Discovery of Small Molecule Splicing Modulators of Survival Motor Neuron-2 (SMN2) for the Treatment of Spinal Muscular Atrophy (SMA)

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Supporting Information: data and experimentals.

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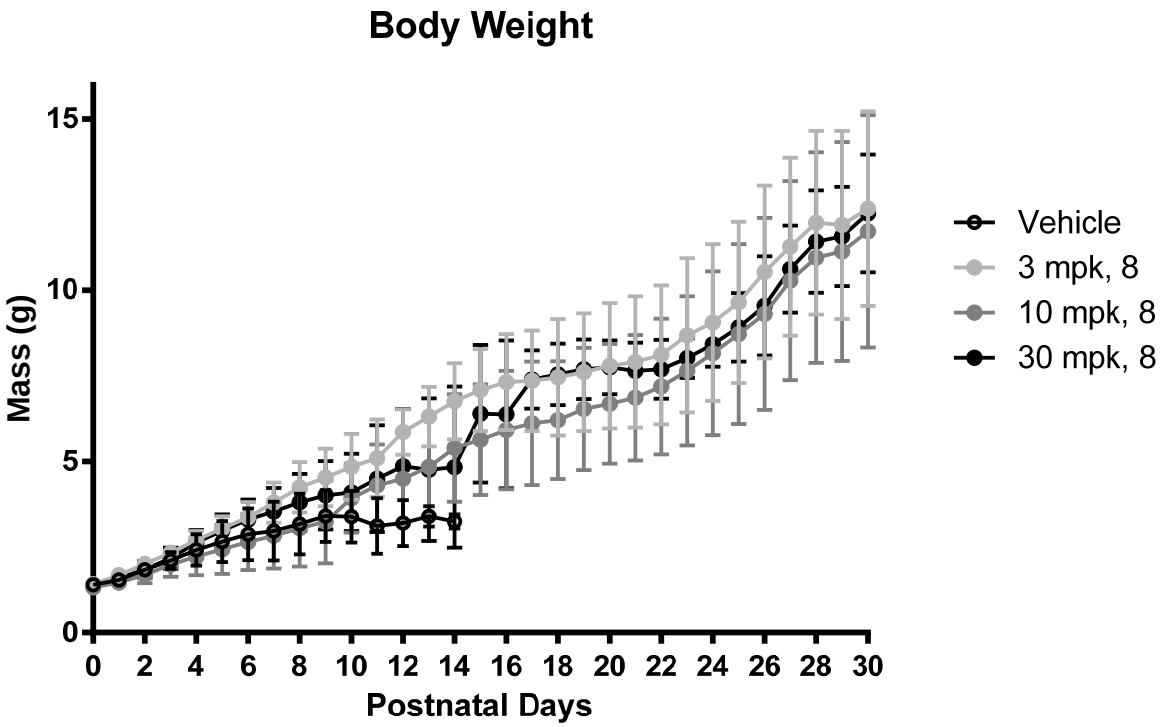
Supplemental Figure 1 (S.Fig. 1). Body weight results for **8** *in vivo* PD and efficacy study in SMNdelta7 mice.



ADME and Safety assays:

Liver Microsomal Clearance Assay. For determination of hepatic CYP450-mediated metabolic stability, test compounds (1 μM), were incubated with 0.5 mg/ml of liver microsomes suspended in 50 mM potassium phosphate buffer at pH 7.4. CYP450 cofactor NADPH (1 mM) was added to initiate bulk s (300 μL final volume). Following 0*, 5, 15 and 30 min of incubation at 37 °C shaking at 400 rpms, 50 μL aliquots were removed and s were terminated using 2 volumes of cold acetonitrile containing 0.4 μM glyburide as an LC-MS/MS internal standard. *(Initial T0 timepoint made from microsomes added to quench first and cofactor added second.) The quenched s were centrifuged for 10 min at 4000xg to pellet precipitated protein, and supernatant analyzed by mass spectrometry. The LC-MS/MS system consists of a Sciex API 4000 mass spectrometer, an H-ESI ion source, a CTC-HTS Pal auto-sampler, and an Agilent LC

Pump. Samples were separated on a Waters XTerra C18 column, 2.1x20 mm, 3.5 micron using the fast mobile phase gradient outlined in Table 1.

Table 1. Mobile phase gradient for HPLC

Time (min)	%B
0.0	2
0.5	98
0.75	98
1.02	2
2.23	2

Mobile phase A consists of purified water containing 0.1% formic acid. Mobile phase B consists of acetonitrile containing 0.1% formic acid. The flow rate is 1.0 mL/min. The injection volume is 10 µL. Compounds are detected using Agilent Analyst software which collects peak intensity data using pre-validated MRM methods for ions and ion fragments specific to the test compound. The ratio of analyte peak area to internal standard peak area, also referred to as peak area ratio (PAR) is used for calculating kinetic parameters.

The in vitro clearance half-life, t(1/2), was calculated based on the elimination rate constant

$$[\text{Obach 1997}]: t_{1/2} = \frac{0.693}{-k_{mic}} \qquad \text{Eq. (1)}$$

where k_{mic} is the *in vitro* elimination rate constant. Experimentally, each microsomal elimination rate, k_{mic}, is based on a 4-point elimination curve tested for each compound. The time-dependent decrease in PAR is expressed as "% remaining" compound at each sampled time-point, where PAR at T=0 min is theoretical 100%. The plot of "% remaining" versus time (min) is used to estimate the slope ($-k_{mic}$) using non-linear regression. This value, in turn, can be used to calculate $t_{1/2}$ according to Eq. (2). An *in vitro* intrinsic clearance value, CL_{int} (µL/min/mg microsomal protein), is calculated to standardize the observed $t_{1/2}$ for *in vitro* conditions, *V*, the incubation volume (µL), and *M*, the microsomal protein content in the incubation (mg):

$$CL_{\text{int}} = \frac{0.693}{t_{1/2}} \bullet \frac{V}{M} \qquad \text{Eq. (2)}$$

[Obach RS, Baxter JG, Liston TE, Silber BM, Jones BC, MacIntyre F, Rance DJ, Wastall P (1997) The prediction of human pharmacokinetic parameters from preclinical and *in vitro* metabolism data. J Pharmacol Exp Ther; 283:46-58.]

PAMPA assay. All buffers solutions (pH 4.0, pH 6.8 and pH 8.0) are purchased from Fluka ready to use. All solutions were prepared with 100 mM KCl and 5% DMSO. To perform the assay, two 96-well plates (donor and acceptor) are prepared separately and assembled to get a "sandwich". This "sandwich" is placed on the shaker and incubated for 4 hours. The donor plate is a Multiscreen Permeability filterplate, 0.4 µm PCTE membrane. The filterplate is coated with 5 µl of a solution of 15% hexadecane in hexane. The coating step is followed by a 15 minute incubation time to evaporate the hexane. At the end of the coating process, each filterwell is coated with 0.75 µl of hexadecane. Keeping membrane volume to a minimum helps to reduce membrane retention in the permeability experiments. In a second step, 300 µl of buffer is added. In a third step, 4.5µl of the compound solution taken from the mother plate is distributed to get a final loading concentration of 5 µM. During the incubation of the coating step, the acceptor plate is prepared and placed on the shaker. The acceptor plate is teflon made to minimize non-specific binding. The last step is the assembly of both plates on the shaker to connect wells of the donor plate to the corresponding acceptor wells. This construct is then shaken at 150 RPM for 4 hours. Trans-wells with electrical resistance lower than 25 kΩ are discarded. To decrease the run time during the quantification step, two compounds are pooled together in the analytical plate. This plate is prepared with the Tecan robot using a cherry-picking process. For each compound at pH 4.0, 6.8 and 8.0, the well with the highest resistivity out of each triplicate is selected. Then 37.5 µl of this well is transferred to the analytical plate and mixed with its complementary compound. Compounds from the reference plate are processed the same way except the selection based on resistivity does not take place. 75 µl of each selected well are transferred to the analytical plate for analysis by LCMS.

