Supporting Information

Discovery of Pyridinyl Acetamide Derivatives as Potent, Selective, and Orally Bioavailable Porcupine Inhibitors

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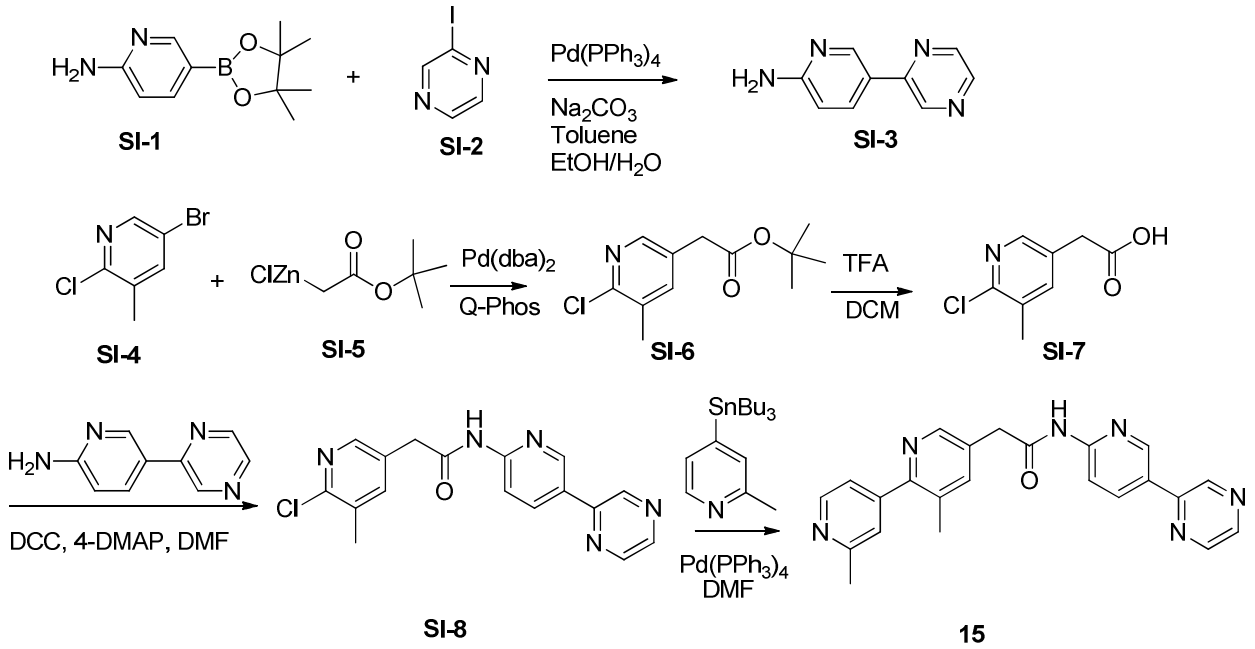
Synthetic schemes, procedure and analytical data for **15** and **19**; analytical data of **1-14**, **16-18**, and **20-21**; general animal study protocols; high throughput solubility assay protocols.

Scheme SI-2. Synthesis of compound **15**



Step 1: To a sealed tube were added 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-amine **SI-1** (2.2 g, 10 mmol), 2-iodopyrazine **SI-2** (2.06 g, 10 mmol), $Pd(PPh_3)_4$ (577 mg, 0.5 mmol), toluene (70 mL), ethanol (15 mL) and 2M Na_2CO_3 (15 mL). The reaction mixture was bubbled with nitrogen for 2 minutes and stirred at 90 °C for 10 hours. After cooled down to room temperature, the solvents were evaporated and the residue was redissovled in dichloromethane (200 ml), treated with 1M HCl aqueous solution (50 mL). The two layers were separated and the aqueous layer was treated with 10% NaOH aqueous solution to adjust the pH to around 13. The

resulting suspension was extracted with ethyl acetate (100 mL x 3). The combined organic phases were washed with H_2O (50 mL) and brine (50 mL), dried over Na_2SO_4, and concentrated to give 5-(pyrazin-2-yl)pyridin-2-amine **SI-3** as white solid (1.2 g, 70%). MS *m/z* 173.1 (M + 1); ^{1}H NMR 400 MHz (DMSO-d_6) δ 9.12(d, 1H, J = 1.6 Hz), 8.73(m, 1H), 8.60(m, 1H), 8.46(d, 1H, J = 2.8 Hz), 8.12(dd, 1H, J_1 = 8.8Hz, J_2 = 2.4 Hz), 6.55(d, 1H, J = 8.8 Hz), 6.46(s, 2H).

Step 2: To a sealed tube were added 5-bromo-2-chloro-3-methylpyridine **SI-4** (4.69 g, 22.72 mmol), 0.5 M (2-tert-butoxy-2-oxoethyl) zinc(II) chloride in ether **SI-5** (50 mL, 25 mmol), $Pd(dba)_2$ (262 mg, 0.45 mmol), Q-phos (320 mg, 0.45 mmol), and THF (75 mL). The reaction mixture was bubbled with nitrogen for 1 minute and stirred at 70 °C for 4 hours. After cooled down to room temperature, all the solvents were evaporated and the residue was redissolved in ethyl acetate, washed with water and brine, dried over Na_2SO_4 and concentrated to dryness by rotary evaporation. The crude product was purified by silica gel flash chromatography, eluted with 20% ethyl acetate in hexane to give tert-butyl 2-(6-chloro-5-methylpyridin-3-yl)acetate **SI-6** as red oil (3.9 g, 71%). MS *m/z* 242.1 (M + 1)

