Discovery of IWP-051, a Novel Orally Bioavailable sGC Stimulator with Once-Daily Dosing Potential in Humans

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SUPPORTING INFORMATION

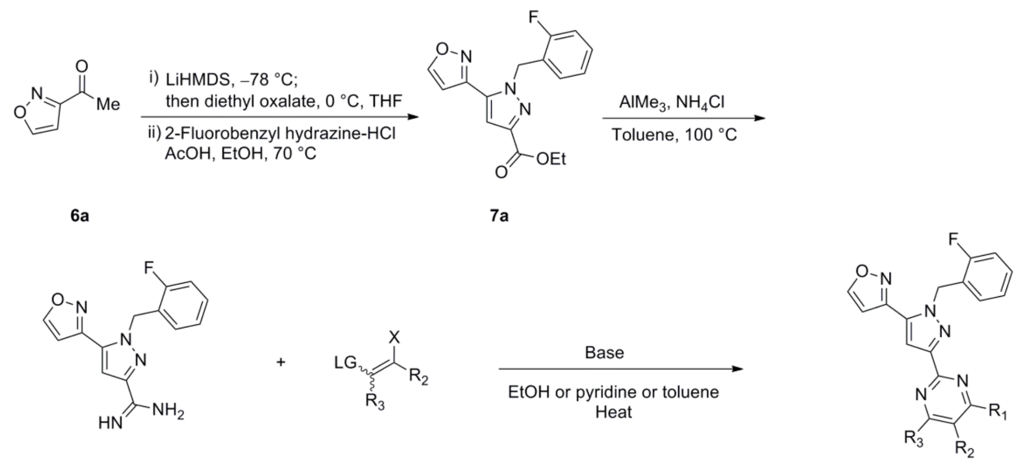
Table of Contents

Synthetic Procedures

Flash chromatography was performed using pre-packed silica gel cartridges compatible with ISCO, Biotage, or Reveleris flash systems. Chemical reactions were monitored by LCMS, and the purity and identity of the reaction products were assayed by LCMS (electrospray ionization) and ^{1}H NMR. LCMS spectra were recorded on a Waters Acquity UPLC equipped with Waters BEH130 C18 column (1.7 µM, 2.1 x 50 mm i.d.) and UV detector. HRMS spectra were recorded on a Waters Synapt G2-S equipped with Thermo Hypersil Gold column (1.9 µm). ^{1}H NMR spectra were recorded on Bruker 500 MHz or Varian 400 MHz spectrometers, and are internally referenced to residual solvent signals. Data for ^{1}H NMR are reported with chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br s = broad singlet), coupling constant (Hz), and integration. All temperatures are degrees Celsius unless otherwise noted. All final compounds with reported biological data were determined to be >80% purity based on LC-MS and NMR data unless otherwise noted.

Detailed procedures for the synthesis of the following starting materials and intermediates have been previously reported:[1-4] (3,3,4,4,4-pentafluorobutyl)hydrazine, 1-(2-fluorobenzyl)-5-(oxazol-2-yl)-1H-pyrazole-3-carboximidamide **(2b)**, 1-(2-fluorobenzyl)-5-(oxazol-4-yl)-1H-pyrazole-3-carboximidamide **(2c)**, 1-(2-fluorobenzyl)-5-(5-methylisoxazol-3-yl)-1H-pyrazole-3-carboximidamide **(2d)**, 1-(2-fluorobenzyl)-5-(pyridin-2-yl)-1H-pyrazole-3-carboximidamide **(2f)**, 5-cyclopropyl-1-(2-fluorobenzyl)-1H-pyrazole-3-carboximidamide **(2g)**, 1-benzyl-5-(isoxazol-3-yl)-1H-pyrazole-3-carboximidamide **(2h)**, 1-(2,3-difluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazole-3-carboximidamide **(2i)**, 1-(3-chloro-2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazole-3-carboximidamide **(2j)**,1-(2-fluoro-3-methylbenzyl)-5-(isoxazol-3-yl)-1H-pyrazole-3-carboximidamide **(2k)**, 1-((3-fluorothiophen-2-yl)methyl)-5-(isoxazol-3-yl)-1H-pyrazole-3-carboximidamide **(2l)z**, 1-(2,4-difluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazole-3-carboximidamide **(2m)**, 1-((3-fluoropyridin-2-yl)methyl)-5-(isoxazol-3-yl)-1H-pyrazole-3-carboximidamide **(2n)**, 5-(isoxazol-3-yl)-1-(3,3,3-trifluoropropyl)-1H-pyrazole-3-carboximidamide **(2o)**, 4-(ethoxymethylene)-2-phenyloxazol-5(4H)-one **(3d)**, ethyl 3-(dimethylamino)-2-fluoroacrylate **(3f)**, 3-(dimethylamino)-2-fluoroacrylonitrile **(3g)**, ethyl 3-(dimethylamino)-2-(methylsulfonyl)acrylate **(3i)**, ethyl 3-(dimethylamino)-2-(phenylsulfonyl)acrylate **(3j)**, ethyl 2-(4-chlorophenylsulfonyl)-3-(dimethylamino)acrylate **(3k)**, ethyl 3-(dimethylamino)-2-(*N*,*N*-dimethylsulfamoyl)acrylate **(3l)**, ethyl 3-(dimethylamino)-2-(*N*-methyl-*N*-phenylsulfamoyl)acrylate **(3m).**

Scheme S1



2a	3b	3-ethoxyacrylonitrile	1	**R_1** = -NH$_2$	**R_2** = -H	**R_3** = -H	
	3a	ethyl 2-cyano-3-ethoxyacrylate	4	**R_1** = -NH$_2$	**R_2** = -CO$_2$Et	**R_3** = -H	
			5	**R_1** = -OH	**R_2** = -CN	**R_3** = -H	
	3c	methyl 3-methoxyacrylate	8	**R_1** = -OH	**R_2** = -H	**R_3** = -H	NCS, DMF, 70 °C
			13	**R_1** = -OH	**R_2** = -Cl	**R_3** = -H	
	3d	4-(ethoxymethylene)-2-phenyloxazol-5(4H)-one	9	**R_1** = -OH	**R_2** = -NHBz	**R_3** = -H	
	3e	diethyl 2-(ethoxymethylene)malonate	10-1	**R_1** = -OH	**R_2** = -CO$_2$Et	**R_3** = -H	MeMgBr, THF, −20 °C
			10	**R_1** = -OH	**R_2** = -Me$_2$OH	**R_3** = -H	
	3f	ethyl 3-(dimethylamino)-2-fluoroacrylate	11	**R_1** = -OH	**R_2** = -F	**R_3** = -H	
	3g	3-(dimethylamino)-2-fluoroacrylonitrile	12	**R_1** = -NH$_2$	**R_2** = -F	**R_3** = -H	
	3i	ethyl 3-(dimethylamino)-2-(methylsulfonyl)acrylate	15	**R_1** = -OH	**R_2** = -SO$_2$Me	**R_3** = -H	
	3j	ethyl 3-(dimethylamino)-2-(phenylsulfonyl)acrylate	16	**R_1** = -OH	**R_2** = -SO$_2$Ph	**R_3** = -H	
	3k	ethyl 2-(4-chlorophenylsulfonyl)-3-(dimethylamino)acrylate	17	**R_1** = -OH	**R_2** = -SO$_2$(*p*-ClPh)	**R_3** = -H	
	3l	ethyl 3-(dimethylamino)-2-(*N*,*N*-dimethylsulfamoyl)acrylate	18	**R_1** = -OH	**R_2** = -SO$_2$N(Me)$_2$	**R_3** = -H	
	3l	ethyl 3-(dimethylamino)-2-(N-methyl-N-phenylsulfamoyl)acrylate	19	**R_1** = -OH	**R_2** = -SO$_2$N(Me)(Ph)	**R_3** = -H	
	3m	ethyl 2-fluoroacetoacetate	20	**R_1** = -OH	**R_2** = -F	**R_3** = -Me	

2-(1-(2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)pyrimidin-4-amine (1): To a cold solution of commercially available 1-(isoxazol-3-yl)ethanone **(6a)** (32.2 g, 275 mmol) in THF (1.0 L) at −78 °C, was added, dropwise, LiHMDS (0.248 L, 248 mmol) as a 1 M solution in toluene over the course of 45 minutes. Then, the yellow solution was immediately warmed to 0 °C and stirred for 45 minutes. To this mixture, was added diethyl oxalate (43.0 mL, 317 mmol). The mixture was stirred at 0 °C for 15 minutes and warmed to 23 °C over the course of 45 minutes. The resulting dark red solution was diluted with ethanol (500 mL) and sequentially treated with (2-fluorobenzyl)hydrazine hydrochloride (58.4 g, 330 mmol) followed by hydrogen chloride (500 mL, 625 mmol) as a 1.25 M solution in EtOH. The solution turned a pale, milky yellow suspension upon addition of all reagents. The mixture was heated to 70 °C for 30 minutes. Upon completion of the reaction, the mixture was cooled to 23 °C. The mixture was carefully treated with sodium bicarbonate (92.4 g) and H_2O (100 ml). The resulting white solid was removed by filtration. The filtrate was concentrated *in vacuo*. The resulting residue was partitioned between DCM (500 ml) and H_2O (500ml). The aqueous layer was extracted with DCM (3 x 500ml). Combined organic layers were washed with brine (100 ml), dried over $MgSO_4$, and concentrated *in vacuo* to give a crude oil. The oil was purified by silica gel column chromatography using a 0-40% EtOAc in hexanes gradient to give ethyl 1-(2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazole-3-carboxylate **(7a)** (56.6 g, 65% yield); LCMS m/z = 316.0 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 8.38-8.50 (m, 1H), 7.11-7.24 (m, 2H), 6.89-7.09 (m, 2H), 6.79 (t, *J*=7.24 Hz, 1H), 6.40-6.64 (m, 1H), 5.94 (s, 2H), 4.34-4.48 (m, 2H), 1.32-1.45 (m, 3H).