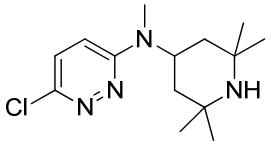
hERG radio-ligand binding assay. Cell membrane preparation. CHO cells expressing functional hERG channels were seeded in 500 cm^2 dishes (Corning #431110) at 12 million cells per dish for 4 days at 30°C with 5% CO_2. The cells were then harvested as follows: The cell

medium was decanted and the cells washed once with 25 m PBS held at room temperature. The PBS was removed before adding 10 ml ice cold lysis buffer (Tris-HCl 20 mM, EDTA 5 mM pH 7.7 at 4 °C). The cells were then scraped off the dishes with a plastic 5" wide beveled taping knife, pipetted up and down several times to fully lyse the cells before transfer to tubes sitting on ice. The cells were then homogenized using a Polytron homogenizer (3 bursts of 10 seconds at setting 8.5) and centrifuged at 17,500 rpm in a SS-34 rotor (about 35ml per tube) for 25 min at 4 °C in a Sorvall Superspeed centrifuge. The pellet of each tube was suspended by vortexing in 10 ml ice cold lysis buffer and homogenized using a teflon/glass homogenizer (1,100 rpm, 10 passes) and re-centrifuged at 17,500 rpm in an SS-34 rotor for 25 min at 4 °C. The final pellets were vortexed in 10 ml (10 dishes) ice cold assay buffer (20mM HEPES, 10 mM KCl, 1 mM MgCl2, pH=7.4 at room temperature) and homogenized using a Teflon/glass homogenizer (1,100 rpm, 10 passes). Protein concentration was measured by the BioRad DC protein assay (Biorad #500-0112, Hercules, CA, USA) using bovine serum albumin as a standard. The crude membrane preparation was aliquoted in 10 ml portions, snap frozen in liquid nitrogen and stored at -80 °C.

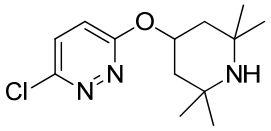
Binding assay. The following components were pipetted into each well of pre-wet 96-well Millipore GF/C filter plates (#MSFCN6B50): 119 µl assay buffer (20 mM HEPES, 10 mM KCl, 1 mM $MgCl_2$, pH=7.4 at rt), 1 µl test compound in 100% DMSO (or 100% DMSO only for total binding), 40 µl [3H] dofetilide (final concentration 2.5 nM; Perkin Elmer NET1144); 40 µl crude membrane suspension (ca. 15 µg protein). The final concentration of DMSO during the incubation was 0.5%. Incubations were performed at room temperature for 90 min. Non-specific binding (NSB) was defined as the binding remaining in the presence of 25 µM terfenadine (Sigma T9652). The incubations were terminated by rapid filtration on a Millipore filtration manifold, followed by three washes of 200 µl ice-cold assay buffer. The plates were left to dry overnight before adding 40 µl scintillant (MicroScint-20). The plates were then sealed (Sealing Tape SI, Nunc 236366) and read in a Wallac MicroBeta Trilux beta-counter for 1.5 min per well.

Compounds were tested as 8-concentration response curves, 0.003, 0.03, 0.1, 0.3, 1, 3, 10, 30 µM, with the two highest concentrations performed in duplicate. Dilution curves were prepared in 100% DMSO. The reference compound (terfenadine) was tested as an eight-concentration response curve, ranging from 10 µM to 0.6 nM in 1:4 dilution steps.

Synthesis of Intermediates:

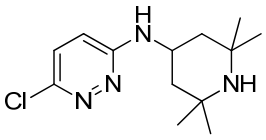


6-Chloro-N-methyl-N-(2,2,6,6-tetramethylpiperidin-4-yl)pyridazin-3-amine (S1). A solution of 3,6-dichloropyridazine (4.00 g, 26.8 mmol) and N,2,2,6,6-pentamethylpiperidin-4-amine (7.32 g, 43.0 mmol) in butan-1-ol (67 mL) was heated at 120 °C for 72 h. The solvent was removed via rotary evaporation, the residue was partitioned between water and dichloromethane, and the water layer was further extracted with dichloromethane. The combined organic layers were washed with water, brine, dried over magnesium sulfate, and concentrated *in vacuo*. The black crude material was stirred in small amount of ethyl acetate overnight, and the resulting off-white solid was collected to provide 6-chloro-N-methyl-N-(2,2,6,6-tetramethylpiperidin-4-yl)pyridazin-3-amine (4.18 g, 14.8 mmol, 55.0 % yield). MS (ESI) *m/z* 283.5 (M + 1). ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 7.40 (d, *J* = 9.60 Hz, 1H), 7.14 (d, *J* = 9.60 Hz, 1H), 4.96-5.13 (m, 1H), 2.93 (s, 3H), 1.59-1.68 (m, 2H), 1.51 (t, *J* = 12.38 Hz, 2H), 1.20 (s, 6H), 1.33 (s, 6H).

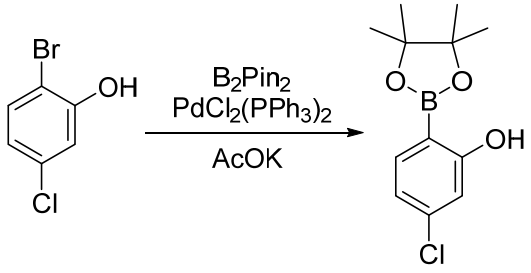


3-Chloro-6-(2,2,6,6-tetramethylpiperidin-4-yloxy)pyridazine (S2). To a solution of 2,2,6,6-tetramethylpiperidin-4-ol (106 mg, 0.67 mmol) in DMF (6.7 mL) was added NaH (60 wt %, 35 mg, 0.87 mmol). The solution was stirred at room temperature for 30 min, then 3,6-dichloropyridazine (100 mg, 0.67 mmol) was added and the mixture was stirred for 1 h. The reaction mixture was diluted with ethyl acetate, washed with water (5x), brine, dried over sodium sulfate, filtered and concentrated under reduced pressure to provide 3-chloro-6-(2,2,6,6-tetramethylpiperidin-4-yloxy)pyridazine (135 mg). The crude material used without further purification. MS (ESI) *m/z* 270.2 (M + 1). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.53 (s,

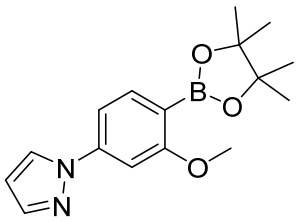
1H), 7.37 (d, *J* = 9.1 Hz, 1H), 6.91 (d, *J* = 9.1 Hz, 1H), 5.68-5.78 (m, 1H), 2.20 (dd, *J* = 12.4, 4.0 Hz, 2H), 1.32 (s, 6H), 1.27-1.29 (m, 2H), 1.20 (s, 6H).



6-Chloro-N-(2,2,6,6-tetramethylpiperidin-4-yl)pyridazin-3-amine (S3). A mixture of 3,6-dichloropyridazine (6.26 g, 42.0 mmol) and 2,2,6,6-tetramethylpiperidin-4-amine (14.7 mL, 84 mmol) was stirred at 120 °C for 1 h, neat. To this mixture was added butan-1-ol (40 mL), and the mixture was stirred at 120 °C for 1 h. The mixture was cooled to room temperature and partitioned between water and dichloromethane. The organic layer was dried over magnesium sulfate, filtered, and concentrated. The crude material was recrystallized from acetonitrile to provide 6-chloro-N-(2,2,6,6-tetramethylpiperidin-4-yl)pyridazin-3-amine (7.3 g, 27.12 mmol, 65 % yield) as an off-white solid. MS (ESI) *m/z* 269.2 (M + 1). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.08 (d, *J* = 9.3 Hz, 1H), 6.54 (d, *J* = 9.3 Hz, 1H), 4.53 (d, *J* = 7.6 Hz, 1H), 4.05-4.26 (m, 1H), 1.98 (dd, *J* = 12.6, 3.8 Hz, 2H), 1.22 (s, 6H), 1.08 (s, 6H), 0.93 (apparent t, *J* = 12.1 Hz, 2H).

