Supporting Information

Mtb PKNA/PKNB Dual Inhibition Provides Selectivity Advantages for Inhibitor Design to Minimize Host Kinase Interactions

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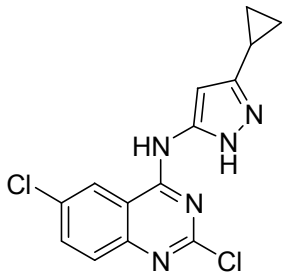
Part 1. Experimental Procedures and Spectroscopic Data

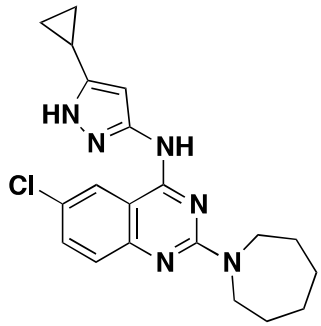
General. All common solvents and chemicals were used as purchased without further purification. Purity of all final compounds was 95% or higher by proton NMR and HPLC. Spots in TLC were visualized by irradiation with ultraviolet light (254 nm). LCMS and HPLC were recorded with Agilent LC (1200)-MS (6110) and Agilent LC (1100)-MS (1956A). Proton (1H) NMR spectra were recorded on Avance III 400 MHz unless otherwise indicated using solvents as indicated in the experimental section. Chemical shifts are given in parts per million (ppm) (δ relative to residual solvent peak for ^{1}H).

2,6-Dichloro-*N*-(3-cyclopropyl-1H-pyrazol-5-yl)quinazolin- 4-amine



To a suspension of 2,4,6-trichloroquinazoline (0.768 g, 3.29 mmol, 1.0 eq.) (prepared according to: *Bioorg. Med. Chem.,* 2003, p2439) in ethanol (80 mL) was added 3-cyclopropyl-1H-pyrazol-5-amine (0.811 g, 6.58 mmol, 2 eq.) in ethanol (30 mL). The suspension was stirred for 8 hours and filtered to afford 2,6-dichloro-*N*-(3-cyclopropyl-1H-pyrazol-5-yl)quinazolin-4- amine (0.9 g, 85%). LC-MS (m/z) = 320.0 $[M + H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 12.38 (s, 1 H), 10.92 (s, 1H), 8.86 (s, 1H), 7.88 (d, J = 8.9 Hz, 1H), 7.71 (d, J = 8.8 Hz, 1H), 6.51 (s, 1H), 1.96 (ddd, J = 13.1, 8.4, 5.0 Hz, 1H), 0.97 (d, J = 6.6 Hz, 2H), 0.74 (d, J = 4.5 Hz, 2H) ppm.

2-(Azepan-1-yl)-6-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)quinazolin-4-mine (1)

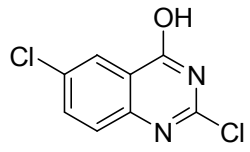


1

To a suspension of 2,6-dichloro-*N*-(3-cyclopropyl-1*H*-pyrazol-5-yl)quinazolin-4-amine (300 mg, 0.938 mmol, 1.0 eq.) in butan-1-ol (10 ml) was added azepane (186 mg, 1.88 mol, 2 eq.) at room temperature. The mixture was stirred at 90 ^{o}C for 4 hours. The solvent was removed and the residue was purified by column chromatography on silica gel to afford the product **1** (250 mg, 70%.) LC-MS (m/z) = 383.2 $[M + H]^+$; ESI-HRMS calcd for 383.1746 $C_{20}H_{23}ClN_6$ (M+H), found 383.1744; ^{1}H NMR (300 MHz, D_2O-d_2) δ 12.21 (s, 1 H), 10.08 (s, 1 H), 8.52 (s, 1 H), 7.51-7.47 (m, 1 H), 7.28 (d, J = 12.0 Hz, 1

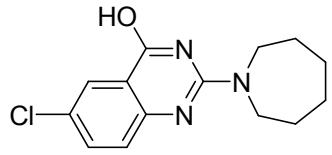
H), 6.42 (s, 1 H), 3.78-3.76 (m, 4 H), 1.95-1.86 (m, 1 H), 1.75 (s, 4 H), 1.50 (s, 4 H), 1.04-0.87 (m, 2 H), 0.74-0.59 (m, 2 H) ppm.

2, 6-Dichloroquinazolin-4-ol



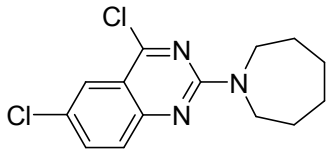
A suspension of 2,4,6-trichloroquinazoline (23.3 g, 0.1 mol, 1 eq.) in sodium hydroxide aqueous solution (2N, 150 ml, 3 eq.) was stirred at room temperature for 3 hours. The reaction mixture was diluted with water (100 ml) and stirred for 10 minutes. The solid was filtered off, washed with water, and dried. The desired compound was crystallized from alcohol to afford 2, 6-dichloroquinazolin-4-ol as a white solid (18.3 g, 85%). LC-MS (m/z) =215.1$[M+H]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.462 (s, 1 H), 8.03 (d, J = 2.1 Hz, 1 H), 7.87 (dd, J = 8.6, 2.1 Hz, 1 H), 7.65 (d, J = 8.7 Hz, 1 H) ppm.

2-(Azepan-1-yl)-6-chloroquinazolin-4-ol



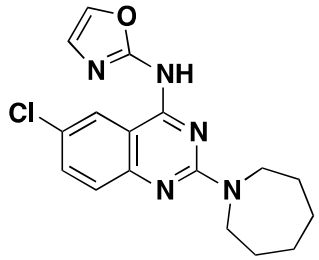
To a suspension of 2, 6-dichloroquinazolin-4-ol (2.15 g, 0.01 mol, 1 eq.) in butan-1-ol (20 ml) was added azepane (1.98 g, 0.02 mol, 2 eq.) at room temperature. Then, the reaction mixture was stirred at 90^{o}C for 3.5 hours. The solution was cooled to 0 ^{o}C and the product was crystallized from butan-1-ol. The solid was filtered, washed with cold ethanol (5ml x 2) and dried under high vacuum at 50 ^{o}C to afford 2-(azepan-1-yl)-6-chloroquinazolin-4-ol (1.94 g, 70%). LC-MS (m/z) =278.0 $[M+H]^+$, ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.21 (s, 1 H), 7.80 (s, 1 H), 7.55 (d, J = 8.7 Hz, 1 H), 7.21 (t, J = 12.8 Hz, 1 H), 3.67 (t, J = 5.8 Hz, 4 H), 1.71 (s, 4 H), 1.49 (s, 4 H) ppm.

2-(Azepan-1-yl)-4, 6-dichloroquinazoline



A suspension of 2-(azepan-1-yl)-6-chloroquinazolin-4-ol (2.77 g, 0.01 mol, 1 eq.) in $POCl_3$ (20 ml) was heated to reflux for one hour. The brown solution was cooled down to 20^oC and poured into ice water (0^oC, 200 ml) while stirring vigorously. The aqueous mixture was maintained at 30^oC during the quench. The cold precipitate was filtered, washed with cold water (3 x 10ml) and dried under high vacuum at 40^oC to afford 2-(azepan-1-yl)-4, 6-dichloroquinazoline (2.1 g, 70%), which was used directly for next step synthesis. LC-MS (m/z) =296.2 $[M+H]^+$.

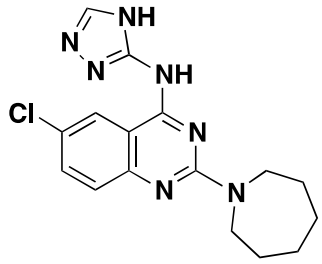
_N_-(2-(azepan-1-yl)-6-chloroquinazolin-4-yl)oxazol-2-amine (2)



2

A mixture of 2-(azepan-1-yl)-4, 6-dichloroquinazoline (1 g, 3.82 mmol, 1 eq.), oxazol-2-amine (0.96 g, 11.46 mmol, 3 eq.), K_2CO_3 (1.054 g, 7.64 mmol, 2 eq.) and DMF (50 mL) was heated to 90^0C for 5 h. The resulting mixture was filtered and the filtrate was concentrated. The residue was purified by silica gel chromatography (petroleum ether / EtOAc: 100:1 to 100:20) to affotrd yellow solid product as *N*-(2-(azepan-1-yl)-6-chloroquinazolin-4-yl)oxazol-2-amine (**2**) (40 mg, 3.38%). LC-MS (m/z) =344.1 $[M+H]^+$,ESI-HRMS calcd for 344.1273 $C_{17}H_{18}ClN_5O$(M+H), found 344.1380; ^1H NMR (300 MHz, DMSO-d_6) δ 10.60 (s, 1 H). 10.09 (bs, 1 H), 8.76 (br s, 1 H), 8.56 (s, 1 H), 7.63 (dd, J = 2.4, 9.3 Hz, 1 H), 7.39 (d, J = 9.0 Hz, 1 H), 3.75-3.64 (m, 4 H), 1.73 (m, 4 H), 1.48 (m, 4 H) ppm.

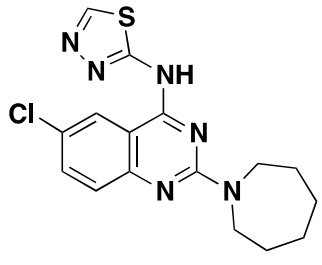
2-(Azepan-1-yl)-6-chloro-*N*-(4*H*-1,2,4-triazol-3-yl)quinazolin-4-amine (3)



3

To 2-(azepan-1-yl)-4,6-dichloroquinazoline (295 mg, 1 mmol, 1 eq.) in acetonitrile (5 ml) was added 4H-1,2,4-triazol-3-amine (252 mg, 3 mmol, 3 eq.). The resulting mixture was heated to reflux for 15 hours. The solvent was removed and the residue was recrystallized from ethanol to afford product 2-(azepan-1-yl)-6-chloro-*N*-(4*H*-1,2,4-triazol-3-yl)quinazolin-4-amine (**3**) (200 mg, 58.4%). LC-MS (m/z) 344.1 [M+H]. ESI-HRMS calcd for 344.1385 $C_{16}H_{18}ClN_7$(M+H), found 344.1380; ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.80 (br s, 1 H), 13.07 (br s, 1 H), 11.08 (br s, 1 H), 8.46 (br s, 1 H), 7.56 (br s, 1 H), 7.33 (br s, 1 H), 3.73 (br s, 4H), 1.71 (m, 4 H), 1.54 (m, 4 H) ppm.

***N*-(2-(azepan-1-yl)-6-chloroquinazolin-4-yl)-1,3,4-thiadiazol-2-amine (4)**

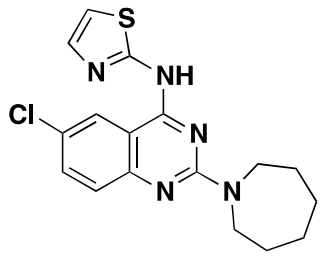


4

To an ice-cooled solution of diisopropylamine (0.24 mL, 1.7 mmol, 2.5 eq.) in THF (3 mL) was added *n*-butyl-lithium (2.5 *M* solution in hexanes, 0.679 mL, 1.7 mmol, 2.5 eq.) under nitrogen atmosphere. The resulting mixture was stirred at 0 ^{0}C for 15 min . The mixture was cooled down to -78 ^{0}C and a solution of 1,3,4-thiadiazol-2-amine (75 mg, 0.877 mmol, 1.3 eq.) in THF (2 mL) was added. The resulting mixture was stirred at -78 ^{0}C for 30 min. A solution of 2-(azepan-1-yl)-4,6-dichloroquinazoline (200 mg, 0.675 mmol, 1 eq.) in THF/HMPA (1/1.2 mL) was added and the resulting mixture was stirred at -78 ^{0}C for 15 min. The cooling bath was removed and the resulting mixture was warmed to room temperature for 3 h. THF was removed by evaporation and residue was dissolved in EtOAc (20 mL). The organic layer was washed with water, brine, dried (Na_2SO_4), filtered and concentrated. The residue was purified by silica gel chromatography (DCM / EtOAc: 1:0 to 12:1) to a crude product, which was crystallized with DCM / ether to afford N-(2-(azepan-1-yl)-6-chloroquinazolin-4-yl)-1,3,4-thiadiazol-2-amine (**4**, 50 mg, 20%). LC-MS (m/z) 361.1 [M+H]. ESI-HRMS calcd for 361.0996 $C_{16}H_{17}ClN_6S$(M+H), found 361.0997; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.40 (br s, 1

H), 9.17 (s, 1H), 8.59(s, 1 H), 7.59 (d, *J* = 8.4Hz, 1 H), 7.40 (d, *J* = 7.8, 1 H), 3.85 (bs, 4 H), 1.80 (bs, 4 H), 1.50 (bs, 4 H) ppm.

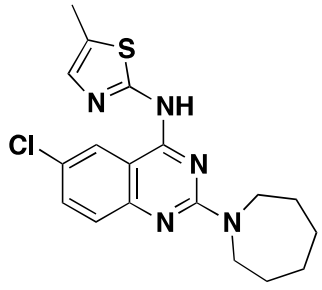
N-(2-(azepan-1-yl)-6-chloroquinazolin-4-yl)thiazol-2-amine (5)



5

To an ice-cooled solution of diisopropylamine (0.12 mL, 0.85 mmol, 2.5 eq.) in THF (2 mL) was added *n*-butyl-lithium (2.5 *M* solution in hexanes, 0.35 mL, 0.85 mmol, 2.5 eq.) under nitrogen atmosphere. The resulting mixture was stirred at 0 ^{0}C for 15 min. The mixture was cooled down to -78^{0}C and a solution of thiazol-2-amine (51.3 mg, 0.508 mmol, 1.5 eq.) in THF (2 mL) was added.. The resulting mixture was stirred at -78^{0}C for 30 min. Then, a solution of 2-(azepan-1-yl)-4,6-dichloroquinazoline (100 mg, 0.338 mmol, 1 eq.) in THF / HMPA (1/1.2 mL) was added and the resulting mixture was stirred at -78 ^{0}C for 45 min. The cooling bath was removed and the resulting mixture was stirred at room temperature for 3 h. THF was removed by evaporation and residue was dissolved in ethyl acetate (20 mL). The organic layer was washed with water, brine, dried (Na_2SO_4), filtered and concentrated. The residue was triturated with dichloromethane to afford N-(2-(azepan-1-yl)-6-chloroquinazolin-4-yl)thiazol-2-amine (**5**, 36 mg, 30%). LC-MS (m/z) 360.1 $[M+H]^+$. ESI-HRMS calcd for 360.1042 $C_{17}H_{18}ClN_5S$(M+H), found 360.1044; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.01 (bs, 1 H), 8.65 (s, 1 H), 7.64 (bt, *J* = 2.7 Hz, 2 H), 7.41 (d, *J* = 9.3Hz, 1 H), 7.33 (d, *J* = 3.3Hz, 1 H), 3.93 (m, 4 H), 1.85 (bs, 4 H), 1.55 (bs, 4 H) ppm.

