LATS2	KINASE	11	>3	<5.52
LCK	LCK	KINASE	14	>3	<5.52
LCK	LCK	KINASE	10	>3	<5.52
LIMK1	LIMK1	KINASE	32	>3	<5.52
LIMK1	LIMK1	KINASE	32	>3	<5.52
LIMK2	LIMK2	KINASE	24	>3	<5.52
LIMK2	LIMK2	KINASE	22	>3	<5.52
LOC100128443	MAST4	KINASE	17	>3	<5.52
LOC100128443	MAST4	KINASE	12	>3	<5.52
LRRK2	LRRK2	KINASE	21	0.0066	8.18
LRRK2	LRRK2	KINASE	24	0.015	7.82
LTK	LTK	KINASE	2	>3	<5.52
LTK	LTK	KINASE	3	>3	<5.52
LYN	LYN	KINASE	45	>3	<5.52
LYN	LYN	KINASE	50	>3	<5.52
MAP2K1	MAP2K1	KINASE	14	>3	<5.52
MAP2K1	MAP2K1	KINASE	16	>3	<5.52
MAP2K2	MAP2K2	KINASE	17	>3	<5.52
MAP2K2	MAP2K2	KINASE	22	>3	<5.52
MAP2K3	MAP2K3	KINASE	16	>3	<5.52
MAP2K3	MAP2K3	KINASE	15	>3	<5.52
MAP2K4	MAP2K4	KINASE	21	>3	<5.52
MAP2K4	MAP2K4	KINASE	19	>3	<5.52
MAP2K5	MAP2K5	KINASE	25	>3	<5.52
MAP2K5	MAP2K5	KINASE	27	>3	<5.52
MAP2K6	MAP2K6	KINASE	19	0.7	6.15

MAP2K6	MAP2K6	KINASE	17	1.1	5.96
MAP2K7	MAP2K7	KINASE	2	>3	<5.52
MAP2K7	MAP2K7	KINASE	3	>3	<5.52
MAP3K1	MAP3K1	KINASE	44	>3	<5.52
MAP3K1	MAP3K1	KINASE	39	>3	<5.52
MAP3K10	MLK2	KINASE	8	>3	<5.52
MAP3K10	MLK2	KINASE	7	>3	<5.52
MAP3K11	MLK3	KINASE	22	>3	<5.52
MAP3K11	MLK3	KINASE	28	>3	<5.52
MAP3K12	DLK	KINASE	3	>3	<5.52
MAP3K13	LZK	KINASE	8	>3	<5.52
MAP3K13	LZK	KINASE	5	>3	<5.52
MAP3K15	MAP3K7	KINASE	29	>3	<5.52
MAP3K15	MAP3K7	KINASE	23	>3	<5.52
MAP3K2	MAP3K2	KINASE	26	>3	<5.52
MAP3K2	MAP3K2	KINASE	23	>3	<5.52
MAP3K3	MAP3K3	KINASE	27	>3	<5.52
MAP3K3	MAP3K3	KINASE	29	>3	<5.52
MAP3K4	MAP3K4	KINASE	84	>3	<5.52
MAP3K4	MAP3K4	KINASE	89	>3	<5.52
MAP3K5	MAP3K5	KINASE	55	>3	<5.52
MAP3K5	MAP3K5	KINASE	47	>3	<5.52
MAP3K6	MAP3K6	KINASE	34	>3	<5.52
MAP3K6	MAP3K6	KINASE	29	>3	<5.52
MAP3K7	TAK1	KINASE	26	>3	<5.52
MAP3K7	TAK1	KINASE	25	>3	<5.52
MAP3K9	MLK1	KINASE	9	>3	<5.52
MAP3K9	MLK1	KINASE	8	>3	<5.52
MAP4K1	HPK1	KINASE	9	>3	<5.52
MAP4K1	HPK1	KINASE	8	>3	<5.52
MAP4K2	GCK	KINASE	25	>3	<5.52
MAP4K2	GCK	KINASE	24	>3	<5.52
MAP4K3	KHS2	KINASE	21	>3	<5.52
MAP4K3	KHS2	KINASE	23	>3	<5.52
MAP4K4	ZC1/HGK	KINASE	48	>3	<5.52
MAP4K4	ZC1/HGK	KINASE	59	>3	<5.52
MAP4K5	KHS1	KINASE	36	>3	<5.52
MAP4K5	KHS1	KINASE	34	>3	<5.52