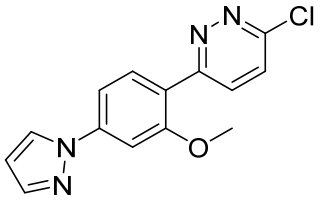


5-Chloro-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (S4). To a 3-L round bottomed flusk was added 2-bromo-5-chlorophenol (45g, 217 mmol), bis(pinacolato)diboron (66.3g, 260 mmol), potassium acetate (42.6g, 434 mmol), $Pd(PPh_3)_2Cl_2$ (4.5g, 6.4 mmol), followed by 524g of 1,4-dioxane under nitrogen. The mixture was degassed with nitrogen for 1 hour. The mixture was then heated at 90~95°C for 24 hours. After completion of reaction indicated by HPLC analysis, the reaction mixture was cooled to 20~30°C before passing through a plug of microcrystalline cellulose. The filtrate (7.3% m/m assay by HPLC with 86% purity by HPLC) was used directly in the following reaction step without further purification.

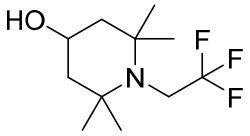


1-(3-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1H-pyrazole (S5). *Step 1:* 2-(2-pyridyl)benzimidazole (0.312 g, 1.598 mmol), cesium carbonate (6.25 g, 19.17 mmol), and copper(I) iodide (0.304 g, 1.598 mmol) were charged to a 250mL round bottom flask under inert atmosphere. Anhydrous DMF (100 ml) was added and the mixture was heated to 60 °C for 1 h. Pyrazole (1.523 g, 22.37 mmol) was added followed by 1-bromo-4-iodo-2-methoxybenzene (5 g, 15.98 mmol) and the mixture was heated to 100 ˚C for 40 h, at which time LC-MS indicated consumption of the iodide. The mixture was cooled for 30 min and was then filtered through celite, washing the filter cake with ethyl acetate. The filtrate was concentrated and the residue was purified by column chromatography using 10-40% ethyl acetate in heptanes, to afford 1-(4-bromo-3-methoxyphenyl)-1H-pyrazole as a light yellow oil which solidified on standing (3.0 g, 11.85 mol, 74% yield). MS (ESI) *m/z* 253.10 (M + 1) and 255.10 (M+1). ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 7.91 (d, *J* = 2.53 Hz, 1H), 7.73 (d, *J* = 1.52, 1H), 7.59 (d, *J* = 8.59 Hz, 1H), 7.43 (d, *J* = 2.02 Hz, 1H), 7.09 (dd, *J* = 8.59, 2.53 Hz, 1H), 6.48 (m, 1H), 3.99 (s, 3H).

1-(3-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1H-pyrazole. *Step 2:* 1-(4-bromo-3-methoxyphenyl)-1H-pyrazole (28.5 g, 113 mmol), bis(pinacolato)diboron (42.9 g, 169 mmol), potassium carbonate (15.56 g, 113 mmol), and PdCl2(dppf).CH2Cl2 adduct (9.20 g, 11.26 mmol) were charged to a 2L 3-neck round bottom flask under inert atmosphere. The solids were dissolved in anhydrous Dioxane (700 ml) and a stream of nitrogen was bubbled through the solution for 10 min. The mixture was heated to 85 °C overnight, at which time LC-MS showed consumption of the bromide. The crude mixture was filtered through a pad of celite and the filter cake washed with ethyl acetate. The residue was purified by column chromatography in 15-30% ethyl acetate in heptanes to afford 1-(3-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1H-pyrazole as an off-white solid (13.9 g, 46.31 mmol, 41% yield). MS (ESI) *m/z* 300.2 (M + 1). ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 1.27 - 1.55 (m, 16 H) 3.88 - 4.04 (m, 3 H) 6.48 (dd, *J*=2.53, 1.52 Hz, 1 H) 7.19 (dd, *J*=8.08, 2.02 Hz, 1 H) 7.38 (d, *J*=2.02 Hz, 1 H) 7.75 (d, *J*=8.76 Hz, 1 H) 7.74 (s, 1 H) 7.96 (d, *J*=2.53 Hz, 1 H).

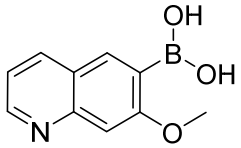


3-Chloro-6-(2-methoxy-4-(1H-pyrazol-1-yl)phenyl)pyridazine (S6). 3,6-dichloropyridazine (11.99 g, 80 mmol), 1-(3-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1H-pyrazole (**S5**) (16.1 g, 53.6 mmol), aqueous sodium carbonate (2 M, 161 mmol, 80 ml) and $PdCl_2(dppf).CH_2Cl_2$ adduct (3.07 g, 3.75 mmol) were charged to a three-neck 1000mL round-bottom flask. The flask was evacuated and backfilled three times with nitrogen. Anhydrous dioxane (274 ml) was added and the mixture was heated to 85 °C overnight. After 19 h the mixture was cooled, filtered through a pad of celite, washing the filter cake with ethyl acetate, and concentrated *in vacuo*. The residue was partitioned between ethyl acetate (250 mL) and water (150 mL). The organic layer was washed with saturated aqueous sodium chloride (100 mL) and then dried over sodium sulfate for 30 min. The mixture was filtered and concentrated *in vacuo*. The residue was purified by column chromatography in 10-30% ethyl actetate in heptanes. The compond-containing fractions were combined and concentrated. The residue was then slurried in 10 volumes of ethyl acetate:heptanes (1:3) and filtered to afford 3-Chloro-6-(2-methoxy-4-(1H-pyrazol-1-yl)phenyl)pyridazine as a white solid (7.0 g, 24.41 mmol, 45% yield). MS (ESI) *m/z* 287.7 (M+1). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.12 - 3.19 (m, 2 H) 3.96 (s, 3 H) 6.59 (s, 1 H) 7.61 (d, *J*=8.47 Hz, 1 H) 7.66 (s, 1 H) 7.79 (d, *J*=1.52 Hz, 1 H) 7.90 (t, *J*=8.59 Hz, 2 H) 8.15 (d, *J*=9.09 Hz, 1 H) 8.61 (d, *J*=2.02 Hz, 1 H)



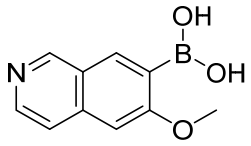
2,2,6,6-tetramethyl-1-(2,2,2-trifluoroethyl)piperidin-4-ol (S7). In a microwave compatible vial, 2,2,2-trifluoroethyl 1,1,2,2,3,3,4,4,4-nonafluorobutane-1-sulfonate (2.48 g, 6.49 mmol) was added to 2,2,6,6-tetramethylpiperidin-4-ol (1.02 g, 6.49 mmol) in acetonitrile (7.5 mL) and the mixture was heated in a Biotage® Initiator microwave reactor at 180 °C for 15 min. After concentration under reduced pressure, the crude material was purified by silica gel chromatography (8-60% ethyl acetate/heptane) to provide th title compound (0.60 g, 39% yield).

^{1}H NMR (400 MHz, DMSO-d_6) δ 4.28 (d, J = 4.9 Hz, 1H), 3.79 (tq, J = 11.3, 4.5 Hz, 1H), 3.25 (q, J = 9.9 Hz, 2H), 1.77-1.68 (m, 2H), 1.23 (t, J = 11.7 Hz, 2H), 1.05 (s, 6H), 1.02 (s, 6H).



(7-methoxyquinolin-6-yl)boronic acid (S8). *Step 1:* In a 100 mL round bottom flask, a solution of sulfuric acid (7.1 mL, 130 mmol) in water (6.5 mL) was treated with 3-nitrobenzenesulfonic acid (7.07 g, 34.8 mmol) and glycerol (8.7 ml, 120 mmol) to give a thick grey suspension. The suspension was heated to 110 °C and 4-bromo-3-methoxyaniline (6.7 g, 33 mmol) was added resulting in an immobile slurry. Additional quantities of water (6 mL), glycerol (6 mL), sulfuric acid (6 mL) were added and the temperature increased to 140 °C. After 3 h, the mixture had become a homogeneous dark brown solution. The solution was cooled to room temperature, poured onto ice, and the pH adjusted to 8 by addition of concentrated ammonium hydroxide. The mixture was extracted with 1:1 ethyl acetate/diethyl ether (5x), dried over magnesium sulfate, filtered, and concentrated to a brown liquid. Silica gel chromatography (0-20% ethyl acetate/dichloromethane) provided impure product which was further purified by silica gel chromatography (0-20% methanol/dichloromethane) to provide 6-bromo-7-methoxyquinoline (3.94 g, 50% yield) as an off-white solid. MS (ESI) *m/z* 240.1 (M + 1). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 8.86 (dd, J = 4.0, 1.5 Hz, 1H), 8.01-8.12 (m, 2H), 7.53 (s, 1H), 7.34 (dd, J = 8.1, 4.5 Hz, 1H), 4.07 (s, 3H).