A 2 L round-bottom flask equipped with an addition funnel was charged with ammonium chloride (22.47 g, 420 mmol) and toluene (449 ml). The suspension was cooled to 0 °C and placed under an atmosphere of nitrogen. To this suspension, was carefully added, trimethylaluminum (210 ml, 420 mmol) as a 2 M solution in toluene through an addition funnel. (Caution: The reaction must be carried out in a well-ventilated flask to accommodate gas generated during the course of the addition). Upon the completion of the addition, the mixture was warmed to 23 °C and stirred until the mixture ceased to generate gas. To this mixture, was added **7a** (28.3 g, 90 mmol). The round-bottom flask was equipped with a reflux condenser and the mixture was heated to 110 °C for 16 h. The mixture was then cooled to 0 °C, diluted with toluene (150 ml), and quenched with MeOH (~50 ml). The resulting mixture was allowed to warm up to 23 °C over 1 h with vigorous stirring and was filtered through a glass-fritted funnel layered with sand and celite. The precipitates were further washed with MeOH (3 x 200 ml). The filtrate was concentrated under vacuum. The resulting residue was re-suspended in a 5:1 EtOAc:isopropanol mixture (600 ml total) and treated with saturated aqueous sodium carbonate (200 ml). The mixture was stirred at 23 °C for 10 minutes, and the layers were separated. The aqueous layer was back-extracted with a 5:1 EtOAc:isopropanol mixture (3 x 500 ml). The organic layers were combined, washed with brine (50 ml), dried over $MgSO_4$, filtered, and concentrated under vacuum to give 1-(2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazole-3-carboximidamide **(2a)** (17 g, 66% yield): LCMS *m*/*z* = 286.3 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 9.14 (d, *J*=1.17 Hz, 1H), 7.52-7.56 (m, 1H), 7.29-7.45 (m, 1H), 7.17-7.28 (m, 1H), 7.12 (t, *J*=7.63 Hz, 1H), 7.09 (d, *J*=1.57 Hz, 1H), 6.87 (t, *J*=7.24 Hz, 1H), 5.87 (s, 2H).

To a solution of **2a** (3.08 g, 10.8 mmol) in pyridine (54 mL), were added **3b** (3.3 mL, 32 mmol) and DBU (1.6 mL, 11 mmol). The reaction mixture was heated to 110 °C for 16 h. The pyridine was removed *in vacuo*. The resulting dark red oil was treated with a 5:1 mixture of DCM/MeOH (200 mL). The precipitate formed was collected by filtration, and rinsed with a minimal amount of diethyl ether. Purification of the solid using a 0-10% 7:1 ACN/MeOH/DCM gradient gave 2-(1-(2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)pyrimidin-4-amine **(1)** (1.8 g, 49% yield) as a brown solid: LCMS *m*/*z* = 337.3 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 9.08 (d, *J*=1.57 Hz, 1H), 8.12 (d, *J*=5.87 Hz, 1H), 7.51 (s, 1H), 7.27-7.41 (m, 1H), 7.19-7.27 (m, 1H), 7.11 (dt, *J*=0.98, 7.53 Hz, 1H), 6.96 (s, 1H), 6.66-6.91 (m, 1H), 6.36 (d, *J*=5.87 Hz, 1H), 5.89 (s, 2H).

Ethyl 4-amino-2-(1-(2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)pyrimidine-5-carboxylate (4) and **2-(1-(2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)-4-hydroxypyrimidine-5-carbonitrile (5):** In a similar manner to **1**, condensation of **2a** and **3a** performed in EtOH gave **4** and **5**.

4 (20 mg, 4% yield): LCMS *m*/*z* = 409.2 $[M+H]^+$; ^1H NMR (500 MHz, $CDCl_3$) δ 9.00 (s, 1H), 8.46 (d, *J*=1.53 Hz, 1H), 7.59 (br s, 1H), 7.18-7.24 (m, 1H), 7.00-7.07 (m, 1H), 6.98 (t, *J*=7.63 Hz, 1H), 6.83 (t, *J*=7.32 Hz, 1H), 6.62 (d, *J*=1.53 Hz, 1H), 6.04 (s, 2H), 4.41 (q, *J*=7.02 Hz, 2H), 1.39-1.48 (m, 3H)

5 (167 mg, 38% yield): LCMS *m*/*z* = 363.0 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 9.13 (d, *J*=1.57 Hz, 1H), 8.41 (s, 1H), 7.78 (s, 1H), 7.30-7.39 (m, 1H), 7.19-7.29 (m, 2H), 7.13 (t, *J*=7.43 Hz, 1H), 6.99 (t, *J*=7.63 Hz, 1H), 5.95 (s, 2H).

2-(1-(2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)pyrimidin-4-ol (8): In a similar manner to **1**, condensation of **2a** and **3c** gave **8** (58 mg, 41% yield): LCMS *m*/*z* = 338.0 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 8.47 (d, *J*=1.57 Hz, 1H), 7.93 (d, *J*=6.65 Hz, 1H), 7.27 (s, 1H), 7.19-7.26 (m, 1H), 6.94-7.10 (m, 3H), 6.56 (d, *J*=1.96 Hz, 1H), 6.35 (d, *J*=7.04 Hz, 1H), 5.86 (s, 2H).

***N*-(2-(1-(2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)-4-hydroxypyrimidin-5-yl)benzamide (9):** In a similar manner to **1**, condensation of **2a** and **3d** gave **9** (24 mg, 10% yield):

LCMS *m/z* = 457.0 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ ppm 9.13 (d, 1 H) 7.95 (d, 2 H) 7.72 (br s, 1 H) 7.59 - 7.68 (m, 1 H) 7.49 - 7.59 (m, 2 H) 7.29 - 7.41 (m, 2 H) 7.19 - 7.29 (m, 2 H) 7.13 (t, 1 H) 6.98 (br s, 1 H) 5.94 (br s, 2 H).

Ethyl 2-(1-(2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)-4-hydroxypyrimidine-5-carboxylate (10-1): In a similar manner to **1**, condensation of **2a** and **3e** gave **10-1** (74 mg, 49% yield): LCMS *m/z* = 410.0 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 13.11 (br s, 1H), 9.05-9.19 (m, 1H), 8.61 (br s, 1H), 7.77 (s, 1H), 7.29-7.56 (m, 1H), 7.18-7.29 (m, 2H), 7.13 (t, *J*=7.63 Hz, 1H), 5.94 (br s, 2H), 4.24 (d, *J*=6.65 Hz, 2H), 1.28 (t, *J*=7.04 Hz, 3H).

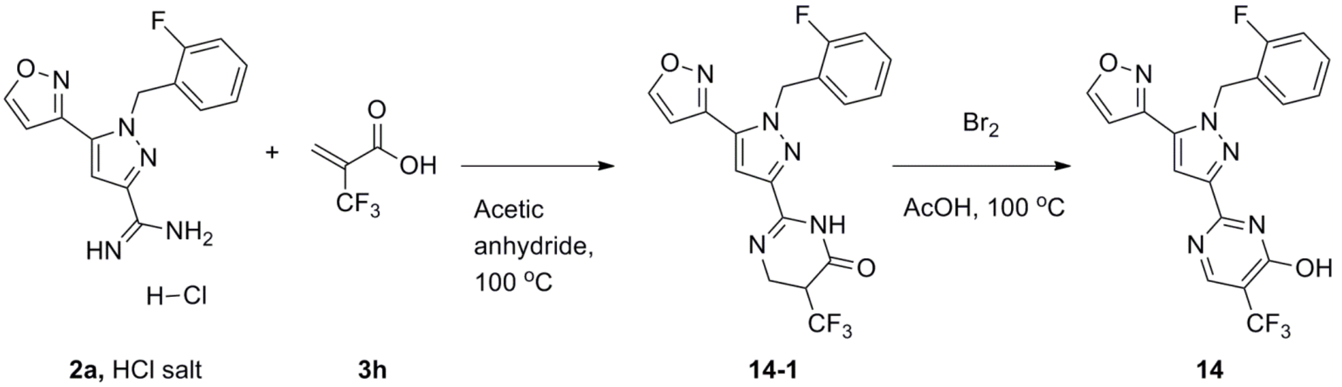
2-(1-(2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)-5-(2-hydroxypropan-2-yl)pyrimidin-4-ol (10): To a suspension of **10-1** (74.3 mg, 0.181 mmol) in THF (1.8 ml) at −20 °C under argon, was slowly added, methylmagnesium bromide (242 µl, 4.0 equiv.) as a 3.0 M solution in diethyl ether. The mixture was then warmed to room temperature and stirred for 30 minutes. The green mixture was poured into ethyl acetate (300 ml) and washed with a saturated solution of ammonium chloride (50 ml). The organic layer was dried, filtered, and evaporated to give a yellow solid. The crude solid was rinsed with a minimal amount of MeOH. The remaining insoluble solid was dried *in vacuo* to give **10** (27 mg, 38% yield) as an off-white solid: LCMS *m/z* = 396.1 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 9.12-9.14 (m, 1H), 7.68 (br s, 1H), 7.31-7.37 (m, 1H), 7.21-7.25 (m, 2H), 7.10-7.15 (m, 2H), 6.96-6.98 (m, 1H), 5.92 (s, 2H), 1.47 (s, 6H).