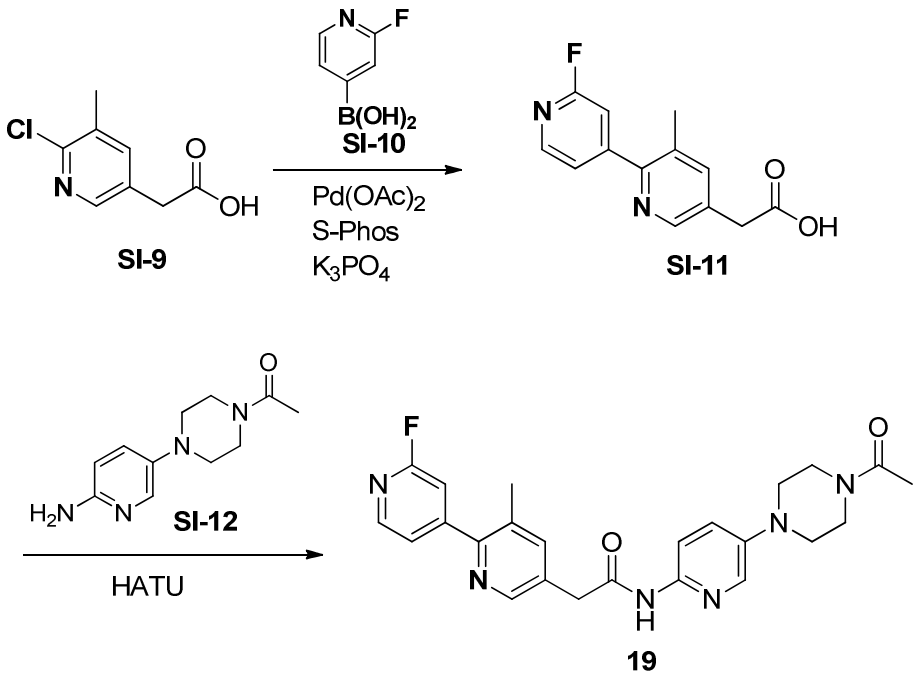
Step 3: A mixture of tert-butyl 2-(6-chloro-5-methylpyridin-3-yl) acetate **SI-6** (7.8 g, 32 mmol) and TFA (32 mL) in DCM (32mL) was stirred at room temperature for 3 hours. The solution was adjusted to pH around 12 by sodium carbonate and extracted with dichloromethane. The aqueous phase was acidified to pH 3 by 1N HCl aqueous solution and stirred for 15 minutes. The suspension was extracted with dichloromethane (100 mL X3). The combined organic phases were washed with water and brine, dried over Na_2SO_4 and then taken to dryness to give 2-(6-chloro-5-methylpyridin-3-yl)acetic acid **SI-7** as pale yellow solid (5.2 g, 88%). MS *m/z* 186.1 (M + 1); ^{1}H NMR 400 MHz (CD_3Cl) δ 8.17(d, 1H, J = 2.0 Hz), 7.55(d, 1H, J = 2.0 Hz), 3.63(s, 2H), 2.38 (s, 3H).

Step 4: A mixture of 2-(6-chloro-5-methylpyridin-3-yl)acetic acid **SI-7 (**3.0 g, 16.2 mmol), 5-(pyrazin-2-yl)pyridin-2-amine **SI-3** (2.80 g, 16.2 mmol),1,3-dicyclohexylcarbodiimide (4 g, 19.44 mmol) and 4-(dimethylamino)pyridine (324 mg, 3.24 mmol) in DMF (45 mL) was stirred at room temperature for 10 hours. The reaction mixture was filtered to remove the solid and the filtrate was diluted with ethyl acetate, washed with water and brine, dried over Na_2SO_4 and

concentrated to dryness by rotary evaporation. The crude product was purified by silica gel flash chromatography, eluted with 5% methanol in dichloromethane to give 2-(6-chloro-5-methylpyridin-3-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide **SI-8** as pale yellow solid (2.75 g, 50 %). MS *m/z* 340.2 (M + 1); ^{1}H NMR 400 MHz (DMSO-d_6) δ 11.09(s 1H), 9.31(d, 1H, J = 1.6 Hz), 9.11(d, 1H, J = 1.6 Hz),8.72(m, 1H), 8.63(m, 1H), 8.51(dd, 1H, J_1 = 8.6Hz, J_2 = 2.4 Hz), 8.21(m, 2H), 7.76(d, 1H, J = 1.6 Hz), 3.82(s, 2H), 2.33(s, 3H).

Step 5: To a reaction flask containing 2-(6-chloro-5-methylpyridin-3-yl)-N-(5-(3-fluorophenyl)pyridin-2-yl)acetamide **SI-8** (3.34 g, 9.4 mmol), 2-methyl-4-(tributylstannyl)pyridine (3.47 g, 9.4 mmol) and $Pd(PPh_3)_4$ (1 g, 0.94 mmol) under Argon was added DMF (45 mL). The mixture was stirred at 120 ^{o}C for 10 hours. 1N KF aqueous solution was added to the mixture and stirred for 15 minutes after it was cooled down to room temperature. The formed solid was collected by filtration and further purified by silica gel flash chromatography, eluted with 10% methanol in dichloromethane to give 2-(2',3-dimethyl-2,4'-bipyridin-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide **15** as white solid (3.6 g, 67%).
^{1}H NMR 400 MHz (DMSO-d_6) δ 11.13(s 1H), 9.31(d, 1H, J = 1.6 Hz), 9.11(d, 1H, J = 1.6 Hz), 8.72(m, 1H), 8.62(d, 1H, J = 2.8 Hz), 8.53(m, 3H), 8.24(d, 1H, J = 8.8 Hz), 7.73(d, 1H, J = 1.6 Hz), 7.42(s, 1H), 7.35(dd, 1H, J_1 = 4.8 Hz, J_2 = 0.8 Hz), 3.87(s, 2H), 2.53(s, 3H), 2.34(s, 3H).
^{13}C NMR 100.6 MHz (DMSO-d_6) δ 19.40, 24.13, 39.51 (under DMSO), 113.16, 120.92, 123.02, 127.25, 130.54, 130.81, 136.45, 139.64, 141.77, 143.56, 144.39, 146.57, 147.63, 147.79, 148.74, 149.21, 152.97, 153.82, 157.88, 169.75.
HRMS: 397.17735 $[M+H]^+$, calculated 397.17712 $[M+H]^+$.