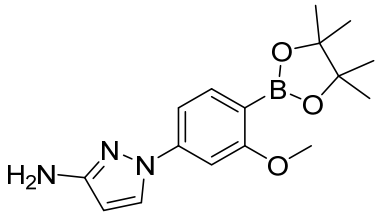
Step 2: n-Butyl lithium (1.6 M in heptane, 4.90 mL, 7.90 mmol) was added dropwise to a solution of 6-bromo-7-methoxyquinoline (1.71 g, 7.18 mmol) cooled to -78 °C. The solution was stirred for 30 min after which time trimethyl borate (2.0 mL, 18 mmol) was added in a single portion. The solution was allowed to warm to room temperature and stir overnight. The crude reaction was rotovapped to dryness and concentrated from heptane (2x). The resulting solids were passed through a plug of silica gel (80 mL, dry measure) eluting with 9:1 dichloromethane/methanol to provide (7-methoxyquinolin-6-yl)boronic acid as an orange solid (778 mg, 46% yield). MS (ESI) *m/z* 204.1 (M + 1).



(6-methoxyisoquinolin-7-yl)boronic acid (S9).

Step 1: 7-Bromo-6-methoxyisoquinoline was prepared as described in WO2007000240. MS (ESI) *m/z* 240.3 (M + 1). ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.08 (s, 1H), 8.49 (d, *J* = 5.8 Hz, 1H), 8.20 (s, 1H), 7.56 (d, *J* = 5.8 Hz, 1H), 7.11 (s, 1H), 4.05 (s, 3H).

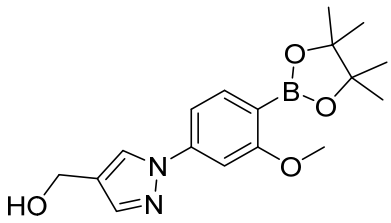
Step 2: To a 50 mL round bottom flask was added 7-bromo-6-methoxyisoquinoline (300 mg, 1.26 mmol), bis(pinacolato)diboron (640 mg, 2.52 mmol), potassium acetate (742 mg, 7.56 mmol), and $PdCl_2$(dppf):dichloromethane complex (103 mg, 0.126 mmol). Dioxane (10 mL) was added and the flask was evacuated/purged with nitrogen (3x). The mixture was stirred at 100 °C for 3 h. The mixture was diluted with ethyl acetate, filtered through celite and concentrated to provide (6-methoxyisoquinolin-7-yl)boronic acid (250 mg, 98% yield) which was used without further purification. MS (ESI) *m/z* 204.4 (M + 1).



1-(3-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1H-pyrazol-3-amine **(S10).** *Step 1*: A mixture of 1-bromo-4-iodo-2-methoxybenzene (3.99 g, 12.75 mmol), 3-nitro-1H-pyrazole (1.730 g, 15.30 mmol), salicylaldoxime (0.350 g, 2.55 mmol), Cu_2O (0.146 g, 1.02 mmol) and cesium carbonate (6.23 g, 19.13 mmol) in DMF (13 mL) was degassed with nitrogen and heated at 95 °C overnight. After cooling to room temperature, the mixture was filtered through celite and rinsed with ethyl acetate. The filtrate was washed with water and brine. The organic phase was dried over sodium sulfate, filtered and concentrated *in vacuo*. The crude product was suspended in 5% methanol/dichloromethane and the solids were isolated by filtration and dried to provide 1-(4-bromo-3-methoxyphenyl)-3-nitro-1H-pyrazole (2.3 g, 61% yield) as a white solid. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.52 (d, *J* = 2.8 Hz, 1H), 7.72 (d, *J* = 8.5 Hz, 1H), 7.60 (d, *J* = 2.4 Hz, 1H), 7.39 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.20 (d, *J* = 2.6 Hz, 1H), 4.02 (s, 3H).

Step 2: To a mixture of 1-(4-bromo-3-methoxyphenyl)-3-nitro-1H-pyrazole (2.3 g, 7.72 mmol) in dichloromethane (24 mL) and acetic acid (6.18 mL, 108 mmol) was added zinc dust (2.52 g, 38.6 mmol) at 0 °C. The mixture was stirred at 0 °C to room temperature overnight. The mixture was filtered through celite, rinsed with ethyl acetate and concentrated *in vacuo*. The residue was purified by silica gel chromotagraphy (10-50% ethyl acetate/heptane) to give a white foam. Concentration from toluene (6 mL) provided 1-(4-bromo-3-methoxyphenyl)-1H-pyrazol-3-amine as a white powder (1.9 g, 92% yield). MS (ESI) *m/z* 270.3 (M + 1). ^{1}H NMR (400 MHz, methanol-d_4) δ 7.98 (d, *J* = 2.7 Hz, 1H), 7.53 (d, *J* = 8.6 Hz, 1H), 7.34 (d, *J* = 2.4 Hz, 1H), 7.10 (dd, *J* = 8.6, 2.3 Hz, 1H), 5.91 (d, *J* = 2.6 Hz, 1H), 3.95 (s, 3H).

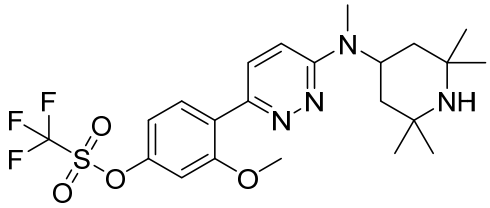
Step 3: A degassed mixture of 1-(4-bromo-3-methoxyphenyl)-1H-pyrazol-3-amine (1 g, 3.73 mmol), bis(pinacolato)diboron (1.989 g, 7.83 mmol), Pd(dppf)Cl_2 (0.273 g, 0.373 mmol), dppf (0.207 g, 0.373 mmol) and potassium acetate (2.56 g, 26.1 mmol) in dioxane (10 mL) was heated at 90 °C overnight. After cooling to room temperature, the mixture was filtered through celite and washed with ethyl acetate. The filtrate was concentrated and the residue was purified by silica gel chromotagraphy (10-60% ethyl acetate/heptane) to provide 1-(3-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1H-pyrazol-3-amine as a white solid (930 mg, 79% yield). MS (ESI) *m/z* 316.2 (M + 1). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.76 (d, *J* = 2.5 Hz, 1H), 7.71 (d, *J* = 8.1 Hz, 1H), 7.24 (d, *J* = 1.7 Hz, 1H), 7.05 (dd, *J* = 8.1, 1.9 Hz, 1H), 5.88 (d, *J* = 2.6 Hz, 1H), 3.93 (s, 3H), 1.38 (s, 12H).



(1-(3-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1H-pyrazol-4-yl)methanol (S11). *Step 1:* A mixture of 4-(hydroxymethyl)pyrazole (500 mg, 5.10 mmol), salicylaldoxime (140 mg, 1.019 mmol), cesium carbonate (4.98 g, 15.29 mmol), cuprous oxide (58.2 mg, 0.306 mmol), 1-bromo-4-iodo-2-methoxybenzene (1.59 g, 5.10 mmol) and DMF (10 mL) were heated under a nitrogen atmosphere at 90 °C overnight. The mixture was cooled to room temperature, filtered through celite, and the filtrate concentrated *in vacuo*. Silica gel

chromatography (10-60% ethyl acetate/heptane) provided (1-(4-bromo-3-methoxyphenyl)-1H-pyrazol-4-yl)methanol (800 mg, 55% yield). MS (ESI) *m/z* 285.3 (M + 1).