5-fluoro-2-(1-(2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)pyrimidin-4-ol (11, IWP-051): A mixture containing ethyl 3-(dimethylamino)-2-fluoroacrylate (1.2 g, 7.9 mmol) and 1-(2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazole-3-carboximidamide (0.75 g, 2.6 mmol) in EtOH (10 ml) was heated to 85 °C for 24 h. The mixture was concentrated *in vacuo* to dryness. The resulting residue was purified by silica gel column chromatography using a 0-70% 7:1 ACN/MeOH in DCM gradient to give **11** (0.45 g 48% yield): HRMS (ESI-Tof) *m/z*: $[M + H]^+$ Calcd for $C_{17}H_{11}F_2N_5O_2$ 356.0959, found 356.0958, PPM Error = 0.30; ^1H NMR (DMSO) δ: 13.28 (br. s., 1H), 9.02-9.22 (m, 1H), 8.15 (br. s., 1H), 7.64 (s, 1H), 7.31-7.41 (m, 1H), 7.18-7.26 (m, 2H), 7.12 (t, J=7.6 Hz, 1H), 6.98 (br. s., 1H), 5.86-5.95 (m, 2H); ^{13}C NMR (DMSO) δ: 160.9, 160.4, 158.5, 152.3, 134.0, 129.9, 129.8, 128.8, 124.7, 124.7, 123.7, 123.5, 115.4, 115.3, 109.5, 104.9, 49.3.

5-fluoro-2-(1-(2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)pyrimidin-4-amine (12): In a similar manner to **1**, condensation of **2a** and **3g** gave **12** (35 mg, 14% yield): LCMS *m/z* = 355.0 $[M+H]^+$; ^1H NMR (500 MHz, DMSO-d_6) δ 9.08 (d, *J*=1.83 Hz, 1H), 8.21 (d, *J*=3.36 Hz, 1H), 7.43-7.54 (m, 1H), 7.29-7.35 (m, 1H), 7.19-7.25 (m, 2H), 7.11 (t, *J*=7.48 Hz, 1H), 6.87 (t, *J*=7.02 Hz, 1H), 5.88 (s, 2H).

5-chloro-2-(1-(2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)pyrimidin-4-ol (13): A mixture containing **8** (31 mg, 0.092 mmol) and *N*-chlorosuccinimide (1.2 mg, 0.092 mmol) in DMF (1.0 ml) was heated to 70 °C for 18 h. The mixture was diluted in ethyl acetate (50 ml) and washed with water (50 ml). The organic layer was dried, filtered and evaporated *in vacuo* to give a crude solid. The solid was rinsed with a minimal amount of diethyl ether and dried *in vacuo* to give **13** (15 mg, 37% yield): LCMS *m/z* = 372.1 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 8.52 (d, *J*=1.57 Hz, 1 H), 7.18 - 7.33 (m, 3 H), 6.99 - 7.12 (m, 3 H), 6.53 - 6.66 (m, 1 H), 5.82 - 5.94 (m, 2 H).

Scheme S2



2-(1-(2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)-5-(trifluoromethyl)pyrimidin-4-ol (14): A mixture of a hydrochloride salt of **2a** (500 mg, 1.6 mmol), **3h** (218 mg, 1.6 mmol), and acetic anhydride (3.0 ml, 31 mmol) was heated to 100 °C for 1 h. The mixture was concentrated *in vacuo* to give a thick oil. The oil was purified by silica gel column chromatography using a 10-40% EtOAc/hexanes gradient to give 2-(1-(2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)-5-(trifluoromethyl)-5,6-dihydropyrimidin-4(1H)-one **(14-1)** (372 mg, 59% yield): LCMS *m*/*z* = 408.1 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 8.51 (d, *J*=1.57 Hz, 1H), 7.16 (s, 1H), 6.97-7.12 (m, 4H), 6.59 (d, *J*=1.57 Hz, 1H), 5.89 (s, 2H), 3.99-4.22 (m, 4H).

A mixture of **14-1** (50 mg, 0.12 mmol) and bromine (6 µl, 0.12 mmol) in AcOH (2.5 ml) in a sealed vial was heated to 100 °C for 2 h. The mixture was cooled and concentrated *in vacuo*. The residue was dissolved in a minimal amount of methanol and precipitated from diethyl ether. The precipitate was collected and dried *in vacuo* to give **14** (20 mg, 40% yield) as a light orange solid: LCMS *m*/*z* = 406.1 $[M+H]^+$; ^1H NMR (400 MHz, CD_3OD) δ 8.81 (d, *J*=1.57 Hz, 1H), 8.38 (s, 1H), 7.59 (s, 1H), 7.27-7.35 (m, 1H), 7.04-7.15 (m, 2H), 6.89-7.00 (m, 2H), 6.03 (s, 2H).

2-(1-(2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)-5-(methylsulfonyl)pyrimidin-4-ol (15): In a similar manner to **1**, condensation of **2a** and **3i** gave **15** (58 mg, 47% yield): LCMS *m*/*z* = 416.0 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 9.06 (d, *J*=1.56 Hz, 1H), 8.24 (s, 1H), 7.54 (s, 1H), 7.30-7.36 (m, 1H), 7.26 (d, *J*=1.96 Hz, 1H), 7.22 (dd, *J*=8.80, 9.98 Hz, 1H), 7.10 (dt, *J*=1.17, 7.43 Hz, 1H), 6.81-6.87 (m, 1H), 5.89 (s, 2H), 3.15 (s, 3H).

2-(1-(2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)-5-(phenylsulfonyl)pyrimidin-4-ol (16): In a similar manner to **1**, condensation of **2a** and **3j** gave **16** (58 mg, 46% yield): LCMS *m*/*z* = 478.0 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 9.02 (d, *J*=1.56 Hz, 1H), 8.42 (d, *J*=1.96 Hz, 1H), 7.90-7.96 (m, 2H), 7.45-7.59 (m, 4H), 7.23-7.31 (m, 1H), 7.14-7.22 (m, 2H), 7.05 (t, *J*=7.63 Hz, 1H), 6.77 (t, *J*=7.04 Hz, 1H), 5.85 (s, 2H).

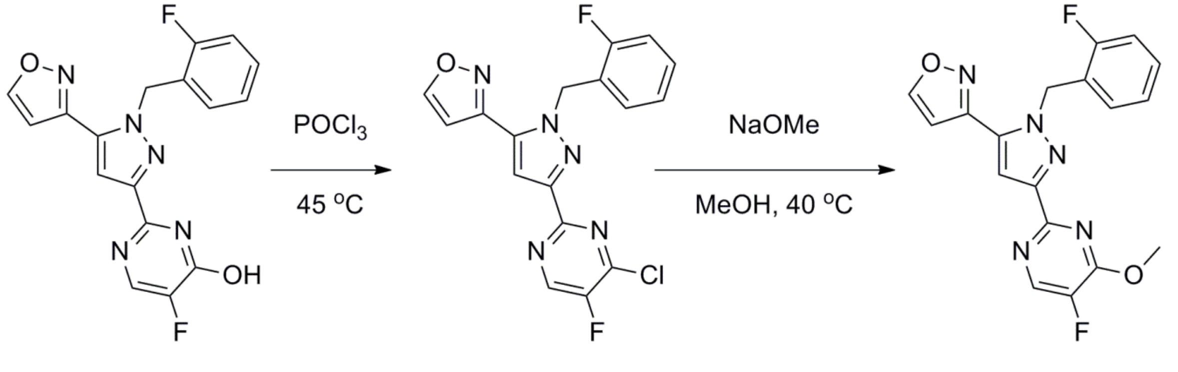
5-(4-chlorophenylsulfonyl)-2-(1-(2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)pyrimidin-4(3H)-one (17): In a similar manner to **1**, condensation of **2a** and **3k** gave **17** (47 mg, 34%): LCMS *m*/*z* = 512.0 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 9.13 (d, *J*=1.96 Hz, 1H), 8.03 (d, *J*=8.61 Hz, 2H), 7.79 (s, 1H), 7.71 (d, *J*=8.61 Hz, 2H), 7.18-7.42 (m, 4H), 7.10 (t, *J*=7.24 Hz, 1H), 6.92 (s, 1H), 5.96 (br s, 2H).