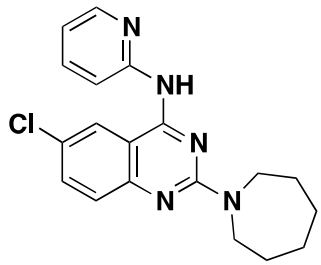
N-(2-(azepan-1-yl)-6-chloroquinazolin-4-yl)-5-methylthiazol-2-amine (6)



6

To 2-(azepan-1-yl)-4,6-dichloroquinazoline (59.2 mg, 0.2 mmol, 1 eq.) in acetonitrile (5 ml) was added K_2CO_3 (55.2 mg, 0.4 mmol, 2 eq.) and 5-methylthiazol-2-amine (45.6 mg, 0.4 mmol, 2 eq.). The resulting mixture was heated to reflux for 12 hours. The solvent was removed and the residue was purified by column chromatography (solvent: dichloromethane) to produce product **6** (37.3 mg, 50%). LC-MS (m/z) = 374.1 $[M + H]^+$; ESI-HRMS calcd for 374.1201 $C_{18}H_{20}ClN_5S$ (M+H), found 374.1199; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.33 (m ,1 H). 7.91 (s, 1 H), 1.59 (m, 4 H), 7.78 (m, 1 H), 7.40, (s, 1 H), 3.36 (s, 4 H), 2.40 (s, 3 H), 1.90 (d, J = 0.9 Hz, 4 H) ppm.

2-(azepan-1-yl)-6-chloro-*N*-(pyridin-2-yl)quinazolin-4-amine (7)

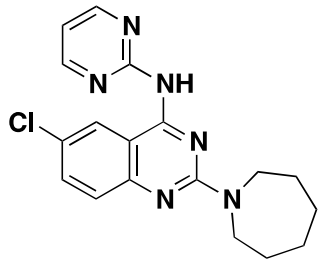


7

To a suspension of 2, 4, 6-trichloroquinazoline (210 mg, 0.90 mmol, 1 eq.) in acetonitrile (5 ml) was added pyridin-2-amine (254 mg, 2.70 mmol, 3 eq.). The resulting mixture was heated to reflux for 12 hours. The solvent was removed and the residue was purified by silica gel chromatography (petroleum ether / ethyl acetate 100:1 to 5:1) to generate the intermediate 2-(azepan-1-yl)-6-chloro-N-(pyridin-2-yl)quinazolin-4-amine (40 mg, 11%).

A mixture of 2-(azepan-1-yl)-6-chloro-N-(pyridin-2-yl)quinazolin-4-amine (301 mg, 1.03 mmol, 1 eq.), azepane (306 mg, 3.09 mmol, 3 eq.) and butan-1-ol (12 mL) was heated to 90 ^{0}C for 14 h. The butan-1-ol was removed by evaporation and the residue was purified by silica gel chromatography (petroleum ether / ethyl acetate from 100 : 1 to 5 : 1) to produce 2-(azepan-1-yl)-6-chloro-*N*-(pyridin-2-yl)quinazolin-4-amine (**7**) (133 mg, 33%). LC-MS (m/z) 354.8 $[M+H]^+$; ESI-HRMS calcd for 354.148 $C_{19}H_{20}ClN_5$(M+H), found 354.1481;^{1}H-NMR (300 MHz, DMSO-d_6) δ 10.13 (s, 1H), 8.62 (d, J = 2.4Hz, 1H), 8.41 (d, J = 3.9 Hz, 1 H), 8.30 (d, J = 8.1Hz, 1 H), 7.87-7.62 (m, 1 H), 7.56 (dd, J = 2.4 Hz, J = 8.7 Hz, 1 H), 7.34 (d, J = 9.3Hz, 1 H), 7.13 (dd, J = 4.5 Hz, 6.9 Hz, 1 H), 3.77 (br s, 4 H), 1.75 (br s, 4 H), 1.50 (br s, 4 H) ppm.

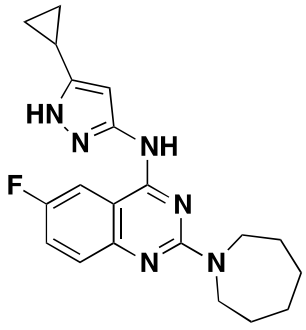
2-(azepan-1-yl)-6-chloro-*N*-(pyrimidin-2-yl)quinazolin-4-amine (8)



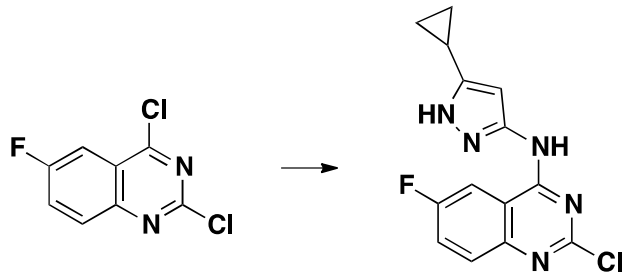
8

To an ice-cooled solution of diisopropylamine (0.12 mL, 0.85 mmol, 2.5 eq.) in THF (2 mL) was added *n*-butyl-lithium (2.5 *M* solution in hexanes, 0.35 mL, 0.85 mmol, 2.5 eq.) under nitrogen atmosphere. The resulting mixture was stirred at 0 ^{0}C for 15 min. The mixture was cooled down to -78 ^{0}C and a solution of pyrimidin-2-amine (48.3 mg, 0.508 mmol, 1.5 eq.) in THF (2 mL) was added. The resulting mixture was stirred at -78^{0}C for 30 min. Then, a solution of 2-(azepan-1-yl)-4,6-dichloroquinazoline (100 mg, 0.338 mmol, 1 eq.) in THF / HMPA (2 / 2.2 mL) was added and the resulting mixture was stirred at -78 ^{0}C for 45 min. Then, cooling bath was removed and the resulting mixture was stirred at room temperature for 3 h. THF was removed by evaporation and residue was dissolved in EtOAc (20 mL). The organic layer was washed with water, brine, dried (Na_2SO_4), filtered and concentrated. The residue was triturated with dichloromethane to afford 2-(azepan-1-yl)-6-chloro-N-(pyrimidin-2-yl)quinazolin-4-amine (**8**) (25 mg, 21%). LC-MS (m/z) 355.1 $[M+H]^+$; ESI-HRMS calcd for 355.1433 $C_{18}H_{19}ClN_6$(M+H), found 355.1429; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.08 (s, 1 H), 8.67 (d, *J* = 3.9 Hz, 2 H), 8.31 (s, 1 H), 7.57 (d, *J* = 8.7 Hz, 1 H), 7.38 (d, *J* = 8.4 Hz, 1 H), 7.15 (s, 1 H), 3.70 (m, 4 H), 1.70 (m, 4 H), 1.46 (m, 4 H) ppm.

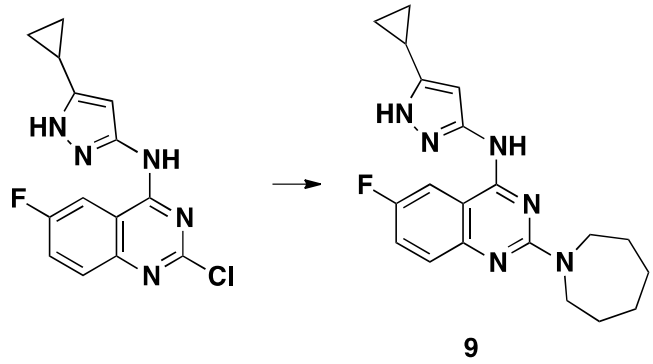
2-(Azepan-1-yl)-*N*-(3-cyclopropyl-1*H*-pyrazol-5-yl)-6-fluoroquinazolin-4-amine (9)



9



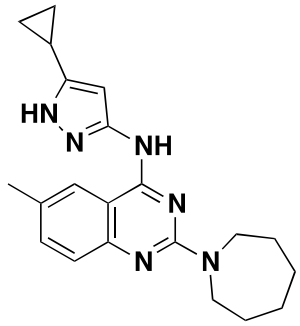
To a solution of 2,4-dichloro-6-fluoroquinazoline (5.0 g, 23 mmol, 1.0 eq.) (prepared according to: *J. Med. Chem.,* 2008, 51, p7855) in ethanol (400 mL) was added 3-cyclopropyl-1*H*-pyrazol-5-amine (5.6 g, 46 mmol, 2.0 eq.). The reaction mixture was stirred at room temperature for 6 h and a precipitate was formed. Filtration afforded 2-chloro-*N*-(3-cyclopropyl-1*H*-pyrazol-5-yl)-6-fluoroquinazolin-4-amine (6.7 g, 96%). LC-MS (m/z) = 304.1 $[M + H]^+$; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.38 (s, 1 H), 10.77 (s, 1 H), 8.53 (s, 1 H), 7.77 (s, 2 H), 6.54 (s, 1 H), 1.96 (s, 1 H), 0.96 (s, 2 H), 0.73 (s, 2 H) ppm.



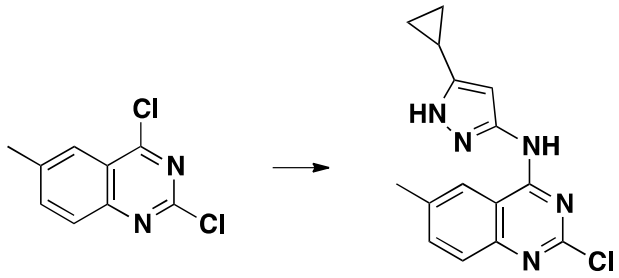
9

To a solution of 2-chloro-*N*-(3-cyclopropyl-1*H*-pyrazol-5-yl)-6-fluoroquinazolin-4-amine (303 mg, 1.0 mmol, 1.0 eq.) in butan-1-ol (10 ml) was added azepane (297 mg, 3.0 mol, 3 eq.) at room temperature. The mixture was stirred at 90 °C for 4 hours. The solvent was removed and the residue was purified by column chromatography on silica gel (dichloromethane / methanol 10 / 1) to obtain 2-(azepan-1-yl)-*N*-(3-cyclopropyl-1*H*-pyrazol-5-yl)-6-fluoroquinazolin-4-amine (**9**) (60 mg, 16%). LC-MS (m/z) = 367.2 $[M + H]^+$; ESI-HRMS calcd for 367.2041 $C_{20}H_{23}FN_6$(M+H), found 367.2040;^{1}H NMR (300 MHz, DMSO-d_6) δ 12.20 (s, 1 H), 9.97 (s, 1 H), 8.24 (d, J = 11.7 Hz, 1 H), 7.46-7.40 (m, 1 H), 7.35-7.30 (m, 1 H), 6.44 (s, 1 H), 3.76 (t, J = 6.0 Hz, 4 H), 1.95-1.87 (m, 1 H), 1.75 (s, 4 H), 1.50 (s, 4 H), 0.98-0.95 (m, 2 H), 0.67-0.64 (m, 2 H) ppm.

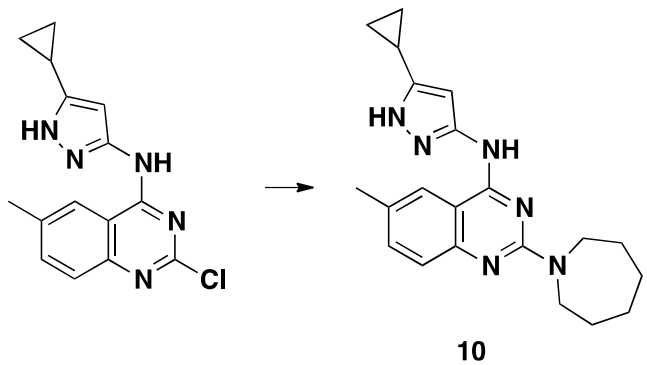
2-(Azepan-1-yl)-*N*-(3-cyclopropyl-1*H*-pyrazol-5-yl)-6-methylquinazolin-4-amine (10)



10



To a solution of 2,4-dichloro-6-methylquinazoline (0.5 g, 4.7 mmol, 1.0 eq.) (prepared according to: *J. Med. Chem.,* 2012, 55, p1346) in ethanol (100 mL) was added 3-cyclopropyl-1*H*-pyrazol-5-amine (0.66 g, 9.4 mmol, 2.0 eq.). The reaction mixture was stirred at room temperature for 6 h. A precipitate was formed. Filtration afforded the product 2-chloro-*N*-(3-cyclopropyl-1*H*-pyrazol-5-yl)-6-methylquinazolin-4-amine (0.44 g, 31%). LC-MS (m/z) = 300.2 $[M + H]^+$; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.33 (s, 1 H), 10.64 (s, 1 H), 8.49 (s, 1 H), 7.67 (s, 1 H), 7.59 (s, 1 H), 6.50 (s, 1 H), 2.46 (s, 3 H), 1.94 (s, 1 H), 0.95 (m, 2 H), 0.72 (m, 2 H) ppm.

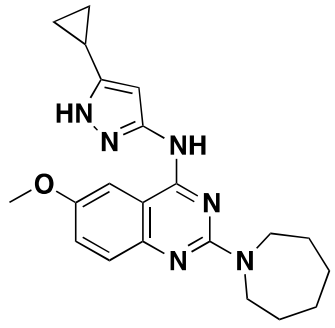


10

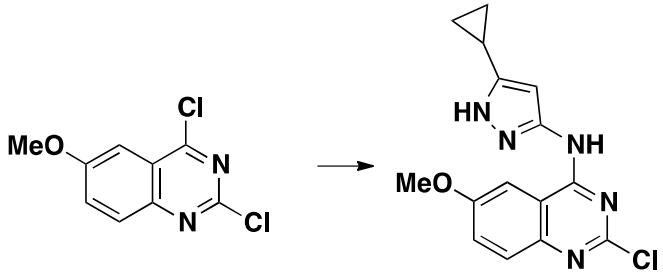
To a solution of 2-chloro-*N*-(3-cyclopropyl-1*H*-pyrazol-5-yl)-6-methylquinazolin-4-amine (300 mg, 1.0 mmol, 1.0 eq.) in butan-1-ol (20 ml) was added azepane (300 mg, 3.0 mol, 3 eq.) at room temperature. Then, the mixture was stirred at 90 °C for 4 h. The

solvent was removed and the residue was purified by column chromatography on silica gel (dichloromethane / methanol 10 / 1) to afford the product 2-(azepan-1-yl)-*N*-(3-cyclopropyl-1*H*-pyrazol-5-yl)-6-methylquinazolin-4-amine (**10**) (52 mg, 14%). LC-MS (m/z) = 363.2 $[M + H]^+$; ESI-HRMS calcd for 363.2292, $C_{21}H_{26}N_6$(M+H), found 3363.2292; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.17 (br s, 1 H), 9.83 (s, 1 H), 8.18 (s, 1 H), 7.35 (d, J = 8.7 Hz, 1 H), 7.22 (d, J = 8.7 Hz, 1 H), 6.44 (s, 1 H), 3.76 (t, J = 5.7 Hz, 4 H), 2.35 (s, 3 H), 1.95-1.84 (m, 1 H), 1.75 (s, 4 H), 1.50 (s, 4 H), 0.97-0.94 (m, 2 H), 0.67-0.65 (m, 2 H) ppm.