Step 2: A mixture of (1-(4-bromo-3-methoxyphenyl)-1H-pyrazol-4-yl)methanol (400 mg, 1.41 mmol), bis(pinacolato)diboron (538 mg, 2.12 mmol), potassium acetate (415 mg, 4.24 mmol), $PdCl_2$(dppf) (103 mg, 0.14 mmol), and dppf (78 mg, 0.14 mmol), in dioxane (6 mL) was heated under an N_2 atmosphere at 90 °C overnight. The mixture was filtered through a disposable filter funnel and concentrated *in vacuo*. Purification by silica gel chromatography (10-60% ethyl acetate/heptane) provided the title compound (300 mg, 64% yield). MS (ESI) *m/z* 331.2 (M + 1).

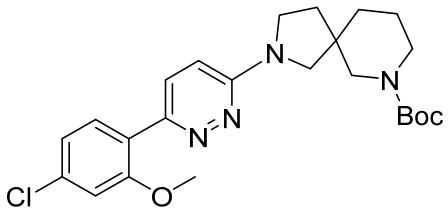


3-Methoxy-4-(6-(methyl(2,2,6,6-tetramethylpiperidin-4-yl)amino)pyridazin-3-yl)phenyl trifluoromethanesulfonate (S12). *Step 1:* To a 25 mL microwave vial was added 4-bromo-3-methoxyphenol (1.0 g, 4.93 mmol), bis(pinacolato)diboron (1.88 g, 7.39 mmol), potassium acetate (2.41 g, 24.63 mmol), $PdCl_2$(dppf) (0.36 g, 0.49 mmol), dppf (0.27 g, 0.49 mmol), and dioxane (10 mL). The solution was purged with nitrogen (3x) and stirred at 90 °C overnight. The mixture was filtered through celite and the filter cake was washed with ethyl acetate. The filtrate was concentrated *in vacuo* to give a brown liquid which was purified by silica gel chromatography (10-50% ethyl acetate/heptane) to provide 3-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (700 mg). MS (ESI) *m/z* 251.4 (M + 1). ^{1}H NMR (400 MHz, chloroform-d) δ 7.57 (d, *J* = 8.1 Hz, 1H), 6.40 (dd, *J* = 8.1, 2.1 Hz, 1H), 6.30 (d, *J* = 2.0 Hz, 1H), 3.62 (s, 3H), 1.33 (s, 12H).

Step 2: 3-Methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (938 mg, 3.75 mmol) and 6-chloro-*N*-methyl-*N*-(2,2,6,6-tetramethylpiperidin-4-yl)pyridazin-3-amine (848 mg, 3 mmol) were coupled following General Procedure E for Suzuki coupling. Purification by silica gel chromatography (0-20% 2 N ammonia in methanol gradient, in dichloromethane) provided 3-methoxy-4-(6-(methyl(2,2,6,6-tetramethylpiperidin-4-yl)amino)pyridazin-3-yl)phenol (420 mg, 38 % yield). MS (ESI) *m/z* 371.3 (M + 1).

Step 3: To a solution of 3-methoxy-4-(6-(methyl(2,2,6,6-tetramethylpiperidin-4-yl)amino)pyridazin-3-yl)phenol (570 mg, 1.539 mmol) in dichloromethane (10 mL) was added

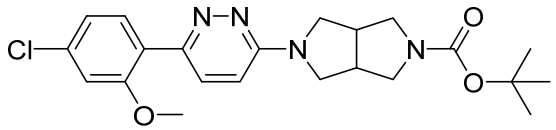
triethylamine (0.54 mL, 3.85 mmol). The mixture was cooled to 0 °C, followed by addition of N-phenyl-trifluoromethanesulfonimide (769 mg, 2.15 mmol). The mixture was warmed to room temperature and stirred for 2 h. The reaction was quenched with saturated aqueous sodium bicarbonate solution and extracted with dichloromethane. The organic layer was dried over sodium sulfate, filtered and concentrated. Silica gel chromatography (0-25% methanol/dichloromethane) provided 3-methoxy-4-(6-(methyl(2,2,6,6-tetramethylpiperidin-4-yl)amino)pyridazin-3-yl)phenyl trifluoromethanesulfonate **(S12**) (270 mg, 35%). MS (ESI) *m/z* 503.4 (M + 1). ^{1}H NMR (400 MHz, methanol-d_4) δ 7.83 (d, *J* = 9.6 Hz, 1H), 7.78 (d, *J* = 8.5 Hz, 1H), 7.18 (d, *J* = 9.6 Hz, 1H), 7.14 (d, *J* = 2.3 Hz, 1H), 7.09 (dd, *J* = 8.5, 2.3 Hz, 1H), 5.48 (tt, *J* = 11.8, 3.8 Hz, 1H), 3.90 (s, 3H), 3.02 (s, 3H), 2.14-1.86 (m, 4H), 1.64 (s, 6H), 1.53 (s, 6H).



2-(6-(4-chloro-2-methoxyphenyl)pyridazin-3-yl)-2,7-diazaspiro[4.5]decane-7-carboxylate (S13). *Step 1*: A mixture of 3,6-dichloropyridazine (97 mg, 0.649 mmol), tert-butyl 2,7-diazaspiro[4.5]decane-7-carboxylate (156 mg, 0.649 mmol), N-ethyl-N-isopropylpropan-2-amine (0.227 mL, 1.298 mmol), and *n*-butanol (2 mL) is heated at 120 °C overnight. The reaction mixture was concentrated, and the residue was purified by silica gel chromatography (0-25% 2N NH3 in MeOH/DCM) to afford tert-butyl 2-(6-chloropyridazin-3-yl)-2,7-diazaspiro[4.5]decane-7-carboxylate as an off-white solid (125 mg, 0.344 mmol, 52.9 % yield). MS (ESI) *m/z* 353.0 (M + 1).

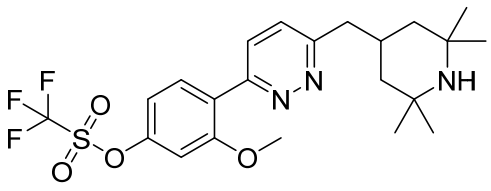
Step 2: A mixture of tert-butyl 2-(6-chloropyridazin-3-yl)-2,7-diazaspiro[4.5]decane-7-carboxylate (125 mg, 0.354 mmol), (4-chloro-2-methoxyphenyl)boronic acid (79 mg, 0.425 mmol), sodium carbonate (113 mg, 1.063 mmol) and $Pd(PPh_3)_4$ (20.47 mg, 0.018 mmol) in 3:1 dioxane/water (4 mL) was purged with nitrogen and heated in microwaver at 90°C for 1 hr. The mixture was diluted with ethyl acetate and filtered through celite. The filtrate was concentrated, and the crude product was purified by silica gel chromatography (0-70% ethyl acetate/heptane) to provide white solid *tert*-butyl 2-(6-(4-chloro-2-methoxyphenyl)pyridazin-3-

yl)-2,7-diazaspiro[4.5]decane-7-carboxylate (130 mg, 0.275 mmol, 78 % yield). MS (ESI) *m/z* 459.0 (M + 1).



***tert*-Butyl 5-(6-(4-chloro-2-methoxyphenyl)pyridazin-3-yl)hexahydropyrrolo[3,4-c]pyrrole-2(1H)-carboxylate (S14).** *Step 1:* A mixture of 3,6-dichloropyridazine (74.5 mg, 0.500 mmol), *tert*-butyl hexahydropyrrolo[3,4-c]pyrrole-2(1H)-carboxylate (106 mg, 0.5 mmol), N-ethyl-N-isopropylpropan-2-amine (0.174 mL, 1.0 mmol), and *n*-butanol (2.5 mL) are heated at 120 °C overnight. The solution is cooled to room temperature and ethyl acetate added. The white solid is filtered and washed with ethyl acetate, dissolved in dichloromethane and washed with water. The organic layer is dried over sodium sulfate, filtered, and concentrated *in vacuo* to afford the crude product which is purified by silica gel chromatography (0-25% ethyl acetate/dichloromethane) to provide *tert*-butyl 5-(6-chloropyridazin-3-yl)hexahydropyrrolo[3,4-c]pyrrole-2(1H)-carboxylate as an off-white solid (138 mg, 81% yield). MS (ESI) *m/z* 325.2 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.44 (d, *J* = 9.6 Hz, 1H), 6.95 (d, *J* = 9.5 Hz, 1H), 3.67 (dd, *J* = 11.1, 7.4 Hz, 2H), 3.54 (dd, *J* = 11.2, 7.4 Hz, 2H), 3.35 (dd, *J* = 11.1, 4.1 Hz, 2H), 3.18 (dd, *J* = 11.2, 4.2 Hz, 2H), 3.01 (m, 2H), 1.39 (s, 9H).

