

Supporting Information

Discovery of a highly selective JAK2 inhibitor, BMS-911543 for the treatment of myeloproliferative neoplasms

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General methods:

1. *In Vitro* biochemical assays:

The inhibitory activity of compounds in biochemical kinase assays using recombinant enzymes has been described previously.¹⁰ In brief, incubation mixtures included: 1.1 nM JAK2, 1.5 μM peptide substrate (5-FAM-KKKKEEIYFFFG-OH for JAK2) and 30 μM ATP. The reaction mixture was analyzed on a Caliper LabChip 3000 (Caliper LifeSciences, Hopinkton, MA, USA) by electrophoretic separation of the fluorescent substrate and phosphorylated product after 180 min. The inhibitory activity of compounds against multiple other recombinant enzymes was evaluated using similar methodology in kinase assays or for interaction with over 450 kinases at 1 μM in collaboration with Ambit Biosciences (now DiscoverX) (San Diego, CA, USA) using competition-binding assays, as described previously.²¹ For enzyme kinetics, BMS-911543 was tested from 42 pM to 8.33 μM against JAK1, JAK2 or JAK3. All kinase reactions were carried out at room temperature with g-[33P]-labeled ATP at 3.75–100 μM for 30 min and terminated by the addition of 1% phosphoric acid. Phosphorylated peptide was captured on 96- well phosphocellulose filter plates using a vacuum manifold and quantified using scintillation counting. K_i was determined from global fits using a competitive inhibition model for JAK1 and JAK3: $v = (V_{max} \times [S]) / (k_m((1 + [I]/k_i)^n) + [S])$, and a mixed type inhibition model for JAK2: $v = V_{max} \times [S] / (k_m \times (1 + ([I]/k_i)^n) + [S](1 + ([I]/k_i)^n))$

2. Protein expression and purification for X-ray structure:

Large scale (40L) expression biomass of Kinase-domain of human JAK2 (residues 839-1132) with hexa-histidine tag at C-terminus expressed in baculovirus was purchased from Blue Sky Biotech, Worcester, MA. After cell lysis, the soluble protein was purified by nickel-agarose affinity chromatography, and gel filtration chromatography (Superdex 75). The protein was concentrated to 10 mg/ml in a buffer containing 20 mM Tris (pH 8.0), 250 mM NaCl, 2 mM dithiothreitol (DTT), and 5% (v/v) glycerol and stored at -80 °C. The C-terminal His tag was not removed for crystallization.

3. Protein crystallization:

Crystals of human JAK2 kinase domain in complex with compounds were obtained at 22°C by the hanging drop vapor diffusion method. The protein (10 mg/ml concentration) was incubated with BMS-865992 (protein:BMS865992 molar ratio of 1:5) at 4°C for 60 mins prior to crystallization setup. The reservoir solution contains 100mM MES buffer (pH -6.5), 100mM NaCl, 28% (w/v) PEG3350, 2mM DTT and 200mM tri-sodium citrate. Crystals were cryoprotected by supplementing the mother liquor with 10% (v/v) PEG400 and in 10 % (v/v) glycerol and harvested by flash-cooling in liquid nitrogen.

4. X-ray structure determination:

Data were collected on Rigaku RU-200 rotating anode with a Saturn92 detector and were reduced with the programs DENZO and SCALEPACK (Otwinowski & Minor, 1997). The previously reported structure of JAK2 (PDB entry 3Q32) was used as the starting model in the refinement. The structure was refined with the program autoBUSTER (Global Phasing Ltd, Cambridge, England) and were examined and manually refitted with the program COOT (ref). The difference electron density clearly defined the position of the inhibitor in the ATP-binding pocket. Additional cycles of refinement were performed to complete the refinement and add solvent molecules. The crystallographic data-collection and reduction statistics reported in PDB.

5. Antiproliferative assays:

The anti-proliferative 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. In certain instances, Ba/F3-

engineered cells were propagated in the presence of recombinant human erythropoietin (EPO, R&D Systems, Minneapolis, MN, USA) or recombinant mouse IL-3 (Invitrogen).

6. Western blot analysis:

Evaluation of BMS-911543 effects on Ba/F3 and SET2 cell lines or tumor xenograft lysates was performed by western blotting. For cell lines, roughly 500 000 cells per mL of media were incubated with compound in dose–response format for 2 h, and subsequently processed for western blotting for p-STAT5 (tyrosine 694, BD Biosciences, 1:400 dilution), total STAT5 protein antibodies (BD Biosciences, 1:400 dilution), p-STAT3 (tyrosine 705, Cell Signaling, Beverly, MA, USA, 1:500 dilution), STAT3 (Cell Signaling, 1:500 dilution), ID1 (R&D Systems, 1:1000 dilution), PIM1 (Cell Signaling, 1:500 dilution), pSTAT1(tyrosine 701, Cell Signaling, 1:1000 dilution) or STAT1(AbCam, Cambridge, MA, USA, 1:500 dilution) at 1:400 dilution. SET2 cells were also treated for 24 h with BMS-911543 for the analysis of STAT1 levels. Protein extracts from snap-frozen SET2 tumors were prepared and similarly processed for pSTAT5/STAT5, as described for the cell line analysis.

7. Pharmaceutics:

In the in vivo studies, BMS-911543 was administered in a polyethylene glycol 400 (PEG-400)/citrate buffer (80:20, v/v) solution. In higher dose oral PK studies, BMS-911543 was administered as a solution in 40% labrasol, 10% pluronic F-68, 40% propylene glycol, 10% water, and 1M equivalent (to drug) of methanesulfonic acid in mice and dogs and as a HCl/PEG-400/polyvinyl pyrrolidinone (20/70/10) solution in rats. The formulation for micro-suspension studies in rats and dogs comprised of 0.5% methyl cellulose, 0.1% tween 80, and 99.4% water.