MAPK1	Erk2	KINASE	25	>3	<5.52
MAPK1	Erk2	KINASE	27	>3	<5.52
MAPK10	JNK3	KINASE	5	>3	<5.52
MAPK10	JNK3	KINASE	4	>3	<5.52
MAPK11	p38b	KINASE	8	>3	<5.52

MAPK11	p38b	KINASE	9	>3	<5.52
MAPK12	p38g	KINASE	2	>3	<5.52
MAPK14	p38a	KINASE	27	>3	<5.52
MAPK14	p38a	KINASE	25	>3	<5.52
MAPK15	Erk7	KINASE	9	>3	<5.52
MAPK15	Erk7	KINASE	8	>3	<5.52
MAPK3	Erk1	KINASE	19	>3	<5.52
MAPK3	Erk1	KINASE	19	>3	<5.52
MAPK4	Erk4	KINASE	2	>3	<5.52
MAPK6	Erk3	KINASE	15	>3	<5.52
MAPK6	Erk3	KINASE	11	>3	<5.52
MAPK7	Erk5	KINASE	26	>3	<5.52
MAPK7	Erk5	KINASE	27	>3	<5.52
MAPK8	JNK1	KINASE	21	>3	<5.52
MAPK8	JNK1	KINASE	23	>3	<5.52
MAPK9	JNK2	KINASE	3	0.98	6.01
MAPK9	JNK2	KINASE	5	1.1	5.96
MAPK9	JNK2	KINASE	21	>3	<5.52
MAPK9	JNK2	KINASE	26	>3	<5.52
MAPKAPK2	MAPKAPK2	KINASE	2	>3	<5.52
MAPKAPK2	MAPKAPK2	KINASE	4	>3	<5.52
MAPKAPK3	MAPKAPK3	KINASE	9	>3	<5.52
MAPKAPK3	MAPKAPK3	KINASE	10	>3	<5.52
MAPKAPK5	MAPKAPK5	KINASE	11	>3	<5.52
MAPKAPK5	MAPKAPK5	KINASE	11	>3	<5.52
MARK1	MARK1	KINASE	24	>3	<5.52
MARK1	MARK1	KINASE	26	>3	<5.52
MARK2	MARK2	KINASE	55	>3	<5.52
MARK2	MARK2	KINASE	55	>3	<5.52
MARK3	MARK3	KINASE	44	>3	<5.52
MARK3	MARK3	KINASE	42	>3	<5.52
MARK4	MARK4	KINASE	26	>3	<5.52
MARK4	MARK4	KINASE	27	>3	<5.52
MAST1	MAST1	KINASE	2	>3	<5.52
MAST1	MAST1	KINASE	2	>3	<5.52
MAST2	MAST2	KINASE	11	>3	<5.52
MAST2	MAST2	KINASE	12	>3	<5.52
MAST3	MAST3	KINASE	27	>3	<5.52
MAST3	MAST3	KINASE	23	>3	<5.52
MASTL	MASTL	KINASE	14	>3	<5.52
MASTL	MASTL	KINASE	19	>3	<5.52
MELK	MELK	KINASE	35	>3	<5.52
MELK	MELK	KINASE	42	>3	<5.52

MERTK	MER	KINASE	32	>3	<5.52
MERTK	MER	KINASE	28	>3	<5.52
MET	MET	KINASE	47	>3	<5.52
MET	MET	KINASE	44	>3	<5.52
MINK1	ZC3/MINK	KINASE	37	>3	<5.52
MINK1	ZC3/MINK	KINASE	35	>3	<5.52
MKNK1	MNK1	KINASE	11	>3	<5.52
MKNK1	MNK1	KINASE	11	>3	<5.52
MKNK2	MNK2	KINASE	11	>3	<5.52
MKNK2	MNK2	KINASE	11	>3	<5.52
MST1R	RON	KINASE	10	>3	<5.52
MST1R	RON	KINASE	15	>3	<5.52
mTOR	FRAP	KINASE	71	>3	<5.52
mTOR	FRAP	KINASE	134	>3	<5.52
mTOR	FRAP	KINASE	63	>3	<5.52
mTOR	FRAP	KINASE	103	>3	<5.52
MYLK	smMLCK	KINASE	54	>3	<5.52
