Supporting Information

Structure-based design of selective Janus kinase 2 imidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridine inhibitors

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Biochemical Assay:

JAK2 Tyrosine Kinase Assay:

The assays were performed in V-bottom 384-well plates. The final assay volume was 30 μL prepared from 15 μL additions of enzyme and substrate (fluoresceinated peptide and ATP) and test compounds in assay buffer (100 nM HEPES pH 7.4, 10 mM $MgCl_2$, 25 mM Beta-Glycerolphosphate, 0.015% Brij35 and 4 mM DTT). The reaction was initiated by the combination of JAK2 with substrates and test compounds. The reaction was incubated at room temperature for 60 min and terminated by adding 45 μL of 35 mM EDTA to each sample. The reaction mixture was analyzed on the Caliper LabChip 3000 by electrophoretic separation of the fluorescent substrate and phosphorylated product after 180 min. Inhibition data were calculated by comparison to no enzyme control reactions for 100% inhibition and vehicle-only reactions for 0% inhibition. The final concentration of reagents in the assay is 1.1 nM JAK2, 1.5 μM peptide substrate (5-FAM-KKKKEEIYFFFG-OH for JAK2), 30 mM ATP and 1.6% DMSO. Dose response curves were generated to determine the concentration required inhibiting 50% of kinase activity (IC_{50}). Compounds were dissolved at 10 mM in dimethylsulfoxide (DMSO) and evaluated at eleven concentrations, each in duplicate. IC_{50} values were derived by non-linear regression. All kinase inhibition data is analyzed by curve fitting software and the IC_{50}, dose response curve and Ymax is routinely reported to chemistry for all compounds tested.

The inhibitory activity of compounds against multiple other recombinant enzymes was evaluated using similar methodology in kinase assays. Key compounds were also tested in intrinsic aqueous solubility assays and kinase nephelometry assays (using the assay conditions reported above) to assess aqueous compatibility (AC) and limit of solubility (LOS).

Cellular Assay:

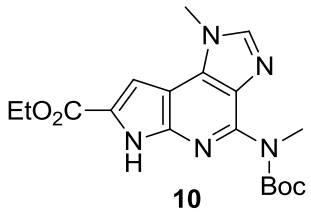
SET-2 Cell Proliferation Inhibition Assay:

The antiproliferative effects of compounds on tumor cell lines were monitored by [3H] thymidine incorporation. Cells were incubated with stepwise dilutions of compound for 72 h in RPMI media supplemented with 10% fetal bovine serum. On day 4, 0.022 mCi/mL of [3H] thymidine was added to each well and allowed to incubate for 3–4 h. Cells were harvested onto filter plates, washed and processed for incorporated radioactivity on a scintillation counter.

General Synthetic Methods:

All experiments were carried out under an inert atmosphere and at room temperature unless otherwise stated. Reactions were monitored using thin-layer chromatography on 250 μm plates, or using HPLC and LCMS with UV detection at 220 or 254 nm. Purification was accomplished by medium pressure liquid chromatography on a CombiFlash Companion (Teledyne Isco) with RediSep normal phase silica gel, or by reverse phase preparative HPLC. All compounds for biological testing had purity analyzed with two orthogonal HPLC conditions: Injection 1: a linear gradient using solvent A (5% acetonitrile, 95% water, 0.05% TFA) and solvent B (95% acetonitrile, 5% water, 0.05% TFA); 10-100% of solvent B over 10 min and then 100% of solvent B over 5 min. Column: Sunfire C18 3.5 μm (4.6 x 150 mm). Flow rate was 2 mL/min and UV detection was set to 220 nm; Injection 2: a linear gradient using solvent A (5% acetonitrile, 95% water, 0.05% TFA) and solvent B (95% acetonitrile, 5% water, 0.05% TFA); 10-100% of solvent B over 10 min and then 100% of solvent B over 5 min. Column: Xbridge Phenyl 3.5 μm (4.6 x 150 mm). Flow rate was 2 mL/min and UV detection was set to 220 nm. The columns were maintained at room temperature. LCMS chromatograms were obtained on a Shimadzu HPLC system running Discovery VP software, coupled with a Waters ZQ mass spectrometer running MassLynx version 3.5 software using: method A: a linear gradient using solvent A (10% acetonitrile, 89.9% water, 0.1% of TFA) and solvent B (89.9% acetonitrile, 10% water, 0.1% of TFA); 0-100% of solvent B over 2 min and then 100% of solvent B over 1 min. Column: PHENOMENEX® Luna 3 μm C18 (2.0 x 30 mm). Flow rate was 5 mL/min and UV detection was set to 220 nm; method B: a linear gradient using solvent A (10% methanol, 89.9% water, 0.1% of TFA) and solvent B (89.9% methanol, 10% water, 0.1% of TFA); 0-100% of solvent B over 4 min and then 100% of solvent B over 1 min. Column: PHENOMENEX® Luna 5 μm C18 (4.5 x 30 mm). Flow rate was 4 mL/min and UV detection was set to 220 nm. The LC columns were maintained at room temperature. ^{1}H NMR spectra were recorded on a Bruker spectrometer (400 or 500 MHz) at ambient temperature.

Experimentals:

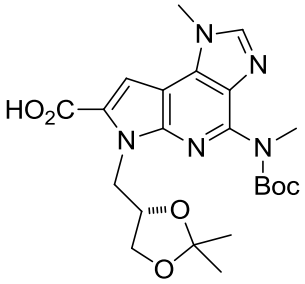


10

Ethyl 4-((tert-butoxycarbonyl)(methyl)amino)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylate (10):

In a 200 mL round bottom flask, ethyl *tert*-butyl 6-amino-1-methyl-1*H*-imidazo[4,5-c]pyridin-4-yl(methyl)carbamate (10 g, 36.1 mmol), synthesized according to WO 2006/122137 was dissolved in acetonitrile (350 mL) and cooled to 0 °C. *N*-Iodosuccinimide (8.52 g, 37.9 mmol) was dissolved in acetonitrile (100 mL) and added to the reaction mixture dropwise over 30 min via addition funnel. The reaction mixture was stirred at 0 °C for 15 min and quenched with 2 M $NaHSO_3$ (200 mL). The temperature and stirring were maintained for 60 min. The mixture was transferred to a separatory funnel. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were washed with water and brine, dried over Na_2SO_4 containing 1 g of charcoal, filtered and concentrated *in vacuo*. MeOH (30 mL) was added and after stirring for 10 min, the slurry was filtered to provide *tert*-butyl 6-amino-7-iodo-1-methyl-1H-imidazo[4,5-c]pyridin-4-yl(methyl)carbamate (13.279 g, 32.9 mmol, 91 % yield) which was used as such in the next reaction.

A 250 mL round bottom flask was charged with *tert*-butyl 6-amino-7-iodo-1-methyl-1H-imidazo[4,5-c]pyridin-4-yl(methyl)carbamate (6.0 g, 14.9 mmol) and $Pd_2(dba)_3$ (1.09 g, 1.19 mmol). The flask was flushed with nitrogen and then DMA (60 mL), *N*-cyclohexyl-*N*-methylcyclohexanamine (5.81 g, 29.8 mmol) and ethyl 2-oxopropanoate (17.3 g, 149 mmol) were added. After stirring at 60 °C for 8 h, the reaction was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution (100 mL) followed by 10% aqueous lithium chloride solution (2 x 100 mL). The organic phase was dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was purified by flash chromatography using an ISCO 330 g column eluting with ethyl acetate to give ethyl 4-(*tert*-butoxycarbonyl(methyl)amino)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylate (4.52 g, 12.10 mmol, 81 % yield) as a yellow foam. MS (ESI) m/z 374.2 (M+H); ^{1}H NMR ($CDCl_3$) δ 9.55 (brs, 1H), 7.75 (s, 1H), 7.34 (s, 1H), 4.37 (q, J = 7.2 Hz, 2H), 4.03 (s, 3H), 3.40 (s, 3H), 1.35 (s, 9H), 1.38-1.34 (m, 3H).

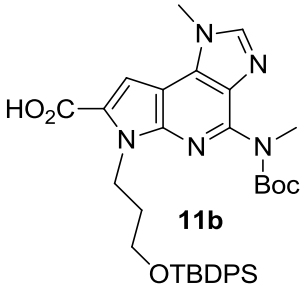


11a

(*S*)-4-((*tert*-Butoxycarbonyl)(methyl)amino)-6-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylic acid (11a):

A 100 mL round bottom flask was charged with ethyl 4-(*tert*-butoxycarbonyl(methyl)amino)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylate (1.0 g, 2.68 mmol) and cesium carbonate (1.74 g, 5.36 mmol). DMF (10 mL) and (*R*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl 4-methylbenzenesulfonate (1.53 g, 5.36 mmol) were added. The reaction mixture was stirred at 90 °C for 0.5 h, and then the brown reaction was cooled to room temperature. The reaction mixture was filtered to remove cesium carbonate. The filtrate was suspended in ethyl acetate and saturated aqueous sodium bicarbonate solution. The layers were separated and the organic layer was dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was purified by flash chromatography using an ISCO 80 g column eluting with ethyl acetate to give (*S*)-ethyl 4-(*tert*-butoxycarbonyl(methyl)amino)-6-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylate (1.3 g, 2.67 mmol, 100 % yield) as a yellow oil. MS (ESI) m/z 488.2 (M+H); ^{1}H NMR ($CDCl_3$) δ 7.83 (s, 1H), 7.49 (s, 1H), 5.02-5.07 (m, 1H), 4.94-4.98 (m, 1H), 4.58-4.63 (m, 1H), 4.44 (q, *J* = 7.2 Hz, 2H), 4.11 (s, 3H), 3.97-4.02 (m, 1H), 3.89-3.93 (m, 1H), 3.50 (s, 3H), 1.42-1.48 (m, 18H).

A 200 mL round bottom flask was charged with (*S*)-ethyl 4-(*tert*-butoxycarbonyl(methyl)amino)-6-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylate (1.3 g, 2.67 mmol). Ethanol (10 mL) and 1N aqueous NaOH solution (10 mL) were added. The reaction mixture was stirred at 80 °C for 1 h and the yellow reaction mixture was cooled to room temperature. The reaction mixture was concentrated to remove ethanol and the resulting aqueous solution was made acidic (pH 4) with 1N aqueous HCl solution. The product was collected by vacuum filtration to give crude (*S*)-4-(*tert*-butoxycarbonyl(methyl)amino)-6-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylic acid (0.94 g, 2.05 mmol, 77 % yield) as a white solid. MS (ESI) m/z 460.2 (M+H).



