Supporting information

Discovery and optimization of selective Nav1.8 modulator series that demonstrate efficacy in preclinical models of pain

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Biology experimental.

Cell culture of sodium channel stable cell lines

HEK293 cells expressing human $Na_V1.8/\beta1$ (Merck Millipore, Billerica, MA, USA and an in house cell line) were grown in DMEM/F12 (with L-glutamine), 10 % FBS, 1 % non-essential amino acids, G418 400 µg ml^{-1}, puromycin 0.625 µg ml^{-1}, hygromycin 100 µg ml^{-1}. HEK293 cells expressing recombinant sodium channels other than $hNa_V1.8$ were grown in DMEM/high-glucose Dulbecco's modified, 10% FBS, 2 mM sodium pyruvate, and G418. All cell culture reagents were from Invitrogen (Life Technologies, Gent, Belgium).

Isolation of human DRG neurons for voltage-clamp recordings

Human DRG neuronal cultures were prepared at AnaBios as described in Davidson, 2014 (Davidson *et al.*, 2014). Breifly, the DRGs were dissected to remove all connective tissue and fat and the ganglia were enzymatically digested at 37°C for 2 h using AnaBios' proprietary enzyme mixture. Samples were then centrifuged for 2 min at 200×g, solution was gently removed, and tissue was washed 3 times, followed by resuspension in DMEM/F12 (Lonza; Allendale, NJ) containing 1% horse serum (Thermo Fisher Scientific; Rockford, IL). Ganglia were mechanically dissociated by gentle trituration through the fire-polished tip of a sterile glass Pasteur pipette. Dissociated cells were seeded on glass coverslips that had been precoated with poly-d-lysine. Cells were maintained in culture at 37°C with 5% CO2 in DMEM/F12 supplemented with 10% horse serum (Thermo Fisher Scientific), 2 mM glutamine, 25 ng/mL hNGF (Cell Signaling Technology, Danvers, MA), 25 ng/mL GDNF (Peprotech)

Rocky Hill, NJ), and penicillin/streptomycin (Thermo Fisher Scientific). Half of the culture media was replaced with fresh media every 3 days.

Isolation of rat DRG neurons for voltage clamp recordings

Rat DRG neuronal cultures were prepared as described in Passmore, 2005 (Passmore G. M 2005). Notable differences were the absence of NGF in the growth medium and cells were plated on pre-coated coverslips (poly-D-lysine/laminin; BD Biosciences, San Jose, CA, USA). Healthy DRG neurons had a visible nucleolus 2-3 hours after isolation and were patched within 12-24 hours of isolation.

Manual Patch Electrophysiological recordings

Coverslips containing either HEK293 cells expressing sodium channels or DRG neurons from rat or human were placed in a recording chamber and perfused (approximately 1 ml min^{-1}) with an extracellular solution containing (in mM): 132 NaCl, 5.4 KCl, 10 HEPES, 5 Glucose, 1.8 $CaCl_2$ and 0.8 $MgCl_2$, pH 7.4 with NaOH for $hNa_V1.1$, $hNa_V1.2$, $hNa_V1.4$, $hNa_V1.6$, $hNa_V1.7$ and $hNa_V1.8$. For $hNa_V1.5$, sodium concentration was reduced by substituting 102 mM Choline Cl. For whole-cell voltage-clamp recordings of tetrodotoxin-resistant (TTX-R) currents from human DRG neurons, ECS contained (in mM): 20 mM NaCl, 112 mM Choline Cl, 5.4 KCl, 10 HEPES, 5 Glucose, 1.8 $CaCl_2$, 0.8 $MgCl_2$, 0.05 $CdCl_2$ and 0.2 TTX, pH 7.4 with NaOH. Patch pipettes were filled with an intracellular solution containing (in mM): 110 CsF, 35 CsCl, 5 NaCl, 10 HEPES and 10 EGTA, pH 7.3 with NaOH, and had a resistance of 1 to 2 MΩ. All recordings were made at room temperature (22-24°C) using Axopatch 200B or Multiclamp 700B amplifiers and PCLAMP software (Molecular Devices, Sunnyvale, CA, USA). All compounds were dissolved in dimethyl sulfoxide (DMSO). The final maximal concentration of DMSO used (<0.3%) was found to have no significant effect on sodium currents. Solutions containing **3** or DMSO control were applied using a perfusion system (MSC-200, Bio-Logic SAS, Claix, France).

Subtype Selectivity.

Selectivity was assessed across the family of human VGSC channels using manual patch clamp electrophysiology. Testing was conducted at the specific half inactivation voltage for each channel to more appropriately assess pharmacological selectivity (n = 5 for the human recombinantly expressed isoforms of the $Na_V1.8$ channel and n = 4 for the TTX-R current in human DRG neurons). Cells were depolarized from a holding potential of -120 mV (or -150 mV for $Na_V1.5$) to a membrane potential that inactivated half of the available channels for 8 seconds followed by a 2 or 20 ms recovery at -120 mV and a 20 ms test pulse to 0 mV.

Analysis of electrophysiology data

Concentration response data was analyzed using nonlinear least squares fit of the Logistic Equation (GraphPad Prism 5, San Diego, CA, USA) to provide half maximal inhibitory (IC_{50}) concentration.

FRET assay for hNav1.8 compounds

HEK293 cells expressing hNav1.8 (in house cell line) and a SHSY5Y neuroblastoma cell line expressing Nav1.2, 1.3 and 1.7 were used to provide data at hNav1.8 and tetrodotoxin-sensitive sodium channels respectively. Cells were grown plates up to 80-90% confluency prior to use. Test compound was diluted in 100 % DMSO at 4 mM and serial dilutions were made (1 to 1.362) to enable profiling over an 11 point concentration range. A 0.75 % DMSO solution was added to determine the zero percent effect (ZPE) and 10 uM propafenone was used as a positive control to determine maximum effect (HPE). This assay was based on fluorescence resonance energy transfer (FRET). When the plasma membrane became depolarized due to the addition of sodium to the extracellular membrane, this caused separation of the FRET pair and a change in the fluorescent signal from red at 580 nm from the acceptor probe to blue at 460 nm from the donor probe. Compounds that inhibited the opening of voltage gated sodium channels were identified by a reduction in the blue fluorescent signal which increased as the concentration of Nav blocker increased. Data was captured at two different time points and two wavelength intensities. It was reported as the ratio of the two intensities normalized to the starting ratio of each well. The data were then

analyzed in the Pfizer proprietary application SIGHTS 0 to generate IC_{50} values. 6 assays were run in total.

IonWorks Quattro selectivity assay

HEK293 cells expressing hNav1.1, 1.7 and 1.5 were used to provide selectivity data against tetrodotoxin-sensitive sodium channels and the cardiac sodium channel Nav1.5. Cells were grown in DMEM, 10 % HIFCS (PAA serum), 2 mM L-glutamine, 500 ug ml-1 G418 (genetecin) and were harvested at 60% confluency prior to use. Test compound was diluted in 100 % DMSO at 30 mM and serial dilutions were made to provide stocks at 3 times the desired assay concentration (stocks: 30, 10, 3, 1, 0.3, 0.1, and 0.03 μM). A 3 % DMSO solution was added as a control to the compound plate and an internal positive control was also used. A voltage step from -55 to 0 mV for Nav1.1, -80 to -20 mV for Nav1.7 and -80 mV to -30 mV for Nav1.5 was applied across the cell plate before and after compound addition using a 10 or 25 pulse protocol. This allowed current readings to be taken from channels in the resting state (pulse 1), and the inactivated state (pulse 10 or 25) as compounds can inhibit the activity of ion channels differently depending on the channels conformational sate and this pulse protocol accommodated this. Current amplitudes at pulse 1 and pulse 10 or 25 pre and post compound were measured and exported into Microscoft EXCEL for analysis and IC50 generation. Each assay was deemed n = 1.