MYLK	smMLCK	KINASE	44	>3	<5.52
MYLK3	CaMLCK	KINASE	21	>3	<5.52
MYLK3	CaMLCK	KINASE	18	>3	<5.52
NEK1	NEK1	KINASE	47	>3	<5.52
NEK1	NEK1	KINASE	49	>3	<5.52
NEK11	NEK11	KINASE	2	>3	<5.52
NEK11	NEK11	KINASE	4	>3	<5.52
NEK2	NEK2	KINASE	30	>3	<5.52
NEK2	NEK2	KINASE	27	>3	<5.52
NEK3	NEK3	KINASE	16	>3	<5.52
NEK3	NEK3	KINASE	14	>3	<5.52
NEK6	NEK6	KINASE	11	>3	<5.52
NEK6	NEK6	KINASE	16	>3	<5.52
NEK7	NEK7	KINASE	13	>3	<5.52
NEK7	NEK7	KINASE	17	>3	<5.52
NEK9	NEK9	KINASE	69	>3	<5.52
NEK9	NEK9	KINASE	66	>3	<5.52
NLK	NLK	KINASE	17	>3	<5.52
NLK	NLK	KINASE	18	>3	<5.52
NRK	ZC4/NRK	KINASE	9	>3	<5.52
NRK	ZC4/NRK	KINASE	7	>3	<5.52
NTRK1	TRKA	KINASE	20	>3	<5.52
NTRK1	TRKA	KINASE	19	>3	<5.52
NUAK1	NuaK1	KINASE	8	1.7	5.77
NUAK1	NuaK1	KINASE	15	2.7	5.57
OXSR1	OSR1	KINASE	19	>3	<5.52

OXSR1	OSR1	KINASE	17	>3	<5.52
PAK2	PAK2	KINASE	5	>3	<5.52
PAK4	PAK4	KINASE	28	>3	<5.52
PAK4	PAK4	KINASE	35	>3	<5.52
PAK6	PAK6	KINASE	6	>3	<5.52
PAK6	PAK6	KINASE	6	>3	<5.52
PASK	PASK	KINASE	2	>3	<5.52
PBK	PBK	KINASE	3	>3	<5.52
PCTK1	PCTAIRE1	KINASE	31	>3	<5.52
PCTK1	PCTAIRE1	KINASE	26	>3	<5.52
PCTK2	PCTAIRE2	KINASE	24	>3	<5.52
PCTK2	PCTAIRE2	KINASE	25	>3	<5.52
PCTK3	PCTAIRE3	KINASE	20	>3	<5.52
PCTK3	PCTAIRE3	KINASE	19	>3	<5.52
PDGFRA	PDGFRa	KINASE	14	>3	<5.52
PDGFRA	PDGFRa	KINASE	10	>3	<5.52
PDGFRB	PDGFRb	KINASE	26	>3	<5.52
PDGFRB	PDGFRb	KINASE	23	>3	<5.52
PDIK1L	CLIK1L	KINASE	6	>3	<5.52
PDIK1L	CLIK1L	KINASE	9	>3	<5.52
PDK2	PDHK2	KINASE	2	>3	<5.52
PDPK1	PDK1	KINASE	28	>3	<5.52
PDPK1	PDK1	KINASE	32	>3	<5.52
PFTK1	PFTAIRE1	KINASE	6	>3	<5.52
PFTK1	PFTAIRE1	KINASE	10	>3	<5.52
PHKG2	PHKg2	KINASE	19	>3	<5.52
PHKG2	PHKg2	KINASE	18	>3	<5.52
PI4K2B	PIK4C2B	KINASE	3	>3	<5.52
PI4K2B	PIK4C2B	KINASE	7	>3	<5.52
PI4K2B	PIK4C2B	KINASE	6	>3	<5.52
PI4KA	PIK4Ca	KINASE	17	>3	<5.52
PI4KA	PIK4Ca	KINASE	52	>3	<5.52
PI4KA	PIK4Ca	KINASE	5	>3	<5.52
PI4KA	PIK4Ca	KINASE	10	>3	<5.52
PI4KA	PIK4Ca	KINASE	49	>3	<5.52
PI4KA	PIK4Ca	KINASE	5	>3	<5.52
PI4KB	PIK4Cb	KINASE	17	>3	<5.52
PI4KB	PIK4Cb	KINASE	21	>3	<5.52
PIK3C2A	PIK3C2a	KINASE	41	>3	<5.52