Step 2: A mixture of *tert*-butyl 5-(6-chloropyridazin-3-yl)hexahydropyrrolo[3,4-c]pyrrole-2(1H)-carboxylate (130 mg, 0.40 mmol), (4-chloro-2-methoxyphenyl)boronic acid (93.0 mg, 0.500 mmol), sodium carbonate (127 mg, 1.20 mmol), $PdCl_2$(dppf):dichloromethane complex (32.7 mg, 0.040 mmol), and 5:1 dioxane/water (6 mL) was purged with nitrogen and heated at 90°C overnight. The mixture was diluted with ethyl acetate and filtered through celite. The filtrate was concentrated, and the crude product was purified silica gel chromatography (0-50% ethyl acetate/dichloromethane) to provide the title compound (140 mg, 77% yield). MS (ESI) *m/z* 431.4 (M + 1).



3-Methoxy-4-(6-((2,2,6,6-tetramethylpiperidin-4-yl)methyl)pyridazin-3-yl)phenyl trifluoromethanesulfonate (S15). *Step 1:* To a 100 mL flask containing methyltriphenylphosphonium bromide (5.2 g, 14.5 mmol) in ether (8 mL) cooled to 0 °C was added potassium *tert*-butoxide (2.2 g, 19.3 mmol). The resulting suspension was stirred for 0.5 h at 0 °C followed by the dropwise addition of 2,2,6,6-tetramethylpiperidin-4-one (1.0 g, 6.44 mmol) in ether (5 mL). The resulting mixture was stirred at room temperature overnight after which time additional potassium *tert*-butoxide (0.72 g, 6.44 mmol) and methyltriphenylphosphonium bromide (1.7 g, 4.83 mmol) were added. The mixture was stirred at room temperature for 4 h then cooled to 0 °C and quenched with water, acidified with aqueous 1 M HCl and washed with ether (3x). The aqueous mixture was adjusted to pH 10 with 2 M aqueous sodium hydroxide and extracted with ether (3x). The organic extract was acidified with trifluoroacetic acid, dried over sodium sulfate and concentrated *in vacuo* to provide 2,2,6,6-tetramethyl-4-methylenepiperidine trifluoroacetate salt (1.7 g,) as a brown oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.53 (br s, 2H), 5.00 (s, 2H), 2.20-2.30 (m, 4H), 1.33 (s, 12H).

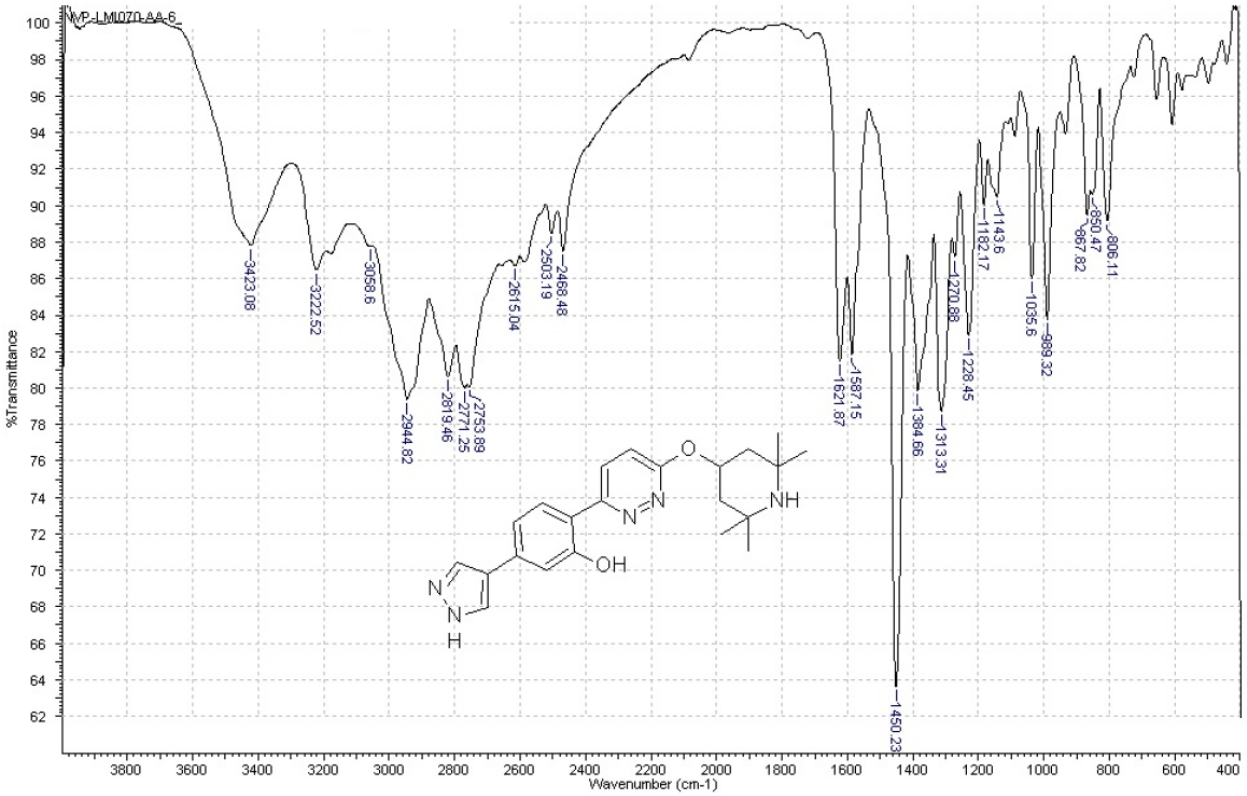
Step 2: To 50 mL flask was added 2,2,6,6-tetramethyl-4-methylenepiperidine trifluoroacetate (1.1 g, 4.12 mmol) and 9-borabicyclo[3.3.1]nonane (0.5 M in tetrahydrofuran, 16.5 mL, 8.23 mmol). The mixture was heated at 65 °C for 1 h. After cooling to room temperature, 3,5-dichloropyridazine (0.61 g, 4.12 mmol), potassium carbonate (1.7 g, 12.35 mmol), $PdCl_2$(dppf):dichloromethane complex (0.17 g, 0.21 mmol), and 5:1 dioxane/water (10 mL) were added and the mixture heated at 60 °C overnight. The mixture was cooled to room temperature, diluted with ethyl acetate, filtered through celite and concentrated *in vacuo*. The crude product was acidified using 12 M HCl aqueous solution and SCX purification (General Procedure F) provided 3-chloro-6-((2,2,6,6-tetramethylpiperidin-4-yl)methyl)pyridazine (1.1 g, 99% yield). MS (ESI) *m/z* 268.2 (M + 1).

Step 3: A mixture of tert-butyl(3-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy)dimethylsilane (408 mg, 1.12 mmol), 3-chloro-6-((2,2,6,6-tetramethylpiperidin-4-yl)methyl)pyridazine (200 mg, 0.75 mmol), sodium carbonate (0.24 g, 2.24 mmol), and $PdCl_2$(dppf):dichloromethane complex (0.06 g, 0.08 mmol) in 3:1 dioxane/water (4 mL) was heated at 80 °C overnight, then cooled to room temperature and diluted with ethyl acetate. The suspension was filtered through celite and concentrated *in vacuo*. SCX purification (General

Procedure F) provided the 3-methoxy-4-(6-((2,2,6,6-tetramethylpiperidin-4-yl)methyl)pyridazin-3-yl)phenol (165 mg, 62% yield). MS (ESI) *m/z* 356.1 (M + 1).