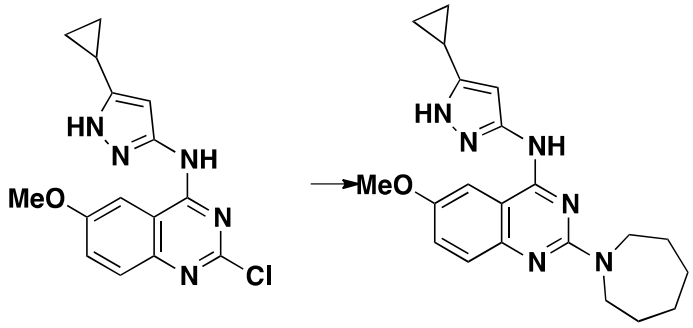
2-(Azepan-1-yl)-*N*-(3-cyclopropyl-1*H*-pyrazol-5-yl)-6-methoxyquinazolin-4-amine (11)



11



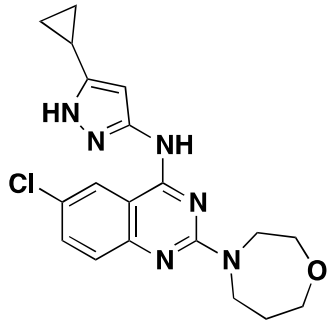
To a solution of 2,4-dichloro-6-methoxyquinazoline (300 mg, 1.31 mmol, 1.0 eq.) in ethanol (53 mL) was added 3-cyclopropyl-1*H*-pyrazol-5-amine (484 g, 3.92 mmol, 3.0 eq.) in anhydrous ethanol (16 mL) dropwise. The reaction mixture was stirred at room temperature for 15 h. Filtration afforded the product 2-chloro-*N*-(3-cyclopropyl-1*H*-pyrazol-5-yl)-6-methoxyquinazolin-4-amine (300 mg, 73%). LC-MS (m/z) = 316.0 $[M + H]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.32 (s, 1H), 10.73 (s, 1H), 8.08 (s, 1H), 7.61 (s, 1H), 7.48 (s, 1H), 6.54 (s, 1H), 3.91 (s, 3H), 1.96 (s, 1H), 0.96 (s, 2H), 0.73 (s, 2H) ppm.



11

To a solution of 2-chloro-*N*-(3-cyclopropyl-1*H*-pyrazol-5-yl)-6-methoxyquinazolin-4-amine (300 mg, 0.95 mmol, 1.0 eq.) in butan-1-ol (12 ml) was added azepane (282 mg, 2.85 mol, 3 eq.) at room temperature. Then, the mixture was stirred at 90 °C for 15 h. The solvent was removed and the residue was purified by column chromatography on silica gel (ethyl acetate / methanol / triethylamine 20 / 1/ 1) to produce the product 2-(azepan-1-yl)-*N*-(3-cyclopropyl-1*H*-pyrazol-5-yl)-6-methoxyquinazolin-4-aminen (**11**) as a solid (200 mg, 56%). LC-MS (m/z) = 379.22 $[M+H]^+$; ESI-HRMS calcd for 379.2241, $C_{21}H_{26}N_6O$(M+H), found 379.2242; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.14 (s, 1 H), 9.93 (s, 1 H), 7.81 (s, 1 H), 7.25-7.14 (m, 2 H), 6.46 (s, 1 H), 3.81 - 3.73 (m, 7 H), 1.91-1.84 (m, 1 H), 1.73 (s, 4 H), 1.48 (s, 4 H), 0.96-0.93 (m, 2 H), 0.66-0.64 (m, 2 H) ppm.

6-Chloro-*N*-(3-cyclopropyl-1*H*-pyrazol-5-yl)-2-(1,4-oxazepan-4-yl)quinazolin-4-amine (12)

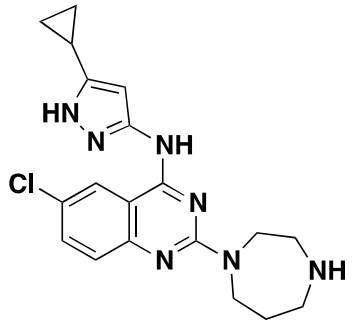


12

To a solution of 2,6-dichloro-*N*-(3-cyclopropyl-1*H*-pyrazol-5-yl)quinazolin-4-amine (300 mg, 0.936 mmol, 1.0 eq.) in butan-1-ol (12 ml) was added K_2CO_3 (382 mg, 2.8 mmol, 3.0 eq) and 1,4-oxazepane hydrochloride (461 mg, 2.8 mmol, 3 eq.) at room temperature. The mixture was stirred at 90 °C for 15 h. The solvent was removed and the residue was purified by column chromatography on silica gel to obtain product 6-chloro-*N*-(3-cyclopropyl-1*H*-pyrazol-5-yl)-2-(1,4-oxazepan-4-yl)quinazolin-4-amine (**12**) as a solid (200 mg, 56%). LC-MS (m/z) = 385.1 $[M+H]^+$; ESI-HRMS calcd for 385.1538, $C_{19}H_{21}ClN_6O$ (M+H), found 385.1537; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.23 (s, 1 H),

10.11 (s, 1 H), 8.55 (s, 1 H), 7.53-7.50 (m, 1 H), 7.31 (d, *J* = 16.0 Hz, 1 H), 6.36 (s, 1 H), 3.91-3.86 (m, 4 H), 3.76-3.73 (m, 2 H), 3.65-3.61 (m, 2 H), 1.96-1.86 (m, 3 H), 0.98-0.92 (m, 2 H), 0.69-0.63 (m, 2 H) ppm.

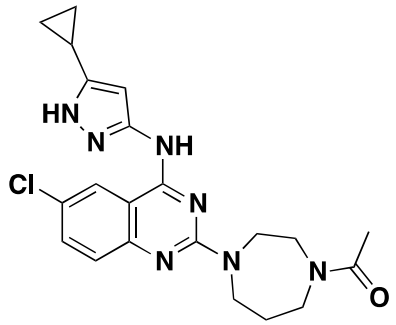
6-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-2-(1,4-diazepan-1-yl)quinazolin-4-amine (13)



13

To a solution of 2,6-dichloro-*N*-(3-cyclopropyl-1*H*-pyrazol-5-yl)quinazolin-4-amine (1 g, 3.12 mmol, 1.0 eq.) in butan-1-ol (12 ml) was added 1,4-diazepane (938 mg, 9.36 mol, 3 eq.) at room temperature. The mixture was stirred at 90 °C for 15 h. The solvent was removed and the residue was purified by column chromatography on silica gel to generate the product methyl 6-chloro-*N*-(3-cyclopropyl-1*H*-pyrazol-5-yl)-2-(1,4-diazepan-1-yl)quinazolin-4-amine (**13**) as a solid (1.04 g, 87%). LC-MS (m/z) = 384.1 $[M + H]^+$. ESI-HRMS calcd for 384.1698 $C_{19}H_{22}ClN_7$(M+H), found 384.1697. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.49 (s, 1 H), 7.89 (m, 2 H), 6.25 (s, 1 H), 4.23 (m, 2 H), 4.00 (m, 2 H), 3.46 (m, 4 H), 2.33 (m, 2 H), 2.02 (m, 1 H), 1.10 (m, 2 H), 0.82 (m, 2 H) ppm.

1-(4-(6-chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)quinazolin-2-yl)-1,4-diazepan-1-yl)ethanone (14)

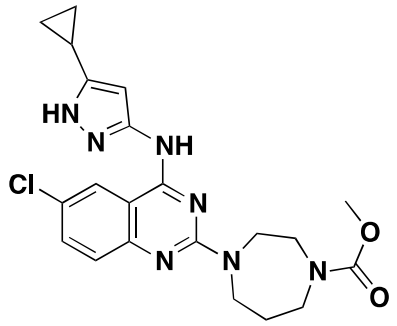


14

To a solution of 6-chloro-*N*-(3-cyclopropyl-1*H*-pyrazol-5-yl)-2-(1,4-diazepan-1-yl)quinazolin-4-amine (1.04 g, 2.7 mmol, 1.0 eq.) in H_2O (4 mL) was added Ac_2O (1.2

mL, 12.5 mmol, 4.6 eq.) in THF (5 mL) dropwise at 0 °C. The reaction mixture was stirred at room temperature for 2 h, the solvent was removed and the residue was purified by column chromatography on silica gel (ethyl acetate /methanol / triethylamine 10 / 1 / 1) to afford the product 1-(4-(6-chloro-4-(3-cyclopropyl-1*H*-pyrazol-5-ylamino) quinazolin-2-yl)-1,4-diazepan-1-yl)ethanone (**14**) as a solid (300 mg, 26%). LC-MS (m/z) = 426.2 $[M + H]^+$; ESI-HRMS calcd for 426.1804 $C_{21}H_{24}ClN_7O$(M+H), found 426.1808; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.24 (s, 1 H), 10.13 (s, 1 H), 8.54 (s, 1 H), 7.54-7.51 (m, 1 H), 7.31 (d, J = 11.2 Hz, 1 H), 6.34 (s, 1 H), 3.94-3.76 (m, 4 H), 3.66-3.60 (m, 2 H), 3.46-3.37 (m, 2 H), 1.99-1.77 (m, 5 H), 1.75-1.72 (m, 1 H), 0.98-0.94 (m, 2 H), 0.69-0.67 (m, 2 H) ppm.

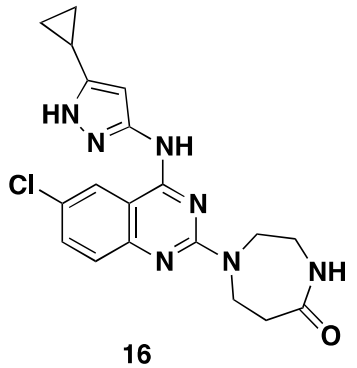
Methyl 4-(6-chloro-4-(3-cyclopropyl-1*H*-pyrazol-5-ylamino)quinazolin-2-yl)-1,4-diazepane-1-carboxylate (15)



15

To a solution of 2,6-dichloro-*N*-(3-cyclopropyl-1*H*-pyrazol-5-yl)quinazolin-4-amine (300 mg, 0.937 mmol, 1.0 eq.) in butan-1-ol (5 ml) was added methyl 1,4-diazepane-1-carboxylate (500 mg, 2.8 mol, 3 eq.) at room temperature. The mixture was stirred at 90 °C for 15 h The solvent was removed and the residue was purified by silica gel to generate the product 1-(4-(6-chloro-4-(3-cyclopropyl-1*H*-pyrazol-5-ylamino)quinazolin-2-yl)-1,4-diazepan-1-yl)ethanone (**15**) as a solid (200 mg, 48%). LC-MS (m/z) = 442.2 $[M + H]^+$; ESI-HRMS calcd for 442.1753 $C_{21}H_{24}ClN_7O_2$(M+H), found 442.1751; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.24 (s, 1 H), 10.12 (s, 1 H), 8.54 (s, 1 H), 7.54-7.51 (m, 1 H), 7.31 (d, J = 5.7 Hz, 1 H), 6.35 (s, 1 H), 3.87-3.78 (m, 4 H), 3.56-3.30 (m, 7 H), 1.92-1.83 (m, 3 H), 1.00-0.96 (m, 2 H), 0.68-0.66 (m, 2 H) ppm.

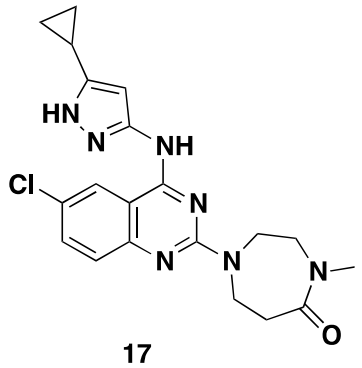
1-(6-Chloro-4-(3-cyclopropyl-1*H*-pyrazol-5-ylamino)quinazolin-2-yl)-1,4-diazepan-5-one (16)



16

To a solution of 2,6-dichloro-*N*-(3-cyclopropyl-1*H*-pyrazol-5-yl)quinazolin-4-amine (100 mg, 0.31 mmol, 1.0 eq.) in butan-1-ol (4 ml) was added 1,4-diazepan-5-one (106 mg, 0.93 mmol, 3 eq.) at room temperature. The mixture was stirred at 90 °C for 15 h. The solvent was removed and the residue was purified by column chromatography on silica gel to provide the product 1-(6-chloro-4-(3-cyclopropyl-1*H*-pyrazol-5-ylamino) quinazolin-2-yl)-1,4-diazepan-5-one (**16**) as a solid (20 mg, 16%). LC-MS (m/z) = 398.1 $[M + H]^+$; ESI-HRMS calcd for 398.1491 $C_{19}H_{20}ClN_7O$(M+H), found 398.1488; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.26 (s, 1 H), 10.17 (s, 1 H), 8.57 (s, 1 H), 7.66 (s, 1 H), 7.55 (m, 1H), 7.37 (m, 1H), 6.27 (s, 1 H), 3.96 (m, 4 H), 3.23 (, 2 H), 2.54 – 2.49 (m, 2 H), 1.94 (m, 1 H), 0.99-0.97 (m, 2 H), 0.69 – 0.67 (m, 2 H) ppm.

1-(6-Chloro-4-(3-cyclopropyl-1H-pyrazol-5-ylamino)quinazolin-2-yl)-4-methyl-1,4-diazepan-5-one (17)

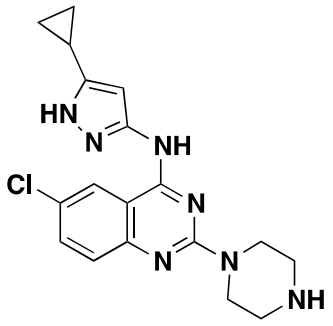


17

To a solution of benzyl 4-methyl-5-oxo-1,4-diazepane-1-carboxylate (1.2 g, 4.57 mmol) in MeOH (50 ml) was added 10% Pd-C (120 mg). The reaction mixture was stirred under H_2 (8 atm) for 24 h. Pd-C was removed by filtration. The filtrate was concentrated to produce the crude 4-methyl-1,4-diazepan-5-one590 mg) which was used directly. LC-MS (m/z) = 129 $[M + H]^+$. To a solution of 2,6-dichloro-*N*-(3-cyclopropyl-1*H*-pyrazol-5-yl)quinazolin-4-amine (100 mg, 0.312 mmol, 1.0 eq.) in butan-1-ol (5 ml) was added crude 4-methyl-1,4-diazepan-5-one (120 mg, 0.936 mol, 3 eq.) at room temperature. The mixture was stirred at 90 °C for 15 h. The solvent was removed and the residue was purified by silica gel to afford the product 1-(6-chloro-4-(3-cyclopropyl-1*H*-pyrazol-5-

ylamino)quinazolin-2-yl)-4-methyl-1,4-diazepan-5-one (**17**) as a solid (25 mg, 20%). LC-MS (m/z) =412.2 $[M + H]^+$; ESI-HRMS calcd for 412.1647 $C_{20}H_{22}ClN_7O$(M+H), found 412.1645; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.23 (s, 1 H), 10.16 (s, 1 H), 8.56 (s, 1 H), 7.55 (d, J = 10.8 Hz, 1 H), 7.34 (d, J = 12.0 Hz, 1 H), 6.31 (s, 1 H), 3.99-3.94 (m, 4 H), 3.54-3.52 (m, 2 H), 2.90 (s, 3 H), 2.65-2.64 (m, 2 H), 1.99-1.83 (m, 1 H), 1.00-0.97 (m, 2 H), 0.71-0.70 (m, 2 H) ppm.