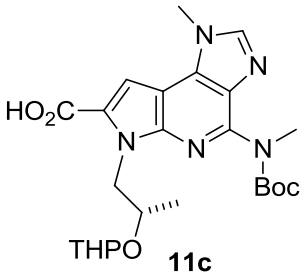
11b

4-((*tert*-Butoxycarbonyl)(methyl)amino)-6-(3-((*tert*-butyldiphenylsilyl)oxy)propyl)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylic acid, sodium salt (11b):

A 100 mL pear shaped flask was charged with ethyl 4-(*tert*-butoxycarbonyl(methyl)amino)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylate (66.7 mg, 0.179 mmol) and cesium carbonate (116 mg, 0.357 mmol) and suspended in DMF (5 mL). 3-Bromopropan-1-ol (24.83 mg, 0.179 mmol) was added and the mixture heated at 70 °C for 0.5 h. The reaction was cooled to RT and filtered to remove any remaining cesium carbonate. The filtrate was suspended in ethyl acetate and washed with saturated aqueous sodium bicarbonate. The organic layer was concentrated *in vacuo*. The crude residue was purified by flash chromatography using an Isco 40g column eluting from 0-100% EtOAc/Hex to give ethyl 4-(*tert*-butoxycarbonyl(methyl)amino)-6-(3-hydroxypropyl)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylate (67.4 mg, 0.156 mmol, 87 % yield) as a yellow oil. MS (ESI) m/z 432.1 (M+H).

In a 100 mL round bottom flask, ethyl 4-(*tert*-butoxycarbonyl(methyl)amino)-6-(3-hydroxypropyl)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylate (67.4 mg, 0.156 mmol) was dissolved in THF (10 mL) and cooled to -78 °C. *n*-Butyllithium (0.098 mL, 0.156 mmol) was added and the reaction allowed to stir for 10 min. *tert*-Butylchlorodiphenylsilane (0.048 mL, 0.187 mmol) was added. After visible reaction had stopped, the reaction mixture was brought to RT. After 30 min, the reaction mixture was heated at 50 °C for 2 h. Imidazole (12.76 mg, 0.187 mmol) was added to the reaction along with an additional equivalent of *tert*-butylchlorodiphenylsilane. After 4 h, the reaction mixture was concentrated to remove THF and partitioned between ethyl acetate and water. The aqueous layer was washed with EtOAc (2 x). The combined organics were dried with anhydrous magnesium sulfate, filtered and concentrated *in vacuo*. The crude residue was purified by flash chromatography using an Isco 40g column eluting from 0-100% EtOAc/Hex to afford ethyl 4-(*tert*-butoxycarbonyl(methyl)amino)-6-(3-(tert-butyldiphenylsilyloxy)propyl)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylate (51.7 mg, 0.077 mmol, 49.4 % yield) as a colorless oil. MS (ESI) m/z 670.3 (M+H).

A 50 mL round bottom flask was charged with ethyl 4-(*tert*-butoxycarbonyl(methyl)amino)-6-(3-(tert-butyldiphenylsilyloxy)propyl)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylate (51.7 mg, 0.077 mmol) and dissolved in ethanol (2 mL). 1 M Sodium hydroxide (0.077 mL, 0.077 mmol) was added to the reaction mixture which was then heated at 50 °C for 3 h. After cooling to RT, the reaction mixture was diluted with water (2 mL) and concentrated *in vacuo* to remove ethanol. The aqueous material was lyophilized ON to yield 4-(*tert*-butoxycarbonyl(methyl)amino)-6-(3-(tert-butyldiphenylsilyloxy)propyl)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylic acid, sodium salt (34.2 mg, 0.053 mmol, 69 % yield). MS (ESI) m/z 642.2 (M+H).



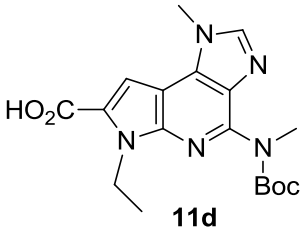
11c

4-((*tert*-Butoxycarbonyl)(methyl)amino)-1-methyl-6-((2*S*)-2-((tetrahydro-2*H*-pyran-2-yl)oxy)propyl)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylic acid, sodium salt (11c):

A round bottom flask was charged with ethyl 4-(*tert*-butoxycarbonyl(methyl)amino)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylate (110 mg, 0.294 mmol), (2*S*)-2-(tetrahydro-2H-pyran-2-yloxy)propyl 4-methylbenzenesulfonate (185 mg, 0.588 mmol), cesium carbonate (192 mg, 0.588 mmol) and DMF (1 mL). The reaction mixture was heated at 60 °C for 3h. After cooling to RT, the reaction mixture was diluted with EtOAc and washed with saturated aqueous sodium bicarbonate. The organics were dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo*. The residue was purified via column chromatography on the ISCO Companion (0%-100% EtOAc/Hex; 12 g column) to give ethyl 4-(*tert*-butoxycarbonyl(methyl)amino)-1-methyl-6-((2*S*)-2-(tetrahydro-2*H*-pyran-2-yloxy)propyl)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylate (165.5 mg, 0.321 mmol, 109 % yield) as a yellow oil. MS (ESI) m/z 516.1 (M+H).

Ethyl 4-(*tert*-butoxycarbonyl(methyl)amino)-1-methyl-6-((2*S*)-2-(tetrahydro-2*H*-pyran-2-yloxy)propyl)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylate (152 mg, 0.295 mmol) was taken up in ethanol (2 mL) and 1 M sodium hydroxide (2 mL). The reaction mixture was heated at 60 °C for 90 min. The reaction mixture was cooled to RT, concentrated *in vacuo* and lyophilized ON to yield 4-(*tert*-butoxycarbonyl(methyl)amino)-1-methyl-6-((2*S*)-2-(tetrahydro-2*H*-pyran-2-yloxy)propyl)-1,6-

dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylic acid, sodium salt (271 mg, 0.531 mmol, 180 % yield) as an off-white solid. MS (ESI) m/z 488.0 (M+H).

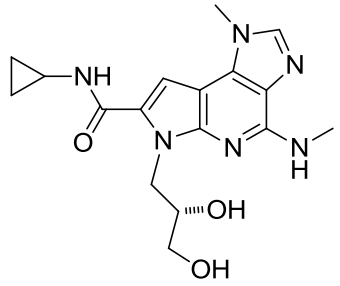


11d

4-((*tert*-Butoxycarbonyl)(methyl)amino)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylic acid (11d):

A 25 mL round bottom flask was charged with ethyl 4-(*tert*-butoxycarbonyl(methyl)amino)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylate (200 mg, 0.536 mmol) and cesium carbonate (349 mg, 1.07 mmol). DMF (1.5 mL) and iodoethane (0.043 mL, 0.536 mmol) were added. The reaction mixture was stirred at 60 °C for 0.5 h and the brown reaction was cooled to room temperature. The reaction mixture was suspended in ethyl acetate and saturated aqueous sodium bicarbonate mixture (1:1, 20 mL). The layers were separated and the organic layer was dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was purified by flash chromatography using an ISCO 80 g column eluting with 80% ethyl acetate/hexanes to give ethyl 4-(*tert*-butoxycarbonyl(methyl)amino)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylate (170 mg, 0.423 mmol, 79 % yield) as a yellow solid. MS (ESI) m/z 402.2 (M+H); ^{1}H NMR ($DMSOd_6$) δ 8.19 (s, 1H), 7.60 (s, 1H), 4.72 (q, *J* = 7.0 Hz, 2H), 4.37 (q, 2H, J = 7.1 Hz), 4.09 (s, 3H), 3.33 (s, 3H), 1.30-1.39 (m, 15H).

A 100 mL round bottom flask was charged with ethyl 4-(*tert*-butoxycarbonyl(methyl)amino)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylate (165 mg, 0.411 mmol). Ethanol (1 mL) and 1N aqueous NaOH solution (1mL) were added. The reaction mixture was stirred at 80 °C for 1 h and the yellow reaction was cooled to room temperature. The reaction was concentrated to remove ethanol and the resulting aqueous solution was made acidic with 1N aqueous HCl solution. The product was collected by vacuum filtration and washed with water to give crude 4-(*tert*-butoxycarbonyl(methyl)amino)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylic acid (145 mg, 0.388 mmol, 94 % yield) as a yellow solid. MS (ESI) m/z 374.2 (M+H).

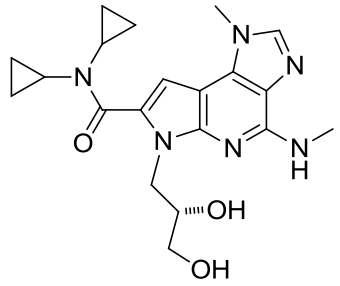


2

(*S*)-*N*-Cyclopropyl-6-(2,3-dihydroxypropyl)-1-methyl-4-(methylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (2):

To a solution of (*S*)-4-(*tert*-butoxycarbonyl(methyl)amino)-6-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylic acid (124.4 mg, 0.271 mmol), HOBT (74.6 mg, 0.487 mmol) and DIPEA (0.142 ml, 0.812 mmol) in acetonitrile (3 ml) was added EDC (122 mg, 0.636 mmol) and cyclopropylamine (0.019 ml, 0.271 mmol). The reaction mixture was heated to 80 °C for 30 min and concentrated to yield a crude product. The crude product was dissolved in EtOAc (100ml) and washed with saturated sodium bicarbonate solution (30 ml), water (30 ml) and brine (30 ml), then dried over anhydrous sodium sulfate. The solution was filtered and concentrated to afford a crude product which was purified on silica gel column (Isco) with EtOAc/MeOH/NH_4OH (100/10/1) to yield (*S*)-*N*-cyclopropyl-6-(2,3-dihydroxypropyl)-1-methyl-4-(methylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (94.7 mg, 0.183 mmol, 67 % yield). MS (ESI) m/z 499.3 (M+H).