Step 4: To a solution 3-methoxy-4-(6-((2,2,6,6-tetramethylpiperidin-4-yl)methyl)pyridazin-3-yl)phenol (165 mg, 0.46 mmol) in dichloromethane (2.8 mL) was added triethylamine (0.162 mL, 1.16 mmol) at room temperature. The mixture was cooled to 0 °C, followed by addition of N-phenyltrifluoromethanesulfonimide (174 mg, 0.49 mmol). The mixture was warmed to room temperature and stirred for 2 h. The reaction was quenched with saturated aqueous sodium bicarbonate solution and extracted with dichloromethane. The organic layer was dried over sodium sulfate, filtered and concentrated *in vacuo* to give the crude product which was adjusted to pH 3 using 1 M HCl aqueous solution. SCX purification (General Procedure F) provided 3-methoxy-4-(6-((2,2,6,6-tetramethylpiperidin-4-yl)methyl)pyridazin-3-yl)phenyl trifluoromethanesulfonate (160 mg, 72% yield). MS (ESI) *m/z* 488.0 (M + 1).

IR, ^{1}H and ^{13}C NMR Spectra for LMI070 (1).



Frequency (MHz)	600.13	*Nucleus*	1H	*Number of Transients*	16
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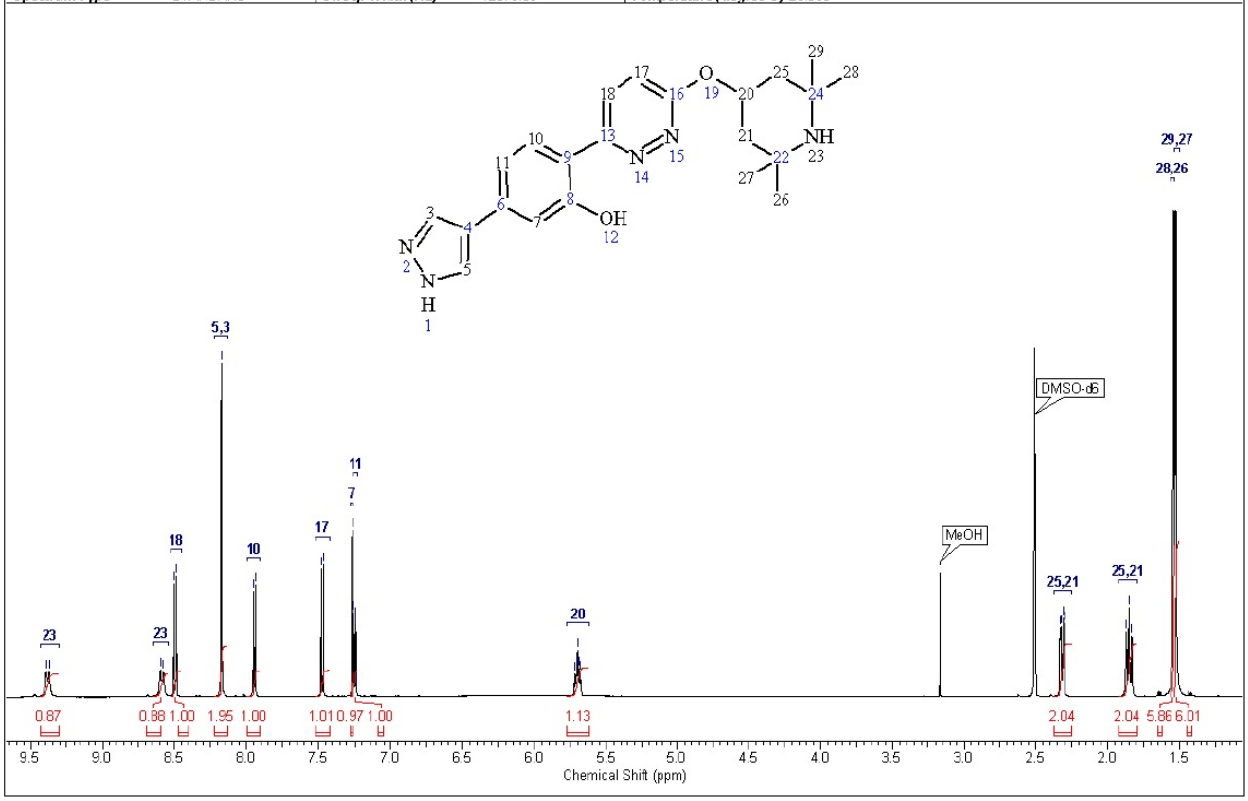
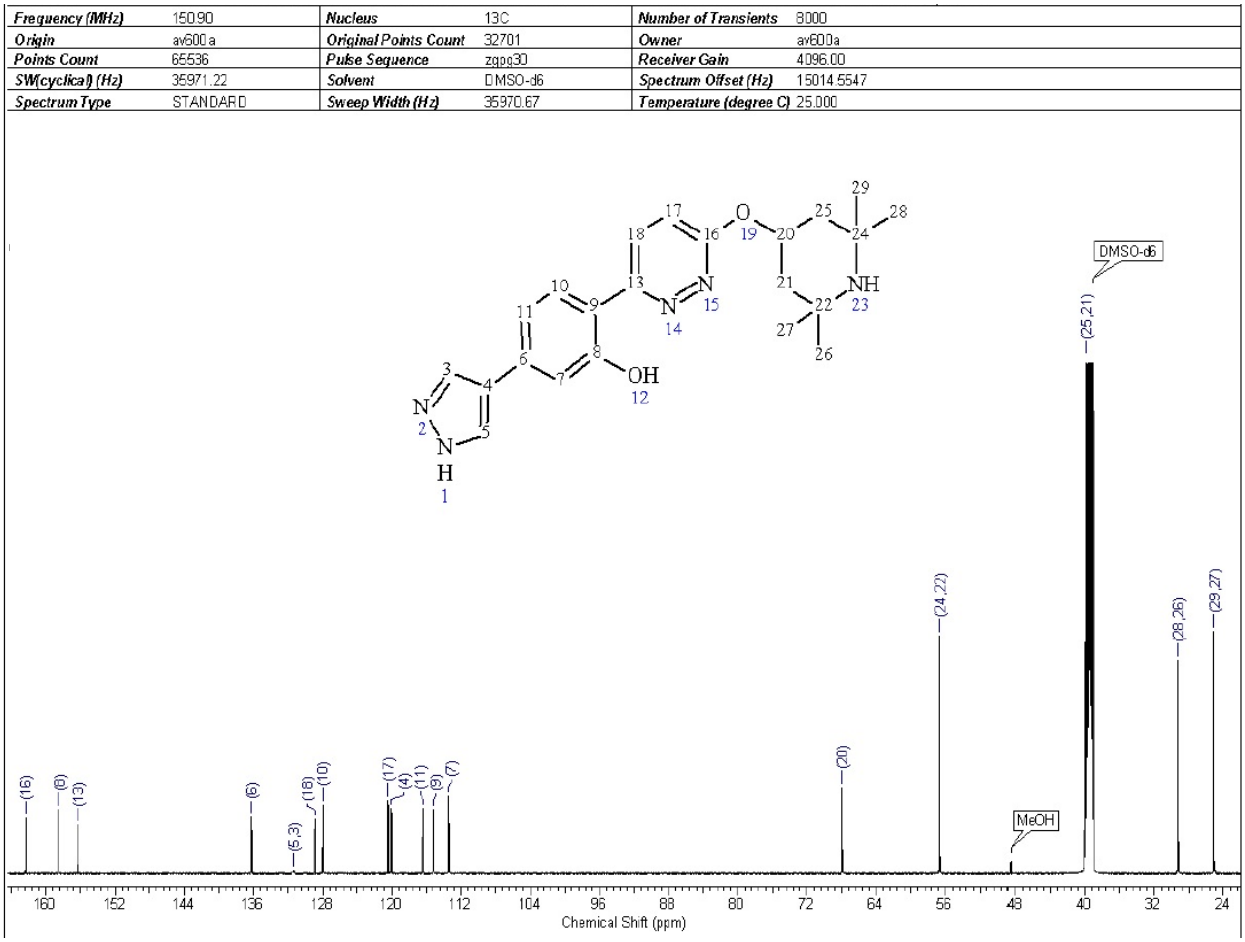
Points Count	32768	*Pulse Sequence*	zg30	*Receiver Gain*	71.80
SW(cyclical) (Hz)	12376.24	*Solvent*	DMSO-d6	*Spectrum Offset (Hz)*	3704.3701
Spectrum Type	STANDARD	*Sweep Width (Hz)*	12375.86	*Temperature (degree C)*	25.000





Crystallography for 8 and 9. Crystallization, data collection and structure determination.