In vivo methodology

170-300g male Sprague Dawley rats (Charles River, Raleigh, NC) had free access to food and water and were maintained on a 12:12 hour light/dark schedule. All experiments conform to the ethical guidelines for investigation of experimental pain in conscious animals and fully complied with UK home office legislation. Compound was formulated in 0.5%MC/0.1%Tween 80 vehicle and dosed via oral gavage prior to behavioral testing.

Statistical analyses were conducted using GraphPad Prism 5 (GraphPad, San Diego, CA, USA) or Excel. Data was analyzed using a one way ANOVA for TNT and a 2-way ANOVA for SNL. Post-hoc analysis was performed using either a Dunnett's or Bonferroni posttests.

Rat tibial nerve transection induced mechanical allodynia

A neuropathic pain condition was induced in rats by ligating the tibial nerve in two places and transecting between the two ligatures under anesthesia as described by Lee *et al.,* 2000. Prior to testing the effect of compound, baseline measurements of allodynia were performed immediately prior to subject selection and compound administration. Paw withdrawal thresholds were measured to different weights of von frey filaments. A baseline reading of 50 % paw withdrawal threshold (PWT) was taken using the up down method to select animals displaying robust allodynia based on the following criteria: ipsilateral paw: 50 % PWT <3 g, contralateral paw: 50 % PWT > 8 g. Following baseline measurements, compound (15 and 40 mg kg^{-1} for compound 13 and 30 mg kg^{-1} for compound 18), vehicle or gabapentin 20 or 10 mg kg^{-1} were administered 1.5 hours prior to testing, (n = 7-8 per group). Test animals were placed in a box separated by walls with a wire mesh floor allowing access to the plantar surface of the paw. Blood samples were collected at the end of the experiment.

Rat SNL hypersensitivity

A neuropathic pain condition was induced in rats using a nerve injury model using a modification of the method described by Kim and Chung (Kim & Chung, 1992). Prior to testing the effect of compound, baseline measurements of allodynia were performed

immediately prior to subject selection and compound administration. Only animals with baseline scores ≤ 6.6 were considered allodynic and utilized in further testing. Following baseline measurements, compound 3 10 or 30 mg kg^{-1}, vehicle or gabapentin 100 mg kg^{-1} were administered 1-2 hours prior to testing, (n = 6-8 per group). Test animals were placed in a box separated by walls with a wire mesh floor allowing access to the plantar surface of the paw. Tactile testing was conducted as described by Chaplan *et al* 1994, A top threshold was set at 15 grams.

Collection of plasma samples for measurement of concentrations of compounds 3, 13 and 18

Terminal blood samples were taken by cardiac puncture and stored on ice. Plasma was prepared by centrifugation within 1 h of blood collection and stored at -20^{o}C until analysis. No terminal blood samples were taken following rat SNL hypersensitivity testing, where exposures were determined in satellite animals as part of the Functional Observation Battery (FOB). For compound 13 plasma samples were taken from 3 or 2 rats (40 mg/kg or 15 mg/kg respectively) for subsequent analysis 2 hours post-dosing. For compound 18 plasma samples were taken from 3 or 2 rats (30 mg/kg) for subsequent analysis 1 hour post-dosing.

Measurement of plasma concentrations of compound 3, 13 and 18.

For the determination of brain tissue concentrations in mice and rats, brain tissue weights were recorded and then treated with 3 x the brain weight of 80:20 acetonitrile: water with 2.5% formic acid containing 75 ng mL^{-1} of the internal standard. Brain tissue was homogenized for 2.5 minutes using the high-energy cell disrupter Mini-Beadbeater-96 (BioSpec Products, Bartlesville, OK, USA). The samples were centrifuged at 3400 rpm at 4^{o}C for 20 minutes and analyzed using LC-MS/MS in the same manner as the diluted plasma samples. The unbound fraction of compound in rat brain homogenate was determined by equilibrium dialysis.

Table 3 has been altered in order to include the doses that gave the exposures cited in the table along with a measure of the error associated with the biological value measured, in this case 95 % confidence interval. This augmented table is shown below as it was deemed too large to be included in the main text and took the manuscript beyond the page limit.

Cmpd	$hNa_v1.8$		hNa_v subtype selectivity		TTX-R Rat DRG		TTX-R human DRG		hERG	Model	Effect	Unbound exposure (at efficacious dose)
	IC_{50} μM (95% CI)	n	IC_{50} μM (95% CI)	n	IC_{50} μM (95% CI)	n	IC_{50} μM (95% CI)	n	IC_{50} μM		significance	μM
3	0.19 (0.15-0.24)	5	$Na_v1.1$ = 13 (12.4-14.7) $Na_v1.2$ = 12.8 (11.4-14.4) $Na_v1.5$ = 9.0 (8.4-9.7) $Na_v1.7$ = 19 (17.5-20.0)	5 5 5 5	0.44 (0.33-0.60)	4	0.31 (0.25-0.38)	4	30	SNL Hyper-sensitivity	*P* <0.05 (equal to 100 mg/kg gabapentin)	0.25 (30 mg/kg)
13	0.19 (0.03-0.93)	3	$Na_v1.1$ = 37 $Na_v1.5$ = 37 $Na_v1.7$ = 36	2 2 2	0.54 (0.30-0.98)	4	0.20 (0.10-0.38)	3	> 30	TNT mechanical allodynia	*P* <0.05 (comparable to 10 mg/kg pregabalin)	0.20 (40 mg/kg)
18	0.26 (0.10-0.66)	4	SHSY5Y* = 10 (6.4-16.0) $Na_v1.5$ = 12 (7.0-20.9)	5 4	0.33 (0.11-0.98)	6	ND	-	> 30	TNT mechanical allodynia	*P* <0.05 (comparable to 10 mg/kg pregabalin)	0.19 (30 mg/kg)

Table 3. $hNa_v1.8$ potency (IC_{50}), selectivity (IC_{50}) and antiallodynic effects in rodent models of neuropathic pain for 3, 13 and 18. IC_{50} values for **3**, **13** and **18** at recombinantly expressed hNav1.8/β1 (Merck Millipore) and at TTX-R in rat and human DRG were determined using manual patch clamp electrophysiology. hNav subtype selectivity for **3** was also measured using manual patch clamp electrophysiology. IC_{50} vlaues determined using manual patch clamp electrophysiology were determined at the respective V0.5 of inactivation for TTX-R and each channel isoform. For **13** and **18** human sodium channel subtype selectivity was measured using IonWorks Quattro and FRET respectively. For the latter SHSY5Y cells expressing hNav1.2, 1.3 and 1.7 were also used. The voltage protocol for the IonWorks Quattro and assay methodology for the FRET assay is detailed in the supplementary information.

Chemistry experimental.

Analytical methods.

^{1}H nuclear magnetic resonance (NMR) spectra were in all cases consistent with the proposed structures. Characteristic chemical shifts (δ) are given in parts-per-million, downfield from tetramethylsilane, using conventional abbreviations for designation of major peaks: e.g. s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. The following abbreviations and chemical formulae have been used for NMR solvents: $CDCl_3$, deuterochloroform; DMSO-d_6, deuterodimethylsulphoxide; CD_3OD, deuteromethanol. Mass spectra (MS) were recorded using either electrospray ionisation (ESI) or atmospheric pressure chemical ionisation (APCI). LCMS indicates liquid chromatography mass spectrometry (t = retention time). Two different methods were used for LCMS. Unless indicated, all LCMS data were obtained using conditions A.