(*S*)-*N*-Cyclopropyl-6-(2,3-dihydroxypropyl)-1-methyl-4-(methylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (33 mg, 0.066 mmol) and HCl, 4N in dioxane (0.4 ml, 1.655 mmol) were stirred at 23 °C for 15 min. The reaction mixture was concentrated and the solid was rinsed with Et_2O to afford (*S*)-*N*-cyclopropyl-6-(2,3-dihydroxypropyl)-1-methyl-4-(methylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide, HCl (23.4 mg, 0.059 mmol, 90 % yield). MS (ESI) m/z 359.2 (M+H). ^{1}H NMR (500 MHz, CD_3OD) δ ppm 8.30 (1 H, s), 7.35 (1 H, s), 4.66 - 4.76 (2 H, m), 4.12 - 4.19 (1 H, m), 4.08 (3 H, s), 3.47 - 3.56 (2 H, m), 3.17 (3 H, s), 2.74 - 2.82 (1 H, m), 0.72 - 0.79 (2 H, m), 0.56 - 0.62 (2 H, m).



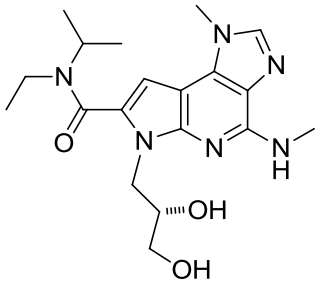
3

(*S*)-*N*,*N*-Dicyclopropyl-6-(2,3-dihydroxypropyl)-1-methyl-4-(methylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (3):

A 25 mL round bottom flask was charged with (*S*)-4-(*tert*-butoxycarbonyl(methyl)amino)-6-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylic acid (70 mg, 0.152 mmol), HOBt (42.0 mg, 0.274 mmol), acetonitrile (1 mL) and *N,N'*-diisopropylethylamine (0.159 mL, 0.914 mmol). EDC (70.1 mg, 0.366 mmol) and dicyclopropylamine hydrochloride (61.1 mg, 0.457 mmol) were added. After stirring at 50 °C 2 h, the reaction mixture was suspended in ethyl acetate and washed with saturated aqueous sodium bicarbonate solution (10 mL), 10% aqueous lithium chloride solution (10 mL), and saturated aqueous bicarbonate solution (10 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. The residue was purified by flash chromatography using an ISCO 40 g column eluting with 1% methanol/ethyl acetate to give (*S*)-*tert*-butyl 7-(dicyclopropylcarbamoyl)-6-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridin-4-yl(methyl)carbamate (44 mg, 0.082 mmol, 54 % yield) as a colorless oil. MS (ESI) m/z 539.4 (M+H). ^{1}H NMR ($CDCl_3$) δ 7.74 (s, 1H), 6.87 (s, 1H), 4.87-4.92 (m, 1H), 4.67-4.70 (m, 1H), 4.42-4.49 (m, 1H), 4.01-4.04 (m, 1H), 3.99 (s, 3H), 3.79-3.81 (m, 1H), 3.40 (s, 3H), 2.70-2.78 (m, 2H), 1.36 (s, 9H), 1.30 (s, 3H), 1.19 (s, 3H), 0.74-0.93 (m, 8H).

A 25 mL round bottom flask was charged with (*S*)-*tert*-butyl 7-(dicyclopropylcarbamoyl)-6-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridin-4-yl(methyl)carbamate (44 mg, 0.082 mmol) and TFA (2 mL) was added. After stirring at room temperature for 1 h, the yellow reaction mixture was concentrated to remove excess TFA. The residue was purified by preperative HPLC using YMC ODS S5 30x250 mm column and eluting with 25-100% methanol/water containing 0.1% TFA. After concentration of the fractions to remove methanol, the aqueous solution was basified with saturated aqueous sodium bicarbonate solution and extracted with ethyl acetate (3 x 20 mL). The combined organic extracts were dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was lyophilized from acetonitrile/water to give (*S*)-*N*,*N*-dicyclopropyl-6-(2,3-dihydroxypropyl)-1-methyl-4-(methylamino)-1,6-dihydroimidazo[4,5-

d]pyrrolo[2,3-b]pyridine-7-carboxamide (15 mg, 0.038 mmol, 46 % yield) as a white solid. MS (ESI) m/z 399.3 (M+H). ^{1}H NMR ($DMSOd_6$) δ 7.86 (s, 1H), 7.04 (s, 1H), 6.87 (q, 1H, J = 5 Hz), 5.38 (d, *J* = 5 Hz, 1H), 4.83 (t, *J* = 6 Hz, 1H), 4.46 (dd, *J* = 14, 5 Hz, 1H), 4.36 (dd, *J* = 14, 5 Hz, 1H), 3.89 (s, 3H), 3.79-3.81 (m, 1H), 3.15-3.20 (m, 1H), 3.09-3.12 (m, 1H), 2.90 (d, 3H, J = 5 Hz), 2.78-2.85 (m, 2H), 0.63-0.70 (m, 8H).

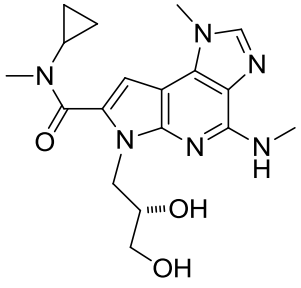


4

(*S*)-6-(2,3-Dihydroxypropyl)-*N*-ethyl-*N*-isopropyl-1-methyl-4-(methylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (4):

Into an I-Kem vial was weighed (*S*)-*N*,*N*-dicyclopropyl-6-(2,3-dihydroxypropyl)-1-methyl-4-(methylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (21 mg, 0.046 mmol) and HATU (52.4 mg, 0.138 mmol). To this vial was added anhdrous DMF (1 mL) and DIPEA (0.048 mL, 0.276 mmol). It was sonicated for 5 mins and then shaken 1 h at RT. *N*-Ethylisopropylamine (0.012 g, 0.138 mmol) was added and the vial shaken ON at RT. The reaction mixture was concentrated *in vacuo* and used crude in the subsequent reaction.

The crude amide was dissolved in 4M HCl in dioxane (1 mL) and sonicated until all solids dissolved into a suspension. The reaction mixture was shaken for 5 h at RT. The reaction mixture was concentrated to dryness using the SpeedVac. The crude residue was purified via prepHPLC to yield (*S*)-6-(2,3-dihydroxypropyl)-*N*-ethyl-*N*-isopropyl-1-methyl-4-(methylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (6.95 mg, 39 % yield). MS (ESI) m/z 389.2 (M+H).

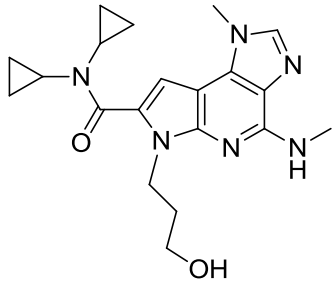


5

(*S*)-*N*-Cyclopropyl-6-(2,3-dihydroxypropyl)-*N*,1-dimethyl-4-(methylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (5):

A 25 mL round bottom flask was charged with (*S*)-4-(*tert*-butoxycarbonyl(methyl)amino)-6-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylic acid (50 mg, 0.109 mmol), HOBt (30.0 mg, 0.196 mmol), acetonitrile (1 ml) and DIPEA (0.076 ml, 0.435 mmol). EDC (50.1 mg, 0.261 mmol) and *N*-methylcyclopropanamine, oxalic acid (35.1 mg, 0.218 mmol) were added. After stirring at 50 °C overnight, the reaction was suspended in ethyl acetate and washed with saturated aqueous sodium bicarbonate solution, 10% aqueous lithium chloride solution, then saturated aqueous bicarbonate solution again. The organic layer was dried over anhydrous sodium sulfate and concentrated *in vacuo* to give (*S*)-*tert*-butyl 7-(cyclopropyl(methyl)carbamoyl)-6-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridin-4-yl(methyl)carbamate (57 mg, 0.111 mmol, 102 % yield) as a yellow oil. The product was used directly without further purification.

A 25 mL round bottom flask was charged with (*S*)-*tert*-butyl 7-(cyclopropyl(methyl)carbamoyl)-6-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridin-4-yl(methyl)carbamate (57 mg, 0.111 mmol). TFA (2 mL) was added. After stirring at RT for 1 h, the yellow reaction was concentrated to remove excess TFA. The residue was diluted with ethyl acetate, washed with saturated aqueous sodium bicarbonate solution, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was triturated with 1:1 ethyl acetate/ether followed by 100% ether to give (*S*)-*N*-cyclopropyl-6-(2,3-dihydroxypropyl)-*N*,1-dimethyl-4-(methylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (33 mg, 0.089 mmol, 80 % yield) as a white solid. ^{1}H NMR (500 MHz, DMSOd_6) δ 7.90 (s, 1H), 7.07 (s, 1H), 6.89 (m, 1H), 5.36 (d, *J* = 5 Hz, 1H,), 4.86 (t, *J* = 5 Hz, 1H), 4.48 (m, 1H), 4.36 (m, 1H), 3.94 (s, 3H), 3.81 (m, 1H), 3.20 (m, 1H), 3.13 (m, 1H), 3.01 (s, 3H), 2.93 (d, *J* = 5 Hz, 3H), 0.66 (m, 2H), 0.59 (m, 2H).



6

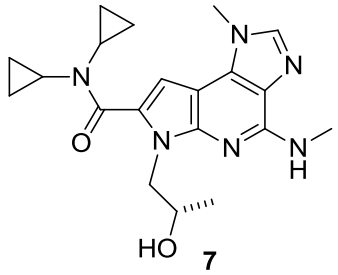
***N*,*N*-dicyclopropyl-6-(3-hydroxypropyl)-1-methyl-4-(methylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (6):**

A 25 mL round bottom flask was charged with 4-(*tert*-butoxycarbonyl(methyl)amino)-6-(3-(*tert*-butyldiphenylsilyloxy)propyl)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylic

acid (34.2 mg, 0.053 mmol) and HOBT (14.69 mg, 0.096 mmol) and dissolved in acetonitrile (1 mL). DIPEA (0.056 mL, 0.320 mmol), EDC (24.52 mg, 0.128 mmol) and dicyclopropylamine, HCl (8.54 mg, 0.064 mmol) were added and the reaction heated at 50 °C 2 d. The reaction was suspended in ethyl acetate and washed with saturated aqueous sodium bicarbonate solution, 10% aqueous lithium chloride solution, then saturated aqueous bicarbonate solution again. The organic layer was dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The residue was purified by flash chromatography using an Isco 40g column eluting with a gradient from 0-10% MeOH/EtOAc to give *tert*-butyl 6-(3-(*tert*-butyldiphenylsilyloxy)propyl)-7-(dicyclopropylcarbamoyl)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridin-4-yl(methyl)carbamate (26.4 mg, 0.037 mmol, 68.7 % yield) as a clear colorless oil. MS (ESI) m/z 721.2 (M+H).