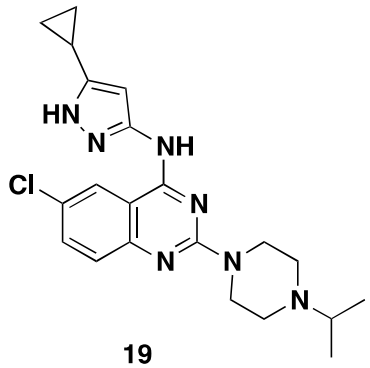
6-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-2-(piperazin-1-yl)quinazolin-4-amine (18)



18

A mixture of 2,6-dichloro-N-(5-cyclopropyl-2H-pyrazol-3-yl)quinazolin-4-amine (80 mg, 0.2499 mmol), tert-butyl piperazine-1-carboxylate (56 mg, 0.3007 mmol) and hunigs base (87 µL, 0.4995 mmol) in n-butanol (2 mL) was heated at 200 ^{o}C for 1 h under a microwave. After removal of solvents, the residiue was stirred with TFA (1 ml) for 30 min. Purification by reverse phase HPLC produced 6-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-2-(piperazin-1-yl)quinazolin-4-amine (**18**) as 2 HCl salt (53 mg, 47%)**.** ESI-MS m/z calc. 369.15, found 370.31 (M+1); ESI-HRMS calcd for 370.1542 $C_{18}H_{20}ClN_7$(M+H), found 370.1540; ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.52 - 12.96 (m, 1 H), 12.60 (s, 1 H), 11.61 - 11.27 (m, 1 H), 8.79 (s, 1 H), 9.42 (s, 2 H), 7.98 (s, 1 H), 7.89 (s, 1 H), 6.31 (s, 1 H), 4.16 (s, 4 H), 3.29 (s, 4 H), 1.98 (ddd, J = 13.4, 8.6, 5.1 Hz, 1 H), 1.03 - 0.90 (m, 2 H), 0.77 - 0.68 (m, 2 H) ppm.

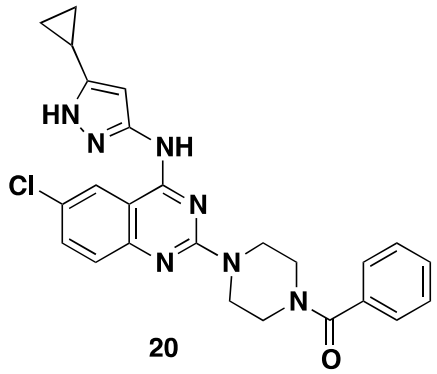
6-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-2-(4-isopropylpiperazin-1-yl)quinazolin-4-amine (19)



19

A mixture of 2,6-dichloro-N-(5-cyclopropyl-2H-pyrazol-3-yl)quinazolin-4-amine (76 mg, 0.2374 mmol), 1-isopropylpiperazine (37 mg, 0.2886 mmol) and hunigs base (83 µL, 0.4765 mmol) in n-butanol (2 mL) was heated at 200 ^{o}C for 1 h under a microwave. After removal of solvent, the residue was purified by reverse phase HPLC to pure HCl salt of **19** (68.2 mg, 0.1518 mmol, 63.9%). LC-MS (m/z) =412.3 $[M + H]^+$; ESI-HRMS calcd for 412.2011 $C_{21}H_{26}ClN_7$ (M+H), found 412.2007; ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.09 (br s, 1H), 11.51 (s, 2 H), 8.84 (d, *J* = 1.5 Hz, 1 H), 8.18 (s, 1 H), 7.92 (d, *J* = 7.5 Hz, 1 H), 6.33 (s, 1 H), 3.94 - 3.45 (m, 8 H), 2.00 (dq, *J* = 8.4, 5.1 Hz, 1 H), 1.31 (d, *J* = 6.6 Hz, 6 H), 1.05 - 0.93 (m, 2 H), 0.81 - 0.63 (m, 2 H) ppm.

(4-(6-chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)quinazolin-2-yl)piperazin-1-yl)(phenyl)methanone (20)

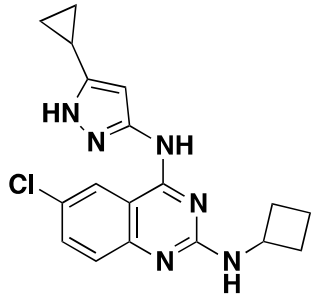


20

A mixture of 2,6-dichloro-N-(5-cyclopropyl-2H-pyrazol-3-yl)quinazolin-4-amine (94 mg, 0.29 mmol), phenyl(piperazin-1-yl)methanone (67 mg, 0.35 mmol) and hunig's base (103 µL, 0.5913 mmol) in n-butanol (2 mL) was heated at 200 ^{o}C for 1 h under a microwave. The crude product was purified by reverse phase HPLC to afford [4-[6-chloro-4-[(3-cyclopropyl-1H-pyrazol-5-yl)amino]quinazolin-2-yl]piperazin-1-yl]-phenyl-methanone (**20**) as HCl salt (64.6 mg, 0.13 mmol, 46.%). LC-MS (m/z) =474.3 $[M + H]^+$; ESI-HRMS calcd for 474.1804 $C_{25}H_{24}ClN_7O$(M+H), found 474.1805; ^{1}H NMR (300

MHz, DMSO-d_6) δ 12.83 (br s, 1 H), 12.60 (s, 1 H), 11.46 (s, 1 H), 8.81 (s, 1 H), 8.05 (s, 1 H), 7.96 - 7.74 (m, 1 H), 7.60-7.43 (m, 5 H), 6.34 (s, 1 H), 4.04 (s, 4 H), 3.56 (s, 4 H), 2.07 - 1.85 (m, 1 H), 0.96 (m, 2 H), 0.71 (m, 2 H) ppm.

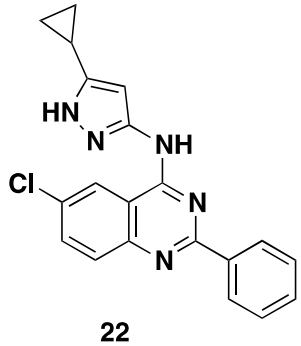
6-Chloro-N^2-cyclobutyl-N^4-(5-cyclopropyl-1*H*-pyrazol-3-yl)quinazoline-2,4-diamine (21)



21

A mixture of 2,6-dichloro-N-(3-cyclopropyl-1H-pyrazol-5-yl)quinazolin-4-amine (320.2 mg, 1 mmol, 1.0 eq.), cyclobutanamine (285 mg, 4 mmol, 4 eq.) in n-BuOH (5 mL)) was heated to 90□ under N for 20 h. The reaction mixture was allowed to cool to room temperature, concentrated *in vacuo* to remove solvent and the residue was directly purified by silica gel column chromatography (eluted with dichloromethane/ methanol, 50 / 1) to produce desired 6-chloro-N^2-cyclobutyl-N^4-(5-cyclopropyl-1*H*-pyrazol-3-yl)quinazoline-2,4-diamine (**21**) (210 mg, 66%). LC-MS (m/z): 355 $[M+H]^+$; ESI-HRMS calcd for 355.1433 $C_{18}H_{19}ClN_6$(M+H), found 355.1431; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.16-12.73 (m, 1 H), 10.01-10.45 (m 1H), 8.36-8.57 (m, 1H), 7.21-7.90 (m, 3 H), 5.78-6.74 (m, 1 H), 4.48-4.51 (m,1 H), 1.63-2.33 (m, 7 H), 0.76-1.02 (m, 4 H) ppm.

6-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-2-phenylquinazolin-4-amine (22)

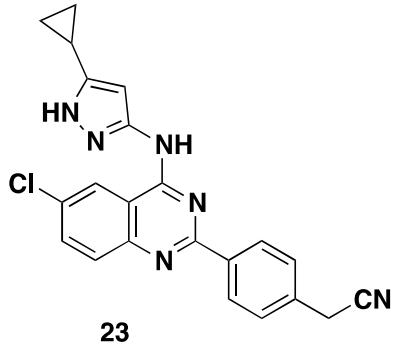


22

A mixture of 2,6-dichloro-N-(3-cyclopropyl-1H-pyrazol-5-yl)quinazolin-4-amine (300 mg, 0.94 mmol, 1.0 eq.), phenylboronic acid (344 mg, 2.82 mmol, 3.0 eq.), $Pd(OAc)_2$ (63 mg, 0.28 mmol, 0.3 eq.), PPh_3 (148 mg, 0.56 mmol, 0.6 eq.) and Na_2CO_3 (300 mg, 2.82

mmol, 3.0 eq.) in dioxane / DMSO / H_2O (20 mL /0.5 mL /1mL)) was heated to 90□ under a nitrogen atmosphere for 3 days. The reaction mixture was allowed to cool to room temperature, concentrated in vacuo to and the residue was directly purified by silica gel column chromatography (eluted with 10% -30% ethyl acetate in petroleum ether) to produce 6-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-2-phenylquinazolin-4-amine (**22**) as pale-yellow solid (30 mg, 10%). LC-MS (m/z): 362.1 $[M+H]^+$; ESI-HRMS calcd for 362.1167 $C_{20}H_{16}ClN_5$(M+H), found 362.1163; ^{1}H NMR(300 MHz, DMSO-d_6); δ 12.30 (s, 1 H), 10.50 (s, 1 H), 8.83 (s, 1 H), 8.44-8.41 (m, 2 H), 7.82 (s, 2 H), 7.51 (dd, *J* = 1.8, 5.4 Hz, 3 H), 6.66 (s, 1 H), 2.01-1.95 (m, 1 H), 1.01-0.97 (m, 2 H), 0.77-0.74 (m, 2 H) ppm.

2-(4-(6-chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)quinazolin-2-yl)phenyl)acetonitrile (23)

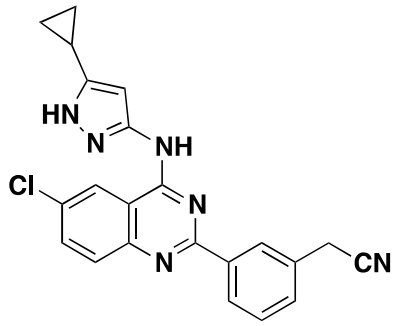


23

Starting from (4-(cyanomethyl)phenyl)boronic acid, **23** was prepared following same procedure as described in **22** in dioxane / DMSO / H_2O (20 / 0.5 / 1)) at 90 ℃ for 3 days. The crude product was purified by silica gel column chromatography (eluted with 10% -30% ethyl acetate in petroleum ether) to afford **23** in 14% yield. LC-MS (m/z): 401.1 $[M+H]^+$; ESI-HRMS calcd for 401.1276 $C_{22}H_{17}ClN_6$(M+H), found 401.1273; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.30 (s, 1 H), 10.53 (s, 1 H), 8.82 (s, 1 H), 8.43 (d, *J* = 8.1Hz, 2 H), 7.82 (s, 2 H), 7.50 (d, *J* = 8.4Hz, 2 H), 6.65 (s, 1 H), 4.14 (s, 2 H), 1.96-2.02 (m, 1 H), 0.96-1.03 (m, 2 H), 0.73-0.79 (m, 2 H) ppm.

.

2-(3-(6-chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)quinazolin-2-yl)phenyl)acetonitrile (24)

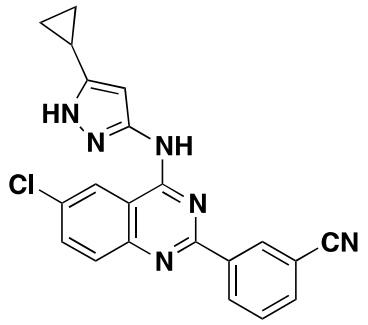


24

To a solution of 2-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acetonitrile (2.5 g, 10.3 mmol, 1.0 eq.) in diethyl ether (50 mL) was added diethanolamine (2.16 g, 20.4 mmol, 2.0 eq.) in 2-propanol (10 mL) and the reaction mixture was stirred at ambient temperature for 72 h. The mixture was then filtered and the solid washed several times with ethyl ether to give a white solid. The solution of this white solid in THF (10 mL) was added 1 *N* HCl (10 mL) and the reaction mixture was stirred for 1h at room temperature. The mixture was then filtered and the white solid was washed several times with water to generate 3-(cyanomethyl)phenylboronic acid (1.2 g, 72.7%), which was directly used in next step.

A mixture of 2,6-dichloro-*N*-(3-cyclopropyl-1H-pyrazol-5-yl)quinazolin-4-amine (1.0 g, 3.13 mmol, 1.0 eq.), 3-(cyanomethyl)phenylboronic acid (1.0 g, 6.27 mmol, 2.0 eq.), $Pd(OAc)_2$ (210 mg, 0.94 mmol, 0.3 eq.), PPh_3 (493 mg, 1.88 mmol, 0.6 eq.) and Na_2CO_3 (996 mg, 9.4 mmol, 3.0 eq.) in dioxane / DMSO / H_2O (40 mL /1 mL /3mL)) was heated to 90 □ under a nitrogen atmosphere and stirred for 3 days. The solvent was removed to give a residue. The residue was purified by silica gel column chromatography (eluted with 10% -30 % ethyl acetate in petroleum ether) to afford 2-(3-(6-chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)quinazolin-2-yl)phenyl)acetonitrile (**24**) as an orange solid (200 mg, 16%). LC-MS (m/z): 401.1 $[M+H]^+$; ESI-HRMS calcd for 401.1276 $C_{22}H_{17}ClN_6$(M+H), found 401.1272; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.28 (s, 1 H), 10.54 (s, 1 H), 8.85 (s, 1 H), 8.44 (s, 1 H), 8.38 (d, *J*=7.8Hz, 1 H), 7.83 (s, 2 H), 7.57-7.46 (m, 2 H), 4.17 (s, 2 H), 6.69 (s, 1 H), 2.00 (m, 1 H), 0.99-0.95 (m, 2 H), 0.81-0.78 (m, 2 H) ppm.