Scheme SI-2. Synthesis of compound **19**



Step 1: To a reaction vial was added 2-(6-chloro-5-methylpyridin-3-yl)acetic acid **SI-9** (185 mg, 1 mmol), 2-fluoropyridin-4-ylboronic acid **SI-10** (220mg, 1.5 mmol), $Pd(OAc)_2$ (12 mg, 0.05 mmol), S-Phos (41 mg, 0.1 mmol) and K_3PO_4 (636 mg, 3 mmol) in 1 mL 2-butanol. The reaction was heated to 100°C and stirred for 2 hours. The reaction was cooled down to room temperature and then diluted to DMSO. The reaction was filtered and the filtrate was purified by reverse-phase HPLC to give 2-(2'-fluoro-3-methyl-2,4'-bipyridin-5-yl)acetic acid **SI-11** as white solid (276 mg, 76 %).

Step 2: To a reaction vial was added 2-(2'-fluoro-3-methyl-2,4'-bipyridin-5-yl)acetic acid **SI-11** (60 mg, 0.17 mmol), 1-(4-(6-aminopyridin-3-yl)piperazin-1-yl)ethanone **SI-12** (50 mg, 0.22 mmol), O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluroniumhexaflurophosphate (HATU) (115 mg, 0.3 mmol) and DIEA (104 μL, 0.58 mmol) in DMF (1 mL) at room temperature. The mixture was stirred at room temperature for 2 hours. The reaction was diluted with DMSO and then purified by reverse-phase HPLC to give N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(2'-fluoro-3-methyl-2,4'-bipyridin-5-yl)acetamide (**19**) as white solid (46 mg, 61%).

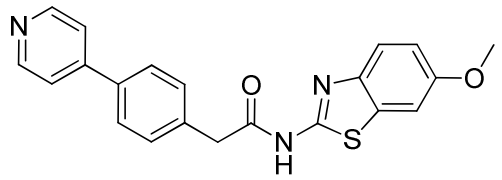
MS *m/z* 449.2 (M + 1);

^{1}H NMR 400 MHz (DMSO-d_6) δ10.58 (s, 1H), 8.42 (d, 1H, J = 1.6 Hz), 8.28 (d, 1H, J = 5.2 Hz), 7.98 (d, 1H, J = 2.8 Hz), 7.87 (d, 1H, J = 9.2 Hz), 7.67 (d, 1H, J = 1.6 Hz), 7.50-7.48 (m, 1H), 7.37 (dd, 1H, J1 = 9.2 Hz, J2 = 3.2 Hz), 7.30 (s, 1H), 3.71 (s, 2H), 3.50 (b, 4H), 3.09 (t, 2H, J = 5.2 Hz), 3.02 (t, 2H, J = 5.2 Hz), 2.30 (s, 3H), 1.97 (s, 3H).

^{13}C NMR 100.6 MHz (DMSO-d_6) δ 168.4, 168.3, 163.2, 153.4, 152.2, 147.8, 147.4, 144.7, 143.5, 139.7, 135.7, 131.9, 130.9, 125.5, 122.2, 113.7, 109.4, 48.7 (2C), 48.3 (2C), 45.3, 21.3, 19.3.

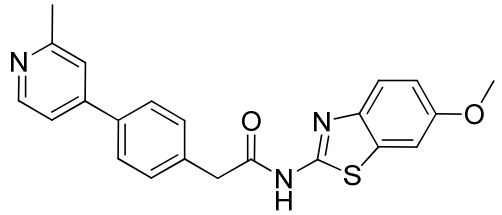
^{1}H NMR and MS data for **1-14, 16-18, 20-21**

N-(6-methoxybenzo[d]thiazol-2-yl)-2-(4-(pyridin-4-yl)phenyl)acetamide (**1**)



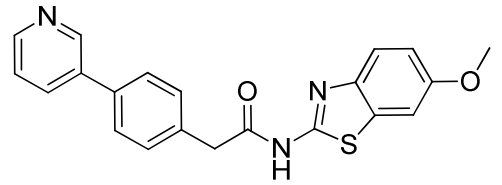
^{1}H NMR (400 MHz, DMSO-d6): δ 12.60(s, 1H), 8.62 (d, 2H, *J* = 5.6 Hz), 7.78 (d, 2H, *J* = 7.6 Hz), 7.70(d, 2H, *J* = 6.0 Hz), 7.64 (d, 1H, *J* = 9.2 Hz), 7.57 ~ 7.53 (m, 1H), 7.49 (d, 2 H, *J* = 7.6 Hz),7.02 (dd, 1H, *J* = 8.8, 2.4 Hz), 3.88 (s, 2H), 3.79(s, 3H). MS *m/z* 376.1 (M+1).

N-(6-methoxybenzo[d]thiazol-2-yl)-2-(4-(2-methylpyridin-4-yl)phenyl)acetamide (**2**)



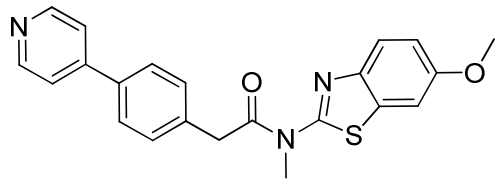
^{1}H NMR (500 MHz, DMSO-d6): δ 12.50 (s, 1H), 8.48 (dd, 1H, *J* = 5.2, 0.8 Hz), 7.82–7.71 (m, 2H), 7.64 (d, 1 H, *J* = 8.8 Hz), 7.56 (dd,2H, *J* = 7.7, 2.4 Hz), 7.52–7.44 (m, 3H), 7.03 (dd, 1H, *J* = 8.8, 2.6 Hz), 3.89 (s, 2H), 3.80 (s, 3H), 2.52 (s, 3H). MS *m/z* 390.2 (M+1).