8. *In vitro* biotransformation studies:

Compounds (10 μ M) were incubated with liver microsomes (1 mg/mL) from human in the presence and absence of NADPH (1 mM) at 37°C for 60 min. The samples were deproteinated by the addition of an equal volume of acetonitrile, followed by centrifugation at 1500xg for 20 min. The supernatant was analyzed by direct injection onto the HPLC/UV/MS system described below.

Chromatographic separations were carried out with an HPLC system that consisted of an Agilent 1100 HPLC (Agilent Technologies, Wilmington, DE) and 5-micron Phenomenex CuroSil-PFP (2.0 x 150 mm for in vitro samples and 4.6 x 250 mm for in vivo samples) column maintained at room temperature. The detectors were an Agilent Photodiode Array Detector and a Finnigan Orbitrap mass spectrometer. The mobile phase consisted of 0.1% formic acid in water (Solvent A) and acetonitrile (Solvent B), with the

following gradient conditions. For in vivo samples, samples were split where $\frac{1}{4}$ went to Mass spectrometer and $\frac{3}{4}$ went to radio-flow detector or fraction collector. For fraction collector, after collection, 96-well plates were dried in SpeedVac overnight, then count radioactivities on topcount for 10 min

Table 1: Solvent Gradient for HPLC Analysis in In Vitro Metabolite Elucidation Studies

Table 7.4: Mobile Phase Gradient for In Vitro Metabolite Elucidation				
Time (min)	% A	% B	Flow Rate (mL/min)	Curve
0 (Initial)	85	15	0.3	isocratic
2	85	15	0.3	isocratic
20	55	45	0.3	linear
21	5	95	0.3	linear
23	5	95	0.3	isocratic
24	85	15	0.3	linear

Table 2: Mobile Phase Gradient for In Vivo Metabolite Elucidation				
Time (min)	% A	% B	Flow Rate (mL/min)	Curve
0 (Initial)	85	15	1	isocratic
2	85	15	1	isocratic
35	55	45	1	linear
36	5	95	1	linear
39	5	95	1	isocratic
40	85	15	1	linear
45	Stop			

UV absorbance was monitored at 340 nm. The HPLC was interfaced to the mass spectrometer with an electrospray atmospheric pressure ionization inlet operating in the positive ionization mode. MS/MS spectra were acquired by data-dependent scanning with relative collision energy of 30%. Metabolite identification was tentatively based on the observed masses and UV spectra compared with those of the parent, obtained under comparable conditions

9. In Vivo pharmacodynamic (PD) assays:

BMS-911543 dosing solutions were administered to BALB/c mice by oral gavage at the indicated dose levels. After BMS-911543 administration, triplicate animals per time point were euthanized and blood was harvested via cardiac puncture for preparation of pharmacokinetic (PK) or PD analyses. Platelets

were stimulated *ex vivo* with murine TPO (mTPO, Peprotech) and stained for CD61 (anti-CD61 FITC, eBiosciences, San Diego, CA, USA). Samples were then processed for p-STAT5 levels, as described above, using anti-pY695 Alexa647-conjugated STAT5 antibody. SET2 cells were inoculated into female athymic mice and propagated as subcutaneous xenografts. Animals with tumors reaching 500 mm³ were administered BMS-911543 or vehicle as described above for the indicated times. Tumors were snap frozen in liquid nitrogen and processed for p-STAT5 for western blot analysis as described above.

KinomeScan (formerly Ambit) Panel Selectivity Data:

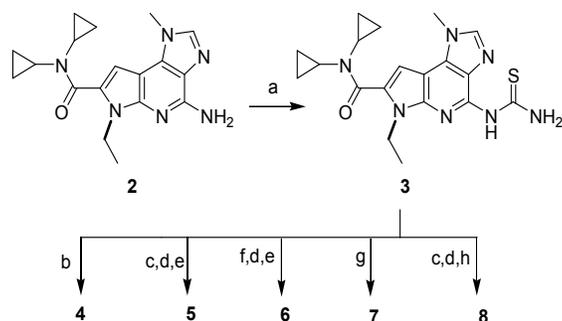
Target	%Ctl %1 uM	Target	%Ctl %1 uM	Target	%Ctl %1 uM	Target	%Ctl %1 uM	Target	%Ctl %1 uM
JAK2	0.2	SRPK1	82.5	HER2/ERBB2	90.0	SYK	95.5	CDC2L5	100.0
FYN	10.3	EGFR	83.0	IRR	90.0	ABL1_Q252H_NPH	96.0	CDK11	100.0
DAPK3	12.5	EGFR_S752DL	83.0	KIT	90.0	CDK8	96.0	CDK2	100.0
ACK	12.8	FLT3_R834Q	83.0	MST1	90.0	EGFR_G719S	96.0	CDK4-CCND1	100.0
SRC	14.5	PKD1	83.0	PIM1	90.0	FGFR4	96.0	CDK4-CCND3	100.0
FLT3_D835H	19.5	PI3KCA	83.5	EPHA3	90.5	INSR	96.0	CDK5	100.0
DAPK2	25.5	PKN2	83.5	GCN2C_S808G	90.5	MAP3K1	96.0	CHK1	100.0
JAK3_D2	26.1	RHOK	83.5	HIPK2	90.5	YSK4	96.0	CK1A	100.0
CAMK4	34.0	TLK1	83.5	MARK3	90.5	DDR1	96.5	CRIK	100.0
TNK1	38.5	YANK2	83.5	PLK1	90.5	EGFR_A750DL	96.5	DCAMKL1	100.0
TYK2	39.5	ABL1_H396P_PH	84.0	QIK	90.5	EPHA2	96.5	DLK	100.0
FLT3_N841I	41.5	AURA	84.0	ABL1_F317I	91.0	EPHB6	96.5	DMPK2	100.0
FLT3	45.5	EGFR_I759DL	84.0	CDKL2	91.0	MSK1_C	96.5	DRAK1	100.0
BLK	46.5	MAP2K1	84.0	DYRK2	91.0	ACTR2	97.0	DYRK1A	100.0
DAPK1	47.5	MAP3K5	84.0	FGFR3	91.0	AMPKA2	97.0	EIF2AK1	100.0
FMS	48.0	MAPKAPK5	84.0	JNK3	91.0	CDK9	97.0	EPHA4	100.0
KIT_D816V	48.5	ROCK2	84.0	KIT_V559DT6	91.0	EPHA8	97.0	EPHA5	100.0
JAK1	51.5	ZC1/HGK	84.0	PAK6	91.0	FGFR3_G697C	97.0	EPHB2	100.0
NUAK1	55.5	EGFR_L858RT	84.5	PI3KC2B	91.0	GPRK4	97.0	ERK1	100.0
FLT3_K663Q	57.0	ERK3	84.5	PKACB	91.0	MNK1	97.0	ERK2	100.0
FLT3_D835Y	57.5	NEK7	84.5	PKN1	91.0	PYK2	97.0	ERK5	100.0
KIT_A829P	59.0	PLK3	84.5	SBK	91.0	TYK2_PSEUDO	97.0	FAK	100.0
LCK	60.5	SRPK2	84.5	ULK1	91.0	BRK	97.5	FER	100.0
CLK2	61.5	IRE1	85.0	YSK1	91.0	CTK	97.5	FES	100.0
ALK1	62.5	JAK1_PSEUDO	85.0	BMPR1B	91.5	IGF1R	97.5	HCK	100.0
ALK	63.5	MARK1	85.0	EPHB4	91.5	KDR	97.5	HER3/ERBB3	100.0
PDGFRB	65.0	PI3KCA_Q546K	85.0	FGFR1	91.5	LIMK2	97.5	HIPK3	100.0
GSK3A	66.0	RAF1	85.0	MAP3K15	91.5	MST3	97.5	HPK1	100.0
RET	66.0	RIOK3	85.0	MELK	91.5	RET_V804L	97.6	ICK	100.0
CAMK2A	66.5	STK16	85.0	P38A	91.5	NIM1	97.5	IRAK3	100.0
CAMK1D	68.0	STK32A	85.0	PKACA	91.5	RSK4_C	97.5	IRAK4	100.0
LRRK2_G2019S	68.0	TAO1	85.0	ROS	91.5	YANK3	97.5	ITK	100.0
LTK	68.0	CAMK1A	85.5	TRKA	91.5	ABL1_M351T	98.0	JNK1	100.0
CAMK2D	69.0	P38G	85.5	JNK2	92.0	CHK2	98.0	KHS2	100.0
GAK	69.5	PI3KCA_E545A	85.5	MLK3	92.0	CLIK1	98.0	KIT_L576P	100.0
SGK110	70.5	AKT3	86.0	YES	92.0	FLT1	98.0	KIT_V559DV6	100.0
GPRK7	71.0	ALK4	86.0	BRAF_V600E	92.5	FUSED	98.0	LATS1	100.0
TGFBR2	71.0	MAP3K3	86.0	CK1E	92.5	MAP2K3	98.0	LOK	100.0
RET_V804M	71.5	MARK2	86.0	CK1G1	92.5	RIPK2	98.0	LRRK2	100.0
EGFR_G719C	72.0	MSK1_N	86.0	FGR	92.5	DYRK1B	98.5	MAK	100.0
MAP3K2	72.0	MUSK	86.0	FRK	92.5	EPHA7	98.5	MAL13P1.279	100.0
PIP5K1C	72.0	TRPM6	86.0	MAP2K4	92.5	FGFR2	98.5	MAP2K5	100.0
LIMK1	72.5	ZC3/MINK	86.0	PCTAIRE3	92.5	NEK9	98.5	MAP2K7	100.0
PDGFRA	72.5	BMPR2	86.5	PIM3	92.5	SLK	98.5	MAP3K4	100.0
CAMKK2	73.5	BRSK2	86.5	ABL1_Y253F	93.0	STK33	98.5	MRCKA	100.0
ERK4	73.5	MAP2K2	86.5	MLK1	93.0	TLK2	98.5	MTOR	100.0
FLT3_ITD	74.0	PAK5	86.5	MSSK1	93.0	ZC2/TNIK	98.5	MYO3B	100.0
PIP5K1A	74.0	PI3KC2G	86.5	MYO3A	93.0	AKT2	99.0	NEK11	100.0
BIKE	75.0	ZAP70	86.5	PAK4	93.0	CDKL1	99.0	NEK3	100.0
CLK1	75.0	CDC2L1_ISO4	87.0	RSK3_C	93.0	DCAMKL2	99.0	NEK4	100.0
CSK	75.0	GSK3B	87.0	SGK288	93.0	EGFR_T751DL	99.0	NEK5	100.0
TYRO3	75.5	PCTAIRE2	87.0	TRKC	93.0	EGFR_T790M	99.0	NEK6	100.0
PI3KCA_H1047Y	76.0	PKCH	87.0	AURB	93.5	EPHB1	99.0	P38B	100.0
QSK	76.0	SMMMLCK	87.0	CK1A2	93.5	KIT_V559D	99.0	P38D	100.0
NUAK2	76.5	ARG	87.5	CLK4	93.5	PFB0815W	99.0	P70S6K	100.0
GCK	77.0	CK1G2	87.5	EPHA1	93.5	PFTAIRE2	99.0	PAK3	100.0
MLK2	77.0	FLT4	87.5	HIPK4	93.5	TESK1	99.0	PCTAIRE1	100.0
PRP4	77.0	HIPK1	87.5	LYN	93.5	AURC	99.5	PFTAIRE1	100.0
MNK2	77.5	PI3KCA_E545K	87.5	MRCKB	93.5	CDKL5	99.5	PHKG1	100.0
NDR1	77.5	PKG2	87.5	NDR2	93.5	MAPKAPK2	99.5	PI3KCA_I800L	100.0
ABL1_Q252H	78.0	RET_V804L	87.5	ALK2	94.0	MER	99.5	PI3KCG	100.0
PI3KCB	78.0	TAO3	87.5	AXL	94.0	MST2	99.5	PIP5K2C	100.0
PAK1	78.5	EGFR_L858R	88.0	CDK3	94.0	TGFBR1	99.5	PKCT	100.0
TIE1	78.5	IKKB	88.0	EGFR_E749DL	94.0	ABL1	100.0	PKD3	100.0
LZK	79.0	MSK2_C	88.0	OSR1	94.0	ABL1_E255K	100.0	PKNB	100.0
ROCK1	79.0	PI3KCA_E542K	88.0	PDK1	94.0	ABL1_E255K_PH	100.0	PLK4	100.0
CAMK1G	79.5	PKCE	88.0	PKCI	94.0	ABL1_F317L_NPH	100.0	PRXK	100.0
DCAMKL3	79.5	PLK2	88.0	PKD2	94.0	ABL1_F317L_PH	100.0	RIOK1	100.0
BTk	80.0	RIOK2	88.0	RSK1_C	94.0	ABL1_F317L	100.0	RIPK1	100.0
CDK7	80.0	TBK1	88.0	TXK	94.0	ABL1_F317L_NPH	100.0	RIPK5	100.0
HER4/ERBB4	80.0	BRAF	88.5	WEE1B	94.0	ABL1_F317L_PH	100.0	RON	100.0
MARK4	80.0	NEK2	88.5	CDC2L2_ISO1	94.5	ABL1_H396P	100.0	RSK1_N	100.0
MAST1	80.0	CK1G3	89.0	CDKL3	94.5	ABL1_H396P_NPH	100.0	RSK2_N	100.0
AAK1	80.5	DDR2	89.0	ERK7	94.5	ABL1_M351T_PH	100.0	RSK3_N	100.0
CAMK2G	80.5	EGFR_L861Q	89.0	KHS1	94.5	ABL1_NPH	100.0	RSK4_N	100.0
PHKG2	81.0	HUNK	89.0	MAP2K6	94.5	ABL1_PH	100.0	SGK085	100.0
PI3KCA_H1047L	81.0	KIT_D816H	89.0	MSK2_N	94.5	ABL1_Q252H_PH	100.0	SGK3	100.0
RET_M918T	81.0	LKB1	89.0	MYT1	94.5	ABL1_T315I	100.0	SIK	100.0
TRKB	81.0	MAP3K6	89.0	PI4KCB	94.5	ABL1_T315L_NPH	100.0	SKMLCK	100.0
CK2A2	81.5	MST4	89.0	CK1D	95.0	ABL1_T315L_PH	100.0	SNRK	100.0
PI3KCD	81.5	PAK2	89.0	CLK3	95.0	ABL1_Y253F_PH	100.0	SRM	100.0
TTK	81.5	PKCD	89.0	DMPK1	95.0	ACTR2B	100.0	STLK3	100.0
CAMK2B	82.0	PKR	89.0	EPHA6	95.0	ADCK3	100.0	TEC	100.0
IKKA	82.0	TAK1	89.0	EPHB3	95.0	ADCK4	100.0	TIE2	100.0
PI3KCA_C420R	82.0	CAMKK1	89.5	NEK1	95.0	AKT1	100.0	TSSK1	100.0
PKG1	82.0	LATS2	89.5	ULK3	95.0	AMPKA1	100.0	VRK2	100.0
TAO2	82.0	PI3KCA_M1043I	89.5	DRAK2	95.5	ANKRD3	100.0	WEE1	100.0
HH498	82.5	PIP5K2B	89.5	MET	95.5	BMPR1A	100.0	ZAK	100.0
IKKE	82.5	ULK2	89.5	MET_M1250T	95.5	BRSK1	100.0		
IRAK1	82.5	BMX	90.0	MET_Y1235D	95.5	CAMLCK	100.0		
PIM2	82.5	CK2A1	90.0	NLK	95.5	CASK	100.0		