PIK3C2A	PIK3C2a	KINASE	25	>3	<5.52
PIK3C2B	complement component C2a, PIK3C2b	PROTEASE, KINASE	18	>3	<5.52
PIK3C2B	PIK3C2b	KINASE	72	>3	<5.52
PIK3C2B	PIK3C2b	KINASE	30	>3	<5.52

PIK3C2B	PIK3C2b	KINASE	66	>3	<5.52
PIK3C3	complement component C2a, PIK3C3	PROTEASE, KINASE	26	>3	<5.52
PIK3C3	PIK3C3	KINASE	60	>3	<5.52
PIK3C3	PIK3C3	KINASE	27	>3	<5.52
PIK3C3	PIK3C3	KINASE	52	>3	<5.52
PIK3CA	PIK3Ca	KINASE	65	>3	<5.52
PIK3CA	PIK3Ca	KINASE	65	>3	<5.52
PIK3CB	PIK3Cb	KINASE	4	>3	<5.52
PIK3CB	PIK3Cb	KINASE	56	>3	<5.52
PIK3CB	PIK3Cb	KINASE	5	>3	<5.52
PIK3CB	PIK3Cb	KINASE	47	>3	<5.52
PIK3CD	PIK3Cd	KINASE	65	>3	<5.52
PIK3CD	PIK3Cd	KINASE	51	>3	<5.52
PIK3CG	PIK3Cg	KINASE	65	>3	<5.52
PIK3CG	PIK3Cg	KINASE	48	>3	<5.52
PIK3R4	PIK3R4	KINASE	25	>3	<5.52
PIK3R4	PIK3R4	KINASE	73	>3	<5.52
PIK3R4	PIK3R4	KINASE	22	>3	<5.52
PIK3R4	PIK3R4	KINASE	67	>3	<5.52
PIM1	PIM1	KINASE	15	>3	<5.52
PIM1	PIM1	KINASE	6	>3	<5.52
PIM1	PIM1	KINASE	18	>3	<5.52
PIM1	PIM1	KINASE	5	>3	<5.52
PIM2	PIM2	KINASE	12	>3	<5.52
PIM2	PIM2	KINASE	12	>3	<5.52
PIM3	PIM3	KINASE	7	>3	<5.52
PIM3	PIM3	KINASE	8	>3	<5.52
PIP4K2A	PIP5K2A	KINASE	17	0.097	7.01
PIP4K2A	PIP5K2A	KINASE	11	0.13	6.89
PIP4K2B	PIP5K2B	KINASE	12	0.062	7.21
PIP4K2B	PIP5K2B	KINASE	15	0.14	6.85
PIP4K2C	PIP5K2C	KINASE	30	0.11	6.96
PIP4K2C	PIP5K2C	KINASE	25	0.16	6.80
PIP5K3	PIP5K3	KINASE	88	>3	<5.52
PIP5K3	PIP5K3	KINASE	61	>3	<5.52
PIP5K3	PIP5K3	KINASE	100	>3	<5.52
PIP5K3	PIP5K3	KINASE	45	>3	<5.52
PKMYT1	MYT1	KINASE	18	>3	<5.52
PKMYT1	MYT1	KINASE	20	>3	<5.52
PKN1	complement component C2a, PKN1	PROTEASE, KINASE	19	>3	<5.52
PKN1	PKN1	KINASE	35	>3	<5.52
PKN2	complement component C2a, PKN2	PROTEASE, KINASE	27	>3	<5.52
PKN2	PKN2	KINASE	36	>3	<5.52

PKN3	complement component C2a, PKN3	PROTEASE, KINASE	26	>3	<5.52
PKN3	PKN3	KINASE	26	>3	<5.52
PLK1	PLK1	KINASE	19	>3	<5.52
PLK1	PLK1	KINASE	25	>3	<5.52
PLK2	PLK2	KINASE	4	>3	<5.52
PLK2	PLK2	KINASE	4	>3	<5.52
PLK4	PLK4	KINASE	24	>3	<5.52
PLK4	PLK4	KINASE	24	>3	<5.52
PRKAA1	AMPKa1	KINASE	42	>3	<5.52
PRKAA1	AMPKa1	KINASE	48	>3	<5.52
PRKAA2	AMPKa2	KINASE	21	>3	<5.52