N-(6-methoxybenzo[d]thiazol-2-yl)-2-(4-(pyridin-3-yl)phenyl)acetamide (**3**)



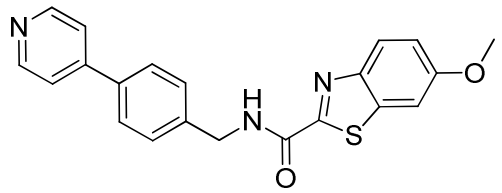
^{1}H NMR 400 MHz (DMSO-d_6) δ 8.90 (s, 1H), 8.57 (d, 1H, *J* = 3.6 Hz), 8.09 (d, 1H, *J* = 8.0 Hz), 7.72 (d, 2H, *J* = 8.0 Hz), 7.65 (d, 1H, *J* = 8.8 Hz), 7.53–7.45 (m, 3 H), 7.03 (dd, 1H, *J* =8.8, 2.4 Hz), 3.88 (s, 2H), 3.80 (s, 3H). MS *m/z* 376.1 (M+1).

N-(6-methoxybenzo[d]thiazol-2-yl)-N-methyl-2-(4-(pyridin-4-yl)phenyl)acetamide (**4**)



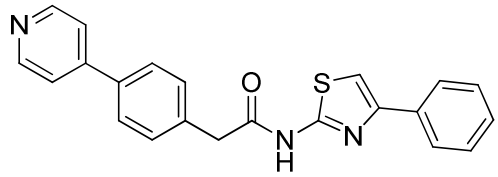
^{1}H NMR (400 MHz) (DMSO-d_6) δ 8.76 (d, 2H, *J* = 5.6 Hz), 8.01 (d, 2H, *J* = 5.6 Hz), 7.87 (d, 2H, *J* = 7.9 Hz), 7.61-7.53 (m, 4H), 7.12 (dd, 1H, *J* = 8.8, 2.9 Hz), 3.98–3.85 (s, 2H), 3.80 (s, 3H), 2.55 (s, 3H). MS *m/z* 390.1 (M+1).

6-methoxy-N-(4-(pyridin-4-yl)benzyl)benzo[d]thiazole-2-carboxamide (**5**)



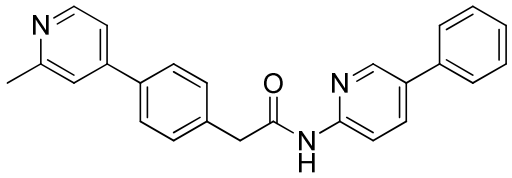
^{1}H NMR (400 MHz) (DMSO-d_6) δ 9.72 (s, 1H), 8.62 (d, 2H, J = 5.2 Hz), 8.02 (d, 1H, J = 8.8 Hz), 7.80-7.76 (m, 3H), 7.70 (d, 2H, J = 5.2 Hz), 7.50 (d, 2H, J = 7.9 Hz), 7.24 (d, 1H, J = 8.8 Hz), 4.55 (s, 2H), 3.87 (s, 3H). MS *m/z* 376.1 (M+1).

N-(4-phenylthiazol-2-yl)-2-(4-(pyridin-4-yl)phenyl)acetamide (**6**)



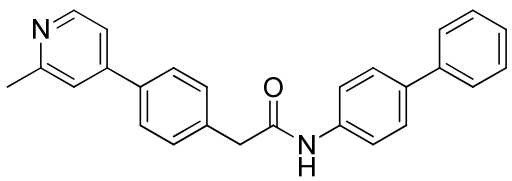
^{1}H NMR (400 MHz,) (DMSO-d_6) δ 12.53 (s, 1H), 8.73 (s, 2H), 8.00 (d, J = 5.7 Hz, 2H), 7.89–7.78 (m, 4H), 7.57 (s, 1H), 7.49 (d, 2H, J = 8.3 Hz), 7.37 (t, 2H, J = 7.6 Hz), 7.26 (t, 1H, J = 7.3 Hz), 3.84 (s, 2H). MS *m/z* 372.2 (M+1).

2-(4-(2-methylpyridin-4-yl)phenyl)-N-(5-phenylpyridin-2-yl)acetamide (**7**)



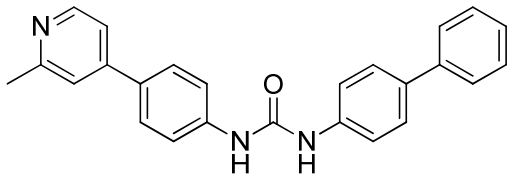
^{1}H NMR 400 MHz (DMSO-d_6) δ 10.84 (s, 1H), 8.60 (d, 1H, J = 2.4Hz), 8.42 (d, 1H, J = 6.4Hz), 8.10 (d, 1H, J =8.8 Hz), 8.04 (dd, 1H, J = 8.8, 2.4 Hz), 7.71-7.67 (m, 2H), 7.66-7.62 (m, 2H), 7.52-7.50 (m, 1H), 7.45-7.38 (m, 5H), 7.34-7.29 (m, 1H), 3.76 (s, 2H), 2.46 (s, 3H). MS *m/z* 380.2 (M+1).

N-([1,1'-biphenyl]-4-yl)-2-(4-(2-methylpyridin-4-yl)phenyl)acetamide (**8**)



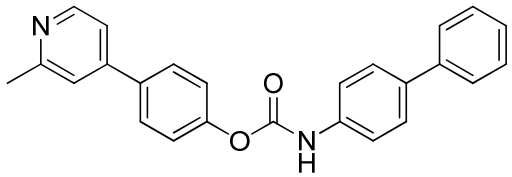
^{1}H NMR 400 MHz (DMSO-d_6) δ 10.33 (s, 1 H), 8.48 (d, 1H, J = 5.2 Hz), 7.79–7.73 (m, 2H), 7.73–7.67 (m, 2H), 7.66–7.60 (m, 4H), 7.58 (bs, 1H), 7.51–7.47 (m, 3H), 7.46–7.40 (m, 2H), 7.35–7.29 (m, 1H), 3.74 (s, 2H), 2.53 (s, 3H). MS *m/z* 379.2 (M+1).