LCMS conditions A

A: 0.1 % formic acid in water
B: 0.1 % formic acid in acetonitrile
Column: C18 phase Phenomenex Gemini 50 × 4.6 mm, 5 µm particle size
Gradient: 95–5% A over 3 min, 1 min hold, 1 mL/min
UV: 210–450 nm DAD
Temperature: 75 °C

LCMS conditions B

A: 0.1 % methanesulfonic acid in water
B: acetonitrile
Column: Waters BEH RP C18 2.1 x 100 mm 1.7 µm
Gradient: 95%–0% A over 8.2 min, 0.5 min hold, 0.5 mL/min
UV: 210 nm
Temperature: 45 °C

Certain examples were purified using automated preparative high-performance liquid chromatography (HPLC):

Reverse-phase HPLC conditions were on FractionLynx systems. Samples were submitted dissolved in 1 mL of DMSO. Depending on the nature of the compounds and the results of pre-analysis, purification was performed under either acidic or basic conditions at ambient temperature. Acidic runs were carried out on a Sunfire Prep C18 OBD column (19 × 50 mm, 5 µm). Basic runs were carried out on an Xterra Prep MS C18 (19 × 50 mm, 5 µm), both from Waters. A flow rate of 18 mL/min was used with mobile phase: A, water + 0.1 % modifier (v/v); B, acetonitrile + 0.1 % modifier (v/v). For acidic runs, the modifier was formic acid; for basic runs, the modifier was diethylamine. A Waters 2525 binary LC pump supplied a mobile phase with a composition of 5% B for 1 min then ran from 5% to 98% B over 6 min, followed by a 2 min hold at 98% B. Detection was achieved using a Waters 2487 dual-wavelength absorbance detector set at 225 nm followed, in series, by a Polymer Labs PL-ELS 2100 detector and a Waters ZQ 2000 4-way MUX mass spectrometer in parallel. The PL 2100 ELSD was set at 30 °C with 1.6 L/min supply of nitrogen. The Waters ZQ MS was tuned with the following parameters:

ES+ Cone voltage: 30 v; Capillary: 3.20 kv

ES- Cone voltage: -30 v; Capillary: -3.00 kv

Desolvation gas: 600 L/h

Source Temp: 120 °C

Scan range: 150–900 Da

The fraction collection was triggered by both MS and ELSD.

Quality control analysis was performed using an LCMS method orthogonal to the preparative method. Acidic runs were carried out on a Sunfire C18 (4.6 × 50 mm, 5 μm); basic runs were carried out on a Xterra C18 (4.6 × 50 mm, 5 μm) - both from Waters. A flow rate of 1.5 mL/min was used with mobile phase: A, water + 0.1 % modifier (v/v); B, acetonitrile + 0.1 % modifier (v/v). For acidic runs, the modifier was formic acid; for basic runs, the modifier was diethylamine. A Waters 1525 binary LC pump ran a gradient elution from 5% to 95% B over 3 min, followed by a 1 min hold at 95% B. Detection was achieved using a Waters MUX UV 2488 detector set at 225 nm followed, in series, by a Polymer Labs PL-ELS 2100 detector and a Waters ZQ 2000 4-way MUX mass spectrometer, in parallel. The PL 2100 ELSD was set at 30 °C with 1.6 L/min supply of nitrogen. The Waters ZQ MS was tuned with the following parameters:

ES+ Cone voltage: 25 v; Capillary: 3.30 kv

ES- Cone voltage: -30 v; Capillary: -2.50 kv

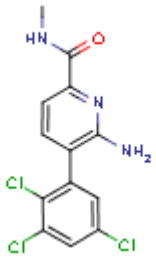
Desolvation gas: 800 L/h

Source Temp: 150 °C

Scan range: 160–900 Da

Purity criteria: Final compounds isolated as singletons >95% based on LCMS and/or 1H NMR. Final compounds isolated via autopurification methods > 90% based on LCMS.

Synthetic procedures.



6-Amino-*N*-methyl-5-(2,3,5-trichlorophenyl)picolinamide (3)

Step 1: *N*-(3-bromo-6-methylpyridin-2-yl)acetamide

Acetic anhydride (21 mL, 223 mmol) was added to a solution of 2-amino-3-bromo-6-picoline (10 g, 53.46 mmol) in dioxan (50 mL) and the mixture stirred at 50 °C for 18 h. The solvent was then evaporated under reduced pressure and the residue diluted with saturated sodium hydrogen carbonate solution (150 mL). The precipitate was filtered, washed with water and

re-dissolved in dichloromethane. The filtrate was neutralised to pH7 with saturated sodium hydrogen carbonate solution and extracted with dichloromethane (3 × 100 mL). The organic layers were combined, washed with water, dried over $MgSO_4$ and concentrated *in vacuo* to give a white solid that was purified by column chromatography on silica gel, eluting with ethyl acetate:heptane, 75:25, to afford the title compound as a white solid (9.2 g, 75% yield). ^{1}H NMR (400 MHz $CDCl_3$) δ: 3.03 (s, 3H), 4.40 (br s, 2H), 7.25 (d, *J* = 2.5Hz, 1H), 7.44 (d, *J* = 7.6Hz, 1H), 7.56 (d, *J* = 2.5Hz, 1H), 7.67 (d, *J* = 7.6Hz, 1H). MS: *m/z* APCI 231 $[M + H]^+$. Microanalysis: $C_8H_9BrN_2O$ requires: C 41.95%; H 3.96%; N 12.23%; found: C 41.92%; H 3.91%; N 12.16%.

Step 2: Methyl 6-amino-5-bromopicolinate

Potassium permanganate (9.77 g, 61.81 mmol) was added portionwise to a solution of *N*-(3-bromo-6-methylpyridin-2-yl)acetamide (Step 1) (4.8 g, 20.95 mmol) in water (100 mL) and pyridine (8 drops) and the mixture heated at 75 °C for 18 h. Further potassium permanganate (3.31 g, 61.81 mmol) was then added to the mixture and stirring continued at 75 °C for 18 h. The reaction mixture was then filtered through Celite® and the filtrate washed with ethyl acetate (6 × 50 mL). The aqueous solution was concentrated *in vacuo* to give a pale yellow solid that was azeotroped with toluene (5 × 50 mL) at 50 °C to afford the crude potassium salt, which was then dissolved in methanol (400 mL) and heated under reflux. Concentrated sulphuric acid (5 mL) was then added to the mixture and heating continued for 48 h. The solvent was then evaporated under reduced pressure, the residue basified to pH8 with a saturated sodium hydrogen carbonate solution (150 mL) and extracted with dichloromethane (3 × 50 mL). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo* to afford the title compound as a pale yellow solid (1.65 g, 34% yield). ^{1}H NMR (400 MHz CD_3OD) δ: 3.90 (s, 3H), 7.25 (d, 1H), 7.88 (d, 1H). LRMS: *m/z* ESI 233 $[M + H]^+$. Microanalysis: $C_7H_7BrN_2O_2$ requires: C 36.39%; H 3.05%; N 12.12%; found: C 36.24%; H 3.08%; N 11.94%.

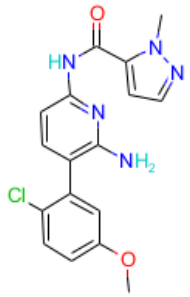
Step 3: 6-Amino-5-bromo-*N*-methylpicolinamide

Methylamine (2 M, in THF, 36.8 mL, 73.64 mmol) was added to a suspension of methyl 6-amino-5-bromopicolinate (Step 2) (1.70 g, 7.36 mmol) in methanol (10 mL) and the mixture stirred for 18 h at room temperature. The reaction mixture was then concentrated *in vacuo* and the residue purified by column chromatography on silica gel, eluting with ethyl acetate:heptane, 75:25, to afford the title compound as a solid (1.63 g, 96% yield). ^{1}H NMR (400 MHz CD_3OD) δ: 2.90 (s, 3H), 7.20 (d, 1H), 7.82 (d, 1H). MS: *m/z* APCI 231 $[M + H]^+$. Microanalysis: $C_7H_8BrN_3O$ requires: C 36.55%; H 3.50%; N 18.26%; found: C 36.50%; H 3.47%; N 18.12%.