Biochemical IC₅₀s of Selected Kinases

JAK2 1.1 nM	FLT3 (ITD) 280 nM	PKA 550 nM	SRC 3,640 nM	AURA >2,000 nM	INSR >50,000
TYK2 66 nM	LYN 300 nM	BMX 1,390 nM	CK2A1 >2,000 nM	BTK >2,000 nM	IRAK4 >50,000
JAK3 75 nM	c-FMS 450 nM	IKKe 2000 nM	c-KIT >2,000 nM	LCK >2,000 nM	AURB >50,000
JAK1 360 nM	FLT3 510 nM	DAPK1 2,320 nM	GSK3 β >2,000 nM	IGFR >50,000	DRAK2 >50,000

Chemistry experimental:

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 μ m plates, or using HPLC and LCMS with UV detection at 220 or 254 nm. Purification was accomplished by medium pressure liquid chromatography on a ISCO (Teledyne Isco) with normal phase silica gel, or by reverse phase preparative HPLC. The purity was 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 μ m (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 μ m (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 μ m 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 μ m 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. ¹H NMR spectra were recorded on a Bruker spectrometer (400 or 500 MHz) at ambient temperature.



Scheme 1: (a) benzoylisothiocyanate, acetone; then 1 N NaOH, ethanol, 60 °C, 72% (b) 3-bromopentan-2-one, 60 °C, 44%; (c) methyl 2-bromo-3-oxobutanoate, ethanol, 65 °C, 84%; (d) 1 N NaOH, methanol, 65 °C, 93%; (e) methylamine, HATU, 2,6-lutidine, DMF, 75%; (f) methyl 3-bromo-2-oxobutanoate, ethanol, 65 °C, 70%; (g) 1-bromo-1-(methylsulfonyl)propan-2-one ethanol, 65 °C, 62%; (h) , 1,1-dioxo-1-thiomorpholine, HATU, 2,6-lutidine, DMF, 62%

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

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 (167 mg, 0.493 mmol) in acetone (1.645 mL) was added benzoyl isothiocyanate (0.067 mL, 0.493 mmol). The reaction mixture was stirred at room temperature overnight. The solution was cooled and water was added. The precipitate was collected by vacuum filtration. Additional product remained in the aqueous filtrate. The aqueous filtrate was extracted with ethyl acetate (2 x 10 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo*. The crude benzoyl urea product was used as such in the next reaction. MS (ESI) *m/z* 502.1 (M+H).