To a solution of *tert*-butyl 6-(3-(*tert*-butyldiphenylsilyloxy)propyl)-7-(dicyclopropylcarbamoyl)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridin-4-yl(methyl)carbamate (26 mg, 0.036 mmol) in THF (1 mL) at RT was added TBAF (0.036 mL, 0.036 mmol). After stirring at RT for 30 min, the reaction was diluted with ethyl acetate, washed with saturated aqueous sodium bicarbonate solution, dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was purified by flash chromatography using an Isco 40g column eluting with 6% methanol / dichloromethane to give *tert*-butyl 7-(dicyclopropylcarbamoyl)-6-(3-hydroxypropyl)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridin-4-yl(methyl)carbamate (26 mg, 0.054 mmol, 149 % yield) as a white solid. MS (ESI) m/z 483.4 (M+H).

A 25 mL round bottom flask was charged with *tert*-butyl 7-(dicyclopropylcarbamoyl)-6-(3-hydroxypropyl)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridin-4-yl(methyl)carbamate (20 mg, 0.041 mmol). TFA (2 mL) was added and the reaction mixture was allowed to stir at RT for 20 min. The mixture was concentrated *in vacuo*. The residue was purified using an Isco 12g column eluting from 0-10% MeOH/CH_2Cl_2. The final product was lyophilized ON to yield *N*,*N*-dicyclopropyl-6-(3-hydroxypropyl)-1-methyl-4-(methylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (4.28 mg, 10.07 µmol, 24 % yield, 90% purity) as an off white solid. MS (ESI) m/z 383.2 (M+H). ^{1}H NMR (400MHz, CD_3OD) δ 7.88 (s, 1H), 7.17 (s, 1H), 4.63 (t, *J* = 6.5 Hz, 2H), 4.03 (s, 3H), 3.42 (t, *J* = 6.0 Hz, 2H), 3.13 (s, 3H), 2.98 - 2.89 (m, 2H), 2.05 (quin, *J* = 6.1 Hz, 2H), 0.90 - 0.82 (m, 4H), 0.78 - 0.72 (m, 4H).



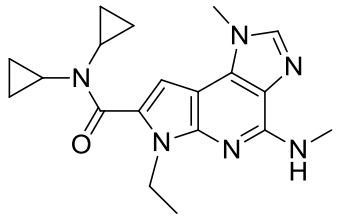
7

(*S*)-*N*,*N*-dicyclopropyl-6-(2-hydroxypropyl)-1-methyl-4-(methylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (7):

A round bottom flask was charged with 4-(*tert*-butoxycarbonyl(methyl)amino)-1-methyl-6-((2*S*)-2-(tetrahydro-2*H*-pyran-2-yloxy)propyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylic acid (171 mg, 0.351 mmol) and HATU (147 mg, 0.386 mmol) and dissolved in DMF (6 mL). DIPEA (0.12 mL, 0.701 mmol) and dicyclopropylamine, HCl (70.2 mg, 0.526 mmol) were added and the reaction heated at 50 °C ON. The reaction was suspended in ethyl acetate and washed with saturated aqueous sodium bicarbonate solution, 10% aqueous lithium chloride solution, then saturated aqueous bicarbonate solution again. The organic layer was dried over anhydrous sodium sulfate and concentrated *in vacuo.* The residue was purified by flash chromatography using an Isco 40g column eluting with a gradient from 0-7% MeOH/CH_2Cl_2 to give *tert*-butyl 7-(dicyclopropylcarbamoyl)-1-methyl-6-((2*S*)-2-(tetrahydro-2*H*-pyran-2-yloxy)propyl)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridin-4-yl(methyl)carbamate (51 mg, 0.090 mmol, 25.7 % yield) as a yellow foam. MS (ESI) m/z 483.1 (M+H-THP).

tert-Butyl 7-(dicyclopropylcarbamoyl)-1-methyl-6-((2*S*)-2-(tetrahydro-2*H*-pyran-2-yloxy)propyl)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridin-4-yl(methyl)carbamate (51 mg, 0.090 mmol) was dissolved in THF (3 mL) and 4N HCl in dioxane (3 mL) was added. The reaction mixture was stirred at RT for 4 h. The reaction mixture was quenched with saturated aqueous sodium bicarbonate solution and extracted with EtOAc. The organics were dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo*. The crude resicue was purified by prep HPLC (2 x) (YMC ODS S5 30x100 mm, 20% to 100% (90%MeOH/H2O with 0.1%TFA), 8 min gradient, 12 min run, 20ml/min flow rate). The final product was converted to the HCl salt by dissolving in dichloromethane (1 mL) and adding 4N HCl in dioxane (33 µL). The solution was concentrated and dried under vacuum to give a yellow solid, (*S*)-*N*,*N*-dicyclopropyl-6-(2-hydroxypropyl)-1-methyl-4-(methylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide, HCl (26 mg, 0.061 mmol, 67.6 % yield). MS (ESI) m/z 383.0 (M+H). ^{1}H NMR (400MHz, $CDCl_3$) δ 8.25 (br s, 1H), 6.91 (s, 1H), 4.64 (br d, *J* = 12.0 Hz, 1H), 4.39 -

4.24 (m, 2H), 4.10 (s, 3H), 3.50 (s, 3H), 2.86 - 2.77 (m, 2H), 1.34 (d, *J* = 6.0 Hz, 3H), 0.92 - 0.68 (m, 8H).

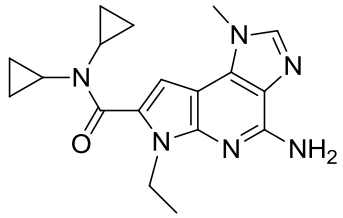


8

***N,N*-Dicyclopropyl-6-ethyl-1-methyl-4-(methylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (8):**

A 25 mL round bottom flask was charged with 4-(*tert*-butoxycarbonyl(methyl)amino)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxylic acid (42 mg, 0.112 mmol) and HOBt (31.0 mg, 0.202 mmol) in acetonitrile (1 mL). This was followed by addition of DIPEA (0.118 mL, 0.675 mmol), EDC (51.7 mg, 0.270 mmol) and dicyclopropylamine, HCl (18.04 mg, 0.135 mmol) and the reaction mixture was stirred at room temperature overnight. The reaction mixture was suspended in ethyl acetate and washed with saturated aqueous sodium bicarbonate solution, 10% aqueous lithium chloride solution, then saturated aqueous bicarbonate solution again. The organic layer was dried over anhydrous magnesium sulfate and concentrated *in vacuo* to afford crude *tert*-butyl 7-(dicyclopropylcarbamoyl)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridin-4-yl(methyl)carbamate (49.2 mg, 0.109 mmol, 97 % yield) as an off white solid. MS (ESI) m/z 453.3 (M+H).

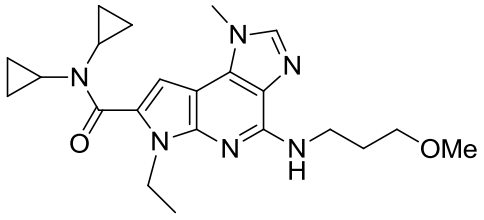
A 25 mL round bottom flask was charged with *tert*-butyl 7-(dicyclopropylcarbamoyl)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridin-4-yl(methyl)carbamate (49.2 mg, 0.109 mmol) and dissolved in TFA (2 mL). The reaction mixture was allowed to stir at at room temperature for 20 min. After concentration *in vacuo* to remove excess TFA, the residue was taken up in MeOH and loaded onto an ISCO 12 g column. The product was eluted using 0-5% MeOH/EtOAc and then lyophilized from 1:1 acetonitrile/water to give *N,N*-dicyclopropyl-6-ethyl-1-methyl-4-(methylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (15.5 mg, 0.042 mmol, 38 % yield) as an off white solid. MS (ESI) m/z 353.2 (M+H); ^{1}H NMR (CD_3OD) δ 7.75 (s, 1H), 7.01 (s, 1H), 4.47 (q, *J* = 8 Hz, 2H), 3.91 (s, 3H), 3.04 (s, 3H), 2.78-2.85 (m, 2H), 1.26 (t, *J* = 8 Hz, 3H), 0.74-0.76 (m, 4H), 0.65-0.68 (m, 4H).



17

4-Amino-*N*,*N*-dicyclopropyl-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (17):

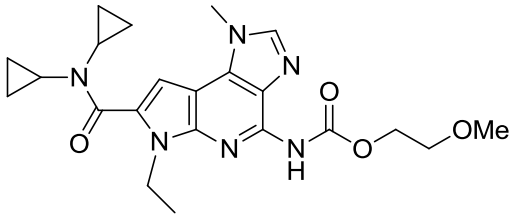
To a solution of trifluoroacetic acid (127 mL, 1.71 mol) in dichloromethane (500 mL) at 0 °C was added *tert*-butyl 7-(dicyclopropylcarbamoyl)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridin-4-yl(2,4-dimethoxybenzyl)carbamate (50.45 g, 86 mmol), synthesized according to U.S. Patent 8202881, in portions over 20 min while keeping the reaction temperature under 5 °C. The red solution was stirred in an ice bath for 4 h. The white solid was removed by filtration. The filtrate was concentrated *in vacuo*. The remaining dark liquid was treated with dichloromethane (100 mL) and concentrated *in vacuo*. This was repeated once. The remaining liquid was dried under vacuum overnight. To the dark liquid was added 1 N NaOH (600 mL). The mixture was stirred at 0 °C, resulting in precipitation of a white solid. The pH of the mixture was 12. After 3 h, the solid was collected by filtration and washed with water until the pH was neutral (800 mL). The solid was dried at 50 °C for 2 h and room temperature for 16 h under vacuum (~20 Torr). The solid was treated with dichloromethane (750 mL) and stirred at at room temperature for 2 h. The undesired solid was removed by filtration. The filtrate was concentrated *in vacuo* to yield 4-amino-*N*,*N*-dicyclopropyl-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (29.3 g, 86 mmol, 101% yield). MS (ESI) m/z 339.3 (M+H). ^{1}H NMR (400 MHz, CD_3OD) δ 7.95 (s, 1 H), 7.15 (s, 1 H), 4.51 (q, *J* = 7.13 Hz, 2 H), 4.04 (s, 3 H), 2.89 - 2.98 (m, 2 H), 1.35 (m, 3 H), 0.82 - 0.91 (m, 4 H), 0.70 - 0.81 (m, 4 H).