Step 4: 6-Amino-*N*-methyl-5-(2,3,5-trichlorophenyl)picolinamide (**3**)

A solution of bis(tri-*tert*-butylphosphine)palladium(0) (135 mg, 0.27 mmol) in THF (11 mL) was added to a mixture of 6-amino-5-bromo-*N*-methylpicolinamide (Step 3) (1.36 g, 5.92 mmol), potassium fluoride (1.14 g, 19.55 mmol), 2,3,5-trichlorobenzeneboronic acid (1.46 g, 6.51 mmol) and *tris*(dibenzylideneacetone)dipalladium(0) (81 mg, 0.09 mmol) in THF (27 mL) and the reaction mixture stirred under nitrogen for 18 h at room temperature. The mixture was then filtered through Arbocel® and washed with THF. The filtrate was concentrated *in vacuo* and purified by column chromatography on silica gel, eluting with heptane:ethyl acetate, 50:50, to afford the title compound as a white solid (1.57 g, 80% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.82 (d, *J* = 4.8 Hz, 3H), 5.91 (s, 2H), 7.25 (d, *J* =

7.4 Hz, 1H), 7.41 – 7.48 (m, 2H), 7.88 (d, *J* = 2.6 Hz, 1H), 8.23 (d, *J* = 5.1 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 25.8, 109.7, 119.8, 129.6, 130.1, 130.4, 132.2, 133.3, 138.9, 139.5, 148.7, 155.2, 164.4; LCMS: *t* = 3.47 min (conditons B); HRMS–ESI (*m/z*): $[M + H]^+$ calcd for $C_{13}H_{11}Cl_3N_3O$, 329.9968; found, 329.9965; Microanalysis: $C_{13}H_{10}Cl_3N_3O$ requires: C 47.23%; H 3.05%; N 12.71%; found: C 47.15%; H 3.18%; N 12.55%.



_N_-(6-amino-5-(2-chloro-5-methoxyphenyl)pyridin-2-yl)-1-methyl-1_H_-pyrazole-5-carboxamide (13)

Step 1: 3-Iodopyridine-2,6-diamine

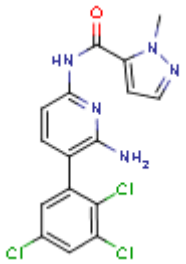
To a solution of 2,6-diaminopyridine (20 g, 0.18 mol) in 2-methyl-THF (400 mL) was added potassium carbonate (25.3 g, 0.18 mol). To this suspension was added a solution of iodine (46.6 g, 0.18 mol) in 2-methyl-THF (100 mL) dropwise over 1 h and the reaction stirred for 2 h at room temperature. The reaction was filtered through a pad of Celite®, and the filtrate washed with water (200 mL) and saturated aqueous sodium thiosulphate solution. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*, azeotroping with dichloromethane to afford a light-brown solid, which was stirred in methanol (500 mL) for 15 minutes. The suspension was filtered and the filtrate concentrated *in vacuo*. The residue was stirred with methanol (50 mL) for another 15 minutes and the solid collected by filtration and dried to furnish an off-white solid (14.3 g). The filtrate was concentrated *in vacuo* and again stirred with methanol (15 mL). The resulting solid was filtered to furnish a solid (12.2 g). These two batches were combined to afford the title compound (26.5 g, 62 % yield). ^{1}H NMR (400 MHz, DMSO-d_6): 5.42 (s, 2H), 5.56 (d, 2H), 5.65 (s, 1H), 7.36 (d, 1H).

Step 2: 3-(2-Chloro-5-methoxyphenyl)pyridine-2,6-diamine

To a suspension of 3-iodopyridine-2,6-diamine (Step 1) (2 g, 8.51 mmol) in 1,4-dioxane (10 mL) and water (5 mL) was added 2-chloro-5-methoxyphenyl boronic acid (0.793 g, 4.25 mmol), caesium carbonate (2.77 g, 8.51 mmol) and palladium tetrakis(triphenylphosphine) (0.123 g, 0.0125 mmol). The reaction was purged with nitrogen and heated at 80 °C for 20 min. Three further portions of palladium tetrakis(triphenylphosphine) (0.123 g, 0.0125 mmol) and 2-chloro-5-methoxyphenyl boronic acid (0.793 g, 4.25 mmol) were added at 20 min intervals. The reaction was heated at 80 °C for 18 h then concentrated *in vacuo*. The residue was taken up in ethyl acetate (20 mL) and washed with a saturated aqueous solution of brine (20 mL), then dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography, eluting with 50:50 to 100:0 ethyl acetate:pentane to afford the title compound as a brown foam (1.157 g, 55% yield). MS *m/z* 250 $[M + H]^+$. ^{1}H NMR (400MHz, $CDCl_3$) δ: 3.79 (s, 3H), 4.23 (br s, 2H), 4.32 (br s, 2H), 6.00 (d, 1H), 6.86 (m, 2H), 7.14 (d, 1H), 7.38 (d, 1H).

Step 3: *N*-(6-amino-5-(2-chloro-5-methoxyphenyl)pyridin-2-yl)-1-methyl-1*H*-pyrazole-5-carboxamide (**13**)

To a solution of 1-methyl-1*H*-pyrazole-5-carboxylic acid (5.28 g, 41.9 mmol) in dichloromethane (55 mL) was added oxalyl chloride (9.14 mL, 104.8 mmol) followed by three drops of DMF. The reaction was stirred at room temperature for 18 h then concentrated *in vacuo*. The residue was dissolved in acetonitrile (42 mL) and added dropwise to a cooled solution of 3-(2-chloro-5-methoxyphenyl)pyridine-2,6-diamine (Step 2) (9.5 g, 38 mmol) and lutidine (6.6 mL, 57.1 mmol) in acetonitrile (650 mL). The reaction was allowed to warm to room temperature and stirred under nitrogen for 2 h, then quenched by the addition of water (300 mL) and concentrated to low volume *in vacuo*. The aqueous residue was washed with dichloromethane (2 × 300 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography eluting with ethyl acetate:heptane 1:4, to give a solid that was recrystallised from toluene (100 mL) to afford the title compound (6.7 g). ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.78 (s, 3H), 4.09 (s, 3H), 5.34 (s, 2H), 6.91 (d, *J* = 2.9 Hz, 1H), 6.99 (dd, *J* = 8.8, 3.0 Hz, 1H), 7.23 (d, *J* = 1.9 Hz, 1H), 7.31 (d, *J* = 8.1 Hz, 1H), 7.40 (d, *J* = 8.0 Hz, 1H), 7.46 (d, *J* = 8.9 Hz, 1H), 7.51 (d, *J* = 2.0 Hz, 1H), 10.32 (s, 1H).; ^{13}C NMR (150 MHz, DMSO-d_6) δ 55.5, 55.5, 102.8, 108.6, 114.5, 115.3, 117.0, 124.2, 130.5, 134.9, 137.0, 137.1, 139.9, 149.7, 155.0, 158.2, 158.3. LCMS: *t* = 2.93 min (conditons B); HRMS–ESI (*m/z*): $[M + H]^+$ calcd for $C_{17}H_{17}ClN_5O_2$, 358.1071; found, 358.1070.



_N_-(6-amino-5-(2,3,5-trichlorophenyl)pyridin-2-yl)-1-methyl-1*H*-pyrazole-5-carboxamide (9)

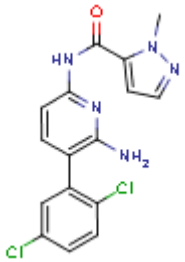
Step 1: 3-(2,3,5-trichlorophenyl)pyridine-2,6-diamine

Prepared using the same method as **13 Step 2**, with 2,3,5-trichlorophenyl boronic acid, 1.5 equivalents of cesium carbonate and 0.1 equivalents of palladium tetrakis(triphenylphosphine) added at 75 °C. The reaction was heated at 75 °C for 22 h. Extra palladium tetrakis(triphenylphosphine) (0.003 equivalents) was added and heating continued for 18 h. The product was purified by recrystallisation from toluene to afford the title compound. MS *m/z* 288 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 5.15 (br s, 2H), 5.61 (br s, 2H), 5.75 (d, 1H), 6.91 (d, 1H), 7.28 (s, 1H), 7.72 (s, 1H).