The intermediate benzoyl urea was stirred in 1 N NaOH (3 mL, 3.00 mmol) for 4.5 h. The reaction mixture was made acidic with 1 N HCl and then basified with saturated aqueous sodium bicarbonate. The solid product was collected by filtration. *N,N*-Dicyclopropyl-6-ethyl-1-methyl-4-thioureido-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (129 mg, 0.325 mmol, 66 % yield) was isolated as a yellow solid, contaminated with small amounts of intermediate (3) and starting aniline. Material used as such in subsequent reactions. MS (ESI) *m/z* 398.0 (M+H).

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 (3, 0.1168 g, 0.294 mmol) in EtOH (1.469 mL) in a vial was added 3-bromopentan-2-one (prepared using the procedure from *Chem. Comm.* **2004**, 470-471) (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/H₂O 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.

Retention Time: 6.51 min

HPLC Purity: 97%

HRMS for C₂₃H₂₇N₇OS:

Calculated - 449.5814

Found 449.5811 [M+H]⁺.

MS (ESI) m/z 504.0 (M+H)

¹H NMR (500 MHz, DMSO-d₆) δ ppm 8.14 (s, 1H), 7.25 (s, 1H), 4.62 (q, 2H, J = 6.97 Hz), 4.04 (s, 3H), 2.89 – 2.98 (m, 2H), 1.40 (t, 3H, J = 6.87 Hz), 0.73 – 0.80 (m, 4H), 0.65 – 0.71 (m, 4H)

2-(7-(dicyclopropylcarbamoyl)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridin-4-ylamino)-N,4-dimethylthiazole-5-carboxamide (5):

Methyl 2-bromo-3-oxobutanoate (213 mg, 1.094 mmol, prepared following the procedure described by Abu T. Khan et al. in *Tet Lett* 2006, 47, 2751-2754) was added to a suspension of *N,N*-dicyclopropyl-6-ethyl-1-methyl-4-thioureido-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (290 mg, 0.730 mmol) in ethanol (5 ml) and the reaction mixture was heated at 65 °C for 2h. LC/MS shows completion of reaction (reaction remained as suspension all the time). The reaction mixture was concentrated to remove ethanol, and taken up in dichloromethane. The solution was washed with saturated sodium bicarbonate and the aqueous layer was back extracted with dichloromethane. The combined organic layers were dried over magnesium sulfate, filtered, and concentrated *in vacuo*. The crude was purified by flash chromatography using an automated ISCO system (40 g column, eluting with 0-5% methanol / dichloromethane) to afford methyl 2-(7-(dicyclopropylcarbamoyl)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridin-4-ylamino)-4-methylthiazole-5-carboxylate (301 mg, 0.610 mmol, 84 % yield) as a white solid.

MS (ESI) m/z 494.4 (M+H).

¹H NMR (400 MHz, CHLOROFORM-d) δ : 7.75 (s, 1H), 6.92 (s, 1H), 4.76 (q, J = 7.0 Hz, 2H), 4.08 (s, 3H), 3.92 (s, 3H), 2.82 - 2.89 (m, 2H), 2.71 (s, 3H), 1.59 (t, J = 7.0 Hz, 3H), 0.85 - 0.93 (m, 4H), 0.75 - 0.82 (m, 4H)

A mixture of methyl 2-(7-(dicyclopropylcarbamoyl)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridin-4-ylamino)-4-methylthiazole-5-carboxylate (example 50A, 288 mg, 0.583 mmol) and 1N NaOH (5.83 mL, 5.830 mmol) in methanol (5 mL) was heated at 65 °C for 18h. Methanol was evaporated and the aqueous solution was acidified to pH 4 with 1N HCl, solids crashed out of solution and were retained after filtration to afford the desired acid in pure form. The aqueous mixture was then extracted with dichloromethane, The organic layer was dried over magnesium sulfate, filtered and concentrated in vacuo. The crude was purified by flash chromatography using an automated ISCO system (40 g column, eluting with 0-8% methanol / dichloromethane). A total of 260 mg of 2-(7-(dicyclopropylcarbamoyl)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridin-4-ylamino)-4-methylthiazole-5-carboxylic acid (93 % yield) was obtained as a yellow solid.

MS (ESI) m/z 480.2 (M+H).

¹H NMR (500 MHz, DMSO-d₆) δ : 8.03 (br. s., 1H), 7.06 (s, 1H), 4.40 (q, J = 7.0 Hz, 2H), 3.86 (s, 3H), 2.96 (s, 1H), 2.69 - 2.76 (m, 2H), 2.36 (s, 3H), 1.20 (t, J = 6.9 Hz, 3H), 0.56 (d, J = 5.5 Hz, 4H), 0.45 - 0.50 (m, 4H).

A mixture of 2-(7-(dicyclopropylcarbamoyl)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridin-4-ylamino)-4-methylthiazole-5-carboxylic acid (30 mg, 0.063 mmol), methanamine, 40% in water (0.022 mL, 0.250 mmol), HATU (30.9 mg, 0.081 mmol) and 2,6-LUTIDINE (0.015 mL, 0.125 mmol) in DMF (1 mL) was stirred at room temperature overnight. LC/MS shows completion of reaction. Solvent was evaporated and the residue was purified by flash chromatography on silica gel using an automated ISCO system (24 g column, eluting with 2-8% methanol / dichloromethane). The product obtained was further purified by prep-HPLC (Phenomenex Luna S5 ODS C18 column, 21.2x100 mm column, RT 9.078 min, 25-100% gradient aqueous methanol over 10 minutes containing 5 mM ammonium acetate, 20 ml/min at 254 nm). 2-(7-(dicyclopropylcarbamoyl)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridin-4-ylamino)-N,4-dimethylthiazole-5-carboxamide (18 mg, 0.035 mmol, 56.1 % yield) was obtained as a off-white solid.