12

***N*,*N*-dicyclopropyl-6-ethyl-4-((3-methoxypropyl)amino)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (12):**

4-Amino-*N*,*N*-dicyclopropyl-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (46.1 mg, 0.136 mmol) was dissolved in MeOH (1 mL) and 4 Å MS were added. 3-Methoxypropanal (2.404 mL, 0.409 mmol), AcOH (7.80 µL, 0.136 mmol) and sodium cyanoborohydride (25.7 mg, 0.409 mmol) were added and the reaction mixture was stirred at RT ON. The reaction mixture was diluted with EtOAc and quenched with 1N NaOH. The aqueous layer was extracted with EtOAc (3x). The organic layers were combined, dried over anhydrous magensium sulfate, filtered and concentrated *in vacuo*. The crude residue was purified by prep HPLC (Sunfire S10 30x250 mm, 20-100% (90%MeOH/H2O+0.1%TFA), 20 ml/min, 20 min gradient, 30 min run). The final compound was converted to its HCl salt, providing *N*,*N*-dicyclopropyl-6-ethyl-4-(3-methoxypropylamino)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide, HCl (20.7 mg, 0.044 mmol, 32.6 % yield). MS (ESI) m/z 411.1 (M+H). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (br s, 1H), 6.87 (s, 1H), 4.55 (q, *J* = 7.0 Hz, 2H), 4.15 (s, 3H), 3.84 (br t, *J* = 6.4 Hz, 2H), 3.57 (t, *J* = 6.1 Hz, 2H), 3.38 (s, 3H), 2.87 - 2.78 (m, 2H), 2.03 (quin, *J* = 6.5 Hz, 2H), 1.38 (t, *J* = 7.2 Hz, 3H), 0.93 - 0.86 (m, 4H), 0.80 - 0.75 (m, 4H).

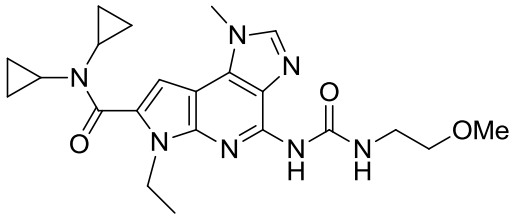


13

2-Methoxyethyl (7-(dicyclopropylcarbamoyl)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridin-4-yl)carbamate (13):

To a cooled (0 °C) solution of 4-amino-*N*,*N*-dicyclopropyl-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (58 mg, 0.171 mmol) and pyridine (69.3 µL, 0.857 mmol) in dichloromethane (1714 µL) was added chloroformic acid 2-methoxyethyl ester (59.4 µL, 0.514 mmol) dropwise. The reaction was stirred at 0 °C for 30 min. Additional pyridine (69.3 µL, 0.857 mmol) and chloroformic acid 2-methoxyethyl ester (59.4 µL, 0.514 mmol) were added and the solution slowly warmed to rt and stirred 40 min. This process was repeated four times. The solution was concentrated and the residue purified by prepHPLC (2 x) (C18 Phen Luna S5 ODS 21.20 x 100 mm, 0-100% (90% MeCN/H2O+5 mM ammonium acetate), 20 ml/min, 12 min gradient, 15 min run) to provide 2-methoxyethyl 7-(dicyclopropylcarbamoyl)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridin-4-ylcarbamate (14.92 mg, 0.033 mmol, 20 % yield). MS (ESI) m/z 441.2 (M+H). ^{1}H NMR (500 MHz, $DMSOd_6$) δ 9.64 (1 H, s), 8.10 (1 H, s), 7.24 (1 H, s), 4.48 (q, *J* = 7.15 Hz, 2H), 4.17 – 4.25

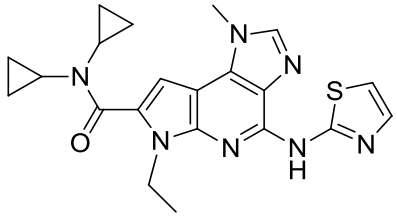
(2 H, m), 4.04 (3 H, s), 3.55 – 3.63 (2 H, m), 3.29 (3 H, s), 2.87 – 3.02 (2 H, m), 1.29 (t, *J* = 7.01 Hz, 3H), 0.78 – 0.82 (4 H, m), 0.62 – 0.71 (4 H, m).



14

***N,N*-Dicyclopropyl-6-ethyl-4-(3-(2-methoxyethyl)ureido)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (14):**

To a solution of 4-amino-*N*,*N*-dicyclopropyl-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (20 mg, 0.059 mmol), 2-methoxyethylamine (5.16 µL, 0.059 mmol) and pyridine (14.34 µL, 0.177 mmol) in dichloromethane (591 µL) at room temperature was added phosgene solution (20% in toluene, 46.7 µL, 0.089 mmol) dropwise. The reaction mixture was stirred at room temperature overnight. Additional pyridine (14.34 µL, 0.177 mmol), 2-methoxyethylamine (5.16 µL, 0.059 mmol) and phosgene solution (20% in toluene, 46.7 µL, 0.089 mmol) were added. After 24 h, The reaction mixture was concentrated and the residue purified by preparative HPLC (YMC ODS S5 20 x 100 mm, 20% to 100% (90% MeOH/H_2O with 0.1% TFA), 8 min gradient, 11 min run, 20 ml/min flow rate) to yield *N*,*N*-dicyclopropyl-6-ethyl-4-(3-(2-methoxyethyl)ureido)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (4.5 mg, 9.52 µmol, 16.11 % yield) along with recovered starting material.MS (ESI) m/z 440.2 (M+H). ^{1}H NMR (500 MHz, DMSOd_6) δ 9.61 (t, *J* = 5.09 Hz, 1 H), 8.17 (s, 1 H), 8.14 (s, 1 H), 7.23 (s, 1 H), 4.50 (q, *J* = 7.15 Hz, 2 H), 4.03 (s, 3 H), 3.52 – 3.59 (m, 2 H), 3.47 – 3.52 (m, 2 H), 3.34 (s, 3H), 2.87 – 2.98 (m, 2 H), 1.31 (t, *J* = 7.01 Hz, 3 H), 0.72 - 0.79 (m, 4 H), 0.62 - 0.69 (m, 4 H).

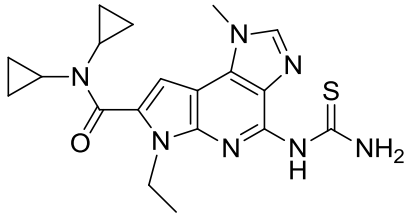


15

***N,N*-Dicyclopropyl-6-ethyl-1-methyl-4-(thiazol-2-ylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (15):**

To a mixture of 4-amino-*N*,*N*-dicyclopropyl-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (30 mg, 0.089 mmol), 2-bromothiazole (11.98 µL, 0.133 mmol), $Pd_2(dba)_3$

(12.18 mg, 0.013 mmol), BINAP (24.84 mg, 0.040 mmol) and sodium *tert*-butoxide (13.63 mg, 0.142 mmol) was added toluene (887 µL). The mixture was sparged with argon gas for 5 min and heated to 85 °C for 5.5 h. The reaction mixture was filtered through a plug of Celite and concentrated *in vacuo*. The crude residue was purified by preparative HPLC (C18 Phen Luna S5 ODS 21.20 x100 mm, 0% to 100% (90% MeCN/H_2O with 5 mM NH_4OAc), 10 min gradient, 20 min run, 20 ml/min flow rate) to provide *N,N*-dicyclopropyl-6-ethyl-1-methyl-4-(thiazol-2-ylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (4 mg, 9.30 µmol, 11 % yield). MS (ESI) m/z 423.0 (M+H). ^{1}H NMR (500 MHz, $DMSOd_6$) δ 8.14 (s, 1H), 7.47 (d, 1H, J = 3.57 Hz), 7.24 (s, 1H), 7.14 (d, 1H, J = 3.57 Hz), 4.63 (q, 2H, J = 6.87 Hz), 4.05 (s, 3H), 2.88 – 2.99 (m, 2H), 1.39 (t, 3H, J = 7.01 Hz), 0.73 – 0.81 (m, 4H), 0.64 – 0.70 (m, 4H).



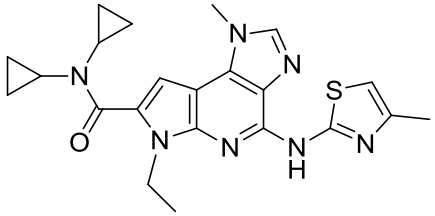
21

***N,N*-Dicyclopropyl-6-ethyl-1-methyl-4-thioureido-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (21):**

A solution of 4-amino-*N,N*-dicyclopropyl-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (234 mg, 0.69 mmol) and benzoyl isothiocyanate (0.1 ml, 0.83 mmol) in acetone (3 ml) was stirred at room temperature for 3h. The reaction mixture was cooled to 0 °C and water was added. The resulting brown solid was collected by filtration and air-dried to give 4-(3-benzoylthioureido)-N,N-dicyclopropyl-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (306 mg, 88% yield). MS (ESI) m/z 502 (M+H). ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 8.44 (br. s., 1 H) 7.99 - 8.07 (m, 2 H) 7.64 - 7.74 (m, 1 H) 7.58 (t, *J* = 7.65 Hz, 2 H) 7.34 (s, 1 H) 4.47 (br s, 2 H) 4.11 (s, 3 H) 2.90 - 2.99 (m, 2 H) 2.08 (s, 1 H) 1.28 (br. s., 3 H) 0.64 - 0.81 (m, 8 H).