PRKAA2	AMPKa2	KINASE	25	>3	<5.52
PRKACA	PKACa	KINASE	6	>3	<5.52
PRKACA	PKACa	KINASE	3	>3	<5.52
PRKACB	PKACb, SgK494	KINASE	5	>3	<5.52
PRKACB	PKACb, SgK494	KINASE	4	>3	<5.52
PRKCA	complement component C2a, PKCa	PROTEASE, KINASE	28	>3	<5.52
PRKCA	PKCa	KINASE	30	>3	<5.52
PRKCB	complement component C2a, PKCb	PROTEASE, KINASE	28	>3	<5.52
PRKCB	PKCb	KINASE	32	>3	<5.52
PRKCD	complement component C2a, PKCd	PROTEASE, KINASE	37	>3	<5.52
PRKCD	PKCd	KINASE	48	>3	<5.52
PRKCE	complement component C2a, PKCe	PROTEASE, KINASE	4	>3	<5.52
PRKCE	PKCe	KINASE	5	>3	<5.52
PRKCI	PKCi	KINASE	7	>3	<5.52
PRKCI	PKCi	KINASE	11	>3	<5.52
PRKCQ	PKCt	KINASE	43	>3	<5.52
PRKCQ	PKCt	KINASE	48	>3	<5.52
PRKCZ	PKCz	KINASE	8	>3	<5.52
PRKCZ	PKCz	KINASE	6	>3	<5.52
PRKD1	PKD1	KINASE	19	>3	<5.52
PRKD1	PKD1	KINASE	20	>3	<5.52
PRKD2	PKD2	KINASE	31	>3	<5.52
PRKD2	PKD2	KINASE	32	>3	<5.52
PRKD3	PKD3	KINASE	24	>3	<5.52
PRKD3	PKD3	KINASE	24	>3	<5.52
PRKDC	DNAPK	KINASE	236	>3	<5.52
PRKDC	DNAPK	KINASE	311	>3	<5.52
PRKDC	DNAPK	KINASE	229	>3	<5.52
PRKDC	DNAPK	KINASE	320	>3	<5.52
PRKG1	PKG1	KINASE	49	>3	<5.52
PRKG1	PKG1	KINASE	47	>3	<5.52
PRKX	PRKX	KINASE	12	>3	<5.52

PRKX	PRKX	KINASE	16	>3	<5.52
PTK2	FAK	KINASE	86	>3	<5.52
PTK2	FAK	KINASE	87	>3	<5.52
PTK2B	PYK2	KINASE	84	>3	<5.52
PTK2B	PYK2	KINASE	95	>3	<5.52
PTK6	BRK	KINASE	4	>3	<5.52
PTK7	CCK4	KINASE	2	>3	<5.52
RAF1	RAF1	KINASE	5	>3	<5.52
RAF1	RAF1	KINASE	7	>3	<5.52
RIOK1	RIOK1	KINASE	2	>3	<5.52
RIOK2	RIOK2	KINASE	4	0.73	6.14
RIOK2	RIOK2	KINASE	4	0.81	6.09
RIOK2	RIOK2	KINASE	39	1.8	5.74
RIOK2	RIOK2	KINASE	35	>3	<5.52
RIPK1	RIPK1	KINASE	8	>3	<5.52
RIPK1	RIPK1	KINASE	11	>3	<5.52
RIPK2	RIPK2	KINASE	29	>3	<5.52
RIPK2	RIPK2	KINASE	17	>3	<5.52
RIPK2	RIPK2	KINASE	29	>3	<5.52
RIPK2	RIPK2	KINASE	11	>3	<5.52
RIPK3	RIPK3	KINASE	7	>3	<5.52
RIPK3	RIPK3	KINASE	8	>3	<5.52
RIPK4	ANKRD3	KINASE	14	>3	<5.52
RIPK4	ANKRD3	KINASE	10	>3	<5.52
RNASEL	RNAseL	KINASE	11	>3	<5.52
RNASEL	RNAseL	KINASE	16	>3	<5.52
ROCK1	ROCK1	KINASE	5	>3	<5.52
ROCK1	ROCK1	KINASE	6	>3	<5.52
ROCK2	ROCK2	KINASE	27	>3	<5.52