1-([1,1'-biphenyl]-4-yl)-3-(4-(2-methylpyridin-4-yl)phenyl)urea (**9**)



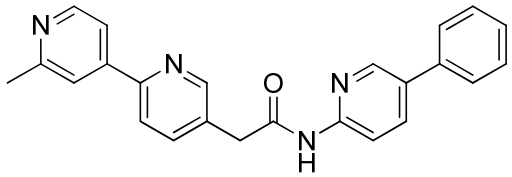
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.97 (s, 1H), 8.89 (s, 1H), 8.46 (d, 1H, J = 5.3 Hz), 7.8 –7.72 (m, 2H), 7.66–7.55 (m, 9H), 7.49 (dd, 1H, J = 5.4, 1.8 Hz), 7.44 (dd, 2H, J = 8.4, 7.0 Hz), 7.36–7.27 (m, 1H), 2.52 (s, 3H). MS *m/z* 380.2 (M+1).

4-(2-methylpyridin-4-yl)phenyl [1,1'-biphenyl]-4-ylcarbamate (**10**)



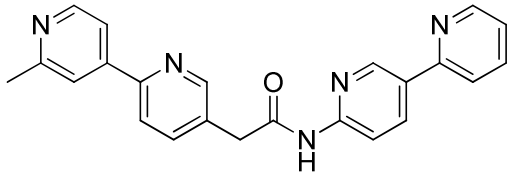
^{1}H NMR (500 MHz, DMSO-d_6) δ 10.41 (s, 1H), 8.51 (d, 1H, J = 5.2 Hz), 7.85 (d, 2H, J = 8.8 Hz), 7.69–7.58 (m, 7H), 7.54–7.50 (m, 1H), 7.45 (t, 2H, J = 7.7 Hz), 7.40 (d, 2H, J = 8.7 Hz), 7.37–7.30 (m, 1H), 2.54 (s, 3H). MS *m/z* 381.2 (M+1).

2-(2'-methyl-[2,4'-bipyridin]-5-yl)-N-(5-phenylpyridin-2-yl)acetamide (**11**)



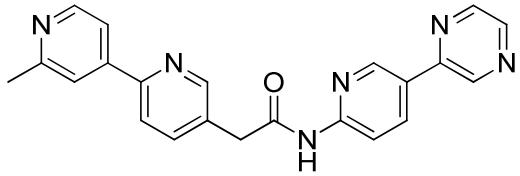
^{1}H NMR (500 MHz, DMSO-d_6) δ 10.95 (s, 1H), 8.68 (ddd, 2H, J = 8.6, 2.4, 0.9 Hz), 8.55 (dd, 1H, J = 5.2, 0.8 Hz), 8.19–8.02 (m, 3H), 7.97–7.88 (m, 2H), 7.85–7.79 (m, 1H), 7.76–7.64 (m, 2H), 7.48 (dd, 2H, J = 8.4, 7.0 Hz), 7.42–7.33 (m, 1H), 3.90 (s, 2H), 2.55 (s, 3H). MS *m/z* 381.2 (M+1).

N-([2,3'-bipyridin]-6'-yl)-2-(2'-methyl-[2,4'-bipyridin]-5-yl)acetamide (**12**)



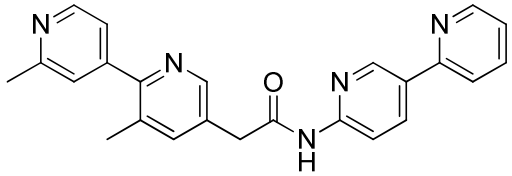
^{1}H NMR (500 MHz, DMSO-d_6) δ 11.03 (s, 1H), 9.06 (dd, 1H, J = 2.5, 0.8 Hz), 8.73–8.62 (m, 2H), 8.55 (dd, 1H, J = 5.2, 0.8 Hz), 8.45 (dd, 1H, J = 8.7, 2.5 Hz), 8.17 (d, 1H, J = 8.7 Hz), 8.07 (dd, 1H, J = 8.2, 0.9 Hz), 8.00 (dt, 1H, J = 8.0, 1.1 Hz), 7.96–7.86 (m, 3H), 7.85–7.78 (m, 1H), 7.36 (ddd, 1H, J = 7.5, 4.8, 1.1 Hz), 3.90 (s, 2H), 2.55 (s, 3H). MS *m/z* 382.2 (M+1).

2-(2'-methyl-[2,4'-bipyridin]-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide (**13**)



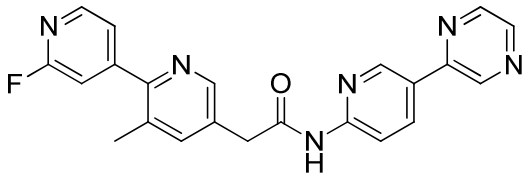
^{1}H NMR 400 MHz (DMSO-d_6) δ 11.11 (s, 1 H), 9.30 (s, 1 H), 9.11 (s, 1 H), 8.70 (d, 2H, J = 11.2 Hz), 8.62 (s, 1H), 8.58–8.48 (m, 2 H), 8.20 (d, 1H, J = 8.0 Hz), 8.08 (d, 1H, J = 8.0 Hz), 7.98–7.88 (m, 2 H), 7.84 (d, 1H, J = 5.2 Hz), 3.92 (s, 2H), 2.56 (s, 3H). MS *m/z* 383.2 (M+1).

N-([2,3'-bipyridin]-6'-yl)-2-(2',3-dimethyl-[2,4'-bipyridin]-5-yl)acetamide (**14**)



^{1}H NMR 400 MHz (DMSO-d_6) δ 10.99 (s, 1 H), 9.02 (s, 1 H), 8.66–8.59 (m, 1H), 8.52–8.38 (m, 2 H), 8.13 (d, 1H, J = 8.8 Hz), 7.97 (d, 1H, J = 7.6 Hz), 7.90–7.80 (m, 1H), 7.69 (s, 1H), 7.42–7.29 (m, 3H), 7.46–7.40 (m, 2H), 7.35–7.29 (m, 1H), 3.81 (s, 2H), 2.46 (s, 3H), 2.30 (s, 3H). MS *m/z* 396.2 (M+1).