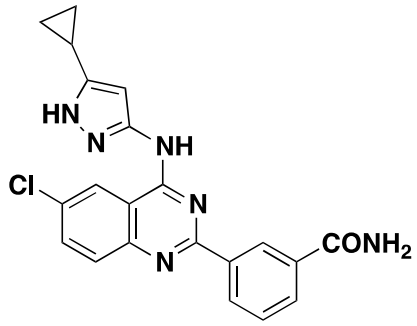
3-(6-chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)quinazolin-2-yl)benzonitrile (25)



25

Starting from 3-(cyanophenyl)boronic acid, **25** was prepared following same procedure as described in **22** in dioxane / DMSO / H_2O (20 / 0.5 / 1)) at 90 □ for 3 days. The crude product was purified by silica gel column chromatography (eluted with 10% -30% ethyl acetate in petroleum ether) to afford the desired product **25** in 13% yield. LC-MS (m/z): 387.1 $[M+H]^+$; ESI-HRMS calcd for 387.1120 $C_{21}H_{15}ClN_6$ (M+H), found 387.1113; ^1H NMR(300 MHz, DMSO-d_6): δ 12.38 (s, 1 H), 10.61 (s, 1 H), 8.82 (s, 1 H), 8.69-8.67 (m, 2 H), 7.98-7.94 (m, 1 H), 7.83 (s, 2 H), 7.73 (t, *J* = 8.7Hz, 1 H), 6.57 (s, 1 H), 1.99-1.93 (m, 1 H), 1.04-0.98 (m, 2 H), 0.80-0.76 (m, 2 H) ppm.

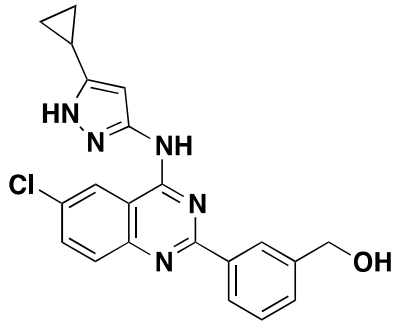
3-(6-chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)quinazolin-2-yl)benzamide (26)



26

Starting from (3-carbamoylphenyl)boronic acid, **26** was prepared following same procedure as described in **22** in dioxane / DMSO / H_2O (20 / 0.5 / 1)) at 90 □ for 3 days in 79% yield. LC-MS (m/z): 371.2 $[M+H]^+$; ESI-HRMS calcd for 405.1225 $C_{21}H_{17}ClN_6O$ (M+H), found 405.1222; ^1H NMR (300 MHz, DMSO-d_6) δ 12.33 (s, 1 H), 10.57 (s, 1 H), 8.95 (s, 1 H), 8.86 (s, 1 H), 8.55 (d, *J*=8.1Hz, 1 H), 8.09 (s, 1 H), 7.97 (d, *J* = 7.8Hz, 1H), 7.84 (s, 2 H), 7.59 (t, *J* = 7.5 Hz, 1 H), 7.48 (s, 1 H), 6.71 (s, 1 H), 2.00-1.94 (m, 1 H), 0.98-0.85 (m, 4 H).

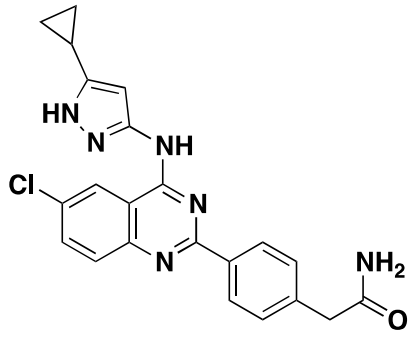
(3-(6-chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)quinazolin-2-yl)phenyl) methanol (27)



27

Starting from (3-(hydroxymethyl)phenyl)boronic acid, **27** was prepared following same procedure as described in **22** in dioxane / DMSO / H_2O (20 / 0.5 / 1)) at 90 □ for 3 day in 14% yield. LC-MS (m/z): 392.1 $[M+H]^+$; ESI-HRMS calcd for 392.1273 $C_{21}H_{18}ClN_5O$ (M+H), found 392.1266; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.32 (s, 1 H), 10.51 (s, 1 H), 8.84 (s, 1 H), 8.44 (s, 1 H), 8.31 (d, J = 6.6Hz, 1 H), 7.82 (s, 2 H),7.45-7.47 (m, 2 H), 6.70 (s, 1 H), 5.29 (t, J = 5.7 Hz, 1 H), 4.61 (d, J = 6.0 Hz, 2 H), 1.96 (m, 1 H), 1.01-0.97 (m, 2 H), 0.80-0.78 (m, 2 H) ppm.

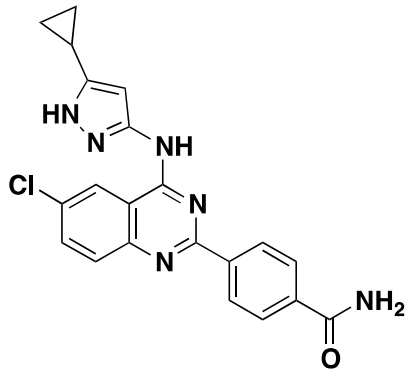
2-(4-(6-chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)quinazolin-2-yl)phenyl) acetamide (28)



28

Starting from (4-(2-amino-2-oxoethyl)phenyl)boronic acid, **28** was prepared following same procedure as described in **22** in dioxane / DMSO / H_2O (20 / 0.5 / 1)) at 90 □ for 3 day in 83% yield. MS (m/z): 419.1 $[M+H]^+$; ESI-HRMS calcd for 419.1382 $C_{22}H_{19}ClN_6O$(M+H), found 419.1374; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.31 (s, 1 H), 10.51 (s, 1 H), 8.82 (s, 1 H), 8.35 (d, J = 7.8Hz, 2 H), 7.81 (s, 2 H), 7.52 (s, 1 H),7.40-7.52 (d, J = 7.5Hz, 2 H), 6.92 (s, 1 H), 6.65 (s, 1 H), 3.46 (s, 2 H), 1.99-1.97 (m, 1 H), 1.01-0.96 (m, 2 H), 0.77-0.76 (m, 2 H) ppm.

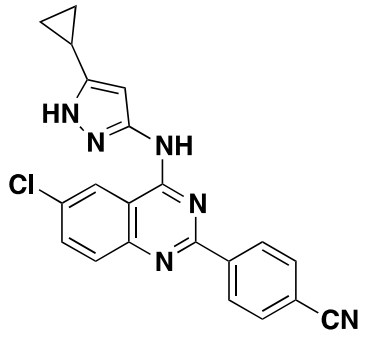
4-(6-chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)quinazolin-2-yl)benzamide (29)



29

Starting from (4-carbamoylphenyl)boronic acid. **29** was prepared following same procedure as described in **22** in dioxane / DMSO / H_2O (20 / 0.5 / 1)) at 90 □ for 3 day in 75% yield. MS (m/z): 405.1 $[M+H]^+$; ESI-HRMS calcd for 405.1225 $C_{21}H_{17}ClN_6O$ (M+H), found 405.1222; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.32 (s, 1 H), 10.56 (s, 1 H), 8.84 (s, 1 H), 8.47 (d, J = 8.4 Hz, 2 H), 8.08 (s, 1 H), 8.01 (d, J = 8.4 Hz, 2 H), 7.84 (s, 2 H), 7.45 (s, 1 H), 6.64 (s, 1 H), 2.00-1.97 (m, 1 H),1.01-0.99 (m, 2 H), 0.77-0.76(m, 2 H) ppm.

4-(6-chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)quinazolin-2-yl)benzonitrile (30)

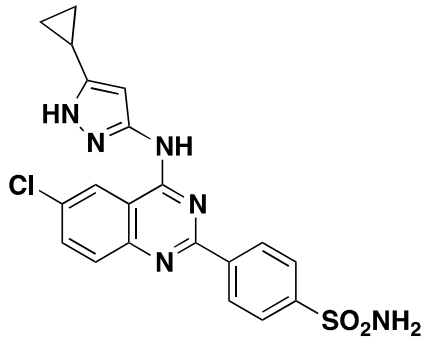


30

A mixture of 2,6-dichloro-*N*-(3-cyclopropyl-1H-pyrazol-5-yl)quinazolin-4-amine (1.0 g, 3.13 mmol, 1.0 eq.), 4-cyanophenylboronic acid (922 mg, 6.26 mmol, 2.0 eq), $Pd(OAc)_2$ (210 mg, 0.94 mmol, 0.3 eq.), PPh_3 (493 mg, 1.88 mmol, 0.6 eq.), Na_2CO_3 (997 mg, 29.40 mmol, 3.0 eq) in dioxane / DMSO / H_2O (40 mL /1 mL /3mL)) was heated to 90 □ under a nitrogen atmosphere and stirred for 3 days. The reaction mixture was allowed to cool down to room temperature and concentrated *in vacuo* to remove the solvent. The residue was directly purified by silica gel column chromatography (eluted with 10% -30

% ethyl acetate in petroleum ether) to afford 4-(6-chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)quinazolin-2-yl)benzonitrile **30** as pale-yellow solid (230 mg, 19%). LC-MS (m/z): 387.1 $[M+H]^+$; ESI-HRMS calcd for 387.1120 $C_{21}H_{15}ClN_6$ (M+H), found 387.1113; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.32 (s, 1 H), 10.59 (s, 1 H), 8.84 (s, 1 H), 8.54 (d, J = 8.4 Hz, 2 H), 7.99 (d, J = 8.1Hz, 2 H), 7.85 (s, 2 H), 6.59 (s, 1 H), 2.01-1.95 (m, 1 H), 1.02-0.96 (m, 2 H), 0.79-0.74 (m, 2 H) ppm

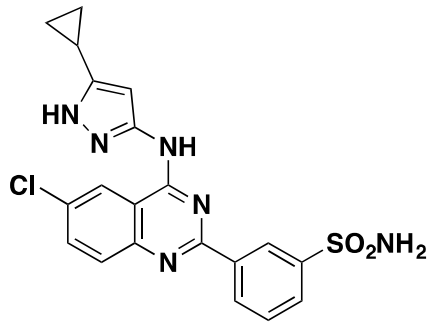
4-(6-chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)quinazolin-2-yl)benzene sulfonamide (31)



31

A mixture of 2,6-dichloro-N-(3-cyclopropyl-1H-pyrazol-5-yl)quinazolin-4- amine (320 mg, 1.0 mmol, 1.0 eq.), 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzene sulfonamide (283 mg, 1.0 mmol, 1 eq.), $Pd_2(dba)_3$ (92 mg, 0.1mmol, 0.1 eq.), PCy_3 (67 mg, 0.24 mmol, 0.24 eq.), K_3PO_4 (736 mg, 3.4 mmol, 3.4 eq.) in dioxane / H_2O (5 mL / 2.7 mL) was heated to 100 ^{0}C under a nitrogen atmosphere and stirred for 14 hours. The reaction mixture was cooled to room temperature, concentrated *in vacuo* to remove solvent and the residue was directly purified by silica gel column chromatography (eluted with 0%-5%methanol in dichloromethane) to afford desired product 4-(6-chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)quinazolin-2-yl)benzene sulfonamide **31** (40 mg, 11%). MS (ESI)**:** 440.91 $[M+H]^+$; ESI-HRMS calcd for 441.0895 $C_{20}H_{17}ClN_6O_2S$(M+H), found 441.0888; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.37 (s, 1 H), 10.63 (s, 1 H), 8.88 (s, 1 H), 8.58 (d, J = 8.1Hz, 2 H), 8.00 (d, J = 8.7Hz, 2 H), 7.88 (s, 2 H), 7.48 (s, 2 H), 6.68 (s, 1 H), 2.07-1.98 (m, 1 H), 1.06-1.01 (m, 2 H), 0.82-0.77 (m, 2 H) ppm.

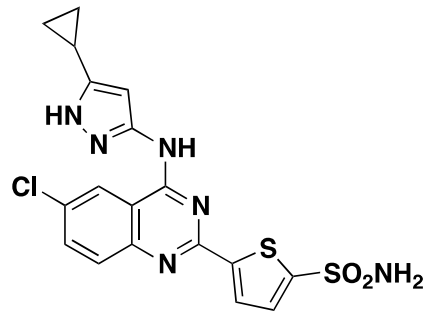
3**-(6-chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)quinazolin-2-yl)benzene sulfinamide (32)**



32

Starting from 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzenesulfonamide, **32** was prepared following same procedure as described in **31** in dioxane /H_2O (2 / 1) at 100 ^{0}C under a nitrogen atmosphere for 14 hours in 11% yield. MS (ESI)**:** 440.91 $[M+H]^+$; ESI-HRMS calcd for 441.0895 $C_{20}H_{17}ClN_6O_2S$(M+H), found 441.0894; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.32 (s, 1 H), 10.59 (s, 1 H), 8.87～8.92 (m, 2 H), 8.61 (d, J = 7.8Hz, 1 H), 7.96 (d, J = 8.1Hz, 1 H), 7.86 (s, 2 H), 7.73 (t, J = 7.5Hz, 1H), 7.42 (s, 2 H), 6.60 (d, J=1.8Hz, 1 H), 2.01-1.92 (m, 1 H), 1.00-0.94 (m, *2* H), 0.86-0.81 (m, *2* H) ppm.

5-(6-chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)quinazolin-2-yl)thiophene-2-sulfonamide (33) hydrochloride



33

Chlorosulfonic acid (3 ml) was added dropwise over 20 min to a cold solution (-78℃) of 2-bromothiophene (2 g, 12.2 mmol) in methylene chloride (7 mL). The reaction mixture was allowed to warm to room temperature slowly (2 h) and cold water (50 ml) was added dropwise. The reaction was extracted with methylene chloride (3 x 20 ml). The combined organic layers were dried ($MgSO_4$), filtered and the solution was concentrated to generate crude 5-bromothiophene-2-sulfonyl chloride (3 g, 94%) which was directly used. LC-MS (m/z) =261.0 $[M+H]^+$.

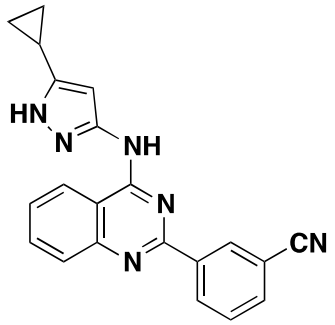
A mixture of 5-bromothiophene-2-sulfonyl chloride obtained above (500 mg, 1.912 mmol) and 2-methylpropan-2-amine (3 mL) in dioxane (10 mL) was stirred at room temperature for 2 h. Water (50 ml) was added and the reaction was extracted with

methylene chloride (3 x 30 ml). The combined organic layers were dried (Na_2SO_4) and filtered. The filtrate was concentrated to afford 5-bromo-N-tert-butylthiophene-2-sulfonamide (520 mg, 91.2%). LC-MS (m/z) =298.0 $[M+H]^+$.