HPLC RT = 6.39 minutes

Purity = 96%.

MS (ESI) m/z 493.1 (M+H).

¹H NMR (400 MHz, CDCl₃) δ: 7.77 (s, 1H), 6.86 (s, 1H), 5.87 - 5.94 (m, J = 4.6 Hz, 1H), 4.66 (q, J = 7.1 Hz, 2H), 4.04 (s, 3H), 3.04 (d, J = 4.6 Hz, 3H), 2.79 - 2.87 (m, 2H), 2.62 (s, 3H), 1.51 (t, J = 7.0 Hz, 3H), 0.83 - 0.90 (m, 4H), 0.73 - 0.80 (m, 4H).

2-(7-(dicyclopropylcarbamoyl)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridin-4-ylamino)-N,5-dimethylthiazole-4-carboxamide (6):

Methyl 3-bromo-2-oxobutanoate (320 mg, 1.642 mmol) was added to a suspension of N,N-dicyclopropyl-6-ethyl-1-methyl-4-thioureido-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (**3**, 435 mg, 1.094 mmol) in EtOH (10 ml) and the reaction mixture was heated at 65 °C for 2h. The solvent was removed in vacuo and the residue purified by silica gel chromatography (2-10% MeOH/dichloromethane) to give methyl 2-(7-(dicyclopropylcarbamoyl)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridin-4-ylamino)-5-methylthiazole-4-carboxylate (380 mg, 70% yield) as yellow solid.

MS (ESI) rt = 0.87 min, m/z 494 (M+H)

¹H NMR (CHLOROFORM-d) δ: 9.41 (br. s., 1H), 7.78 (s, 1H), 6.89 (s, 1H), 4.73 (q, J = 7.0 Hz, 2H), 4.03 (s, 3H), 3.95 (s, 3H), 2.80 - 2.87 (m, 2H), 2.77 (s, 3H), 1.54 (t, J = 7.0 Hz, 3H), 0.83 - 0.90 (m, 4H), 0.73 - 0.80 (m, 4H).

In aqueous NaOH (0.962 mL, 0.962 mmol) was added to a suspension of methyl 2-(7-(dicyclopropylcarbamoyl)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridin-4-ylamino)-5-methylthiazole-4-carboxylate (example 41, 0.19 g, 0.385 mmol) in ethanol (2 mL) and the mixture was heated at 60 °C for 2h. The reaction was cooled to room temperature and acidified with 1N HCl to pH 4. The reaction mixture was concentrated and the residue triturated 5x with methanol. The combined methanol washes were concentrated, and the remaining solid washed 3x with 2:1 ether/dichloromethane provided 2-(7-(dicyclopropylcarbamoyl)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridin-4-ylamino)-5-methylthiazole-4-carboxylic acid (105 mg, 57% yield).

MS (ESI) m/z 480 (M+H)

Example **6** was prepared in analogous manner as **5** from 2-(7-(dicyclopropylcarbamoyl)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridin-4-ylamino)-5-methylthiazole-4-carboxylic acid to give 2-(7-(dicyclopropylcarbamoyl)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridin-4-ylamino)-N,5-dimethylthiazole-4-carboxamide (75% yield).

HPLC RT = 6.42 minutes

Purity = 97%.

MS (ESI) m/z 493 (M+H).

¹H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 7.84 (s, 1 H) 7.40 (d, J=4.99 Hz, 1 H) 6.90 (s, 1 H) 4.73 (q, J=6.94 Hz, 2 H) 4.08 (s, 3 H) 2.99 (d, J=4.99 Hz, 3 H) 2.73 - 2.91 (m, 5 H) 2.14 (s, 1 H) 1.53 (t, J=7.07 Hz, 3 H) 0.66 - 0.97 (m, 8 H).

Preparation of N,N-dicyclopropyl-6-ethyl-1-methyl-4-(4-methyl-5-(methylsulfonyl)thiazol-2-ylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (7):

To a solution of methanesulphonylacetone (0.5 g, 3.67 mmol) in AcOH (7.34 mL) and dichloromethane (11.02 mL) in a round bottom flask was added bromine (0.189 mL, 3.67 mmol) dropwise. The reaction mixture was stirred at rt 90 min. Solvent was removed in vacuo. The residue was taken up in ether and washed with saturated aqueous sodium bicarbonate (2 x). The organics were concentrated in vacuo. 1-Bromo-1-(methylsulfonyl)propan-2-one (0.332 g, 42% yield) was isolated as an oil.

MS (ESI) m/z 215.5 (M+H)

1-bromo-1-(methylsulfonyl)propan-2-one (32.5 mg, 0.151 mmol) was condensed with *N,N*-dicyclopropyl-6-ethyl-1-methyl-4-thioureido-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide in a similar manner as example 4 to provide *N,N*-dicyclopropyl-6-ethyl-1-methyl-4-(4-methyl-5-(methylsulfonyl)thiazol-2-ylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (12.5 mg, 0.023 mmol, 30.6 % yield) as an off-white solid.

HPLC RT = 5.8 minutes

Purity = 95%.