4-(3-Benzoylthioureido)-*N,N*-dicyclopropyl-6-ethyl-1-methyl-1,6-dihydroimidazo [4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (306 mg, 0.61 mmol) and 1N NaOH (10 ml, 10.00 mmol) in 10 ml EtOH were heated at 60°C for 1h. The reaction was cooled to room temperature and concentrated. The remaining aqueous solution was extracted (3x) with ethyl acetate. The organic phase was dried over Na_2SO_4, filtered, and concentrated. Purification by silica gel chromatography (dichloromethane/methanol 0-4%) gave *N,N*-dicyclopropyl-6-ethyl-1-methyl-4-thioureido-1,6-

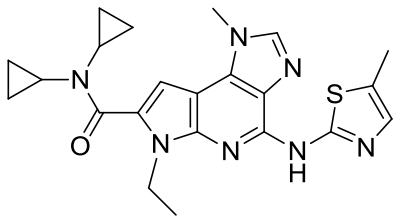
dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide as a yellow solid (199 mg ,82% yield). MS (ESI) m/z 398 (M+H). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 11.02 (br. s., 1 H) 9.27 (s, 2 H) 7.75 (s, 1 H) 6.89 (s, 1 H) 4.52 (q, *J* = 7.12 Hz, 2 H) 4.06 (s, 3 H) 2.77 - 2.90 (m, 2 H) 1.45 (t, *J* = 7.15 Hz, 3 H) 0.73 - 0.97 (m, 8 H).



18

***N,N*-Dicyclopropyl-6-ethyl-1-methyl-4-(4-methylthiazol-2-ylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (18):**

N,N-Dicyclopropyl-6-ethyl-1-methyl-4-thioureido-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (40 mg, 0.10 mmol) and chloroacetone (62.4 mg, 0.40 mmol) in EtOH (2 ml) were heated at 80°C for 20 min. The reaction was cooled to room temperature and the solvent removed in vacuo. Saturated $NaHCO_3$ solution was added and the aqueous layer extracted (3x) with dichloromethane. The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by silica gel chromatography (dichloromethane/methanol 0-7%) to provide *N,N*-dicyclopropyl-6-ethyl-1-methyl-4-(4-methylthiazol-2-ylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (9 mg, 12 % yield) as a white solid. MS (CI) m/z 436.1 (M+H). ^{1}H NMR (400 MHz, $DMSOd_6$) δ ppm 10.70 (br s, 1H), 8.12 (s, 1H), 7.23 (s, 1H), 6.69 (s, 1H), 4.61 (q, 2H, *J* = 7.19 Hz), 4.04 (s, 3H), 2.88 – 2.98 (m, 2H), 2.29 (s, 3H), 1.38 (t, 3H, *J* = 7.15 Hz), 0.72 – 0.81 (m, 4H), 0.62 – 0.70 (m, 4H).

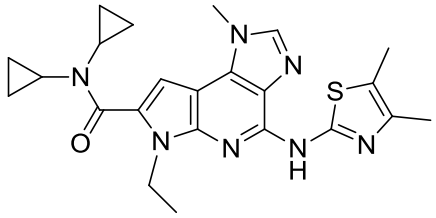


19

***N,N*-Dicyclopropyl-6-ethyl-1-methyl-4-(5-methylthiazol-2-ylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (19):**

4-Amino-*N,N*-dicyclopropyl-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (86.3 mg, 0.255 mmol) was dissolved in acetone and benzoyl isothiocyanate (37.8 µL, 0.281 mmol) was added. The reaction was stirred at room temperature for 2h. The solvent was removed *in vacuo*.

The residue was taken up in ethanol (1500 µL) and K_2CO_3 (49.3 mg, 0.357 mmol) was added. The reaction mixture was warmed to 60 °C for 3 h. 2-Bromopropanal (45.4 mg, 0.332 mmol) was added. After 3 h, additional 2-bromopropanal (~100 mg) was added. The reaction mixture was stirred at 60 °C for 16 h. Additional 2-bromopropanal was added and the reaction mixture was stirred at 60 °C 3 h. The ethanol was removed by concentration *in vacuo*. The crude residue was purified by flash chromatography using an Isco 12 g column eluting with 2 - 10% MeOH/CH_2Cl_2. Additional purification was accomplished via preparative HPLC (YMC S5 ODS 20 x100 mm, 30% to 100% (90% MeCN/H_2O with 0.1% TFA), 10 min gradient, 15 min run, 20 ml/min flow rate). *N*,*N*-Dicyclopropyl-6-ethyl-1-methyl-4-(5-methylthiazol-2-ylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (20 mg, 17.47 % yield) was isolated as a white solid. MS (ESI) m/z 436.0 (M+H). ^{1}H NMR (500 MHz, DMSOd_6) δ ppm 10.56 (br s, 1H), 8.12 (s, 1H), 7.23 (s, 1H), 7.12 (s, 1H), 4.62 (q, 2H, *J* = 6.78 Hz), 4.04 (s, 3H), 2.87 – 2.99 (m, 2H), 2.39 (s, 3H), 1.35 – 1.46 (m, 3H), 0.72 – 0.81 (m, 4H), 0.59 – 0.71 (m, 4H).



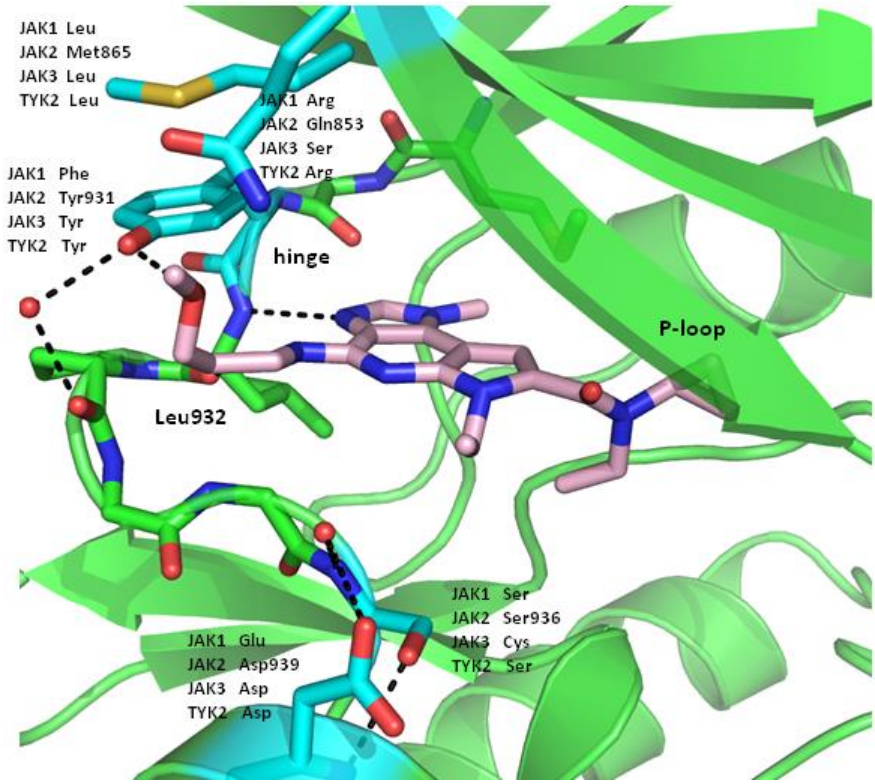
20

***N*,*N*-Dicyclopropyl-4-(4,5-dimethylthiazol-2-ylamino)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (20):**

To a mixture of *N*,*N*-dicyclopropyl-6-ethyl-1-methyl-4-thioureido-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (0.1168 g, 0.294 mmol) in EtOH (1.469 mL) in a vial was added 3-bromo-2-butanone (0.034 mL, 0.323 mmol). The vial was capped and the reaction mixture was warmed to 60 °C for 4 h. The reaction mixture was concentrated *in vacuo*. The residue was taken up in methanol and purified by prep HPLC (YMC ODS S5 20 x 100 mm, 30% to 100% (90% MeOH/H_2O with 0.1% TFA), 10 min gradient, 12 min run, 20 ml/min flow rate). The appropriate fractions were concentrated *in vacuo*. The product residue was basified with saturated aqueous sodium bicarbonate and the aqueous layer was extracted with ethyl acetate (3 x 10mL). The combined organics were dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo*. *N*,*N*-Dicyclopropyl-4-(4,5-dimethylthiazol-2-ylamino)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (59.17 mg, 0.130 mmol, 44 % yield) was isolated as a pale yellow solid. MS (ESI) m/z 450.1 (M+H). HRMS calcd for $C_{23}H_{28}N_7OS$ 450.2076, found 450.2065 $[M+H]^+$. ^{1}H NMR (400 MHz,

DMSOd_6) δ 8.15 (s, 1H), 7.23 (s, 1H), 4.61 (q, *J* = 7.03 Hz, 2H), 4.04 (s, 3H), 2.88 – 2.98 (m, 2H), 2.29 (s, 3H), 2.19 (s, 3H), 1.38 (t, *J* = 7.03 Hz, 3H), 0.73 – 0.80 (m, 4H), 0.62 – 0.71 (m, 4H).

Crystal Structure of 12 Bound to the JAK2 Catalytic Domain:



Crystal structure of **12** (PDB ID 5CF4) bound to the kinase catalytic domain of JAK2. The carbons of **12** are colored in pink and the carbons for JAK2 are colored in green except for the residues near the C-4 alkyl group which differ in the JAK family (carbons are colored cyan). Oxygens are colored red, nitrogens blue and sulfurs yellow. Hydrogen bonds are indicated with dashed lines.