A mixture of 5-bromo-N-tert-butylthiophene-2-sulfonamide (400 mg, 1.34 mmol, 1 eq.), bis(pinacolato)diborn (409 mg, 1.61 mmol, 1.2 eq.), KOAc (328.8 mg, 3.35 mmol, 2.5 eq.), $PdCl_2(dppf)CH_2Cl_2$ (109.5 mg, 0.134 mmol, 0.1 eq.) and DMSO (1 mL) was flushed with nitrogen. 1, 4-dioxane (20 mL) was added and the reaction mixture was stirred at 90 □ for 1h. After cooling, the reaction mixture was filtered. $Pd_2(dba)_3$ (123 mg, 0.134 mmol, 0.1 eq.), 2-bromo-6-chloro-N-(5-cyclopropyl-1H-pyrazol-3-yl) quinazolin-4-amine (488 mg, 1.34 mmol, 1 eq.), Pcy_3 (90.26 mg, 0.322 mmol, 0.24 eq.) and aqueous K_3PO_4 (3.6 mL) was added to the filtrate. The reaction mixture was heated to 100 □ for 2 h under nitrogen atmosphere. The reaction mixture was cooled down to room temperature and extracted with THF. The combined layers was purified by silica gel chromatography (CH_2Cl_2 / THF / Et_3N 40 : 3 : 1) to afford N-tert-butyl-5-(6-chloro-4-(5-cyclopropyl-1H-pyrazol-3-ylamino)quinazolin-2-yl)thiophene-2-sulfonamide as a solid (200 mg, 29.7%); LC-MS (m/z) =503.1 $[M+H]^+$; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.38 (s, 1 H), 10.73 (s, 1 H), 8.88 (s, 1 H), 7.90-7.84 (m, 3 H), 7.79 (d, J = 8.7Hz, 1 H), 7.63 (d, J = 3.6Hz, 1 H), 6.69 (s, 1 H), 2.03-1.94 (m, 1 H), 1.21 (s, 9 H), 1.03-1.00 (m, 2 H), 0.81-0.78 (m, 2 H) ppm.

The mixture of N-tert-butyl-5-(6-chloro-4-(5-cyclopropyl-1H-pyrazol-3-ylamino) quinazolin-2-yl) thiophene-2-sulfonamide (200 mg, 0.397 mmol) in 4 *N* HCl (8 mL) was heated to 100 □ for 8 h. The reaction mixture was extracted with THF. The combined organic layers were dried (Na_2SO_4) and filtered. The filtrate was concentrated by evaporation. The resulting residue was crystallized by methanol to afford crude product. The crude product was re-crystallized by methanol to afford 5-(6-chloro-4-(5-cyclopropyl-1H-pyrazol-3-ylamino)quinazolin-2-yl)thiophene-2-sulfonamide hydrochloride **(33)** (30 mg, 15.7%). LC-MS (m/z) =447.0 $[M+H]^+$; ESI-HRMS calcd for 447.0459 $C_{18}H_{15}ClN_6O_2S_2HCl$ (M+H), found 447.0456; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.23 (s, 1 H), 10.90 (s, 1 H), 8.89 (d, J = 1.8 Hz, 1 H), 7.97 (d, J = 4.2Hz, 1 H), 7.88-7.83 (m, 4 H), 7.64 (d, J = 4.5 Hz, 1H), 6.67 (s, 1 H), 2.04-1.95 (m, 1 H), 1.04-0.99 (m, 2 H), 0.83-0.79 (m, 2 H) ppm.

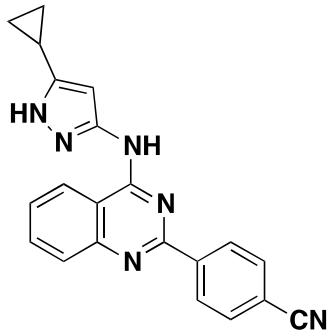
3-(4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)quinazolin-2-yl)benzonitrile (34)



34

Starting from 2-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)quinazolin-4-amine and 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile, **34** was prepared following same procedure as described in **31** in 17% yield in dioxane / H_2O (2 / 1) at 100 ^{0}C under a nitrogen atmosphere for 14 hours. MS (m/z): 353.2 $[M+H]^+$; ESI-HRMS calcd for 353.1509 $C_{21}H_{16}N_6$(M+H), found 353.1505; ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.85 (s, 1 H), 8.77 (d, *J* = 8.1Hz, 1 H), 8.62 (d, *J* = 6.9Hz, 1 H), 8.16 (s, 1 H), 8.00-7.97 (m, 1 H), 7.92-7.87 (m, 1 H), 7.83-7.77 (m, 1 H), 7.75 (m, 1 H), 7.65-7.61 (m, 1 H), 7.58-7.53 (m, 1 H), 6.75 (bs, 1 H), 2.01-1.92 (m, 1 H), 1.08-1.06 (m, 2 H), 0.91-0.85 (m, 2 H) ppm.

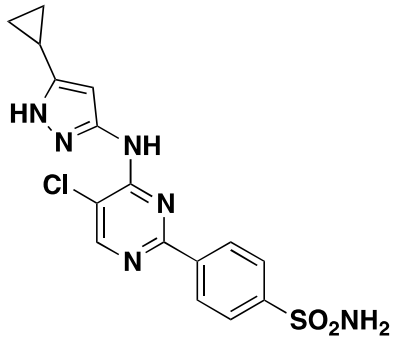
4-(4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)quinazolin-2-yl)benzonitrile (35)



35

Starting from 2-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)quinazolin-4-amine and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile, **35** was prepared following same procedure as described in **31** in 16% yield in dioxane / H_2O (2 / 1) at 100 ^{0}C under a nitrogen atmosphere for 14 hours. MS (m/z): 353.2 $[M+H]^+$; ESI-HRMS calcd for 353.1509 $C_{21}H_{16}N_6$(M+H), found 353.1505; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.29 (s, 1 H), 10.47 (s, 1 H), 8.64 (d, *J* = 8.4Hz, 1 H), 8.56 (d, *J* = 8.4Hz, 2 H), 8.00 (d, *J* = 8.4Hz, 2 H), 7.85 (m, 2 H), 7.56-7.58 (m, 1H), 6.60 (s, 1 H), 1.97-2.00 (m, 1 H), 0.98-1.02 (m, 2 H), 0.76-0.78 (m, 2 H) ppm.

4-(5-chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)benzene sulfonamide (36)



36

A mixture of 4-bromobenzene-1-sulfonyl chloride (4.89g, 19.1 mmol, 1.0 eq.), DIPEA (10 mL) and 2-methylpropan-2-amine (30 mL) in DCM (100 mL) was stirred at room temperature for 1 h. Water (200 mL) was added and the reaction was extracted with EA (3 x 100 mL). The combined organic layers were dried (Na_2SO_4) and filtered. The filtrate was concentrated to afford 4-bromo-*N*-(*tert*-butyl)benzenesulfonamide (5.1 g, 91%) used directly. LC-MS (m/z) = 293 $[M + H]^+$.

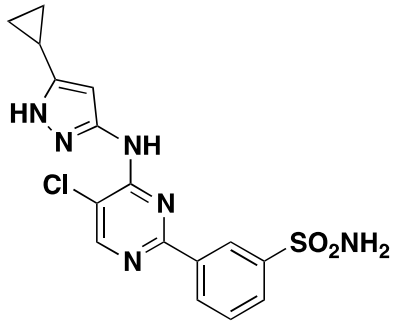
A mixture of 4-bromo-*N*-(*tert*-butyl)benzenesulfonamide (2.7 g, 9.05 mmol, 1.0 eq.), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (3 g, 10.86 mmol, 1.2 eq.), Pd(dppf)Cl_2 (0.74 g, 0.905 mmol, 0.1 eq.), CH_3COOK (3.59 g, 36.7 mmol, 4.0 eq.), 1,4-dioxane (30 ml) was stirred at 95 ^oC under a nitrogen atmosphere for 2 h. The reaction mixture was allowed to cool to room temperature and concentrated to give a residue. The residue was purified by silica gel chromatography (petroleum ether / ethyl acetate 300 : 1 to 40 : 1 as eluent) to afford *N*-(*tert*-butyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzenesulfonamide (2.1 g, 68%). LC-MS (m/z) = 340.1 $[M + H]^+$.

A mixture of *N*-(*tert*-butyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzene sulfonamide (441 mg, 1.30 mmol, 1.3 eq.), 2-bromo-5-chloro-N-(3-cyclopropyl-1H-pyrazol-5-yl)pyrimidin-4-amine (314 mg, 1.0 mmol, 1.0 eq.), Pd (dppf)Cl_2 (88 mg, 0.107 mmol, 0.1 eq.), Na_2CO_3 (420 mg, 4.0 mmol, 4.0 eq.), 1,4-dioxane (20 ml) and water (5 ml) was stirred at 95 ^oC under a nitrogen atmosphere for 2 h. The reaction mixture was allowed to cool to room temperature and concentrated to give a residue. The residue was purified by silica gel chromatography (dichloromethane / methanol 500 : 1 to 50 : 1 as eluent) to afford *N*-(*tert*-butyl)-4-(5-chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino) pyrimidin-2-yl)benzenesulfonamide (325.6 mg, 73%) as light yellow solid. LC-MS (m/z) = 448.0 $[M + H]^+$.

A mixture of *N*-(*tert*-butyl)-4-(5-chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino) pyrimidin-2-yl)benzenesulfonamide (320 mg, 0.72 mmol, 1.0 eq.), BCl_3 / CH_2Cl_2 (1 M ,

7.17 ml, 7.17 mmol, 10 eq.) in dichloromethane (20 ml) was stirred at 0 ^{o}C under a nitrogen atmosphere for 0.5 h. Water (50 ml) was added into the mixture and the mixture was extracted with ethyl acetate (3 X 50 ml) The combined organic phases was washed with saturated aqueous $NaHCO_3$, brine, dried (Na_2SO_4), filtered and concentrated. The crude product was purified by silica gel chromatography (ethyl acetate / petroleum ether 1 : 2) and washing with ether to afford 4-(5-chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)benzenesulfonamide (**36**) (126 mg, 45.1%) as a light yellow solid. LC-MS (m/z) = 391.6 $[M + H]^+$; ESI-HRMS calcd for 391.0739 $C_{16}H_{15}ClN_6O_2S$ (M+H), found 391.0735; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.32 (s, 1H), 9.39 (s, 1 H), 8.55 (s, 1 H), 8.40 (d, J = 8.8 Hz, 2 H), 7.95 (d, J = 8.4 Hz, 2 H), 7.47 (s, 2 H), 6.41 (d, J = 1.6 Hz, 1 H), 1.96-2.01 (m, 1 H),0.98-1.02 (m, 2 H), 0.74-0.78 (m, 2 H) ppm.

3-(5-chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)benzene sulfonamide (37)



37

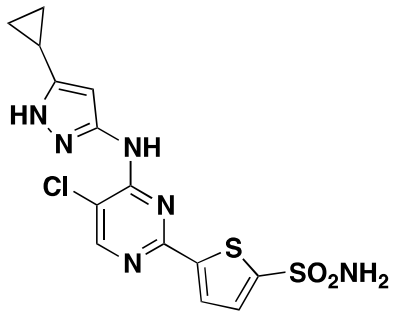
A mixture of 5-chloropyrimidine-2,4-diol (20 g, 136.5 mmol), N,N-dimethyl benzenamine (35ml, 273.0 mmol, 2 eq) and toluene (500 ml) was stirred at r.t for 5 minutes, then $POBr_3$ (117.4g, 409.5 mmol, 3 eq) was added portionwise at 0 ^{o}C. After 10 min, the reaction was heated at 90 ^{o}C for 2 h. The mixture was cooled to room temperature, the toluene layer was poured onto crushed ice (500 g), ethyl acetate (300 ml) was added and the separated organic layer was washed with brine, dried (Na_2SO_4), filtered, evaporated and purified by silica gel chromatography (ethyl acetate / petroleum ether 10 : 1 to 5 : 1) to afford the compound 2,4-dibromo-5-chloropyrimidine (**40**) (31g, 83%) as white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.88 (s, 1H) ppm./

A mixture of compound 2,4-dibromo-5-chloropyrimidine (5 g , 18.4 mmol) and 5-cyclopropyl-1H-pyrazol-3-Amine (3.4 g, 27.6mmol, 1.5 eq) in ethanol(50 ml) was stirred at room temperature for 4 h. The reaction mixture was filtered, washed with ethanol to afford 2-bromo-5-chloro-N-(5-cyclopropyl-1H-pyrazol-3-yl)pyrimidin-4-amine (**41**) (4.6 g, 78.0%). LC-MS (m/z) =314.0 $[M+H]^+$, ^{1}H-NMR (500 MHz, DMSO-d_6) δ 12.35 (s, 1

H), 9.69 (s, 1 H), 8.26 (s, 1 H), 6.19 (s, 1 H), 1.92 (s, 1 H), 0.95 (d, *J* = 6.5 Hz, 2 H), 0.70 (d, *J* = 3.2 Hz, 2 H) ppm.

From **41**, **37** (8.5%) was prepared following procedures as described in **31** in dioxane / H_2O (5 mL / 2.7 mL) at 100 ^{0}C under a nitrogen atmosphere for 14 hours.. LC-MS (m/z) = 391.0 $[M+H]^+$; ESI-HRMS calcd for 391.0739 $C_{16}H_{15}ClN_6O_2S$ (M+H), found 391.0735; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.31 (s, 1 H), 9.36 (s, 1 H), 8.76 (s, 1 H), 8.54 (s, 1 H), 8.43 (d, *J* = 8.0Hz, 1 H), 7.94 (d, *J*=7.6Hz, 1 H), 7.71 (t, *J*=7.2Hz, 1 H), 6.41 (s, 2 H), 7.43 (s, 2 H), 1.93 (s, 1 H), 0.95 (d, *J* = 7.2 Hz, 2 H), 0.81 (s, 2 H) ppm.

5-(5-Chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thiophene-2-sulfonamide (38) hydrochloride



38

A mixture of 5-bromothiophene-2-sulfonyl chloride **42** (500 mg, 1.91 mmol, 1.0 eq.), DIPEA (1 mL) and 2-methylpropan-2-amine (3 mL) in dioxane (10 mL) was stirred at room temperature for 4 h. Water (30 mL) was added and the reaction was extracted with ethyl acetate (3 x 10 mL). The combined organic layers were dried (Na_2SO_4), filtered. The filtrate was concentrated to afford 5-bromo-*N*-(*tert*-butyl)thiophene-2-sulfonamide (**43**) (535 mg, 94%) used directly for next step. LC-MS (m/z) =299.0 $[M+H]^+$.

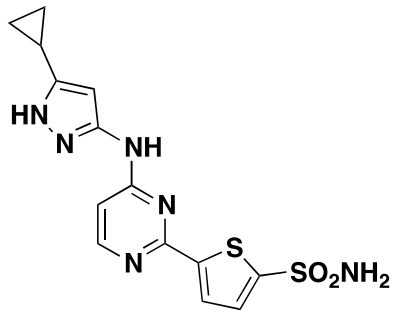
A mixture of 5-bromo-*N*-(*tert*-butyl)thiophene-2-sulfonamide **43** (2.7 g, 9.05 mmol, 1.0 eq.), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (3 g, 10.86 mmol, 1.2 eq.), Pd(dppf)Cl_2 (0.74 g, 0.905 mmol, 0.1 eq.), CH_3COOK (3.59 g, 36.7 mmol, 4.0 eq.) in 1,4-dioxane (30 ml) was stirred at 100 ^{o}C under a nitrogen atmosphere for 3 h. The reaction mixture was allowed to cool to room temperature and concentrated to give a residue. The residue was purified by silica gel chromatography (petroleum ether / ethyl acetate 300 : 1 to 40 : 1 as eluent) to produce *N*-(*tert*-butyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiophene-2-sulfonamide (**44**) (2.2 g, 70%), which was used directly.