MS (ESI) m/z 514.1 (M+H)

¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 12.00 (br s, 1H), 8.18 (s, 1H), 7.27 (s, 1H), 4.59 (q, 2H, J = 6.78 Hz), 4.06 (s, 3H), 3.29 (s, 3H), 2.88 – 2.98 (m, 2H), 2.53 (s, 3H), 1.41 (t, 3H, J = 6.90 Hz), 0.72 – 0.81 (m, 4H), 0.62 – 0.71 (m, 4H)

N,N-dicyclopropyl-4-((5-((1,1-dioxido-4-thiomorpholinyl)carbonyl)-4-methyl-1,3-thiazol-2-yl)amino)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (8):

A 25 mL round bottom flask was charged with 2-(7-(dicyclopropylcarbonyl)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridin-4-ylamino)-4-methylthiazole-5-carboxylic acid (intermediate from second step of example 5, 63 mg, 0.131 mmol), HATU (100 mg, 0.263 mmol) and DMF (1 mL). 2,6-

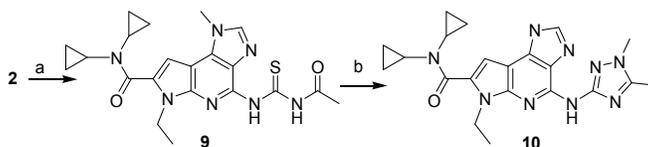
Lutidine (0.040 mL, 0.342 mmol) was added followed by thiomorpholine 1,1-dioxide (53.3 mg, 0.394 mmol), and the reaction mixture was heated at 50 °C for 1.5 h. After cooling to rt, the reaction mixture was diluted with CH₂Cl₂ and washed sequentially with saturated sodium bicarbonate, 10% LiCl solution and saturated sodium bicarbonate. The organics were dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo. The crude residue was purified twice by flash chromatography using an Isco 40 g column eluting from 0-6% MeOH/CH₂Cl₂ to yield the final product (35 mg, 0.058 mmol, 44.2 % yield) as a pale yellow solid.

HPLC RT = 3.4 minutes

Purity = 98%.

MS (ESI) m/z 597.2 (M+H).

¹H NMR (400MHz, CHLOROFORM-d) δ 7.75 (s, 1H), 6.91 (s, 1H), 4.72 (q, J=7.0 Hz, 2H), 4.21 (br d, J=5.1 Hz, 4H), 4.07 (s, 3H), 3.17 - 3.10 (m, 4H), 2.88 - 2.80 (m, 2H), 2.45 (s, 3H), 1.54 (t, J=7.0 Hz, 3H), 0.91 - 0.85 (m, 4H), 0.80 - 0.74 (m, 4H).



Scheme 2: (a) acetyl isocyanate, acetone, 35 °C, 44%; (b) methyl hydrazine, AcOH, 80 °C, 31%;

N,N-dicyclopropyl-4-(1,5-dimethyl-1H-1,2,4-triazol-3-ylamino)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (10):

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 (**3**, 60 mg, 0.177 mmol) in acetone (1.182 mL) was added acetyl isothiocyanate (0.020 mL, 0.230 mmol). The reaction mixture was stirred at room temperature for 6h. LC/MS showed still half of the starting material left. Another 0.5 equivalent of acetyl isothiocyanate was added and the reaction mixture was heated at 35 °C overnight. Solvent was evaporated and the crude product was purified by flash chromatography on silica gel using an automated ISCO system (24 g column, eluting with 2-8% methanol / dichloromethane). 4-(3-acetylthioureido)-N,N-dicyclopropyl-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (**9**, 34 mg, 43.6 % yield) was obtained as a yellow solid.

MS (ESI) m/z 440.1 (M+H).

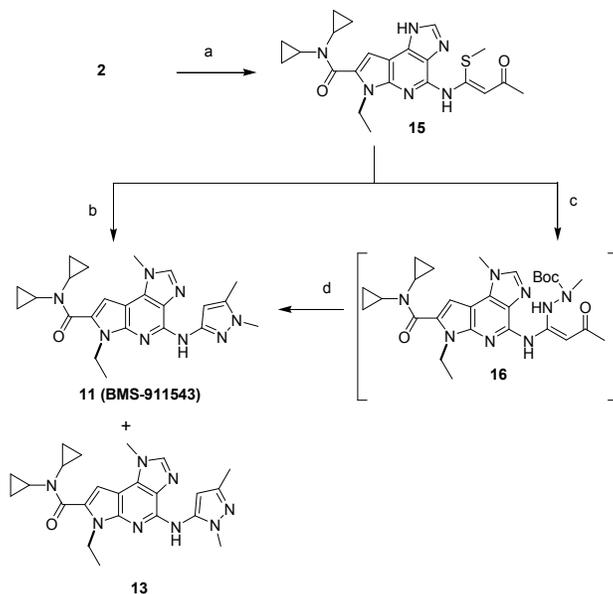
A mixture of 4-(3-acetylthioureido)-N,N-dicyclopropyl-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (example 125A, 17 mg, 0.039 mmol) and methylhydrazine (2.486 μ L, 0.046 mmol) in acetic acid (0.5 mL) was heated at 80 $^{\circ}$ C for 4h, LC/MS showed still starting material remaining, more methylhydrazine (2.486 μ L, 0.046 mmol) was added and heating continued for another 2h, reaction complete. Solvent was evaporated and the crude was purified by preparative HPLC to furnish N,N-dicyclopropyl-4-(1,5-dimethyl-1H-1,2,4-triazol-3-ylamino)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (5.2 mg, 30.7 % yield) as an off-white solid.

HPLC RT = 7.4 minutes

Purity = 96%.

MS (ESI) m/z 434.3 (M+H).

1 H NMR (CDCl₃) δ : 7.96 (s, 1H), 7.65 (s, 1H), 6.83 (s, 1H), 4.67 (q, J = 7.1 Hz, 2H), 4.01 (s, 3H), 3.82 (s, 3H), 2.77 - 2.84 (m, 2H), 2.43 (s, 3H), 1.48 (t, J = 7.2 Hz, 3H), 0.79 - 0.86 (m, 4H), 0.71 - 0.77 (m, 4H).