Crystals of compounds **8** and **9** were obtained by dissolving in a minimum amount of solvents from which the solvent was allowed to slowly evaporate at room temperature: for compound **8** methanol was used, for compound **9** ethyl methyl ketone.

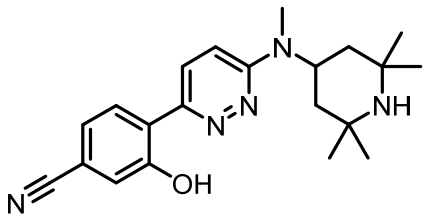
Diffraction data were collected at 100 K on a Bruker AXS MicroStar diffractometer using a SMART 6000 CCD detector on a three-circle platform goniometer with Cu(K_α) radiation (λ = 1.54178 Å) from a microfocus rotating anode generator with a Helios MX confocal mirror system in case of **8** and from a microfocus sealed tube generator equipped with Incoatec multilayer optics (QUAZAR focussing mirror system)in case of **9**. 16 ω-scans at different ϕ-positions were performed to ensure appropriate data redundancy (10.6 for **8** and 10.8 for **9** in the monoclinic space group $P2_1/c$, Friedel pairs merged, respectively).

Crystal data, data collection and structure refinement details are summarized in supplemental material, respectively. The crystal structures were solved by dual space-recycling methods and refined based on full-matrix least-squares on F^2 using the SHELXTL program suite (Sheldrick GM (2001)). The structure of **8** consists of two independent molecules and is a mono methanol solvate In molecule 2, all numbers used for molecule 1 have been increased by 30. The structure of **9** consists of one independent molecules. Data will be deposited at the Cambridge Crystallographic Data Centre CCDC (CCDC-1876001, **9**; CCDC-1876002, **8**).

Reference Citation (SHEXLTL and PLATON)

1. Sheldrick, G.M. SHELXTL Version 6.14, Bruker Analytical Instruments Inc. Madison, WI, USA, (2001).
2. Spek, A.L. (2003) Single-crystal structure validation with the program PLATON. J. Appl. Cryst.; 36: 7-13.

Table S1. Crystal data and structure refinement for 8 (CCDC-1876002).



Empirical formula	C22 H31 N5 O2	
Formula weight	397.52	
Temperature	100(2) K	
Wavelength	1.54178 Å	
Crystal system	Monoclinic	
Space group	P21/c	
Unit cell dimensions	a = 39.963(11) Å	α= 90°
	b = 11.639(3) Å	β= 96.158(11)°
	c = 9.199(3) Å	γ = 90°

Volume	4254(2) $Å^3$
Z	8
Density (calculated)	1.241 g/cm^3
Absorption coefficient	0.653 mm^{-1}
F(000)	1712
Crystal size	0.34 x 0.20 x 0.07 mm^3
Theta range for data collection	3.34 to 68.30°
Index ranges	-48<=h<=48, -14<=k<=14, -10<=l<=10
Reflections collected	84143
Independent reflections	7719 [R(int) = 0.0366]
Completeness to theta = 68.30°	99.2 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9557 and 0.8086
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	7719 / 0 / 539
Goodness-of-fit on F^2	1.098
Final R indices [I>2sigma(I)]	R1 = 0.0484, wR2 = 0.1306
R indices (all data)	R1 = 0.0516, wR2 = 0.1330
Largest diff. peak and hole	0.384 and -0.301 $e.Å^{-3}$

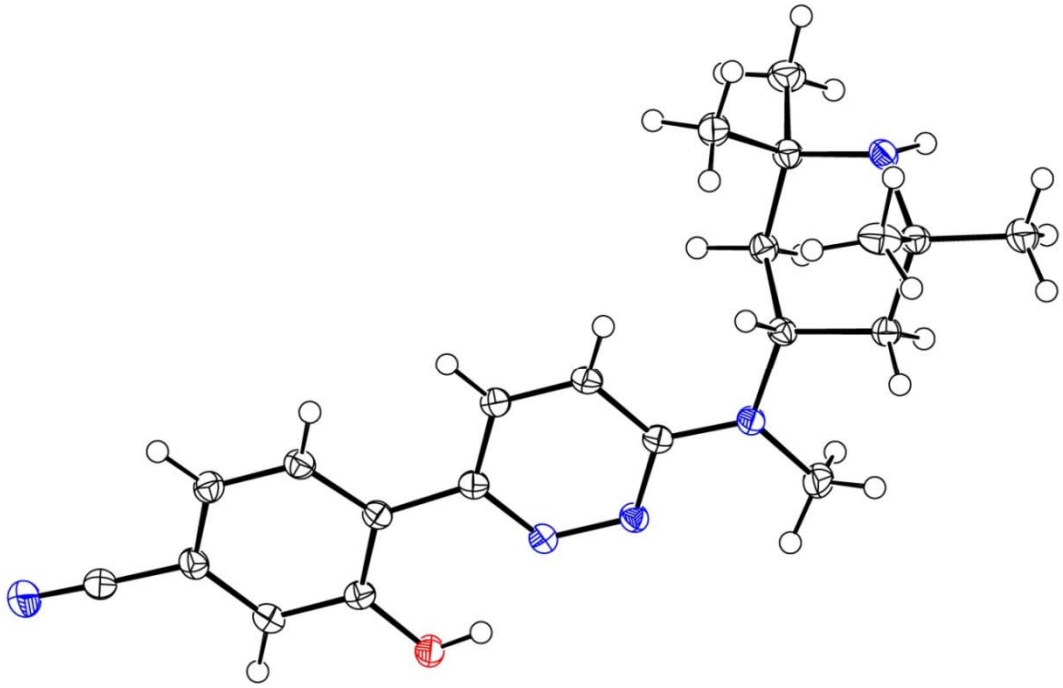
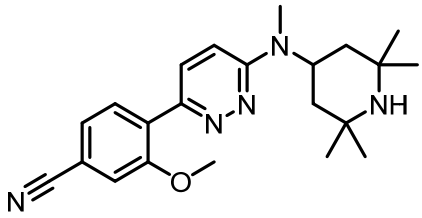


Table S2. Crystal data and structure refinement for 9 (CCDC-1876001).



Empirical formula	C22 H29 N5 O	
Formula weight	379.50	
Temperature	100(2) K	
Wavelength	1.54178 Å	
Crystal system	Monoclinic	
Space group	P2(1)/c	
Unit cell dimensions	a = 7.995(2) Å	α= 90°
	b = 11.225(3) Å	β= 96.080(12)°

	c = 22.292(6) Å γ = 90°
Volume	1989.3(9) $Å^3$
Z	4
Density (calculated)	1.267 g/cm^3
Absorption coefficient	0.637 mm^{-1}
F(000)	816
Crystal size	0.12 x 0.09 x 0.04 mm^3
Theta range for data collection	3.99 to 68.33°
Index ranges	-9<=h<=9, -13<=k<=13, -26<=l<=26
Reflections collected	40639
Independent reflections	3663 [R(int) = 0.0406]
Completeness to theta = 68.33°	99.8 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9750 and 0.9275
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3663 / 0 / 259
Goodness-of-fit on F^2	1.023
Final R indices [I>2sigma(I)]	R1 = 0.0359, wR2 = 0.0910
R indices (all data)	R1 = 0.0405, wR2 = 0.0947
Largest diff. peak and hole	0.214 and -0.190 $e.Å^{-3}$