2-(1-(2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)-4-hydroxy-*N*,*N*-dimethylpyrimidine-5-sulfonamide (18): In a similar manner to **1,** condensation of **2a** and **3l** gave **18** (68 mg, 44% yield): LCMS *m*/*z* = 445.4 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 9.13 (d, *J*=1.57 Hz, 1H), 7.79 (s, 1H), 7.31-7.39 (m, 2H), 7.28 (br s, 1H), 7.20-7.26 (m, 2H), 7.13 (dt, *J*=0.98, 7.53 Hz, 1H), 5.96 (s, 2H), 3.32-3.36 (m, 6H).

2-(1-(2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)-4-hydroxy-*N*-methyl-*N*-phenylpyrimidine-5-sulfonamide (19): In a similar manner to **1**, condensation of **2a** and **3m** gave **19** (19 mg, 11% yield): LCMS *m*/*z* = 507.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.13 (d, *J*=1.96 Hz, 1H), 7.77 (s, 1H), 7.30-7.38 (m, 6H), 7.21-7.28 (m, 3H), 7.12 (t, *J*=7.43 Hz, 2H), 5.95 (s, 2H), 3.34 (s, 3H).

5-fluoro-2-(1-(2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)-6-methylpyrimidin-4-ol (20): In a similar manner to **1**, condensation of **2a** and **3n** gave **20** (20 mg, 20% yield) as a white solid: LCMS *m*/*z* = 370.0 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.11 (d, *J*=1.96 Hz, 1H), 7.62 (s, 1H), 7.30-7.39 (m, 1H), 7.26 (d, *J*=1.56 Hz, 1H), 7.17-7.25 (m, 1H), 7.12 (s, 1H), 6.96 (d, *J*=6.65 Hz, 1H), 5.92 (s, 2H), 2.28 (d, *J*=3.13 Hz, 3H).

Scheme S3

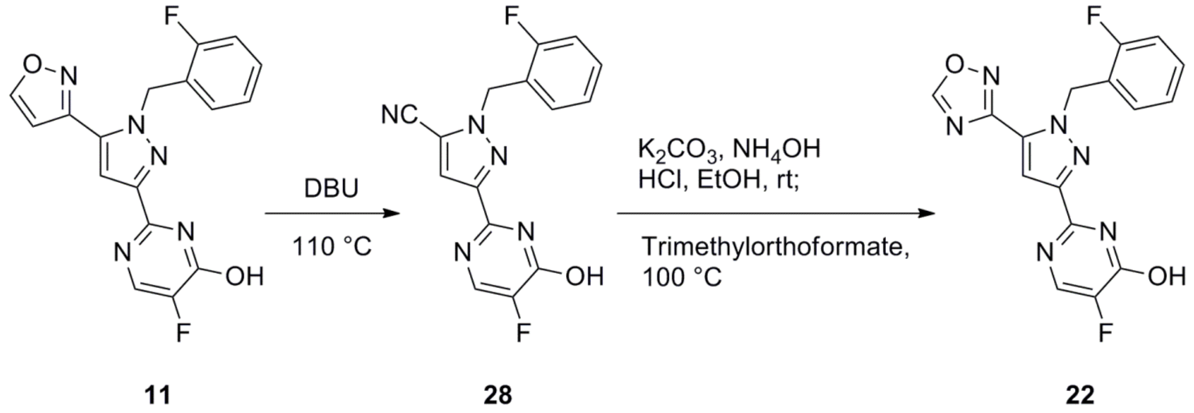


11 **21-1** **21**

3-(3-(5-fluoro-4-methoxypyrimidin-2-yl)-1-(2-fluorobenzyl)-1H-pyrazol-5-yl)isoxazole (21): A flask containing **11** (50.0 mg, 0.14 mmol) was charged with $POCl_3$ (1.0 ml, 11 mmol) and the resulting mixture was heated to 45 °C for 1 h. The reaction was diluted with DCM (25 ml) and carefully quenched with water (50 ml). The two phases were separated and the aqueous layer was back extracted with additional DCM (25 ml). The organic layers were combined, dried with sodium sulfate, filtered, and then concentrated *in vacuo*. Purification of the crude oil using silica gel chromatography employing a 0-75% EtOAc/hexanes gradient afforded **21-1** (45 mg, 86% yield) as a white solid: LCMS *m*/*z* = 374.0 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (s, 1H), 8.48 (d, *J*=1.57 Hz, 1H), 7.44 (s, 1H), 7.18-7.25 (m, 1H), 7.01-7.07 (m, 1H), 6.96-7.01 (m, 1H), 6.83-6.88 (m, 1H), 6.03 (s, 2H).

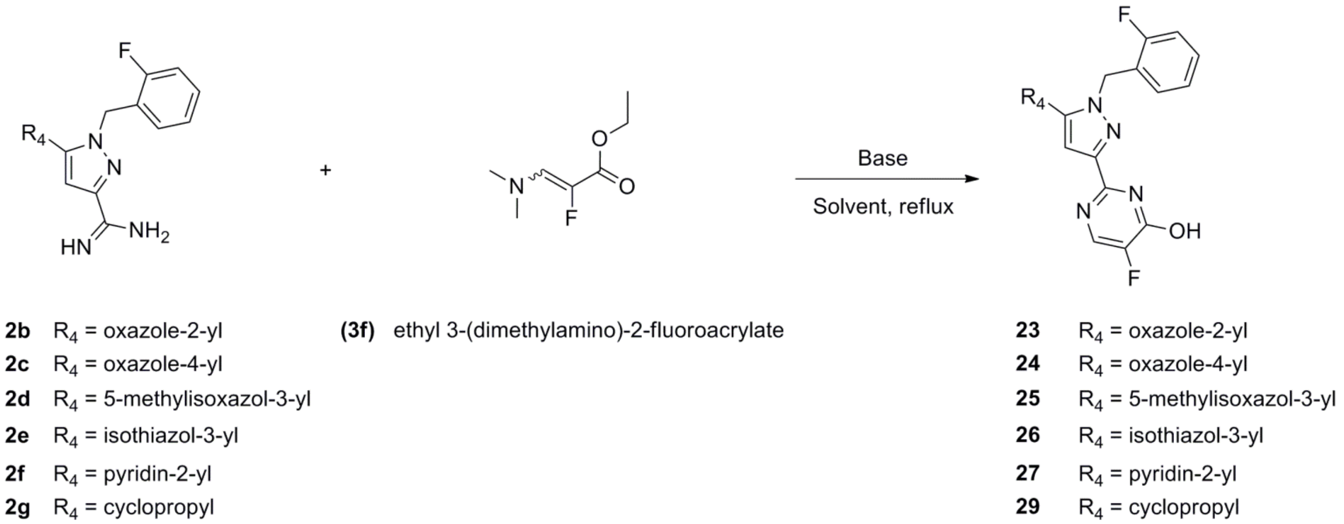
To a solution of **21-1** (40 mg, 0.11 mmol) in MeOH (1.0 ml) was added sodium methoxide (0.024 ml, 0.107 mmol) as a 25%wt solution in MeOH. The reaction was heated to 40 °C for 30 minutes. The reaction mixture was concentrated *in vacuo*, and the resulting crude oil was purified using silica gel chromatography employing a 0-100% EtOAc/hexanes gradient to afford **21** (23 mg, 95% yield): LCMS *m*/*z* = 370.1 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44-8.53 (m, 1H), 8.42 (d, *J*=2.35 Hz, 1H), 7.37 (s, 1H), 7.17-7.25 (m, 1H), 6.93-7.09 (m, 2H), 6.87 (dt, *J*=1.57, 7.63 Hz, 1H), 6.60 (d, *J*=1.56 Hz, 1H), 5.99 (s, 2H), 4.20 (s, 3H).

Scheme S4



5-fluoro-2-(1-(2-fluorobenzyl)-5-(1,2,4-oxadiazol-3-yl)-1H-pyrazol-3-yl)pyrimidin-4-ol (22): 28 (0.260 g, 0.83 mmol) was diluted with ethanol (10 mL) and consecutively charged with potassium carbonate (344 mg, 2.49 mmol) and ammonium hydroxide (72 mg, 1.04 mmol). After stirring the mixture for 18 h at room temperature, the reaction was diluted with EtOAc (50 mL) and filtered. The filtrate was concentrated and the crude material was charged with trimethylorthoformate (5 mL) and heated to 100 °C for 24 h. The reaction mixture was concentrated and the crude material was purified via silica gel chromatography using a 0-10% MeOH/DCM gradient to afford the desired **22** (35 mg, 11% yield): LCMS *m*/*z* = 357.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.87 (s, 1H), 8.15 (br s, 1H), 7.62 (s, 1H), 7.36 (d, *J*=8.22 Hz, 1H), 7.19-7.30 (m, 1H), 7.14 (t, *J*=7.63 Hz, 1H), 7.05 (d, *J*=7.43 Hz, 1H), 5.95 (s, 2H).

Scheme S5



5-fluoro-2-(1-(2-fluorobenzyl)-5-(oxazol-2-yl)-1H-pyrazol-3-yl)pyrimidin-4-ol (23): In a similar manner to **1**, condensation of **2b** and **3f** gave **23** (20 mg, 6% yield): LCMS *m*/*z* = 356.1 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD) δ 8.03 (s, 2H), 7.53 (s, 1H), 7.24-7.39 (m, 2H), 7.04-7.16 (m, 2H), 6.96 (t, *J*=7.04 Hz, 1H), 6.14 (s, 2H).