ROCK2	ROCK2	KINASE	26	>3	<5.52
RP6-213H19 1	MST4	KINASE	6	>3	<5.52
RP6-213H19 1	MST4	KINASE	7	>3	<5.52
RPS6KA1	RSK3	KINASE	38	>3	<5.52
RPS6KA1	RSK3	KINASE	39	>3	<5.52
RPS6KA2	RSK1	KINASE	15	>3	<5.52
RPS6KA2	RSK1	KINASE	13	>3	<5.52
RPS6KA3	RSK2	KINASE	38	>3	<5.52
RPS6KA3	RSK2	KINASE	39	>3	<5.52
RPS6KA4	MSK2	KINASE	41	>3	<5.52
RPS6KA4	MSK2	KINASE	42	>3	<5.52
RPS6KA5	MSK1	KINASE	38	1.5	5.82
RPS6KA5	MSK1	KINASE	38	2.5	5.60
RPS6KA6	RSK4	KINASE	21	>3	<5.52

RPS6KA6	RSK4	KINASE	14	>3	<5.52
RPS6KB1	p70S6K	KINASE	11	>3	<5.52
RPS6KB1	p70S6K	KINASE	14	>3	<5.52
RPS6KC1	RSKL1	KINASE	2	>3	<5.52
SBK2	SgK069, SgK110	KINASE	2	>3	<5.52
SCYL1	SCYL1	KINASE	2	>3	<5.52
SGK1	SGK1	KINASE	4	>3	<5.52
SGK1	SGK1	KINASE	4	>3	<5.52
SGK3	SGK3	KINASE	14	>3	<5.52
SGK3	SGK3	KINASE	13	>3	<5.52
SIK1	SIK	KINASE	4	>3	<5.52
SIK1	SIK	KINASE	6	>3	<5.52
SIK2	QIK	KINASE	43	>3	<5.52
SIK2	QIK	KINASE	39	>3	<5.52
SLK	SLK	KINASE	97	>3	<5.52
SLK	SLK	KINASE	99	>3	<5.52
SMG1	SMG1	KINASE	2	>3	<5.52
SMG1	SMG1	KINASE	2	>3	<5.52
SNRK	SNRK	KINASE	13	>3	<5.52
SNRK	SNRK	KINASE	13	>3	<5.52
SRC	SRC	KINASE	19	>3	<5.52
SRC	SRC	KINASE	24	>3	<5.52
STK10	LOK	KINASE	67	>3	<5.52
STK10	LOK	KINASE	80	>3	<5.52
STK11	LKB1	KINASE	12	>3	<5.52
STK11	LKB1	KINASE	14	>3	<5.52
STK16	MPSK1	KINASE	14	>3	<5.52
STK16	MPSK1	KINASE	16	>3	<5.52
STK17A	DRAK1, SgK085	KINASE	8	>3	<5.52
STK17A	DRAK1, SgK085	KINASE	11	>3	<5.52
STK17B	DRAK2	KINASE	10	>3	<5.52
STK17B	DRAK2	KINASE	9	>3	<5.52
STK24	MST3	KINASE	2	>3	<5.52
STK25	YSK1	KINASE	3	>3	<5.52
STK25	YSK1	KINASE	6	>3	<5.52
STK3	MST2	KINASE	22	>3	<5.52
STK3	MST2	KINASE	18	>3	<5.52
STK33	STK33	KINASE	25	1	6.00
STK33	STK33	KINASE	26	>3	<5.52
STK35	CLIK1	KINASE	18	>3	<5.52
STK35	CLIK1	KINASE	23	>3	<5.52
STK36	Fused	KINASE	5	>3	<5.52
STK36	Fused	KINASE	2	>3	<5.52

STK38	NDR1	KINASE	23	>3	<5.52
STK38	NDR1	KINASE	20	>3	<5.52
STK38L	NDR2	KINASE	15	>3	<5.52
STK38L	NDR2	KINASE	10	>3	<5.52
STK39	STLK3	KINASE	14	>3	<5.52
STK39	STLK3	KINASE	14	>3	<5.52
STK4	MST1	KINASE	28	>3	<5.52
STK4	MST1	KINASE	29	>3	<5.52
STRADA	STLK5	KINASE	14	>3	<5.52