Full Ambit Profile for 20:

Target	% Ctl	% Ctl N of M	% Ctl SD	Target	% Ctl	% Ctl N of M	% Ctl SD
FYN	4.35	2 of 2	2.76	JNK2	100.00	2 of 2	0.00
HPK1	99.50	2 of 2	0.71	KIT	75.50	2 of 2	4.95
OSR1	100.00	2 of 2	0.00	HIPK4	86.00	2 of 2	19.80
p38b	97.00	2 of 2	4.24	MET (M1250T)	100.00	2 of 2	0.00
SRMS	100.00	2 of 2	0.00	MAST1	82.50	2 of 2	16.26
TBK1	100.00	2 of 2	0.00	PKN2	57.50	2 of 2	20.51
INSR	92.00	2 of 2	11.31	PLK4	100.00	2 of 2	0.00
CRIK	97.00	2 of 2	4.24	FES	88.00	2 of 2	16.97
MER	100.00	2 of 2	0.00	FMS	23.50	2 of 2	2.12
EGFR (L861Q)	79.50	2 of 2	28.99	HCK	96.00	2 of 2	5.66
EGFR-T751DL	92.50	2 of 2	10.61	PI3KCA E545K	100.00	2 of 2	0.00
MLK3	86.00	2 of 2	19.80	PAK6	86.00	2 of 2	8.49
MAP3K2	81.50	2 of 2	26.16	EGFR-I759DL	100.00	2 of 2	0.00
MELK	100.00	2 of 2	0.00	MLK1	100.00	2 of 2	0.00
PI3KCG	100.00	2 of 2	0.00	PAK3	94.50	2 of 2	7.78
PI3KCD	100.00	2 of 2	0.00	PFTAIRE2	94.00	2 of 2	8.49
AAK1	74.00	2 of 2	8.49	IKKB	97.50	2 of 2	3.54
ZC1/HGK	84.50	2 of 2	21.92	LRRK2 (G2019S)	100.00	1 of 1	0.00
EGFR A750del	94.50	2 of 2	7.78	TAK1	100.00	2 of 2	0.00
CK1G2	99.00	2 of 2	1.41	BLK	18.50	2 of 2	7.78
EPHA4	85.00	2 of 2	21.21	CAMLCK	97.50	2 of 2	3.54
PFTAIRE1	92.50	2 of 2	10.61	TLK1	99.50	2 of 2	0.71
PDGFRA	95.50	2 of 2	6.36	ERK3	88.00	2 of 2	16.97
SGK3	100.00	1 of 1	0.00	KIT (D816V)	35.00	2 of 2	7.07
RIPK2	88.00	2 of 2	16.97	LRRK2/ RIPK7	88.00	1 of 1	0.00
ROS	77.50	2 of 2	4.95	DMPK 2	100.00	2 of 2	0.00
CDC2L2	84.00	2 of 2	22.63	EGFR G719S	100.00	2 of 2	0.00
AXL	100.00	2 of 2	0.00	MST3	100.00	2 of 2	0.00
BRAF	95.50	2 of 2	3.54	CK1D	100.00	2 of 2	0.00
DCAMKL2	99.00	2 of 2	1.41	CAMKK1	86.00	2 of 2	18.38
CK1G3	82.50	2 of 2	12.02	EPHB4	86.50	2 of 2	19.09
BTK	100.00	2 of 2	0.00	EPHA3	79.50	2 of 2	26.16
p38a	98.00	2 of 2	2.83	EPHA5	100.00	2 of 2	0.00
NIM1	100.00	2 of 2	0.00	MST4	100.00	2 of 2	0.00
SKMLCK	85.50	2 of 2	20.51	MRCKA	100.00	2 of 2	0.00
SLK	100.00	2 of 2	0.00	PKN1	66.00	2 of 2	14.14
ABL1 (Q252H)	90.00	1 of 1	0.00	RIOK2	67.50	2 of 2	0.71
ACK	9.40	2 of 2	7.92	PKCD	77.00	2 of 2	32.53
FGFR4	95.50	2 of 2	6.36	ALK	48.50	2 of 2	9.19
BRAF (V600E)	100.00	2 of 2	0.00	ABL1 (F 317I)	61.00	1 of 1	0.00
BRSK1	100.00	2 of 2	0.00	ADCK4	100.00	2 of 2	0.00
BMPR1A	100.00	2 of 2	0.00	FGFR2	100.00	2 of 2	0.00
MYO3B	91.00	2 of 2	12.73	EGFR S752DL	100.00	2 of 2	0.00
CDK4-cyclinD1	100.00	1 of 1	0.00	TA01	100.00	2 of 2	0.00
CDK5	100.00	2 of 2	0.00	DMPK 1	82.50	2 of 2	21.92
CAMK4	29.50	2 of 2	0.71	PI3KCA (E542K)	100.00	2 of 2	0.00
PI3KCA (Q546K)	100.00	2 of 2	0.00	RSK4-D2	100.00	2 of 2	0.00
PI3KCA (C420R)	96.00	2 of 2	5.66	STK16	77.50	2 of 2	0.71
TGFBR1	100.00	2 of 2	0.00	SRC	6.50	2 of 2	4.95
EPHA8	80.00	2 of 2	19.80	CDK11	97.00	2 of 2	4.24
EPHA7	88.00	2 of 2	16.97	CK2A1	100.00	2 of 2	0.00
PI3KC2B	100.00	2 of 2	0.00	CLIK1	81.50	2 of 2	26.16
PIM2	93.50	2 of 2	9.19	SGK288/ANKK1	100.00	2 of 2	0.00

p38D	98.50	2 of 2	2.12	ABL1	100.00	1 of 1	0.00
JAK3	18.80	2 of 2	17.25	ABL1 (phos)	84.00	1 of 1	0.00
MAP3K5	100.00	2 of 2	0.00	ROCK2	80.00	2 of 2	0.00
CLK2	43.00	2 of 2	26.87	RET M918T	63.50	2 of 2	13.44
DAPK1	13.00	2 of 2	1.41	RSK3-D2	100.00	2 of 2	0.00
CDKL5	96.50	2 of 2	4.95	ABL1 (E255K)	100.00	1 of 1	0.00
KIT (V559DT6)	97.00	2 of 2	4.24	ADCK3	87.50	2 of 2	17.68
KIT (V559D)	79.50	2 of 2	23.33	ALK2	91.50	2 of 2	12.02
PDK1	100.00	2 of 2	0.00	CDK9	100.00	2 of 2	0.00
MNK2	98.50	2 of 2	2.12	CDKL2	94.50	2 of 2	7.78
PCTAIRE2	84.00	2 of 2	22.63	CDC2L1	87.00	2 of 2	12.73
CDKL1	100.00	1 of 1	0.00	EPHB6	100.00	2 of 2	0.00
CK1A2	92.50	2 of 2	10.61	EPHA2	100.00	2 of 2	0.00
CK2A2	92.50	2 of 2	10.61	TA03	100.00	2 of 2	0.00
CLK1	64.00	2 of 2	24.04	SGK110	26.50	2 of 2	7.78
DYRK1A	97.00	2 of 2	4.24	TA02	100.00	2 of 2	0.00
ACTR2	69.00	2 of 2	5.66	EGFR	93.50	2 of 2	9.19
RAF1	100.00	2 of 2	0.00	FGFR3G697C	92.00	2 of 2	11.31
ROCK1	91.00	2 of 2	12.73	MARK3	100.00	2 of 2	0.00
SMMLCK	99.50	2 of 2	0.71	MST1	100.00	2 of 2	0.00
YES	84.00	2 of 2	22.63	PIP5K1C	100.00	1 of 1	0.00
WEE1B	95.00	2 of 2	7.07	PKG2	89.00	2 of 2	15.56
VRK2	98.00	1 of 1	0.00	PI3KCA (H1047L)	100.00	2 of 2	0.00
YANK1/ STK32A	100.00	1 of 1	0.00	STLK3	80.50	2 of 2	14.85
MTOR/FRAP1	100.00	1 of 1	0.00	ABL1 (F 317L)	70.00	1 of 1	0.00
p70S6K	84.00	1 of 1	0.00	TEC	91.00	2 of 2	12.73
PYK2	87.00	2 of 2	18.38	LIMK1	61.50	2 of 2	54.45
JAK2	1.80	2 of 2	1.56	CDKL3	94.00	2 of 2	8.49
IRR	85.50	2 of 2	20.51	DCAMKL1	100.00	2 of 2	0.00
KIT (V559DV6)	100.00	2 of 2	0.00	CSK	80.00	2 of 2	5.66
LCK	45.50	2 of 2	26.16	CTK	99.00	2 of 2	1.41
ULK3	100.00	2 of 2	0.00	IKKE	77.00	2 of 2	32.53
YANK3	100.00	2 of 2	0.00	ERK7	90.00	2 of 2	14.14
TYRO3	72.50	2 of 2	38.89	NUAK1	63.00	2 of 2	21.21
CAMK2B	80.00	2 of 2	28.28	MYT1	79.00	2 of 2	29.70
AURB	100.00	2 of 2	0.00	NLK	76.00	2 of 2	33.94
TRKB	62.50	2 of 2	6.36	RSK3	100.00	2 of 2	0.00
ZC2/TNIK	82.00	2 of 2	24.04	RSK1-D2	92.50	2 of 2	10.61
TRKC	84.00	2 of 2	22.63	AURC	100.00	2 of 2	0.00
SIK	100.00	2 of 2	0.00	AMPKA1	65.50	2 of 2	6.36
FUSED	82.00	2 of 2	25.46	TSSK1	99.00	2 of 2	1.41
FGR	82.00	2 of 2	25.46	SNRK	97.00	1 of 1	0.00
FLT3 (K 663Q)	37.50	2 of 2	13.44	PHKG1	100.00	2 of 2	0.00
CDK3	84.50	2 of 2	21.92	ABL1 (M351T)	79.00	1 of 1	0.00
FLT3 (N841I)	6.65	2 of 2	8.98	YSK4	100.00	2 of 2	0.00
EGFR (L858R)	98.50	2 of 2	2.12	AKT3	86.00	2 of 2	19.80
PKD2	92.50	2 of 2	10.61	AKT2	100.00	2 of 2	0.00
PKCT	100.00	2 of 2	0.00	MAP3K15	100.00	2 of 2	0.00
KIT (L576P)	67.50	2 of 2	16.26	PKCI	100.00	1 of 1	0.00
LYN	66.50	2 of 2	27.58	PKD1	63.50	2 of 2	9.19
EPHB3	93.50	2 of 2	9.19	PLK3	95.50	2 of 2	4.95
PRP4	88.00	2 of 2	14.14	ERK1	100.00	2 of 2	0.00
PRKX	97.00	2 of 2	4.24	LOK	100.00	2 of 2	0.00
MARK1	82.50	2 of 2	24.75	MAP2K4	100.00	2 of 2	0.00
MET (Y1235D)	82.50	2 of 2	4.95	MAP3K6	97.50	2 of 2	3.54
MSK1	93.00	2 of 2	9.90	MLK2	81.00	2 of 2	19.80