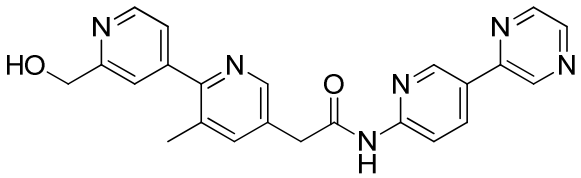
2-(2'-fluoro-3-methyl-[2,4'-bipyridin]-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide (**16**)



^{1}H NMR 400 MHz (DMSO-d$_6$) δ11.07 (s, 1H), 9.25 (d, 1H, J = 1.6 Hz), 9.06-9.04 (m, 1H), 8.67-8.65 (m, 1H), 8.57 (d, 1H, J = 2.4 Hz), 8.48-8.44 (m, 2H), 8.28 (d, 1H, J = 5.2 Hz), 8.16 (d,

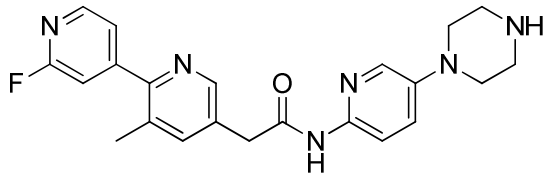
1H, *J* = 8.8 Hz), 7.71 (d, 1H, *J* = 1.6 Hz), 7.51–7.49 (m, 1H), 7.31 (s, 1H), 3.82 (s, 2H), 2.31 (s, 3H). MS *m/z* 401.2 (M+1).

2-(2'-(hydroxymethyl)-3-methyl-[2,4'-bipyridin]-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide (**17**)



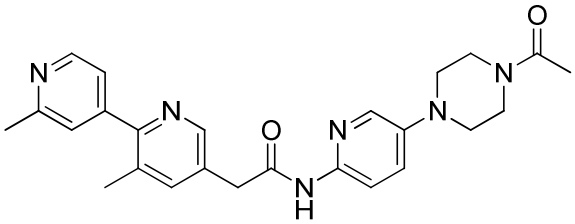
^{1}H NMR 500 MHz (DMSO-d_6) δ11.12 (s, 1H), 9.33 (d, 1H, J = 1.5 Hz), 9.14 (dd, 1H, *J* = 2.0, 0.5 Hz), 8.74 (dd, 1H, *J* = 2.5,1.5 Hz), 8.65 (d, 1H, *J* = 2.5 Hz), 8.58 (dd, 1H, J = 5.0, 0.5 Hz), 8.54 (dd, 1H, J=8.5, 2.5 Hz), 8.52 (d, 1H, *J* = 8.5 Hz), 8.24 (d, 1H, *J* = 8.5 Hz), 7.76 (d, 1H, *J* = 1.5 Hz), 7.65 (dd, 1H, *J* = 1.5, 0.5 Hz), 7.45 (dd, 1H, *J* = 5.0, 1.5 Hz) 5.47 (t, 1H, *J* = 6.0 Hz), 4.65 (d, 2H, *J* = 6.0 Hz), 3.90 (s, 2H), 2.38 (s, 3H). MS *m/z* 413.2 (M+1).

2-(2'-fluoro-3-methyl-[2,4'-bipyridin]-5-yl)-N-(5-(piperazin-1-yl)pyridin-2-yl)acetamide (**18**)



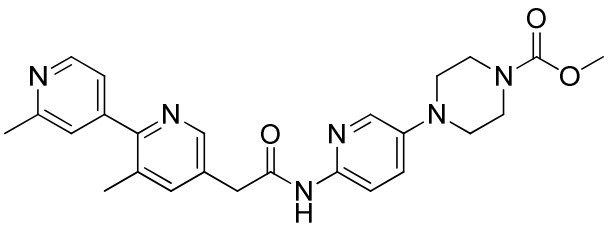
^{1}H NMR (400 MHz) (DMSO-d_6) δ 10.60 (s, 1H), 8.49 (s, 1H), 8.36-8.32 (m, 1H), 8.02 (s, 1H), 7.94-7.88 (m, 1H), 7.74 (s, 1H), 7.55 (s, 1H), 7.41-7.34 (m, 2H), 3.77 (s, 2H), 3.12 (b, 4H), 2.97 (b, 4H), 2.37 (s, 3H). MS *m/z* 407.2 (M+1).

N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(2',3-dimethyl-[2,4'-bipyridin]-5-yl)acetamide (**20**)



^{1}H NMR 400 MHz (DMSO-d_6) δ10.57 (s, 1H), 8.49 (d, 1H, J = 5.0 Hz), 8.40 (d, 1H, J = 1.8 Hz), 7.98 (d, 1H, J = 2.9 Hz), 7.87 (d, 1H, J = 9.0 Hz), 7.64 (d, 1H, J = 1.5 Hz), 7.42 (s, 1H), 7.36 (dd, 2H, J = 9.0, 3.0 Hz), 3.70 (s, 2H), 3.53-3.48 (m, 4H), 3.12-3.05 (m, 2H), 3.04-2.97 (m, 2H), 2.49 (s, 3H), 2.28 (s, 3H), 1.97 (s, 3H). MS *m/z* 445.2 (M+1).

Methyl 4-(6-(2-(2',3-dimethyl-[2,4'-bipyridin]-5-yl)acetamido)pyridin-3-yl)piperazine-1-carboxylate (**21**)



^{1}H NMR 400 MHz (DMSO-d_6) δ 10.77 (s, 1H), 8.86-8.80 (m, 1H), 8.57 (s, 1H), 8.10 (s, 1H), 8.06-8.01 (m, 2H), 7.95–7.85 (m, 1H), 7.82 (s, 1H), 7.5-7.43 (m, 1H), 3.84 (s, 2H), 3.16-3.08 (b, 4H), 2.82-2.75 (b, 4H), 2.55 (s, 3H), 2.54 (s, 3H), 2.42 (s, 3H). MS *m/z* 461.2 (M+1).

HT Solubility Assays.

One –hundred microliter aliquots of 1 mM DMSO solutions were added to each of three glass vials and evaporated to dryness prior to addition of 500 microliter of pH 6.8 buffer. Following 24 h of shaking, the solutions were vacuum filtered through MultiScreen Solubility 96-well plates with 0.4 μm modified PCTE membrane (Millipore, MSSLBPC10), and an aliquot of each filtrate is transferred to a UV plate for quantification.