Step 2: *N*-(6-amino-5-(2,3,5-trichlorophenyl)pyridin-2-yl)-1-methyl-1*H*-pyrazole-5-carboxamide (**9**)

Oxalyl chloride (0.088 g, 0.693 mmol) was added to a slurry of 1-methyl-1*H*-pyrazole-5-carboxylic acid (0.066 g, 0.523 mmol) in dichloromethane (2 mL). One drop of DMF was added and the reaction left to stir at room temperature for 2 h. The reaction was concentrated *in vacuo* and azeotroped with dichloromethane. The residue was dissolved in THF (2 mL) and to this was added diisopropylethylamine (0.0956 g, 0.693 mmol) and 4-pyrrolidinopyridine (0.005 g, 0.035 mmol). The solution was cooled to 0 °C and 3-(2,3,5-trichlorophenyl)pyridine-2,6-diamine (Step 1) (0.100 g, 0.347 mmol) was added portionwise over 1 min. The reaction was then warmed to room temperature and stirred for 18 h. The mixture was diluted with dichloromethane and washed with a saturated aqueous solution of NH_4Cl (10 mL), followed by a saturated aqueous solution of $NaHCO_3$ (10 mL) and then

water (10 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to afford a brown gum, which was purified by trituration with pentane to afford the title compound as a yellow oil. MS *m/z* 396 $[M + H]^+$. ^1H NMR (400MHz, DMSO-d_6) δ: 4.09 (s, 3H), 5.60 (br s, 2H), 7.22 (d, 1H), 7.33 (d, 1H), 7.38 (d, 1H), 7.42 (d, 1H), 7.50 (d, 1H), 7.84 (d, 1H), 10.30 (br s, 1H); HRMS–ESI (*m/z*): $[M + H]^+$ calcd for $C_{16}H_{13}Cl_3N_5O$, 396.0180; found, 396.0178.



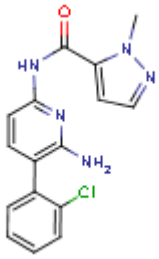
_N_-(6-amino-5-(2,5-dichlorophenyl)pyridin-2-yl)-1-methyl-1_H_-pyrazole-5-carboxamide (11)

Step 1: 3-(2,5-Dichlorophenyl)pyridine-2,6-diamine

Prepared using the same method as **13 Step 2**, with 2,5-dichlorophenyl boronic acid. MS *m/z* 254 $[M + H]^+$. ^1H NMR (400MHz, $CDCl_3$) δ: 4.23 (br s, 2H), 4.34 (br s, 2H), 6.00 (d, 1H), 7.13 (d, 1H), 7.24-7.26 (m, 1H), 7.33 (s, 1H), 7.43 (d, 1H).

Step 2: *N*-(6-amino-5-(2,5-dichlorophenyl)pyridin-2-yl)-1-methyl-1*H*-pyrazole-5-carboxamide (**11**)

Oxalyl chloride (4.53 g, 35.7 mmol) was added to a slurry of 1-methyl-1*H*-pyrazole-5-carboxylic acid (3.00 g, 23.8 mmol) in dichloromethane (150 mL). Five drops of DMF were added and the reaction left to stir at room temperature for 4 h. The reaction was concentrated *in vacuo* to half the volume of dichloromethane. A portion (0.825 mL) of this acid chloride solution in dichloromethane was added to a cooled solution of 3-(2,5-dichloro)pyridine-2,6-diamine (Step 1) (0.376 g, 1.485 mmol) in anhydrous pyridine (5 mL) and stirred at room temperature for 16 h. The reaction was concentrated *in vacuo*, then partitioned between saturated $NaHCO_3$ solution (20 mL) and dichloromethane (20 mL). The dichloromethane layer was washed with a saturated solution of brine (20 mL), dried over Na_2SO_4 and concentrated *in vacuo.* The residue was purified by silica gel column chromatography eluting with 50:50 ethyl acetate:heptane to afford the title compound (0.146 g, 27% yield). LCMS: *t* = 3.37 min; ^1H NMR (400MHz, $CDCl_3$): 4.26 (s, 3H), 4.32 (br s, 2H), 6.71 (s, 1H), 7.31–7.51 (m, 5H), 7.72 (d, 1H), 8.13 (br s, 1H); HRMS–ESI (*m/z*): $[M + H]^+$ calcd for $C_{16}H_{14}Cl_2N_5O$, 362.0570; found, 362.0565.



_N_-(6-amino-5-(2-chlorophenyl)pyridin-2-yl)-1-methyl-1_H_-pyrazole-5-carboxamide (15)

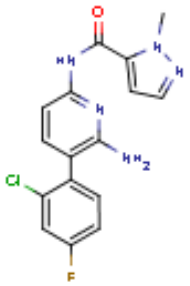
Step 1: 3-(2-Chlorophenyl)-pyridine-2,6-diamine

Prepared using the same method as **13 Step 2**, with 1.2 equivalents of 2-chlorophenyl boronic acid and 0.08 equivalents of palladium tetrakis(triphenylphosphine), added at 80 °C. The

product was purified by silica gel column chromatography, eluting with 80:20 to 100:0 ethyl acetate:heptane. LCMS: *t* = 1.68 min. MS *m/z* 220 $[M + H]^+$. ^{1}H NMR (400MHz, $CDCl_3$) δ: 4.15 (br s, 2H), 4.25 (br s, 2H), 5.99 (d, 1H), 7.12 (d, 1H), 7.22–7.37 (m, 3H), 7.49 (d, 1H).

Step 2: *N*-(6-amino-5-(2-chlorophenyl)pyridin-2-yl)-1-methyl-1*H*-pyrazole-5-carboxamide (**15**)

Oxalyl chloride (0.453 g, 3.57 mmol) was added to a slurry of 1-methyl-1*H*-pyrazole-5-carboxylic acid (0.150 g, 1.19 mmol) in dichloromethane (7 mL). One drop of DMF was added and the reaction left to stir at room temperature for 2.5 hours. The reaction was concentrated *in vacuo* and azeotroped with dichloromethane. The residue was dissolved in CH_3CN to make a 1 M solution. A portion (0.260 mL) of this 1 M solution of acid chloride (0.260 mmol) in CH_3CN was added to a cooled solution of 3-(2-chlorophenyl)pyridine-2,6-diamine (Step 1) (0.055 g, 0.250 mmol) and lutidine (0.035 mL, 0.300 mmol) in CH_3CN (2 mL). The reaction was warmed to room temperature and stirred for 18 h. A further 0.130 mL of the 1 M solution of acid chloride (0.130 mmol) in CH_3CN, and lutidine (0.017 mL, 0.15 mmol) were added to the reaction, and stirred at room temperature for a further 18 h, then concentrated *in vacuo.* The residue was taken up in ethyl acetate (60 mL) and washed with saturated aqueous $NaHCO_3$ solution (20 mL), then dried over Na_2SO_4 and concentrated *in vacuo* to afford a golden oil. The residue was purified by preparative HPLC. LCMS: *t* = 3.03 min; ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.10 (s, 3H), 5.30 (s, 2H), 7.22 (d, *J* = 2.1 Hz, 1H), 7.30 (d, *J* = 7.9 Hz, 1H), 7.39 – 7.35 (m, 1H), 7.45 – 7.39 (m, 3H), 7.50 (d, *J* = 2.0 Hz, 1H), 7.60 – 7.56 (m, 1H), 10.32 (s, 1H); HRMS–ESI (*m/z*): $[M + H]^+$ calcd for $C_{16}H_{15}ClN_5O$, 328.0960; found, 328.0963.