5-fluoro-2-(1-(2-fluorobenzyl)-5-(oxazol-4-yl)-1H-pyrazol-3-yl)pyrimidin-4-ol (24): In a similar manner to **1**, condensation of **2c** and **3f** gave **24** (5 mg, 3% yield): LCMS *m*/*z* = 356.1 $[M+H]^+$; ^{1}H NMR

(400 MHz, DMSO-d_6) δ 8.70 (s, 1H), 8.61 (d, *J*=0.78 Hz, 1H), 7.31-7.37 (m, 1H), 7.29 (s, 1H), 7.17-7.24 (m, 1H), 7.12 (dt, *J*=1.17, 7.63 Hz, 1H), 6.95-7.04 (m, 1H), 5.87 (s, 2H).

5-fluoro-2-(1-(2-fluorobenzyl)-5-(5-methylisoxazol-3-yl)-1H-pyrazol-3-yl)pyrimidin-4(3H)-one (25): In a similar manner to **1**, condensation of **2d** with sodium (*E*)-3-ethoxy-2-fluoro-3-oxoprop-1-en-1-olate **(3o)** gave **25** (8 mg, 3% yield): LCMS *m*/*z* = 370.4 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, *J*=2.74 Hz, 1H), 7.27-7.35 (m, 1H), 7.19 (s, 1H), 7.03-7.12 (m, 3H), 6.24 (s, 1H), 5.88 (s, 2H), 2.52 (s, 3H).

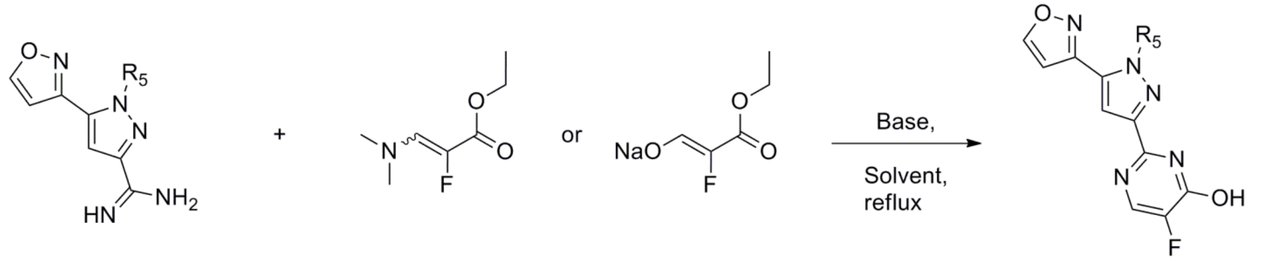
5-fluoro-2-(1-(2-fluorobenzyl)-5-(isothiazol-3-yl)-1H-pyrazol-3-yl)pyrimidin-4(3H)-one (26): In a similar manner to **1**, condensation of **2e** (prepared from **2e-1** as described in Scheme 7) with **3o** gave the desired **26** (53 mg, 33% yield): LCMS *m*/*z* = 372.2 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD) δ 9.00 (d, *J*=4.70 Hz, 1H), 8.04 (d, *J*=3.13 Hz, 1H), 7.74 (d, *J*=4.70 Hz, 1H), 7.45 (s, 1H), 7.22-7.32 (m, 1H), 7.05-7.13 (m, 1H), 7.02 (t, *J*=8.02 Hz, 1H), 6.89 (t, *J*=7.24 Hz, 1H), 6.17 (s, 2H).

5-fluoro-2-(1-(2-fluorobenzyl)-5-(pyridin-2-yl)-1H-pyrazol-3-yl)pyrimidin-4-ol (27): In a similar manner to **1**, condensation of **2f** and **3f** gave **27** (30 mg, 12% yield): LCMS *m*/*z* = 366.0 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60-8.67 (m, 1H), 8.15 (br s, 1H), 7.86-7.99 (m, 2H), 7.53 (s, 1H), 7.37-7.47 (m, 1H), 7.25-7.33 (m, 1H), 7.16 (s, 1H), 7.04-7.12 (m, 1H), 7.01 (br s, 1H), 6.09 (s, 2H).

3-(5-fluoro-4-hydroxypyrimidin-2-yl)-1-(2-fluorobenzyl)-1H-pyrazole-5-carbonitrile (28): 11 (1.0 g, 2.81 mmol) was directly charged with DBU (0.53 mL, 3.52 mmol) and subsequently heated to 110 °C for 24 h. The mixture was diluted with DCM (100 mL) and washed with 1N HCl (50 ml). The organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo*. The crude material was purified by silica gel chromatography using a 0-10% MeOH/DCM gradient to afford **28** (400 mg, 43% yield): LCMS *m*/*z* = 314.3 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (br s, 1H), 7.77 (s, 1H), 7.36-7.56 (m, 2H), 7.15-7.35 (m, 2H), 5.68 (s, 2H).

2-(5-cyclopropyl-1-(2-fluorobenzyl)-1H-pyrazol-3-yl)-5-fluoropyrimidin-4(3H)-one (29): In a similar manner to **1**, condensation of **2g** and **3f** gave **29** (75 mg, 30% yield): LCMS *m*/*z* = 329.2 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (dd, *J*=1.17, 2.74 Hz, 1H), 7.30-7.39 (m, 1H), 7.02-7.19 (m, 3H), 6.51 (s, 1H), 5.48 (s, 2H), 1.68-1.83 (m, 1H), 0.87-1.08 (m, 2H), 0.70 (q, *J*=5.35 Hz, 2H).

Scheme S6



2h	R_5 = benzyl	**3f**	**3o**	**30**	R_5 = benzyl
2i	R_5 = 2,3-difluorobenzyl			**31**	R_5 = 2,3-difluorobenzyl
2j	R_5 = 3-chloro-2-fluorobenzyl			**32**	R_5 = 3-chloro-2-fluorobenzyl
2k	R_5 = 2-fluoro-3-methylbenzyl			**33**	R_5 = 2-fluoro-3-methylbenzyl
2l	R_5 = thiophen-2-ylmethyl			**34**	R_5 = thiophen-2-ylmethyl
2m	R_5 = 2,4-difluorobenzyl			**35**	R_5 = 2,4-difluorobenzyl
2n	R_5 = 3-fluoropyridin-2-yl			**36**	R_5 = 3-fluoropyridin-2-yl
2o	R_5 = 3,3,3-trifluoropropyl			**37**	R_5 = 3,3,3-trifluoropropyl
2p	R_5 = 3,3,4,4,4-pentafluorobutyl			**38**	R_5 = 3,3,4,4,4-pentafluorobutyl

2-(1-benzyl-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)-5-fluoropyrimidin-4-ol (30): In a similar manner to **1**, condensation of **2h** and **3f** gave **30** (19 mg, 20% yield): LCMS m/z = 338.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.12 (d, J=1.57 Hz, 1H), 8.16 (br s, 1H), 7.62 (s, 1H), 7.20-7.35 (m, 6H), 5.85 (s, 2H).

2-(1-(2,3-difluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)-5-fluoropyrimidin-4-ol (31): In a similar manner to **1**, condensation of **2i** and **3f** gave **31** (715 mg, 8% yield): LCMS m/z = 374.0 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.13 (d, J=1.96 Hz, 1H), 8.15 (br s, 1H), 7.66 (s, 1H), 7.31-7.45 (m, 1H), 7.25 (s, 1H), 7.08-7.18 (m, 1H), 6.84 (br s, 1H), 5.95 (s, 2H).

2-(1-(3-chloro-2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)-5-fluoropyrimidin-4-ol (32): In a similar manner to **1**, condensation of **2j** and **3f** gave **32** (49 mg, 38% yield): LCMS m/z = 390.0 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.06-9.20 (m, 1H), 8.14 (br s, 1H), 7.66 (s, 1H), 7.49-7.59 (m, 1H), 7.25 (s, 1H), 7.16 (t, J=7.83 Hz, 1H), 6.99 (t, J=6.65 Hz, 1H), 5.95 (s, 2H).

5-fluoro-2-(1-(2-fluoro-3-methylbenzyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)pyrimidin-4-ol (33): In a similar manner to **1**, condensation of **2k** and **3f** gave **33** (111 mg, 58% yield): LCMS m/z = 370.4 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.12 (d, J=1.57 Hz, 1H), 8.13 (br s, 1H), 7.64 (s, 1H), 7.15-7.31 (m, 2H), 7.00 (t, J=7.63 Hz, 1H), 6.74 (t, J=6.85 Hz, 1H), 5.90 (s, 2H), 2.23 (s, 3H).

5-fluoro-2-(1-((3-fluorothiophen-2-yl)methyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)pyrimidin-4-ol (34): In a similar manner to **1**, condensation of **2l** and **3o** gave **34** (86 mg, 33% yield): LCMS m/z = 362.4 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.18 (d, J=1.57 Hz, 1H), 8.16 (br s, 1H), 7.53-7.64 (m, 1H), 7.42-7.53 (m, 1H), 7.23 (br s, 1H), 6.95 (d, J=5.48 Hz, 1H), 5.86-6.04 (m, 2H).