A mixture of *N*-(*tert*-butyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiophene-2-sulfonamide (**44**) (448 mg, 1.30 mmol, 1.3 eq.), 2-bromo-5-chloro-N-(3-cyclopropyl-1H-pyrazol-5-yl)pyrimidin-4-amine (314 mg, 1.0 mmol, 1.0 eq.), Pd (dppf)Cl_2 (88 mg, 0.107 mmol, 0.1 eq.), Na_2CO_3 (420 mg, 4.0 mmol, 4.0 eq.) in 1,4-dioxane (20 ml) and water (5 ml) was stirred at 100 ^{o}C under a nitrogen atmosphere for 15 h. The reaction mixture was

allowed to cool to room temperature and concentrated to give a residue. The residue was purified by silica gel chromatography (dichloromethane / methanol 500 : 1 to 50 : 1) to afford *N*-(*tert*-butyl)-5-(5-chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thiophene-2-sulfonamide (**45**) (302 mg, 67%) as white solid. LC-MS (m/z) =454.1 $[M+H]^+$

A mixture of compound **45** (325 mg, 0.717 mmol, 1.0 eq.), BCl_3 / CH_2Cl_2 (7.17 ml, 7.17 mmol, 10 eq. 1 M) in CH_2Cl_2 (20 ml) was stirred at 20 ^oC under a nitrogen atmosphere for 6 h. Water (50 ml) was added into the mixture, the mixture was extracted by ethyl acetate (3 X 50 ml) The combined organic phases were washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), filtered and concentrated. The residue was purified by recrystallization (ethyl acetate /petroleum ether / diisopropyl ether) to afford title compound 5-(5-chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl) amino) pyrimidin-2-yl)thiophene-2-sulfonamide hydrochloride (**38**) (111 mg, 39.1%) as a yellow solid. LC-MS (m/z) =397.2 $[M+H]^+$; ESI-HRMS calcd for 397.0303$C_{14}H_{13}ClN_6O_2S_2$ (M+H), found 397.0298; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.33 (br s, 1 H), 9.65 (s, 1 H), 8.50 (s, 1 H), 7.84 (s, 2 H), 7.78 (d, *J*=3.6Hz, 1 H), 7.59 (d, *J*=4.4Hz, 1 H), 6.42 (s, 1 H), 1.99-1.97(m, 1 H), 1.02-1.00 (m, 2 H), 0.79-0.78 (m, 2 H) ppm.

5-(4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thiophene-2-sulfonamide (46)



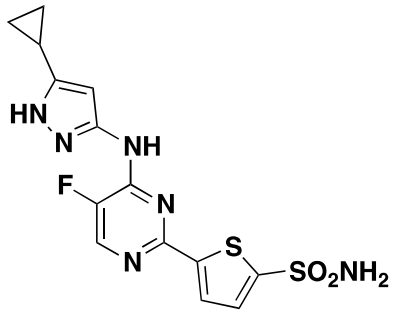
- **46**

A mixture of 2-bromo-N-(5-cyclopropyl-1H-pyrazol-3-yl)pyrimidin-4-amine (300 mg, 1.071 mmol, 1.0 eq.), *N*-(*tert*-butyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiophene-2-sulfonamide (443.7 mg, 1.285 mmol, 1.2 eq.), Pd(dppf)Cl_2 (87.5 mg, 0.1071 mmol, 0.1 eq.), Na_2CO_3 (454.1 mg, 4.284 mmol, 4 eq.), 1,4-dioxane (20 ml) and water (4 ml) was stirred at 100 ^oC under a nitrogen atmosphere for 15 h. The reaction mixture was allowed to cool to room temperature and concentrated to give a residue, which was purified by silica gel chromatography (dichloromethane / methanol 500 : 1 to 50 :1) to afford *N*-(*tert*-butyl)-5-(4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thiophene-2-sulfonamide (300 mg, 66.9%) as solid. LC-MS (m/z) =419.1 $[M+H]^+$.

A mixture of *N*-(*tert*-butyl)-5-(4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thiophene-2-sulfonamide (300 mg, 0.717 mmol, 1.0 eq.), BCl_3 / CH_2Cl_2 (7.17 ml, 7.17

mmol, 10 eq. 1 M) in dichloromethane (20 ml) was stirred at 20 ^{o}C under a nitrogen atmosphere for 6 h. Water (50 ml) was added into the mixture. The mixture was extracted by ethyl acetate (3 x 50 ml). The combined organic phases were washed (with saturated aqueous $NaHCO_3$ followed by brine), dried (Na_2SO_4), filtered and concentrated. The residue was purified by recrystallization (ethyl acetate / petroleum ether / diisopropyl ether) to afford 5-(4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thiophene-2-sulfonamide (**46**) (50.9 mg, 19.6%) as a yellow solid. LC-MS (m/z) =363.0 $[M+H]^+$; ESI-HRMS calcd for 363.0692 $C_{14}H_{14}N_6O_2S_2$(M+H), found 363.0686; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.72-0.74 (m, 2 H), 0.94-0.99 (m, 2 H), 1.89-1.94 (m, 1 H), 6.41 (s, 1 H), 6.84 (s, 1H), 7.58 (d, J = 4Hz, 1 H), 7.49-7.81 (m, 3 H),8.29 (d, J = 5.2Hz, 1 H), 10.08 (s, 1 H), 12.14 (s, 1 H) ppm.

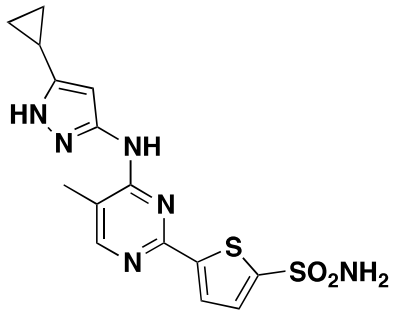
5-(4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)-5-fluoropyrimidin-2-yl)thiophene-2-sulfonamide (47)



47

Starting from 2-bromo-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-5-fluoropyrimidin-4-amine and *N*-(*tert*-butyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiophene-2-sulfonamide, **47** was prepared as described in **46** by two steps in 17.4% yield in 1,4-dioxane (20 ml) and water (4 ml) at 100 ^{o}C for15 h (step 1) and in dichloromethane at 20^{o}C under N for 6 h (step 2). LC-MS (m/z) =381.0 $[M+H]^+$; ESI-HRMS calcd for 381.0598 $C_{14}H_{13}FN_6O_2S_2$(M+H), found 381.0591; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.75-0.76 (m, 2 H), 0.98-0.99 (m, 2 H), 1.94-1.97 (m, 1 H), 6.49 (s, 1 H), 7.57 (d, J=4Hz, 1 H), 7.71 (d, J=3.6Hz, 1 H), 7.82 (s, 2 H), 8.36(s,1 H), 10.23 (s, 1 H), 12.25 (s, 1 H) ppm.

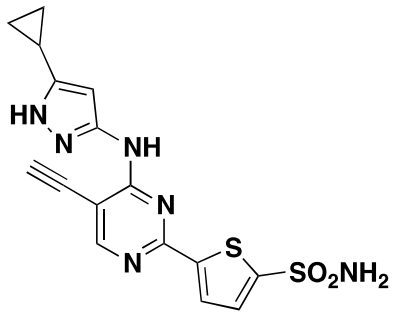
5-(4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)-5-methylpyrimidin-2-yl)thiophene-2-sulfonamide (48)



48

Starting from 2-bromo-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-5-methyl-pyrimidin-4-amine and *N*-(*tert*-butyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiophene-2-sulfonamide, **48** was prepared as described in **46** by two steps in 24.8% yield in 1,4-dioxane (20 ml) and water (4 ml) at 100 ^{o}C for 15 h (step 1) and in dichloromethane at 20 ^{o}C under N for 6 h. (step 2)**.** LC-MS (m/z) =377.0 $[M+H]^+$; ESI-HRMS calcd for 377.0849 $C_{15}H_{16}N_6O_2S_2$(M+H), found 377.0844; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.75-0.76 (m, 2 H), 0.97-0.99 (m, 2 H), 1.93-1.95 (m, 1 H), 2.18 (s, 3 H), 6.51 (s, 1 H), 7.57 (d, J = 3.6Hz, 1 H), 7.73 (d, J = 3.2Hz, 1 H), 7.78 (s, 2 H), 8.14 (s, 1 H), 9.13 (s, 1 H), 12.17 (s, 1 H) ppm.

5-(4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)-5-ethynylpyrimidin-2-yl)thiophene-2-sulfonamide (49)



49

A mixture of pyrimidine-2,4-diol (**50)** (5 g, 44.6 mmol, 1.0 eq.), N-iodosuccinimide (10 g, 44.6 mmol, 1.0 eq.) in AcOH (60 ml) was stirred at 20 ^{o}C under a nitrogen atmosphere for 7 h. The white solid was obtained by filtration and washing with ethyl acetate (50 ml) as 5-iodopyrimidine-2,4-diol (**51)** (7.3 g, 68.7%). LC-MS (m/z) =239.1 $[M+H]^+$.

A mixture of 5-iodopyrimidine-2,4-diol (**51)** (7 g, 29.4 mmol, 1.0 eq.), trimethylsilylacetaylene (3.5 g, 35.2 mmol, 1.2 eq.), $(Ph_3P)_2PdCl_2$ (5.15 g, 7.35 mmol, 0.25 eq.), CuI (1.4 g, 7.35 mmol, 0.25 eq.), Et_3N (100 ml) , EtOAc (10 ml) was stirred at 50 ^{o}C under a nitrogen atmosphere for 10 h. The reaction was evaporated to a residue which was washed with ethyl acetate / ether (5 ml). The solid was stirred with THF (30 ml) and was filtered. The filtrate was evaporated to small volume and was recrystallized

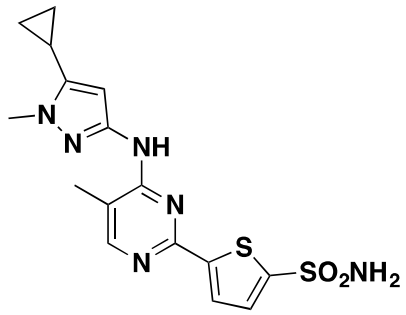
from ethanol to afford 5-((trimethylsilyl)ethynyl) pyrimidine-2,4-diol (**52)** (2.8 g, 46%) as a white solid. LC-MS (m/z) =209.0 $[M+H]^+$

A mixture of compound **52** (10g, 48 mmol, 1.0 eq.), dimethyl aniline(11.6g, 96 mmol, 2.0 eq.), $POBr_3$ (55g, 192 mmol, 4 eq.) in toluene (150 ml) was stirred at 100 ^oC under a nitrogen atmosphere for 50 min. After cooling to RT, the reaction was poured to crashed ice (500 g), extracted with ethyl acetate (2 x 100 ml). The combined organic phases were washed with brine, dried (Na_2SO_4), filtered and concentrated to a crude product which was recrystallized from EtOAc / ether to afford 2,4-dibromo-5-((trimethylsilyl)ethynyl) pyrimidine (**54)** (8.2 g, 51%) as a light yellow solid, which was used directly. A mixture of 2,4-dibromo-5-((trimethylsilyl)ethynyl) pyrimidine (**54** (16.7 g, 50 mmol, 1.0 eq.), 5-cyclopropyl- 1H-pyrazol-3-amine (9.2 g, 75 mmol, 1.5 eq.) in ethanol (200 ml) was stirred at RT under a nitrogen atmosphere for 4 h, when large amount of solid was generated. The solid was obtained by filtration and wash with ethanol (30 ml) as 2-bromo-*N*-(3-cyclopropyl-1*H*-pyrazol-5-yl)-5-((trimethylsilyl)ethynyl)pyrimidin-4-amine (**55)** (13.4 g, 71%) as a light yellow solid. LC-MS (m/z) =378.0 $[M+H]^+$.

A mixture of 2-bromo-*N*-(3-cyclopropyl-1*H*-pyrazol-5-yl)-5-((trimethylsilyl)ethynyl) pyrimidin-4-amine (**55)** (376 mg, 1.0 mmol, 1.0 eq.), N-tert-butyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiophene-2-sulfonamide (448 mg, 1.30 mmol, 1.3 eq.), Pd (dppf)Cl_2 (88 mg, 0.107 mmol, 0.1 eq.), Na_2CO_3 (420 mg, 4.0 mmol, 4.0 eq.) in 1,4-dioxane (20 ml) and water (5 ml) was stirred at 100 ^oC under a nitrogen atmosphere for 15 h. The reaction mixture was allowed to cool to room temperature and concentrated to give a residue. The residue was purified by silica gel chromatography (dichloromethane / methanol 500 : 1 to 50 : 1) to afford *N*-(*tert*-butyl)-5-(4-((3-cyclopropyl-1*H*-pyrazol-5-yl)amino)-5-ethynylpyrimidin-2-yl)thiophene-2-sulfonamide (**56)** (287 mg, 65%) as a white solid. LC-MS (m/z) =443.1 $[M+H]^+$.

A mixture of com afford *N*-(*tert*-butyl)-5-(4-((3-cyclopropyl-1*H*-pyrazol-5-yl)amino)-5-ethynylpyrimidin-2-yl)thiophene-2-sulfonamide (**56)** (280 mg, 0.63 mmol, 1.0 eq.), BCl_3/CH_2Cl_2 (1 M ,7.17 ml, 7.17 mmol, 11.4 eq.) in dichloromethane (20 ml) was stirred at 20 ^oC under a nitrogen atmosphere for 6 h. Water (50 ml) was added into the mixture and the mixture was extracted with ethyl acetate (3 X 50 ml). The combined organic phases were washed (saturated aqueous $NaHCO_3$ followed by brine), dried (Na_2SO_4), filtered and concentrated. The residue was purified by silica gel chromatography (dichloromethane/ methanol 500 : 1 to 50 : 1 as eluent) to afford 5-(4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)-5-ethynyl -pyrimidin-2-yl)thiophene-2-sulfonamide (**49**) (109 mg, 44.8%) as light yellow solid. LC-MS (m/z) =386.9 $[M+H]^+$; ESI-HRMS calcd for 387.0692 $C_{16}H_{14}N_6O_2S_2$(M+H), found 387.0686; ^1H NMR (400 MHz, DMSO-d_6) δ 12.30 (bs, 1 H), 8.80 (bs, 1 H), 8.52 (s, 1 H), 7.84 (s, 2 H), 7.81 (d, *J* = 4.0Hz, 1 H), 7.60 (d, *J* = 4.0Hz, 1 H), 6.44 (s, 1 H), 4.90 (s, 1 H), 1.91-1.98 (m, 1 H), 0.97-0.99 (m, 2 H), 0.73-0.77 (m, 2 H) ppm.