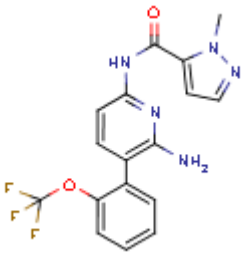


_N_-(6-amino-5-(2-chloro-4-fluorophenyl)pyridin-2-yl)-1-methyl-1_H_-pyrazole-5-carboxamide (21)

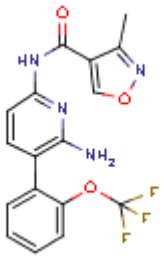
Step 1: *N*-(6-amino-5-iodopyridin-2-yl)-1-methyl-1*H*-pyrazole-5-carboxamide

To a suspension of 1-methyl-1*H*-pyrazole-5-carboxylic acid (0.400 g, 3.15 mmol) in thionylchloride (15 mL, 3.15 mmol) was added two drops of DMF. The reaction was left to stir at room temperature for 20 h. The reaction was then concentrated *in vacuo* and azeotroped with dichloromethane (10 mL). The residue was dissolved in CH_3CN to make a 0.25 M solution. A portion (7.12 mL) of this 0.25 M solution of acid chloride (1.78 mmol) in CH_3CN was added to a cooled solution of 3-iodopyridine-2,6-diamine (**13** Step 1) (0.380 g, 1.62 mmol) and lutidine (0.272 mL, 2.43 mmol) in CH_3CN (20 mL). The reaction was warmed to room temperature and stirred for 24 h, then concentrated *in vacuo.* The residue was taken up in ethyl acetate and washed with water, then dried over Na_2SO_4 and concentrated *in vacuo.* The residue was triturated with dichloromethane to afford the title compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.06 (s, 3H), 5.84 (br s, 2H), 7.15 (d, 1H), 7.19 (s, 1H), 7.48 (s, 1H), 7.89 (d, (s, 1H), 7.89 (d, 1H).

Step 2: *N*-(6-amino-5-(2-chloro-4-fluorophenyl)pyridin-2-yl)-1-methyl-1*H*-pyrazole-5-carboxamide (**21**).
N-(6-amino-5-iodopyridin-2-yl)-1-methyl-1*H*-pyrazole-5-carboxamide (Step 1) (0.040 g, 0.12 mmol) was combined with 2-chloro-4-fluorophenyl boronic acid (0.044 g, 0.232 mmol) and caesium carbonate (0.038 g, 0.116 mmol) and suspended in a mixture of 1,4-dioxane (2 mL) and water (1 mL). The reaction was heated to 80 °C in a small, sealed, reaction vial (Reacti-vial™), then palladium tetrakis(triphenylphosphine) (0.010 g, 0.0087 mmol) was added. The reaction was heated for 4 h then cooled to room temperature and concentrated *in vacuo*. The residue was partitioned between dichloromethane and a saturated aqueous solution of Na_2CO_3, then filtered through a phase separation cartridge and concentrated *in vacuo*. The residue was purified by preparative HPLC to afford the title compound. LCMS: *t* = 3.13 min; HRMS–ESI (*m/z*): $[M + H]^+$ calcd for $C_{16}H_{14}ClFN_5O$, 346.0865; found, 346.0864.



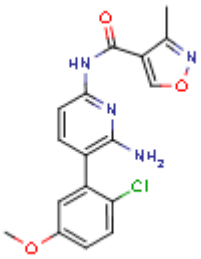
_N_-(6-amino-5-[2-(trifluoromethoxy)phenyl]pyridin-2-yl)-1-methyl-1*H*-pyrazole-5-carboxamide (17)
Prepared using the same method as **21** Step 2, using *N*-(6-amino-5-iodopyridin-2-yl)-1-methyl-1*H*-pyrazole-5-carboxamide (**21** Step 1), 2-(trifluoromethoxy)phenyl boronic acid and 0.074 equivalents of palladium tetrakis(triphenylphosphine), stirred for 5 h. LCMS: *t* = 3.59 min; ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.09 (s, 3H), 5.38 (s, 2H), 7.23 (d, *J* = 2.0 Hz, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 7.41 (d, *J* = 8.0 Hz, 1H), 7.49 – 7.44 (m, 3H), 7.56 – 7.49 (m, 2H), 10.32 (s, 1H), HRMS–ESI (*m/z*): $[M + H]^+$ calcd for $C_{17}H_{15}F_3N_5O_2$, 378.1178; found, 378.1176.



_N_-(6-amino-5-[2-(trifluoromethoxy)phenyl]pyridin-2-yl)-3-methylisoxazole-4-carboxamide (18)
Step 1: 3-(2-(Trifluoromethoxy)phenyl)pyridine-2,6-diamine
Prepared using the same method as **13** Step 2, in a small, sealed reaction vial (Reacti-vial™), using 1.4 equivalents of 2-(trifluoromethoxy)phenyl boronic acid, 1 equivalent of caesium carbonate and 0.07 equivalents of palladium tetrakis(triphenylphosphine), added at 75 °C, stirred for 5 h. A further 0.035 equivalents of catalyst, 0.6 equivalents of boronic acid and 0.6 equivalents of caesium carbonate were added and the reaction stirred for 1.5 h. LCMS: *t* = 1.58 min. MS: *m/z* 270 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.87 (br s, 2H), 5.55 (br s, 2H), 5.78 (m, 2H), 6.92 (d, 1H), 7.33–7.64 (m, 3H).

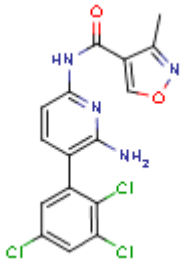
Step 2: *N*-(6-amino-5-[2-(trifluoromethoxy)phenyl]pyridin-2-yl)-3-methylisoxazole-4-carboxamide (**18**).

Oxalyl chloride (1.46 g, 11.5 mmol) was added to a slurry of 3-methylisoxazole-4-carboxylic acid (0.50 g, 3.93 mmol) in dichloromethane (30 mL). Two drops of DMF were added and the reaction left to stir at room temperature for 18 h. The reaction was concentrated *in vacuo* and azeotroped with dichloromethane. The residue was dissolved in CH_3CN to make a 1 M solution. A portion (2.5 mL) of this 1 M solution of acid chloride (2.50 mmol) in CH_3CN was added to a cooled solution of the 3-(2-(trifluoromethoxy)phenyl)pyridine-2,6-diamine (Step 1) (0.50 g, 1.86 mmol) and lutidine (0.33 mL, 2.97 mmol) in CH_3CN (30 mL). The reaction was warmed to room temperature and stirred for 19 h, then concentrated *in vacuo.* The residue was taken up in ethyl acetate and washed with a saturated aqueous solution of $NaHCO_3$, then concentrated *in vacuo.* The residue was purified by silica gel column chromatography eluting with 15:85 to 50:50 ethyl acetate:heptane to afford the title compound (0.565 g, 80% yield); ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.43 (s, 3H), 5.35 (s, 2H), 7.31 (d, *J* = 8.1 Hz, 1H), 7.42 (d, *J* = 8.0 Hz, 1H), 7.44 – 7.50 (m, 3H), 7.51 – 7.56 (m, 1H), 9.56 (s, 1H), 10.41 (s, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 10.5, 102.4, 111.2, 115.3, 119.9 (q, *J* = 256.8 Hz), 121.5, 127.9, 129.5, 131.0, 132.3, 140.3, 146.2, 150.0, 155.2, 158.6, 159.3, 161.0; LCMS: *t* = 3.17 min (conditons B); HRMS–ESI (*m/z*): $[M + H]^+$ calcd for $C_{17}H_{14}F_3N_4O_3$, 379.1018; found, 379.0971.