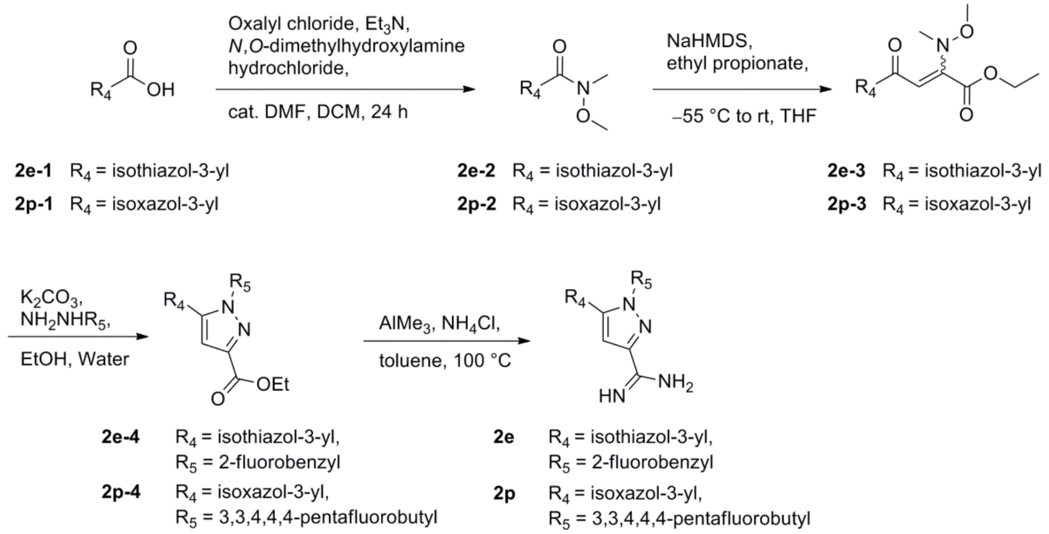
2-(1-(2,4-difluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)-5-fluoropyrimidin-4-ol (35): In a similar manner to **1**, condensation of **2m** and **3f** gave **35** (31 mg, 51%): LCMS m/z = 374.0 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.13 (d, J=1.96 Hz, 1H), 8.16 (br s, 1H), 7.63 (s, 1H), 7.21-7.35 (m, 2H), 7.14 (br s, 1H), 7.03 (dt, J=1.96, 8.61 Hz, 1H), 5.87 (s, 2H).

5-fluoro-2-(1-((3-fluoropyridin-2-yl)methyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)pyrimidin-4-ol (36): In a similar manner to **1**, condensation of **2n** and **3o** gave **36** (224.4 mg, 60% yield): LCMS *m*/*z* = 357.1 $[M+H]^+$; ^1H NMR (500 MHz, DMSO-d_6) δ 9.07 (s, 1H), 8.23 (d, *J*=4.58 Hz, 1H), 8.12 (br s, 1H), 7.76 (t, *J*=9.31 Hz, 1H), 7.63 (s, 1H), 7.37-7.49 (m, 1H), 7.23 (br s, 1H), 6.05 (s, 2H).

5-fluoro-2-(5-(isoxazol-3-yl)-1-(3,3,3-trifluoropropyl)-1H-pyrazol-3-yl)pyrimidin-4-ol (37): In a similar manner to **1**, condensation of **2o** and **3o** gave **37** (36 mg, 10% yield): LCMS *m*/*z* = 344.2 $[M+H]^+$; ^1H NMR (500 MHz, CD_3OD) δ 7.27 (d, *J*=1.53 Hz, 1H), 6.47 (d, *J*=3.05 Hz, 1H), 5.84 (s, 1H), 5.39 (d, *J*=1.83 Hz, 1H), 3.36-3.45 (m, 2H), 1.29-1.42 (m, 2H).

5-fluoro-2-(5-(isoxazol-3-yl)-1-(3,3,4,4,4-pentafluorobutyl)-1H-pyrazol-3-yl)pyrimidin-4-ol (38): In a similar manner to **1**, condensation of **2p** (prepared from **2p-1** as described in Scheme 7) and **3f** gave **38** (4.5 mg, 53% yield): LCMS *m*/*z* = 394.0 $[M+H]^+$; ^1H NMR (400 MHz, CD_3OD) δ 8.86 (d, *J*=1.57 Hz, 1H), 8.05 (d, *J*=3.52 Hz, 1H), 7.43 (s, 1H), 6.98 (d, *J*=1.57 Hz, 1H), 5.00-5.09 (m, 2H), 2.83-3.01 (m, 2H).

Scheme S7



1-(2-fluorobenzyl)-5-(isothiazol-3-yl)-1H-pyrazole-3-carboximidamide (2e): To a suspension of isothiazole-3-carboxylic acid (**2e-1**) (2.0 g, 15.5 mmol) in DCM (52 ml) at 0 °C was added oxalylchloride (1.7 ml, 20 mmol) followed by three drops of DMF. The mixture was stirred at room temperature for 3 h. The mixture was cooled to 0 °C. To this mixture, was added *N*,*O*-dimethylhydroxylamine hydrochloride (1.96 g, 20.1 mmol) followed by triethylamine (7.6 ml, 54 mmol) over 10 minutes. It was warmed to room temperature and stirred for the total of 15 h. The mixture was diluted with aqueous 1N HCl (75 mL) and DCM (100 mL). The layers were separated and the aqueous layer was extracted with DCM (2 x 50 mL). The organic layers were combined, dried over magnesium sulfate, filtered, and the solvent was removed *in vacuo*. Purification by silica gel chromatography using a 0 to 50% EtOAc in hexanes gradient gave *N*-methoxy-*N*-methylisothiazole-3-carboxamide **(2e-2)** (1.0 g, 38% yield) as a pale yellow solid: LCMS *m*/*z* = 173.1 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 8.68 (d, *J*=4.70 Hz, 1H), 7.70 (br s, 1H), 3.81 (s, 3H), 3.35-3.66 (m, 3H).

To a solution of **(2e-2)** (200 mg, 1.2 mmol) and ethyl propiolate (0.18 mL, 1.7 mmol) in THF (7 mL) at −55 °C, was added sodium bis(trimethylsilyl)amide (1.64 mL, 1.6 mmol) as a 1N solution in THF over the course of 5 minutes. The mixture was warmed to room temperature and stirred for 30 minutes. The pH of the mixture was lowered to pH 2 by the addition of 10% aqueous citric acid. The mixture was partitioned between DCM (50 ml) and water (50 ml). The aqueous layer was extracted with dichloromethane (2x30 mL) and ethyl acetate (30 mL). The organic layers were combined, dried over $MgSO_4$, filtered, and the solvent was removed in vacuum. Purification by silica gel column chromatography using a 0-50% EtOAc/hexanes gradient gave ethyl 4-(isothiazol-3-yl)-2-(methoxy(methyl)amino)-4-oxobut-2-enoate **(2e-3)** (234 mg, 75% yield) as an oil: LCMS *m/z* = 271.3 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, *J*=4.70 Hz, 1H), 7.26 (s, 1H), 5.91 (s, 1H), 3.90 (d, *J*=7.04 Hz, 2H), 3.18 (s, 3H), 2.64 (s, 3H), 0.84 (t, *J*=7.04 Hz, 3H).

To a solution of (2-fluorobenzyl)hydrazine hydrochloride (168 mg, 0.95 mmol) in EtOH (2 mL) and water (0.3 mL), were added potassium carbonate (65 mg, 0.48 mmol) as a solution in water (0.4 mL) and **2e-3** (234 mg, 0.87 mmol) as a solution in EtOH (8 mL). The mixture was stirred at room temperature for 3 h. The solution was diluted with DCM (100 mL) and aqueous 1N HCl (30 mL). The layers were separated and the aqueous layer was extracted with dichloromethane (2 x 30 mL). The organic layers were combined, dried over magnesium sulfate, filtered, and the solvent was removed *in vacuo*. Purification by silica gel chromatography using a 0-100% EtOAc in hexanes gradient provided ethyl 1-(2-fluorobenzyl)-5-(isothiazol-3-yl)-1H-pyrazole-3-carboxylate **(2e-4)** (166 mg, 58% yield) as a white solid: LCMS *m/z* = 332.2 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 8.75 (d, *J*=4.70 Hz, 1H), 7.47-7.59 (m, 1H), 7.22-7.28 (m, 2H), 6.97-7.13 (m, 2H), 6.73-6.97 (m, 1H), 6.19 (s, 2H), 4.50 (q, *J*=7.04 Hz, 2H), 1.48 (t, *J*=7.24 Hz, 3H).