Wnt3a co-culture reporter gene assay.

Mouse leydig cell TM3 cells (American Type Culture Collection) were cultured in a 1:1 mixture of Ham's F-12 medium and DMEM (Gibco/Invitrogen) supplemented with 2.5% (vol/vol) FBS (Gibco/Invitrogen), 5% (vol/vol) horse serum (Gibco/Invitrogen), 50 unit/mL penicillin, and 50 μg/mL streptomycin (Gibco/Invitrogen). TM3 cells in a 10-cm dish were cotransfected with 8 μg STF-reporter plasmid containing a luciferase gene driven by Wnt-responsive elements and 2 μg pcDNA3.1-Neo (Gibco/Invitrogen) with 30 μL FuGENE6 (Roche Diagnostics) following the manufacturer's protocol. Stable cell lines (TM3 Wnt-Luc) were selected with 400 μg/mL G418 (Gibco/Invitrogen). The TM3 Wnt-Luc cells and L-cell Wnt3A cells (American Type Culture Collection) were cocultured in a 384-well plate with DMEM supplemented with 2% (vol/vol) FBS and treated with different concentrations of compounds. After 24 h, the firefly luciferase activities were assayed with the Bright-Glo Luciferase Assay System (Promega). The IC_{50} is measured when the effect of the compound reduces the luminescence signal by 50%. All compounds are tested in triplicates.

Radio ligand binding assay of GNF-1331.

a. Membrane preparation: 10^8 cells were transfected with pcDNA 3.1 constructs (Invitrogen) bearing human PORCN using Fugene 6 (Roche). After 48 h, cells were harvested by scraping in PBS and centrifuging at 1,000 × g for 10 min. Cell pellets were frozen in a dry ice bath and then gently resuspended in 10 mL 50 mM Tris (pH 7.5) and 250 mM sucrose buffer containing an EDTA-free protease inhibitor mixture (Sigma). Cells were lysed using a polytron (Brinkman).

Lysed cells were centrifuged at 1,600 × g for 20 min at 4 °C, and supernatant was transferred and centrifuged at 20,000 rpm in an SS34 rotor for 20 min at 4 °C. Supernatants were discarded, and the pellets were resuspended in 10% (wt/vol) sucrose, 50 mM Tris (pH 7.5), 5 mM $MgCl_2$, and 1 mM EDTA solution using three 10-s pulses with a polytron. ^{3}H-radiolabeled GNF-1331 was made by AmBioslabs through a hydrogenationreaction. GNF-1331 binds to PORCN and serves as a hot radioligand in the in vitro biochemical PORCN binding assay for competition with cold testing compound GNF-1331.

b. Radioligand binding assay: using the aforementioned membrane preps, filtration binding assays were performed. To reduce nonspecific binding, 96-well filtration plates (PerkinElmer) were precoated as suggested by the manufacturer with 0.1% BSA and then washed four times with 0.1% BSA. Membrane preps (50 μg total protein) were incubated in polypropylene 96-well plates with 6.6 nM ^{3}H-GNF-1331 in the presence or absence of a testing compound in binding buffer (50 mM Tris, pH 7.5, 5 mM MgCl2, 1 mM EDTA, 0.1% BSA) plus EDTA-free protease inhibitor mixture (Sigma) in a final volume of 150 μL for 3 h at room temperature. Binding reaction mixtures were then transferred to the precoated 96-well filtration plates (PerkinElmer), filtered, and washed using a 96-pin FilterMate Harvester (PerkinElmer). Radioactive signals were obtained using a Microplate Scintillation Counter TopCount (PerkinElmer).

Protocol of mouse PK Study.

Testing compound was formulated as a solution in 75% PEG300/25% D5W and administered to male Balb/c mice intravenously via tail vein at 2 mg/kg (n = 3) and orally via gavage at 5 mg/kg (n = 3). Blood samples were collected serially at scheduled times over 24 h after dosing.

In vivo PK/PD and efficacy Studies.

a. All animal studies were conducted at the Genomics Institute of the Novartis Research Foundation. The experimental protocols were in compliance with animal welfare regulations and approved by the Institutional Animal Care and Use Committee at Genomics Institute of the Novartis Research Foundation.

b. Efficacy: Nude mice bearing the mouse mammary tumor virus–Wnt1 were randomized according to tumor volume. The average tumor volume when starting treatment is about 100 mm^3. Compound **19** was formulated in 20% (vol/vol) PEG300 and 30% (vol/vol) 10% ETPGS in water and administered by oral gavage at a dosing volume of 10 μL/g animal body weight. Body weight was monitored daily, and tumor sizes were assessed three times per week after the tumors were palpable. Tumor sizes were determined by using caliper measurements. Tumor volumes were calculated with a formula (length × width × height)/2.

c. PK/PD: The plasma and tumor concentrations of **19** in the tumor-bearing nude mice (n = 3 per time point) were determined at 1, 5, 7, 10, and 24 h after a single oral dose of 3 mg/kg by liquid chromatography/MS/MS. TaqMan analyses were performed using TaqMan Universal Master Mix (Applied Biosystems) and AXIN2 and GAPDH probes (Applied Biosystems) according to the manufacturer's instructions. AXIN2 mRNA expression levels were normalized to GAPDH mRNA levels.