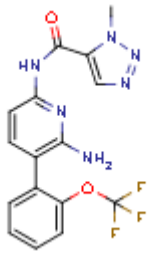
***N*-[6-amino-5-(2-chloro-5-methoxyphenyl)pyridin-2-yl]-3-methylisoxazole-4-carboxamide (12)**

To a suspension of 3-methylisoxazole-4-carboxylic acid (2.73 g, 21.48 mmol) in dichloromethane (10 mL) was added oxalyl chloride (2.62 mL, 30.1 mmol) followed by two drops of DMF. The reaction was stirred at room temperature for 18 h then concentrated *in vacuo.* The residue was azeotroped with dichloromethane, dissolved in acetonitrile (15 mL) and added dropwise to a cooled solution of 3-(2-chloro-5-methoxyphenyl)pyridine-2,6-diamine (**13**, Step 2) (5.2 g, 20.82 mmol) and lutidine (3.15 mL, 27.1 mmol) in acetonitrile (150 mL). The reaction was allowed to warm to room temperature and stirred under nitrogen for 30 min. The reaction was quenched by the addition of water (100 mL), extracted into ethyl acetate (200 mL), dried ($MgSO_4$) and concentrated *in vacuo.* The residue was purified by silica gel column chromatography eluting with ethyl acetate:heptane 1:2, to furnish a pale yellow solid. This was triturated with *t*-butyl methyl ether, filtered, and recrystallised from ethyl acetate to give the title product as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.40 (s, 3H), 3.95 (s, 3H), 5.30 (br s, 2H), 6.48-6.53 (m, 1H), 6.90-6.95 (m, 1H), 7.28-7.32 (m, 1H), 7.40–7.45 (m, 2H), 9.55 (s, 1H), 10.40 (br s, 1H); LCMS: *t* = 2.92 min; HRMS–ESI (*m/z*): $[M + H]^+$ calcd for $C_{17}H_{16}ClN_4O_3$, 359.0905; found, 359.0908.



_N_-(6-amino-5-(2,3,5-trichlorophenyl)pyridin-2-yl)-3-methylisoxazole-4-carboxamide (10)

Prepared using the same method as **9 Step 2** using the acid chloride prepared from 3-methylisoxazole-4-carboxylic acid. Purified by preparative HPLC. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.42 (s, 3H), 5.55 (br s, 2H), 7.31 (d, 1H), 7.39 (m, 2H), 7.83 (s, 1H), 9.54 (s, 1H), 10.38 (br s, 1H); HRMS–ESI (*m/z*): $[M + H]^+$ calcd for $C_{16}H_{12}Cl_3N_4O_2$, 397.0020; found, 397.0017.



_N_-(6-amino-5-[2-(trifluoromethoxy)phenyl]pyridin-2-yl)-1-methyl-1*H*-1,2,3-triazole-5-carboxamide (19)

Step 1: 1-Methyl-1*H*-1,2,3-triazole

Sodium methoxide, prepared from sodium (1.8 g, 79.8 mmol) and methanol (30 mL) was added to a cooled solution of 1*H*-1,2,3-triazole (5 g, 72.5 mmol) and stirred at 0 °C for 30 min. Iodomethane (5 mL, 79.8 mmol) was then added dropwise and the reaction warmed to room temperature and stirred for 24 h. The reaction was concentrated *in vacuo* and the residue partitioned between dichloromethane and 1 M aqueous NaOH. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to afford the title compound as a yellow oil (2.5 g, 42% yield). ^{1}H NMR (400MHz, $CDCl_3$) δ: 4.11 (s, 3H), 7.53 (s, 1H), 7.69 (s, 1H).

Step 2: 1-Methyl-1*H*-1,2,3-triazole-5-carbaldehyde

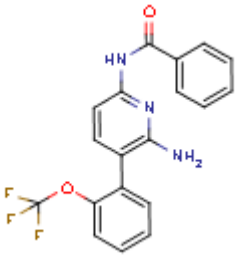
To a solution of 1-methyl-1*H*-1,2,3-triazole (Step 1) (0.1 g, 1.2 mmol) in THF (10 mL) at -78 °C was added dropwise 1.6 M *n*-butyl lithium (0.9 mL, 1.4 mmol), maintaining the temperature below -60 °C. The reaction was stirred at -78 °C for 30 min, then DMF (0.14 mL, 1.8 mmol) was added. The reaction was warmed to room temperature and stirred for 1 h. The reaction was then quenched with water and extracted with ethyl acetate (3 × 10 mL). The combined organic extracts were washed with water (3 × 10 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography, eluting with 95:5 to 100:0 dichloromethane:methanol, to afford the title compound as a yellow oil (0.04 g, 30% yield). ^{1}H NMR (400MHz, $CDCl_3$) δ: 4.10 (s, 3H), 7.88 (s, 1H), 9.55 (s, 1H).

Step 3: 1-Methyl-1*H*-1,2,3-triazole-5-carboxylic acid

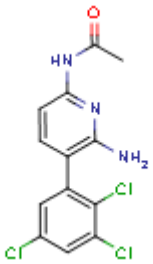
To a solution of 1-methyl-1*H*-1,2,3-triazole-5-carbaldehyde (Step 2) (5 g, 45 mmol) and sodium hydroxide (8.59 g, 215 mmol) in water (120 mL) at 15 °C was added dropwise a solution of potassium permanganate (5.83 g, 36.9 mmol) in water (120 mL). The reaction was stirred at room temperature for 30 min and then heated to reflux for 1 h. The reaction was

filtered and the filtrate acidified to pH3 with concentrated HCl, then extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were washed with saturated aqueous brine, dried over Na_2SO_4 and concentrated *in vacuo* to afford the title compound. LCMS: *t* = 1.72 min. MS: *m/z* 128 $[M + H]^+$. ^{1}H NMR (400MHz, CD_3OD) δ: 4.31(s, 3H), 8.14 (s, 1H).

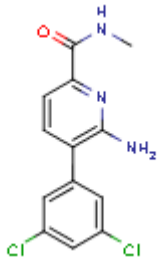
Step 4: *N*-(6-amino-5-[2-(trifluoromethoxy)phenyl]pyridin-2-yl)-1-methyl-1*H*-1,2,3-triazole-5-carboxamide (**19**)
Prepared using the same method as **11** Step 2, with 3-(2-(trifluoromethoxy)phenyl)pyridine-2,6-diamine (**18**, Step 1) and 1 equivalent of the acid chloride prepared from 1-methyl-1*H*-1,2,3-triazole-5-carboxylic acid (Step 3), stirred at 60 °C for 1 h. The product was purified by preparative HPLC to afford the title compound. LCMS: *t* = 3.44 min. MS: *m/z* 379 $[M + H]^+$; HRMS–ESI (*m/z*): $[M + H]^+$ calcd for $C_{16}H_{14}F_3N_6O_2$, 379.1125; found, 379.1128.



***N*-(6-amino-5-[2-(trifluoromethoxy)phenyl]pyridin-2-yl)benzamide (20)**
Prepared using the same method as **11** Step 2, with 3-(2-(trifluoromethoxy)phenyl)pyridine-2,6-diamine (**18**, Step 1) and 1.2 equivalents of benzoyl chloride, stirred for 18 h. The product was purified by preparative HPLC to afford the title compound. LCMS: *t* = 3.35 min; HRMS–ESI (*m/z*): $[M + H]^+$ calcd for $C_{19}H_{14}F_3N_3O_2$, 374.1116; found, 374.1089.