To a suspension of ammonium chloride (147 mg, 2.8 mmol) in toluene (2.5 ml), was added over the course of 5 minutes, trimethylaluminum (1392 µl, 2.5 mmol) as a 2 M solution in toluene. After 30 minutes, **2e-4** (166 mg, 0.5 mmol) was added as a solution in toluene (2.5 ml) and heated to 100 °C for 3 h. The solution was cooled to 0 °C and quenched with methanol (200 uL). The mixture was diluted with toluene and the insoluble material was removed by filtration through celite. The filtrate was concentrated *in vacuo* to give **(2e)** (130 mg, 86% yield) as a yellow solid: LCMS *m/z* = 302.2 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 9.26 (d, *J*=4.70 Hz, 1H), 7.84 (s, 1H), 7.67-7.78 (m, 1H), 7.31-7.40 (m, 1H), 7.17-7.27 (m, 1H), 7.10 (dt, *J*=1.17, 7.63 Hz, 1H), 6.91 (dt, *J*=1.57, 7.83 Hz, 1H), 6.08 (s, 2H).

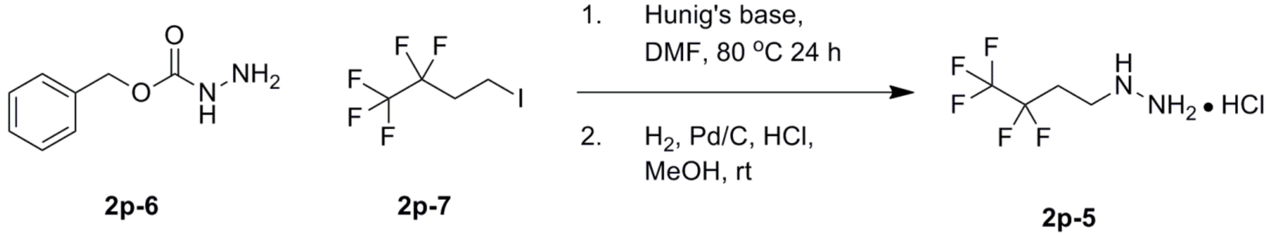
5-(isoxazol-3-yl)-1-(3,3,4,4,4-pentafluorobutyl)-1H-pyrazole-3-carboximidamide hydrochloride (2p): In a similar manner to **2e-2**, isoxazole-3-carboxylic acid **(2p-1)** was converted to *N*-methoxy-*N*-methylisoxazole-3-carboxamide (**2p-2)** (2.0 g, 71% yield); LCMS *m/z* = 179.0 $[M+Na]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 8.46-8.51 (m, 1H), 6.67-6.76 (m, 1H), 3.80 (br s, 3H), 3.39 (br s, 3H).

In a similar manner to **2e-3**, **2p-2** was converted to ethyl 4-(isoxazol-3-yl)-2-(methoxy(methyl)amino)-4-oxobut-2-enoate (**2p-3)**: (769 mg, 43% yield); LCMS *m/z* = 255.1 $[M+H]^+$; ^1H NMR (500 MHz, $CDCl_3$) δ 8.42 (d, *J*=1.83 Hz, 1H), 6.77 (d, *J*=1.83 Hz, 1H), 6.19 (s, 1H), 4.47 (q, *J*=7.32 Hz, 2H), 3.76 (s, 3H), 3.22 (s, 3H), 1.41 (t, *J*=7.17 Hz, 3H).

In a similar manner to **2e-4**, condensation of **2p-3** and (3,3,4,4,4-pentafluorobutyl)hydrazine hydrochloride **(2p-5)** (Scheme 8) gave ethyl 5-(isoxazol-3-yl)-1-(3,3,4,4,4-pentafluorobutyl)-1H-pyrazole-3-carboxylate **(2p-4)** (49 mg, 53% yield); LCMS *m/z* = 354.1 $[M+H]^+$; ^1H NMR (500 MHz, $CDCl_3$) δ 8.54 (d, *J*=1.53 Hz, 1H), 7.27 (s, 1H), 6.62 (d, *J*=1.83 Hz, 1H), 4.96-5.01 (m, 2H), 4.42-4.48 (m, 2H), 2.73-2.86 (m, 2H), 1.41-1.45 (m, 3H).

In a similar manner to **2e**, **2p-4** was converted to **2p** (230 mg, quantitative yield); LCMS *m/z* = 324.0 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ ppm 9.18 (d, 1 H) 7.54 (s, 1 H) 7.08 (d, 1 H) 4.88 (t, 2 H) 2.76 - 3.04 (m, 2 H).

Scheme S8



(3,3,4,4,4-pentafluorobutyl)hydrazine, hydrochloride (**2p-5)**: A mixture containing Hunig's base (31.5 ml, 181 mmol), carbobenzoxyhydrazide **(2p-6)** (10.0 g, 60.2 mmol), and 3,3,4,4,4-pentafluoro-1-iodobutane **(2p-7)** (14.5 ml, 102 mmol) in DMF (30 ml) was heated to 80 °C for 24 h. The mixture was diluted in ethyl acetate (150 ml) and washed with water (100 ml x 3). The organic layer was dried, filtered and evaporated to give a crude oil. The oil was purified by silica gel column chromatography using a 0-100% EtOAc/hexanes gradient to give benzyl 2-(3,3,4,4,4-pentafluorobutyl)hydrazinecarboxylate (10.8 g, 58% yield) as a white solid: LCMS *m*/*z* = 313.0 $[M+H]^+$; ^1H NMR (500 MHz, CD_3OD) δ 7.28-7.41 (m, 5H), 5.06-5.21 (m, 2H), 2.98-3.12 (m, 2H), 2.20-2.43 (m, 2H).

A mixture containing HCl (28 ml, 35 mmol) as a 1.25 M solution in ethanol, 10% Pd/C (0.4 g, 3.5 mmol) and benzyl 2-(3,3,4,4,4-pentafluorobutyl)hydrazinecarboxylate (10.8 g, 35 mmol) in MeOH (173 ml) was stirred under an atmosphere of hydrogen at room temperature for 24 h. The mixture was filtered through a pad of celite and the filtrate was concentrated *in vacuo* to give **(2p-5)** (7.1 g, 96% yield): LCMS *m*/*z* = 179.0 $[M+H]^+$; ^1H NMR (500 MHz, CD_3OD) δ 3.21-3.29 (m, 2H), 2.41-2.57 (m, 2H).

cGMP Generation Assay Using HEK-293 Cells.

HEK-293 cells were purchased from ATCC (#CRL-1573) and maintained in DMEM containing 10% FBS, 100U/mL penicillin, and 100µg/mL streptomycin. For sGC activity assays, cells were seeded in 384 well poly-D-lysine coated flat bottom plates. Cells were incubated 24 hours at 37°C in a humidified chamber supplemented with 5% CO_2. Compounds were diluted in 100% DMSO to 100x the final assay concentration. Immediately prior to the assay, compound was diluted 20-fold into HBSS (with calcium and magnesium) containing 50µM DETA-NONOate (5x the final assay concentration). The compound/HBSS plate was placed on a shaker platform and allowed to mix for 15 minutes. Medium was removed and cells were washed once with 40µL of HBSS. Cells were then incubated with 40µL of a solution containing 0.5mM IBMX in HBSS for 15 minutes at 37°C. 10µL from the compound/HBSS/DETA-NONOate place was added to the cells, which were incubated for a further 20 minutes at 37°C. Final DETA-NONOate concentration was 10µM. Final DMSO concentration was 1%. Final compound concentrations in nM were: 30,000, 7500, 1875, 468.75, 117.19, 29.29, 7.32, 1.83, 0.46, 0.114, and 0.029.Following incubation with compound, assay buffer was removed and 50µL of ice cold 10% acetic acid + 150ng/mL internal standard (^{13}C cGMP; Toronto Research Chemicals) was added to each well. Samples were incubated on ice for 30 minutes. Following centrifugation at 4°C for 5 minutes at 1000xg to pellet cell debris, the supernatant was transferred to a clean plate and the samples analyzed for cGMP content by LC-MS/MS. Data were fit using a 4-parameter fit (log (agonist) vs. response – variable slope using GraphPad Prism Software v.5. The EC_{50} was interpolated from the curve fit and is defined as the concentration at which the compound elicits 50% of its maximal response.

Determination of cGMP Concentrations Using LC-MS/MS.

Cell lysates were analyzed for cGMP levels using the LC-MS/MS conditions below (Table 1). A standard curve (1, 5, 10, 50, 100, 250, 500, 1000, 2000 3000 ng/mL) was prepared in 10% acetic acid (aq) containing 150ng/mL ^{13}C-labeled cGMP (Toronto Research Chemicals, P/N G837962) as internal standard. Final values were converted to nM upon data analysis.

Table S1. LC-MS/MS conditions for cGMP analysis.

MS:	Thermo Vantage				
Ion mode:	ESI$^+$				
Scan type	MRM				
Compound:	**Transition**	**Dwell time (msec)**	**Collision energy (V)**	**S Lens**	**Retention time (min)**
cGMP	346>152	100	32	75	0.6
+3 cGMP	349>155	100	32	75	0.6
HPLC	Waters Acuity UPLC				
Column	Thermo Hypersil Gold 2.1 x 50mm 3 micron particle size				
Flow rate	0.750mL/minute				
Column Temperature	25°C				
Injection Volume	20ul				
Mobile Phases: **Gradient:**	A=100% water + 0.1% formic acid B=100% acetonitrile + 0.1% formic acid				
	Time (minutes)	**%A**		**%B**	
	0	100		0	
	0.2	100		0	
	0.3	50		50	
	0.7	50		50	
	0.8	100		0	

Table S2. Potency Measured in Human Embryonic Kidney (HEK) Cells in the Presence of 10 µM DETA-NO.