STRADA	STLK5	KINASE	13	>3	<5.52
STRADB	STLK6	KINASE	4	>3	<5.52
STRADB	STLK6	KINASE	2	>3	<5.52
SYK	SYK	KINASE	35	>3	<5.52
SYK	SYK	KINASE	36	>3	<5.52
TAOK1	TAO1	KINASE	8	>3	<5.52
TAOK1	TAO1	KINASE	13	>3	<5.52
TAOK2	TAO2	KINASE	15	>3	<5.52
TAOK2	TAO2	KINASE	22	>3	<5.52
TAOK3	TAO3	KINASE	28	>3	<5.52
TAOK3	TAO3	KINASE	36	>3	<5.52
TBK1	TBK1	KINASE	59	>3	<5.52
TBK1	TBK1	KINASE	60	>3	<5.52
TEC	TEC	KINASE	56	>3	<5.52
TEC	TEC	KINASE	62	>3	<5.52
TEK	TIE2	KINASE	30	>3	<5.52
TEK	TIE2	KINASE	28	>3	<5.52
TESK1	TESK1	KINASE	15	>3	<5.52
TESK1	TESK1	KINASE	12	>3	<5.52
TESK2	TESK2	KINASE	20	>3	<5.52
TESK2	TESK2	KINASE	18	>3	<5.52
TGFBR1	TGFbR1	KINASE	29	>3	<5.52
TGFBR1	TGFbR1	KINASE	30	>3	<5.52
TGFBR2	TGFbR2	KINASE	23	>3	<5.52
TGFBR2	TGFbR2	KINASE	28	>3	<5.52
TLK1	TLK1	KINASE	4	>3	<5.52
TLK1	TLK1	KINASE	2	>3	<5.52
TLK2	TLK2	KINASE	6	>3	<5.52
TNIK	ZC2/TNIK	KINASE	62	>3	<5.52
TNIK	ZC2/TNIK	KINASE	62	>3	<5.52
TNK1	TNK1	KINASE	30	>3	<5.52
TNK1	TNK1	KINASE	30	>3	<5.52
TNK2	ACK	KINASE	37	>3	<5.52
TNK2	ACK	KINASE	31	>3	<5.52

TP53RK	PRPK	KINASE	13	1.4	5.85
TP53RK	PRPK	KINASE	14	2	5.70
TRIM33	TIF1g, TRIM33	KINASE, PHD, BROMO	3	>3	<5.52
TRIO	Trio	KINASE	5	>3	<5.52
TRRAP	TRRAP	KINASE	43	>3	<5.52
TRRAP	TRRAP	KINASE	29	>3	<5.52
TRRAP	TRRAP	KINASE	2	>3	<5.52
TTK	TTK	KINASE	25	0.078	7.11
TTK	TTK	KINASE	36	0.11	6.96
TTN	TTN	KINASE	5	>3	<5.52
TYK2	TYK2	KINASE	68	>3	<5.52
TYK2	TYK2	KINASE	68	>3	<5.52
TYRO3	TYRO3	KINASE	14	>3	<5.52
TYRO3	TYRO3	KINASE	16	>3	<5.52
UHMK1	KIS	KINASE	4	>3	<5.52
UHMK1	KIS	KINASE	3	>3	<5.52
ULK1	ULK1	KINASE	26	>3	<5.52
ULK1	ULK1	KINASE	27	>3	<5.52
ULK2	ULK2	KINASE	2	>3	<5.52
ULK3	ULK3	KINASE	29	>3	<5.52
ULK3	ULK3	KINASE	34	>3	<5.52
WEE1	Wee1, Wee1B	KINASE	43	>3	<5.52
WEE1	Wee1, Wee1B	KINASE	40	>3	<5.52
WNK1	Wnk1	KINASE	14	>3	<5.52
WNK1	Wnk1	KINASE	12	>3	<5.52
WNK4	Wnk4	KINASE	5	>3	<5.52
WNK4	Wnk4	KINASE	3	>3	<5.52
YES1	YES	KINASE	25	>3	<5.52
YES1	YES	KINASE	24	>3	<5.52
ZAK	ZAK	KINASE	28	>3	<5.52
ZAK	ZAK	KINASE	29	>3	<5.52

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