MSSK1	100.00	2 of 2	0.00	SBK1	100.00	2 of 2	0.00
MYO3A	88.50	2 of 2	16.26	RIOK3	84.00	2 of 2	22.63
ZAK	80.00	2 of 2	28.28	ABL1 (H396P)	67.00	1 of 1	0.00
ABL1(nonph)	100.00	1 of 1	0.00	YSK1	88.50	2 of 2	16.26
ULK1	87.50	2 of 2	17.68	PDGFRB	16.00	2 of 2	7.07
DRAK1	100.00	2 of 2	0.00	PI3KCA (H1047Y)	97.00	2 of 2	4.24
DAPK3	6.55	2 of 2	2.05	MNK1	100.00	2 of 2	0.00
LATS2	100.00	2 of 2	0.00	PI3KCA (E545A)	100.00	2 of 2	0.00
LATS1	100.00	2 of 2	0.00	IGF1R	100.00	2 of 2	0.00
PIM3	96.00	2 of 2	5.66	QIK	79.00	2 of 2	29.70
PIM1	98.00	2 of 2	2.83	PLK1	100.00	2 of 2	0.00
DYRK2	95.50	2 of 2	6.36	TrkA	94.50	2 of 2	3.54
DYRK1B	100.00	2 of 2	0.00	TTK	82.00	2 of 2	18.38
FLT3	21.00	2 of 2	2.83	CDK4-cyclinD3	100.00	1 of 1	0.00
FLT3 (ITD)	75.50	2 of 2	14.85	TIE1	85.00	2 of 2	21.21
FLT4	91.00	2 of 2	12.73	FLT3 (D835H)	14.20	2 of 2	9.62
GSK3A	64.00	2 of 2	19.80	ICK	100.00	2 of 2	0.00
GPRK7	96.50	2 of 2	4.95	HIPK3	100.00	2 of 2	0.00
GPRK4	97.00	2 of 2	4.24	CHK1	100.00	2 of 2	0.00
HIPK1/ MAP4K1	100.00	2 of 2	0.00	DAPK2	14.65	2 of 2	7.57
EPHB1	85.00	2 of 2	21.21	MAP4K5/KHS1	99.50	2 of 2	0.71
HUNK	100.00	2 of 2	0.00	MSK2	78.00	2 of 2	31.11
IKKA	93.00	2 of 2	9.90	MET	100.00	2 of 2	0.00
JAK1	63.00	2 of 2	49.50	MUSK	85.00	2 of 2	21.21
IRAK1	100.00	2 of 2	0.00	DLK	100.00	2 of 2	0.00
CAMK1A	68.50	2 of 2	24.75	DDR 1	100.00	2 of 2	0.00
CAMK2G	77.50	2 of 2	21.92	KDR	100.00	2 of 2	0.00
CDK2	96.50	2 of 2	4.95	JNK1	100.00	2 of 2	0.00
CLK4	78.50	2 of 2	30.41	JNK3	100.00	2 of 2	0.00
DDR2	97.00	2 of 2	4.24	PI3KCA	100.00	2 of 2	0.00
AURA	97.00	2 of 2	4.24	NUAK2	79.00	2 of 2	5.66
TESK1	77.00	2 of 2	32.53	EGFR G719C	91.00	2 of 2	11.31
SYK	97.50	2 of 2	0.71	PKCH	98.50	2 of 2	2.12
TRPM6	80.00	1 of 1	0.00	RON	97.50	2 of 2	3.54
SRPK1	67.50	2 of 2	6.36	PI4KCB	100.00	2 of 2	0.00
CAMK2A	65.00	2 of 2	38.18	GCN2 (S808G)	80.00	2 of 2	28.28
BIKE	85.00	2 of 2	1.41	HER3/ERBB3	94.00	2 of 2	8.49
BMX	71.50	2 of 2	28.99	ERK2	96.50	2 of 2	4.95
EGFR (L858R, T790M)	94.00	2 of 2	8.49	ERK5	100.00	2 of 2	0.00
GSK3B	100.00	2 of 2	0.00	ITK	89.50	2 of 2	9.19
PKR	78.50	2 of 2	24.75	MSK1(D2)	83.00	2 of 2	24.04
PLK2	97.50	2 of 2	2.12	LZK	90.50	2 of 2	13.44
PKG1	83.00	2 of 2	11.31	QSK	100.00	2 of 2	0.00
TGFBR2	73.50	2 of 2	10.61	CLK3	68.00	2 of 2	15.56
MAP2K6	85.50	2 of 2	4.95	ABL 1 (Y253F)	94.00	1 of 1	0.00
MAP2K2	100.00	2 of 2	0.00	EPHA6	88.50	2 of 2	16.26
RIPK5/ DSTYK	92.00	1 of 1	0.00	PCTAIRE3	99.50	2 of 2	0.71
RET	63.50	2 of 2	10.61	MSK2_D2	99.50	2 of 2	0.71
RET V804L	69.00	2 of 2	4.24	GCK/MAK4K2	96.00	2 of 2	5.66
TIE2	100.00	2 of 2	0.00	FLT3 (D835Y)	21.00	2 of 2	8.49
AKT1	100.00	2 of 2	0.00	CHK2	92.00	2 of 2	11.31
ACTR2B	84.50	2 of 2	21.92	MAP2K3	100.00	2 of 2	0.00
FAK	99.50	2 of 2	0.71	MAP2K1	92.50	2 of 2	2.12
DRAK2	77.00	2 of 2	1.41	PCTAIRE1	100.00	2 of 2	0.00
LKB1	97.50	2 of 2	3.54	PAK4	100.00	2 of 2	0.00

LTK	21.00	2 of 2	1.41	PAK5	84.00	2 of 2	14.14
LIMK2	100.00	2 of 2	0.00	WEE1	92.50	2 of 2	10.61
PIP5K2B	97.00	2 of 2	4.24	ZAP70	100.00	2 of 2	0.00
PIP5K2C	100.00	1 of 1	0.00	TXK	81.50	2 of 2	4.95
PIP5K1A	99.50	2 of 2	0.71	EGFR A749DL	99.00	2 of 2	1.41
ANKRD3/ RIPK4	100.00	2 of 2	0.00	FGFR1	76.00	2 of 2	28.28
ULK2	100.00	2 of 2	0.00	CDK8	100.00	2 of 2	0.00
PI3KCB	98.00	2 of 2	2.83	CHED (CDC2L5)	100.00	1 of 1	0.00
PI3KC2G	100.00	2 of 2	0.00	CDK7	100.00	2 of 2	0.00
PI3KCA (M1043I)	97.50	2 of 2	3.54	ARG	70.00	2 of 2	35.36
MST2	100.00	2 of 2	0.00	ALK1	66.00	2 of 2	0.00
p38G	92.00	2 of 2	11.31	EPHB2	99.00	2 of 2	1.41
PAK2	95.50	2 of 2	6.36	ERK4	98.50	2 of 2	2.12
PHKG2	97.00	2 of 2	4.24	EPHA1	96.50	2 of 2	4.95
HIPK2	100.00	2 of 2	0.00	RIPK1	95.00	2 of 2	7.07
TNK1	31.50	2 of 2	20.51	RET V804M	73.00	2 of 2	5.66
SRPK2	97.00	2 of 2	4.24	AMPKA2	84.00	2 of 2	22.63
STK33	100.00	2 of 2	0.00	YANK2	91.50	2 of 2	12.02
BRK	94.50	2 of 2	7.78	IRAK4	100.00	1 of 1	0.00
ABL1 (T315I)	100.00	1 of 1	0.00	MAP3K4	100.00	2 of 2	0.00
MRCKB	83.50	2 of 2	23.33	MAP KAPK5	94.00	2 of 2	8.49
MAP3K3	100.00	2 of 2	0.00	MARK2	81.50	2 of 2	7.78
SGK085	100.00	2 of 2	0.00	MAP4K3 (GLK/KHS2)	73.00	2 of 2	16.97
BMPR1B	100.00	2 of 2	0.00	TYK2 Pseudo- kinase	100.00	2 of 2	0.00
CK1G1	80.50	2 of 2	27.58	TYK2	37.25	2 of 2	40.66
CK1e	90.00	2 of 2	14.14	JAK1 Pseudokinase	100.00	2 of 2	0.00
RIOK1	95.50	2 of 2	6.36	IRAK3	100.00	2 of 2	0.00
RSK2	100.00	2 of 2	0.00	CAMKK2	68.50	2 of 2	23.33
ZC3/MINK	100.00	2 of 2	0.00	CASK	100.00	1 of 1	0.00
TLK2	94.50	2 of 2	7.78	BRSK2	78.50	2 of 2	30.41
CAMK2D	58.00	2 of 2	18.38	FRK	80.50	2 of 2	26.16
BMPR2	100.00	2 of 2	0.00	GAK	47.50	2 of 2	27.58
CAMK1G	81.00	2 of 2	16.97	DCAMKL3	27.50	2 of 2	16.26
HER2/ERBB2	100.00	2 of 2	0.00	HER4/ERBB4	90.50	2 of 2	13.44
FER	100.00	2 of 2	0.00	HH498	51.00	2 of 2	35.36
FLT1	100.00	2 of 2	0.00	IRE1	93.50	2 of 2	9.19
MAP3K1	100.00	2 of 2	0.00	RSK4	100.00	2 of 2	0.00
MAP KAPK2	97.00	2 of 2	4.24	RSK1	100.00	2 of 2	0.00
MAK	100.00	2 of 2	0.00	FGFR3	100.00	2 of 2	0.00
PKCE	79.50	2 of 2	6.36	PKACA	96.00	2 of 2	5.66
PKD3	100.00	2 of 2	0.00	MARK4	78.50	2 of 2	12.02
PKACB	81.00	2 of 2	26.87	PAK1	92.50	2 of 2	10.61

Pharmacokinetics:

In the *in vivo* studies, compound **20** was administered in a polyethylene glycol 400 (PEG-400)/citrate buffer (80:20, v/v) solution. Measurements were made at 0.5, 1, 2, 4 and 8 h and averaged over 2 rats.