The geometric mean of at least two repeated tests was used to determine the potency (EC_{50}), unless stated otherwise. When the concentration response was incomplete, the potency was reported as ND or "not determined" with maximal efficacy (%E_{max}) at 30 µM with respect to the reference standard. The average of at least two repeated tests was used to determine maximal efficacy (%E_{max}). The column titled "n" indicates the number of test performed. The geometric mean and the geometric standard deviation were calculated using Excel. The reference compound Bay 41-2272 was obtained from Sigma-Aldrich.

Compound	**EC_{50} (nM)**	**%E_{max}**	**n**
Bay 41-2272	55±2.1		3
1	240±110		8
5	500±280		5
8	ND	92	2
9	ND	53	2
10	ND	40	2
11	290±320		6
12	190±160		11
13	590±300		4
14	ND	54	2
15	900±40		2
16	350±100		4
17	160±180		10
18	ND	87	2
19	730±200		2
20	ND	70	2
21	320±190		4
22	ND	68	2
23	1500±2800		4
24	ND	78	2
25	ND	23	2
26	180±70		4
27	ND	35	2
28	ND	73	2
29	ND	26	2
30	880		1
31	370±250		5
32	380±70		4
33	240		1
34	280±210		4
35	530±870		3
36	ND	44	2
37	ND	83	2
38	1500		1

Rat Liver Microsomal and Human Liver Microsomal Stability Assays.

Compound(s) were incubated at 2 µM with 0.5 mg/mL of male SD rat and or mixed gender human liver microsomes. The reaction was initiated by adding the cofactors NADPH (2mM) plus 3 mM $MgCl_2$, and was maintained at 37°C with constant mixing on a plate shaker. At 0, 10, 20, and 30 minutes, reactions were stopped using ice-cold acetonitrile containing an internal standard. Sample extracts were diluted with water then analyzed by LC/MS/MS to measure the amount of compound remaining in each sample. These data were used to calculate the intrinsic clearance and predicted hepatic extraction ratio in all species tested.

Data handling was conducted using Excel software. The quantities of compound remaining in the samples were determined using LC-MS/MS, and the initial rate constant (k) of NADPH dependent degradation was calculated as the negative slope from a linear regression fit of log-transformed peak area ratios (relative to internal standard) over time. The half-life ($t_{1/2}$), intrinsic clearance (CL_{int}), predicted in vivo hepatic clearance (CLint, hepatic), and predicted hepatic extraction ratio (ERH) were calculated using the in vitro $t_{1/2}$ method and well-stirred model without protein binding parameters. The equation to scale to in vivo hepatic clearance (CLint, hepatic) incorporates species specific liver blood flow rates and liver mass-to body weight ratio.

Pharmacokinetics (PK) in Nonclinical Species.

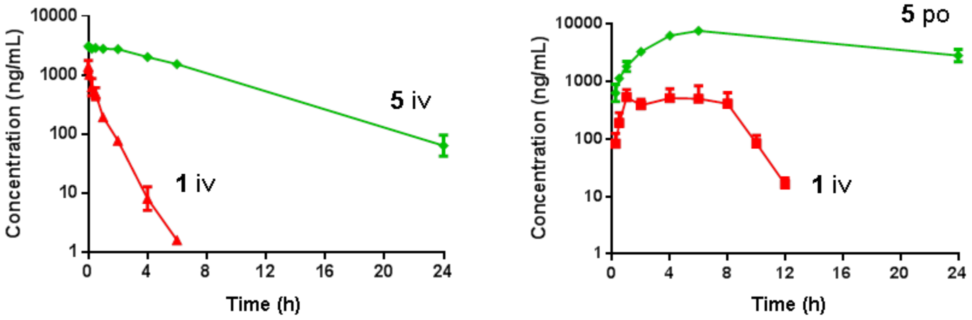
All procedures performed on animals were in accordance with regulations and established guidelines and were reviewed and approved by Ironwood Institutional Animal Care and Use Committee. Pharmacokinetic studies were conducted in CD-1 Mice, Sprague-Dawley rats, beagle dogs to assess the oral (po) and intravenous (iv) exposure for compound IWP-051. Several PK parameters, including the calculated oral bioavailability, were also determined. The iv doses were administered at 0.1 mg/kg in a standard 10% (v/v) Dimethylimidazolidinone (DMI) / 25% (v/v) Propylene Glycol / 15% (v/v) Ethanol (200 proof) / 50% (v/v) D5W (5% Dextrose in Water) formulation. Oral doses were administered at 1 mg/kg in a standard vehicle (100% PEG400). Samples were collected, processed to plasma, and analyzed by LC−MS/MS. The PK data are summarized in Table 4 of the original manuscript. Following iv administration, the mean terminal elimination half-life ($t_{1/2}$) of IWP-051 ranged from approximately 2 to 6 h, depending on the species. Clearance was low for all species where values were less than hepatic blood flow in each respective species. The T_{max} ranged from approximately 1.7 to 5.3 h for all species. The oral bioavailability for mice, rats, dogs was >100%, 96 ± 26%,and 45 ± 3%, respectively.

Table S3. Pharmacokinetics (PK) of Compounds 1 and 5 Sprague-Dawley Rats.

The pharmacokinetics studies were performed as described above except, oral (po) doses of compounds **1** and **5** administered as suspensions in 0.5% methylcellulose at 10 mg/kg; intravenous (iv) doses administered 10% DMI, 35% propylene glycol, 15% EtOH, 40% D5W (5% dextrose in water) at 1 mg/kg in male Sprague-Dawley rats. For **5**, all animals reached C_{max} at 6 hours. Therefore, error is not reported for T_{max}.

Compound	**1**	**5**
Half-life (min.)	26 ± 7.6	234±27
C_o (ng/ml)	1400 ± 370	3100±160
Vol of Dist.	820 ± 260	214±16
Clearance (ml/min/kg)	22±3.2	0.59±0.080
AUC_{0-120} (min*ng/ml)	41000±6400	340000±35000
AUC_{0-last}(min*ng/ml)	47000±7600	1700000±220000
C_{max}(ng/ml)	660±240	7500±730
T_{max}(min.)	300±160	360
AUC_{0-120}(min*ng/ml)	41000±12000	210000±27000
AUC_{0-last}(min*ng/ml)	250000±83000	7200000±740000
Bioavailability %	53±38	43±16

Figure S1. Pharmacokinetics (PK) of Compounds 1 and 5.



Determination of Hemodynamic Effects Using normotensive male Sprague-Dawley Rats.

Male Sprague-Dawley rats surgically implanted with femoral arterial catheters (Harlan, 200–350 g) were fitted with harnesses (Instech Laboratories, Plymouth Meeting, PA), and tethers (IInstech Laboratories, Plymouth Meeting, PA), and connected to pressure transducers (Hugo Sachs Electonik – Harvard Apparatus, March-Hugstetten, Germany). Pressure transducers were connected to an 8-channel amplifier (Harvard Apparatus) which was connected to a data acquisition system (PowerLab, ADInstruments, Sydney, Australia). Hemodynamic parameters were measured continuously over a 24 hr. period following oral dosing with vehicle (PEG400) or IWP-051 dosed in PEG400 in a dosing volume of 3 mL/kg for or vehicle (0.5% methylcellulose) or Bay 41-2272 (Sigma-Aldrich) dosed in 0.5% methylcellulose in a dosing volume of 1 ml/kg. Data is represented as the change from their own vehicles. Data was sampled using LabChart 7.0 software (ADInstruments, Sydney, Australia). Data analysis was done using Microsoft Excel 2010 and Graphpad Prism 5.0. Prior to treatment mean arterial pressure and heart rate of treated and untreated control groups was in the range of 126 + 8.2 mmHg and 420 + 37.0 beats min−1, respectively.

References

1. For preparation of **2b-d**, **2f, 2h-k, 2m-n, 3d-m**, see Kim, C.; Nakai, T.; Moore, J.; Perl, N. R.; Im, G-Y. J.; Barden, T. C.; Iyengar, R. R.; Zimmer, D. P.; Fretzen, A.; Renhowe, P. A. 2-Benzyl, 3-(pyrimidin-2-yl) substituted pyrazoles useful as sgc stimulators. WO 2013/101830 A1;
2. For preparation of **2g**, see Kim, C.; Nakai, T.; Lee, T. W-H.; Moore, J.; Perl, N. R.; Rhode, J. Sgc stimulators. WO 2012/064559 A1;
3. For preparation of **2l**, see Im, G-Y. J.; Iyengar, R.; Moore, J.; Fretzen, A. Sgc stimulators. WO 2014/047111 A1;
4. For preparation of **2o**, see Nakai, T.; Moore, J.; Perl, N. R.; Iyengar, R. R.; Mermerian, A.; Im, G-Y. J.; Lee, T. W.-H.; Hudson, C.; Rennie, G. R.; Jia, J.; Renhowe, P. A.; Barden, T. C.; Yu, X. Y.; Sheppeck, J. E.; Iyer, K.; Jung, J. Sgc stimulators. WO 2014/144100 A2