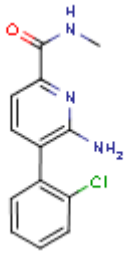


***N*-(6-amino-5-(2,3,5-trichlorophenyl)pyridin-2-yl)acetamide (2)**
To a solution of 3-(2,3,5-trichlorophenyl)pyridine-2,6-diamine (**9**, Step 1) (1.0 g, 3.47 mmol) in THF (45 mL), cooled to 0 °C, was added diisopropylethylamine (0.621 mL, 3.99 mmol) and the mixture stirred for 5 min. A solution of acetyl chloride (0.259 mL, 3.64 mmol) in THF (5 mL) was then added dropwise over 30 min, maintaining the temperature at 0 °C. Following complete addition, the reaction was stirred at 0 °C for 2 h, then partitioned between water (200 mL) and ethyl acetate (200 mL). The organic layer was separated and washed with water (100 mL), brine (100 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* and the residue purified by column chromatography over silica gel to afford the title compound as a pale solid (500 mg). ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.06 (s, 3H), 5.53 (s, 2H), 7.24 (d, *J* = 7.9 Hz, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 7.38 (d, *J* = 2.5 Hz, 1H), 7.83 (d, *J* = 2.5 Hz, 1H), 10.01 (s, 1H); LCMS: *t* = 2.81 min (conditions B); HRMS–ESI (*m/z*): $[M + H]^+$ calcd for $C_{13}H_{11}Cl_3N_3O$, 329.9968; found, 329.9963.



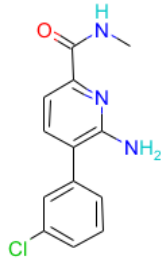
6-Amino-5-(3,5-dichlorophenyl)-*N*-methylpicolinamide (8)

To a suspension of 6-amino-5-bromo-*N*-methylpicolinamide (**3**, Step 3) (0.600 g, 2.61 mmol) in 1,4-dioxane (10 mL) and water (2 mL) was added 3,5-dichlorophenyl boronic acid (0.697 g, 3.65 mmol), caesium carbonate (0.850 g, 2.61 mmol) and palladium tetrakis(triphenylphosphine) (0.300 g, 0.26 mmol). The reaction was purged with nitrogen and heated at 80 °C for 18 h. The mixture was concentrated *in vacuo* and the residue partitioned between ethyl acetate (10 mL) and water (10 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel, eluting with heptane:ethyl acetate, 1:2, then by preparative HPLC, to afford the title compound as a white solid (204 mg, 26% yield). LCMS: *t* = 3.57–3.60 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.80 (d, *J* = 5.2Hz, 3H), 7.35–7.60 (m, 5H), 8.40 (br s, 1H).



6-Amino-5-(2-chlorophenyl)-*N*-methylpicolinamide (4)

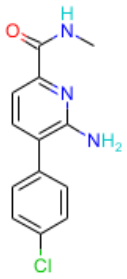
Prepared using the same method as **8**, with 2-chlorophenyl boronic acid. The catalyst was added at 80 °C and the reaction heated for 4.5 h in a small, sealed, reaction vial (Reacti-vial™). The product was purified by column chromatography on silica gel, eluting with heptane:ethyl acetate, 4:1 to 0:1 to afford title compound as a pale brown solid (341 mg, 86% yield). LCMS: *t* = 1.21 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.80 (d, *J* = 5.3Hz, 3H), 5.60 (br s, 2H), 7.27 (d, *J* = 7.5Hz, 1H), 7.35–7.45 (m, 4H), 7.58-7.62 (m, 1H), 8.25 (br s, 1H); HRMS–ESI (*m/z*): $[M + H]^+$ calcd for $C_{13}H_{13}ClN_3O$, 262.0747; found, 262.0739.



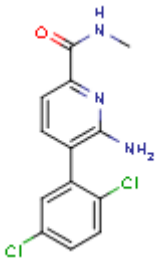
6-Amino-5-(3-chlorophenyl)-*N*-methylpicolinamide (5)

Prepared using the same method as **8**, with 3-chlorophenyl boronic acid, heated for 2 hours. The product was purified by column chromatography on silica gel, eluting with heptane:ethyl acetate, 2:1 to 1:9 to give an off-white solid that was triturated in diethyl ether. The solid was filtered to give the title compound as a white solid (43 mg, 50% yield); ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.82 (d, *J* = 5.0 Hz, 3H), 5.82 (s, 2H), 7.30 (d, *J* = 7.5 Hz, 1H), 7.42 – 7.48 (m,

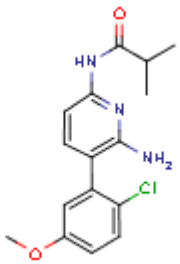
2H), 7.48 – 7.54 (m, 3H), 8.27 (d, *J* = 5.1 Hz, 1H); LCMS: *t* = 2.25 min (conditons B); HRMS–ESI (*m/z*): $[M + H]^+$ calcd for $C_{13}H_{13}ClN_3O$, 262.0747; found, 262.0739.



6-Amino-5-(4-chlorophenyl)-*N*-methylpicolinamide (6)
Prepared using the same method as **8**, with 4-chlorophenyl boronic acid, heated for 2 hours. The product was purified by column chromatography on silica gel, eluting with heptane:ethyl acetate, 2:1 to 1:9 to give an off-white solid that was triturated in diethyl ether. The solid was filtered to give the title compound as a white solid (41 mg, 47% yield). LCMS: 94.3% purity. MS: *m/z* ESI 262 $[M + H]^+$.

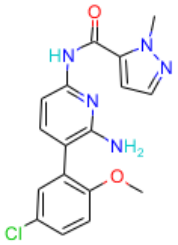


6-Amino-5-(2,5-dichlorophenyl)-*N*-methylpicolinamide (7)
Prepared using the same method as **8**, with 2,5-dichlorophenyl boronic acid, heated at 70 °C. Additional catalyst (0.03 equivalents.), cesium carbonate (0.6 equivalents) and boronic acid (0.6 equivalents) were added and heating continued for 10 h. The product was purified by column chromatography on silica gel, eluting with heptane:ethyl acetate, 3:2 to 2:3 to give an oil/solid that was recrystallized from diethyl ether to give the title compound as a white solid (57 mg, 22% yield). LCMS: *t* = 2.10 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.02-3.04 (d, *J* = 5.1Hz, 3H), 7.36 (s, 1H), 7.36-7.40 (m, 1H), 7.46-7.50 (m, 2H), 7.64-7.68 (m, 1H); HRMS–ESI (*m/z*): $[M + H]^+$ calcd for $C_{13}H_{12}Cl_2N_3O$, 296.0352; found, 296.0350.



***N*-(6-amino-5-(2-chloro-5-methoxyphenyl)pyridin-2-yl)isobutyramide (16)**
Prepared using the same method as **15** Step 2, with 3-(2-chloro-5-methoxyphenyl)pyridine-2,6-diamine (**13**, Step 2) and isobutyryl chloride, followed by purification by preparative

HPLC, to give the title compound. LCMS: *t* = 2.95–2.99 min. MS: *m/z* ESI 320 $[M + H]^+$; HRMS–ESI (*m/z*): $[M + H]^+$ calcd for $C_{16}H_{19}ClN_3O_2$, 320.1160; found, 320.1156.



***N*-[6-amino-5-(2-methoxy-5-chlorophenyl)pyridin-2-yl]-1-methyl-1*H*-pyrazole-5-carboxamide (14)**

Step 1: 3-(2-Methoxy-5-chlorophenyl)pyridine-2,6-diamine

Prepared using the same method as **13**, Step 2, with 2-methoxy-5-chlorophenyl boronic acid. The product was purified by trituration in heptane to give the title compound as a grey solid (2.2 g). LCMS: 100% purity.

Step 2: *N*-[6-amino-5-(2-methoxy-5-chlorophenyl)pyridin-2-yl]-1-methyl-1*H*-pyrazole-5-carboxamide (**14**).

Prepared using the same method as **13** Step 3, using 3-(2-methoxy-5-chlorophenyl)pyridine-2,6-diamine (Step 1) in pyridine. The title compound was isolated as a yellow solid (192 mg) LCMS: *t* = 3.41 min; HRMS–ESI (*m/z*): $[M + H]^+$ calcd for $C_{17}H_{17}ClN_5O_2$, 358.1065; found, 358.1064.