5-(4-(5-cyclopropyl-1-methyl-1*H*-pyrazol-3-ylamino)-5-methylpyrimidin-2-yl)thiophene-2-sulfonamide(57)



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To a solution of 18-crown-6 (528 mg, 2 mmol, 0.1 eq.) in ether (40 mL) was added t-BuOK (2.68 g, 24 mmol, 1.2 eq.). The mixture was stirred for 30 minutes. 3-Cyclopropyl-1*H*-pyrazol-5-amine (2.46 g, 10 mmol, 1.0 eq.) was added and the reaction mixture was stirred at room temperature for 30 minutes. Methyl iodide (1.4 mL, 24 mmol, 1.2 eq.) dissolving in ether (20 mL) was added dropwise to the reaction mixture in an ice bath. The reaction mixture was stirred at room temperature for 3 h. Water was added to the reaction mixture and the layers were separated. The aqueous layers were extracted with ether. The combined organic layers were washed with brine, dried (Na_2SO_4) and filtered. The filtrate was concentrated and the residue was purified by silica gel chromatography (petroleum ether / ethyl acetate from 10 : 1 to 1 :2) to afford 5-cyclopropyl-1-methyl-1*H*-pyrazol-3-amine (**58**) (1.2 g, 43%). LC-MS (m/z) =138 $[M + H]^+$.

From 5-cyclopropyl-1-methyl-1*H*-pyrazol-3-amine (**58**), 2-bromo-*N*-(5-cyclopropyl-1-methyl-1*H*-pyrazol-3-yl)-5-methylpyrimidin-4-amine (**59**) was prepared using the procedure for synthesis of **55** in ethanol at RT under nitrogen atmosphere for 4 h (70%)**.** LC-MS (m/z) =308 $[M + H]^+$.

N-(*tert*-butyl)-5-(4-((5-cyclopropyl-1-methyl-1*H*-pyrazol-3-yl)amino)-5-methylpyrimidin-2-yl)thiophene-2-sulfonamide Compound(**60**) (27.7%) was prepared using the procedure for synthesis of **56** in 1,4-dioxane and water at 100 ^oC under nitrogen atmosphere for 15 h. LC-MS (m/z) = 447.0 [M + H]; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.21 (s, 1 H), 8.13 (d, J = 0.8 Hz, 1 H), 7.86 (s, 1 H), 7.69 (d, J = 4.0 Hz, 1 H), 7.58 (d, J = 4.4 Hz, 1 H), 6.46 (s, 1 H), 3.79 (s, 3 H), 2.17 (s, 3 H), 1.91-1.98 (m, 1 H), 1.00-1.03 (m, 2 H), 1.20 (s, 9 H), 0.68-0.72 (m, 2 H) ppm.

5-(4-(5-cyclopropyl-1-methyl-1*H*-pyrazol-3-ylamino)-5-methylpyrimidin-2-yl)thiophene-2-sulfonamide (**57**) was prepared using the procedure for synthesis of **49.** (71.7%) in dichloromethane at 20 ^oC under a nitrogen atmosphere for 6 h. LC-MS (m/z) = 390.9 $[M + H]^+$; ESI-HRMS calcd for 391.1005 $C_{16}H_{18}N_6O_2S_2$ (M+H), found 391.1000 ^{1}H-NMR (400 MHz,DMSO-d_6) δ 9.21 (s, 1 H), 8.14 (d, J = 0.8 Hz, 1H), 7.78 (s, 2 H), 7.71 (d, J = 3.6 Hz, 1 H), 7.56 (d, J = 3.6 Hz, 1 H), 6.46 (s, 1 H), 3.79 (s, 3 H), 2.17 (s, 3 H), 1.98-1.91 (m, 1 H), 1.03-1.00 (m, 2 H), 0.71-0.70 (m, 2 H) ppm.

Part 2. Table for Ki (nM) and TB MIC (μM)

Compounds	PknA	PknB	TB-MIC	GSK3β	CDK2	SRC
1	>33300	150	33.3	900	12	950
2	>33000	>33000	>100	>4000	>4000	>4000
3	>33000	>33000	>100	>4000	>4000	>4000
4	>33000	>33000	>100	>4000	>4000	>4000
5	>33000	>33000	>100	>4000	>4000	>4000
6	>33000	>33000	>100	>4000	>4000	>4000
7	>33000	>33000	>100	>4000	>4000	>4000
8	>33000	>33000	100	>4000	>4000	>4000
9	>33000	81	33.3	110	2	130
10	>33000	98	33.3	610	4	800
11	>33000	82	33.3	960	5	2500
12	>33000	150	33.3	1400	48	520
13	ND	960	100	>4000	590	1500
14	>33000	150	100	2200	180	900
15	>33000	230	33.3	2500	190	880
16	>33000	190	>100	1300	140	750
17	>33000	310	>100	2300	220	1200
18	720	990	>33.3	3500	330	1000
19	>4000	1400	33.3	>4000	970	2400
20	ND	310	ND	>4000	400	2200
21	>33000	70	33.3	1100	27	1300
22	>30000	69	>100	>1600	860	180
23	>4000	5	>100	44	35	67
24	ND	38	>100	44	35	67

25	>4000	50	ND	>4000	750	200
26	>4000	15	>100	>4000	38	44
27	2300	23	25	44	35	69
28	1800	23	>100	1800	250	59
29	>4000	29	>100	>4000	270	88
30	>4000	88	>100	190	170	710
31	22	24	>100	910	140	39
32	<8	6	32.5	150	2	6
33	<8	<1	>100	170	3	27
34	>33000	40	>100	9	11	22
35	>33000	46	33.3	70	140	18
36	2900	1	>100	340	99	250
37	<8	17	8.3	32	4	58
38	9	<1.3	6.25	102	17	150
46	18	4	4.2	20	7	120
47	12	<1	3.1	110	25	330
48	18	4	4.7	98	18	175
49	11	<2	4.7	1675	147	200
57	>4000	>4000	>100	>4000	>4000	>4000

(ND: Not determined)

Part 3. Pkn A and Pkn B Expression

The PknA sequence (M1-A296) was inserted into the *E. coli* expression vector pBev10-TOPO, incorporating an N-terminal His6 purification tag and thrombin cleavage site. The clone was then transfected into *E. coli* BL21 (DE3) pLysS cells that were grown in BHI media at 37°C. The cells were induced with IPTG at OD 1.0 (600 nm) and grown overnight (~16 hrs) at 30°C. After harvest, the cells were resuspended in 5 mL of breaking buffer (50 mM Hepes pH 7.0, 500 mM NaCl, 5 mM imidazole, 10% glycerol, 5 mM □-mercaptoethanol, 0.1% Tween 20 plus protease inhibitors) per 1 g of cells. They

were then lysed twice in a microfluidizer and centrifuged for 1 hour at 4°C at 54,000 X g. The supernatant was decanted and combined with Ni-IMAC resin at 1 mL of resin per 30 g of cellular wet weight, and incubated overnight at 4°C. The protein was then eluted using breaking buffer plus 350 mM imidazole, concentrated and loaded onto S200 sizing column equilibrated in SEC buffer (50 mM hepes pH 7.0, 300 mM NaCl, 5 mM □ -mercaptoethanol and 5% glycerol) to remove any protein aggregates. Typical yields after sizing were 0.52 milligrams of protein per gram of cellular wet weight.

The PknB sequence (M1-G279) was inserted into the *E. coli* expression vector pET21b, incorporating a C-terminal His6 purification tag and thrombin cleavage site. The clone was then transfected into *E. coli* BL21 (DE3) cells that were grown in BHI media at 27°C. The cells were induced with IPTG at OD 0.6 (600 nm) and grown for 3 hrs at 20°C. After harvest, the cells were resuspended in 5 mL of breaking buffer (50 mM hepes pH 7.5, 250 mM NaCl, 10% glycerol, 5 mM beta-mercaptoethanol plus protease inhibitors) per 1 g of cells. They are then lysed twice in a microfluidizer and centrifuged for 1 hour at 4°C at 54,000 X g. The supernatant was decanted and combined with Ni-IMAC resin at 1 mL of resin per 30 g of cellular wet weight. This was incubated overnight at 4°C. The protein was then eluted using breaking buffer plus 300 mM imidazole, concentrated and loaded onto S200 sizing column equilibrated in SEC Buffer (50 mM hepes pH 7.5, 100 mM NaCl and 5 mM □ -mercaptoethanol) to remove any protein aggregates. Typical yields after sizing were 6.7 milligrams of protein per gram of cellular wet weight.

Part 4. *In vitro* Pkn A and Pkn B Kinase Assays

Inhibition of PknA and PknB by test compounds was determined by following the residual kinase activity using a radioactive phosphate incorporation assay. Assays were carried out in a mixture of 100mM HEPES (pH 7.5), 10mM $MgCl_2$, 25mM NaCl, 1mM $MnCl_2$, and 1mM DTT. Final substrate concentrations were 15μM [□-33P]ATP for PknA or 3μM [□-33P]ATP for PknB, both at (100 mCi 33P ATP/ mmol ATP, Perkin Elmer) and 6uM GarA protein (purified recombinant, Vertex Pharmaceuticals). Final enzyme concentrations were 50nM PknA or 6nM PknB (purified recombinant kinase domains, Vertex Pharmaceuticals).

A 1.5μL aliquot of DMSO stock containing serial dilutions of each test compound was placed in each well followed by the addition of 50μL of 2x ATP solution. The reaction was initiated with 50μL of 2x enzyme/GarA solution. The reactions were stopped by the addition of 50μL 30% trichloroacetic acid (TCA) containing 10mM cold ATP. The reaction time for PknA and PknB were120 minutes and 50 minutes respectively. The entire quenched reactions were transferred to 96-well glass fiber filter plates (Millipore, Cat no. MAFBNOB50). The plates were washed with 3 x 5% TCA. After drying, 50μL of Ultima Gold liquid scintillation cocktail (Perkin Elmer) was added to the well prior to scintillation counting in a PerkinElmer TopCount.

After removing mean background values for all of the data points, Ki(app) data were calculated from non-linear regression analysis of the initial rate data using the Prism software package (GraphPad Prism, GraphPad Software, San Diego California, USA). Compound potencies are determined in singlicate. Average Kis may be reported for compounds that have multiple determinations. The Robust Average MSRs for PknA and PknB assays have been determined to be 5.2 and 3.8 respectively. (REFERENCES: JBiomolScreen_MSR, AMC_Robust_Statistics)

Part 5. Other Kinase Assays

Compounds of interest were also assayed for their selectivity against CDK2, GSK3b and SRC kinases (purified recombinant, Vertex Pharmaceuticals). The residual activity for these kinases was followed using spectrophotometric detection of a standard coupled-enzyme assay used for kinases. In this assay, each mole of ADP produced by the kinase reaction was coupled to the generation of one mole of NAD from NADH using pyruvate kinase (PK) and lactate dehydrogenase (LDH). The final concentrations of the components in the assay were as follows: 100 mM HEPES (pH 7.5), 10 mM $MgCl_2$, 1 mM DTT, 2.5 mM phosphoenolpyruvate, 200 µM NADH, 50 µg/mL PK, 10 µg/mL LDH, and ATP and peptide substrate as described in the table below. The activity is measured by reading the reduction of NADH at 340nm. Compound potencies are determined in singlicate. Average Kis may be reported for compounds that have multiple determinations. The Robust Average MSRs for CDK2, GSK3b, and SRC assays have been determined to be 2.5, 2.9, and 3.1 respectively.

ATP and Peptide Substrate Concentrations Used in Vertex Kinase Selectivity Assays

Kinase	**[ATP], µM**	**[peptide]**	**Peptide**
CDK2/cyclin A	200	300 µM	MAHHRSPRKRAKKK
GSK3ß	20	300 µM	HSSPHQ(Sp)EDEEE
SRC	50	0.3 mg/mL	polyE4Y

Part 6. *Mycobacterium* sp. Isolates and MIC assay

ATCC 25177(strain H37Ra) colony was used for routine MIC analysis. All compounds were dissolved in 100% dimethyl sulfoxide (DMSO) and stored as frozen stocks at a concentration of 10 mM or 1 mg/ml. Before evaluation *in vitro*, compounds were diluted into DMSO that did not exceed 1% and then re-suspended into culture medium consisting of 7H10 broth supplemented with 10% OADC (BBL Microbiology Systems, Cockeysville, Maryland) and 0.05% Tween 80.

For microdilution MIC assays, the Mtb strains were routinely cultured on 7H11 agar, picked and prepared at 1 X 10^8 CFU/ml, and diluted 200x to a final concentration of 5 X

10^5 CFU/ml in 7H9 broth supplemented with 10% ADC. Compound stock solutions were prepared in DMSO at 100-fold for each test concentration and 1 µl of each stock was dispensed into a microtiter well. Compounds were diluted by the addition of 100 µl of the bacterial cell-suspension in culture medium. The 96-well plates were then incubated at 37^0C in ambient air. For MIC determination using the Mtb H37Ra isolate, 30µl of Resazurin detection buffer added into each well and the plate was returned to the incubator (Franzblau et al). After 24 h incubation with Resazurin, the fluorescence was read using a Biotek Synergy2, at a gain of 35 with excitation wavelength 492nm and emission wavelength 595 nm. For MIC of other isolates, wells were visually inspected after 9 days of incubation. The MIC was defined as the lowest concentration of a compound that inhibited reduction of Resazurin to its fluorescent (emission 595 nm) species by ≥90%. All active compounds were repeated a minimum of 3 times, and average MIC was reported.

Part 7. Crystallization Condition

PknB crystallization – PknB was crystallized by the vapor diffusion method. Ligand dissolved in 100% DMSO was added to the protein to a final concentration of 1mM. Hanging drops containing a 1:1 mixture of 10 mg / mL protein and 25% (w/v) PEGMME 2000, 0.1 M Tris-HCI pH 8.5, 400 mM magnesium chloride. For data collection, crystals were equilibrated for ≈ 1 minute in a solution containing well solution plus 25% glycerol.

PknA crystallization - PknA was crystallized by the vapor diffusion method. 2,6-dimethyl-4-heptyl-ß-D-maltopyranoside was added to a final concentration of 27.5mM. Ligand dissolved in 100% DMSO was added to the protein to a final concentration of 1mM. Hanging drops containing a 1:1 mixture of 10 mg / mL protein and 3M sodium formate, 0.1 M buffer (ADA, cacodylate, or sodium citrate) pH 6.5. For data collection, crystals were equilibrated for ≈ 1 minute in a solution containing well solution plus 25% glycerol.

Summary of Data Collection and Atomic Model Refinement Statistics for compound 36 in PknB & PknA.

PDB	6B2Q	6B2P
Protein	PknB	PknA
Space group	$C222_1$	$C222_1$
Cell dimensions		
a, b, c	117.952 123.146 45.193	79.065 227.432 158.606

Resolution (Å)	59.0-3.0	113.7-2.88
Resolution-high (Å)	3.1-3.0	2.92-2.88
Rmerge	0.061 (0.60)	0.063 (0.67)
Rpim	0.030 (0.295)	0.029 (0.30)
CC 1⁄2	0.999 (0.944)	0.999 (0.781)
I/sigI	15.1 (2.4)	18.6 (2.4)
Completeness (%)	97.8 (100.0)	94.8 (99.6)
Redundancy	4.9 (5.0)	5.7 (5.9)
Refinement		
resolution (Å)	59.0-3.0	113.7-2.88
unique reflections	6695	31096
Rwork/Rfree (%)	20.1/25.5	22.8/26.5
# atoms		
Protein	1967	7964
Ligand	25	144
r.m.s. deviations		
Bond length (Å)	0.009	0.008
Bond angle (°)	1.03	1.01