Figure SI-1 ^{1}H NMR spectrum of **15** in DMSO-d_6

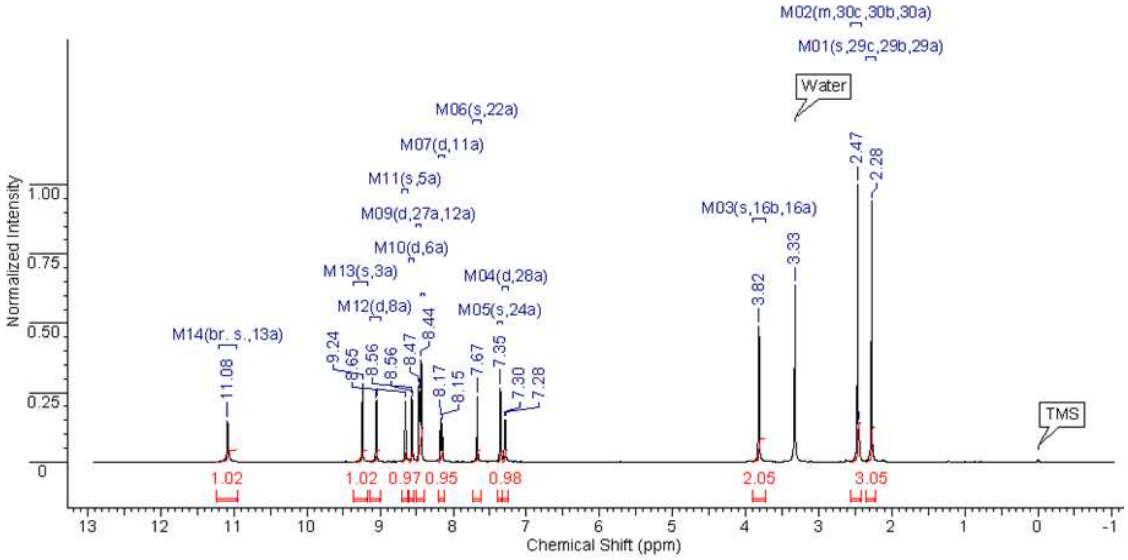


Figure SI-2 ^{13}C NMR spectrum of **15** in DMSO-d_6

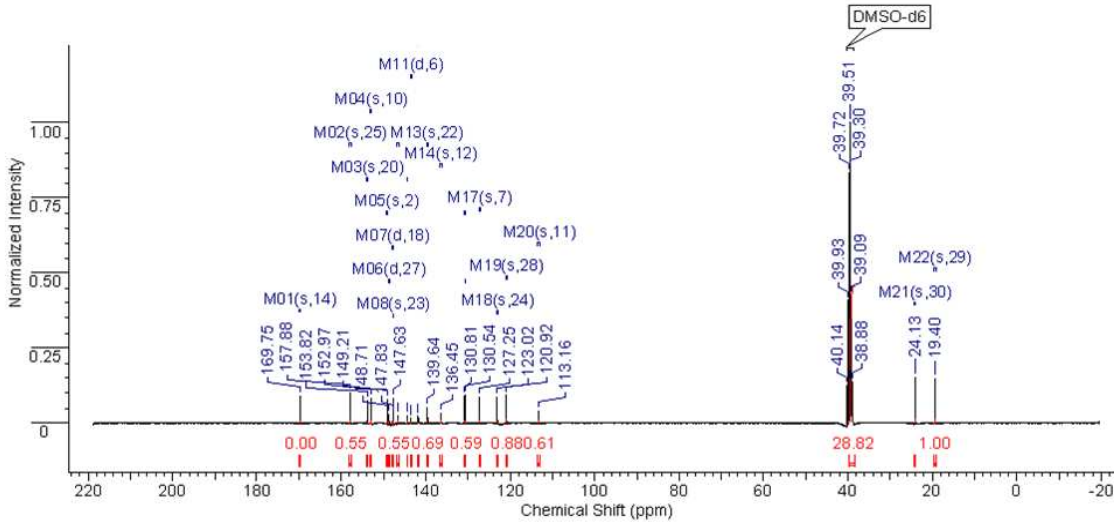


Figure SI-3 ^{1}H NMR spectrum of **19** in DMSO-d_6

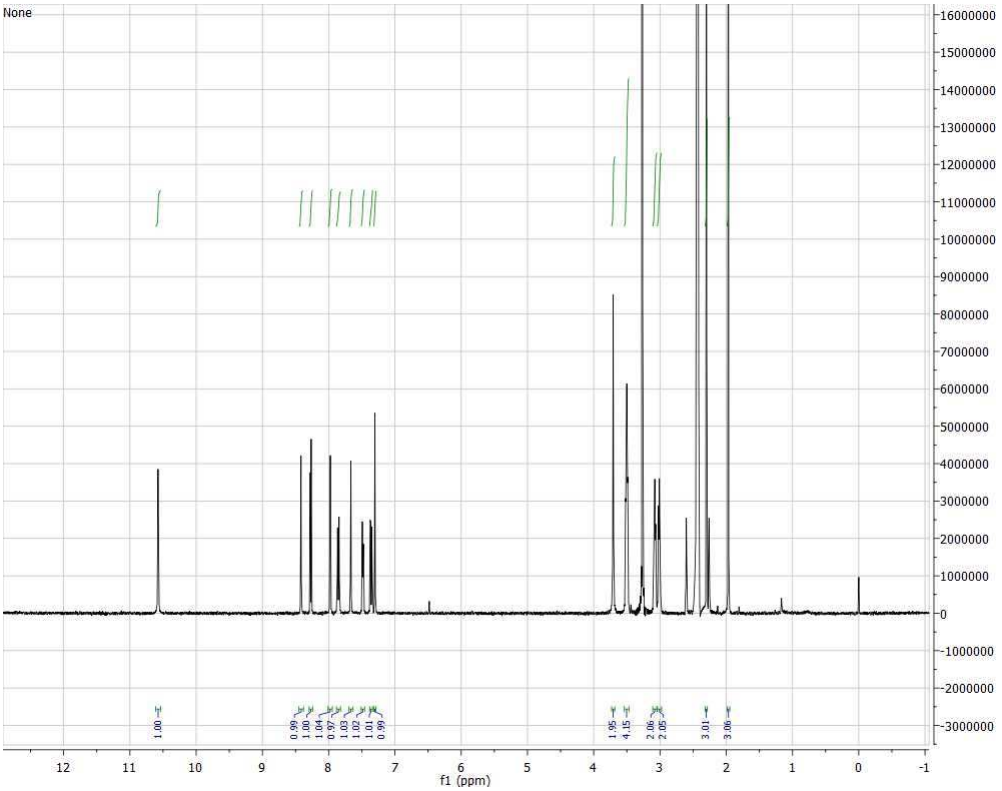


Figure SI-2 ^{13}C NMR spectrum of **19** in DMSO-d_6