Scheme 3: (a) NaH, 4,4-bis(methylthio)but-3-en-2-one, DMF, RT, 75%; (b) methyl hydrazine, EtOH, 85 $^{\circ}$ C, 30%; (c) tert-butyl 1-methylhydrazinecarboxylate; AcOH, 50 $^{\circ}$ C (d) formic acid, 60 $^{\circ}$ C, 65%

(Z)-N,N-dicyclopropyl-6-ethyl-1-methyl-4-(1-(methylthio)-3-oxobut-1-enylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (15)

A round bottom flask was charged with 4-amino-N,N-dicyclopropyl-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (**2**) (29.3 g, 86 mmol) in DMF (200 mL). Sodium hydride (10.28 g, 257 mmol) was added and the reaction mixture was stirred for 1 h at room

temperature. 4,4-Bis(methylthio)but-3-en-2-one (27.8 g, 171 mmol) was dissolved in DMF (50 mL) and added to the reaction mixture. The reaction mixture was stirred at room temperature for 6 h. Additional sodium hydride (2 eq) and 4,4-Bis(methylthio)but-3-en-2-one (1 eq) were added. The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with water and then concentrated in vacuo to remove half of the DMF. Ethyl acetate (100 mL) was added, followed by water (2 L). Additional ethyl acetate (400 mL) was added, at which point a yellow solid precipitated out of solution. The solid was isolated by filtration and dissolved in dichloromethane (300 mL). The organic layer was washed with saturated aqueous sodium bicarbonate (2 x 100 mL) and brine. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated in vacuo to provide (Z)-N,N-dicyclopropyl-6-ethyl-1-methyl-4-(1-(methylthio)-3-oxobut-1-enylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (31 g, 68.5 mmol, 80% yield) as a yellow solid.

MS (ESI) m/z 453.4 (M+H)

¹H-NMR (400 MHz, CDCl₃) δ 7.78 (s, 1 H), 6.87 (s, 1 H), 5.41 (s, 1 H), 4.64 (q, 2 H, J = 7.11 Hz), 4.03 (s, 3 H), 2.77 - 2.87 (m, 2 H), 2.43 (s, 3 H), 2.22 (s, 3 H), 1.48 (t, 3 H, J = 7.03 Hz), 0.82 - 0.90 (m, 4 H), 0.70 - 0.79 (m, 4 H).

Compound 11 (BMS-911543) and 13:

Methylhydrazine (3.11 μL, 0.058 mmol) was added to the solution of (Z)-N,N-dicyclopropyl-6-ethyl-1-methyl-4-(1-(methylthio)-3-oxobut-1-enylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (**15**) (25 mg, 0.055 mmol) in ethanol (1 mL) and the resulting reaction solution was heated at 85 °C for 4h. LC/MS showed completion of reaction with formation of two regioisomers. The reaction mixture was diluted with methanol and purified by preparative HPLC using Waters Xbridge Phenyl 250 x 20 mm ID, 5μm column with 25-100% methanol/water containing 0.1% TFA. The fractions were concentrated to afford two regioisomers. The structures were determined by NMR studies (¹H, ¹³C, ROESY, ¹H-¹³C-HMQC, ¹H-¹³C-HMBC, ¹H-¹⁵N-HMBC (j=4 and 10Hz)).

Major isomer: N,N-dicyclopropyl-4-(1,5-dimethyl-1H-pyrazol-3-ylamino)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (**13**) (9 mg, 24% yield).

Retention time = 5.66 min

HPLC purity: 94%

MS (ESI) m/z 433.2 (M+H)

¹H NMR (400MHz, CDCl₃) δ 7.68 (s, 1H), 7.29 (s, 1H), 6.85 (s, 1H), 6.45 (s, 1H), 4.58 (q, J=7.0 Hz, 2H), 4.04 (s, 3H), 3.84 (s, 3H), 2.86 - 2.76 (m, 2H), 2.31 (s, 3H), 1.42 (t, J=7.0 Hz, 3H), 0.88 - 0.80 (m, 4H), 0.79 - 0.71 (m, 4H);

Minor isomer: N,N-dicyclopropyl-4-(1,3-dimethyl-1H-pyrazol-5-ylamino)-6-ethyl-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (**11**, **BMS-911543**) (5 mg, 13% yield).

Retention time = 9.339 min.

HPLC purity: 99.9%,

HRMS for C₂₃H₂₉N₈O:

Calculated - 433.24588

Found 433.24568 [M+H]⁺.

¹H-NMR (400 MHz, CDCl₃) δ 8.06 (s, 1 H), 7.62 (s, 1 H), 6.94 (s, 1 H), 6.85 (s, 1 H), 4.66 (q, 2 H, J = 7.12 Hz), 3.98 (s, 3 H), 3.72 (s, 3 H), 2.78 - 2.88 (m, 2 H), 2.33 (d, 3 H, J = 0.44 Hz), 1.49 (t, 3 H, J = 7.15 Hz), 0.81 - 0.89 (m, 4 H), 0.71 - 0.78 (m, 4 H).

¹³C NMR (126MHz, CDCl₃) δ 167.4, 147.7, 144.4, 144.1, 139.1, 138.8, 133.5, 126.4, 124.6, 101.0, 98.3, 96.1, 38.9, 35.4, 32.6, 31.3, 15.8, 11.5, 9.0

Modified procedure for BMS-911543-

A mixture of (Z)-N,N-dicyclopropyl-6-ethyl-1-methyl-4-(1-(methylthio)-3-oxobut-1-enylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (**17**) (30 g, 66.3 mmol) and tert-butyl 1-methylhydrazinecarboxylate (13.57 g, 93 mmol) in acetic acid (110 mL) was stirred at 35 °C with a bleach trap to collect methanethiol. LC/MS shows formation of the intermediate with complete conversion of the starting material after 70 min. Formic acid (110 mL) was added and the reaction mixture was stirred at 60 °C overnight. The solvent was evaporated and the residue was dissolved in dichloromethane. The organic layer was washed with saturated aqueous sodium bicarbonate, dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo. The crude product was purified by recrystallization in EtOH/water (1:5) to yield. N,N-dicyclopropyl-4-(1,5-dimethyl-1H-pyrazol-3-ylamino)-6-ethyl-1-methyl-1,6-dihydroimidazo [4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (21 g, 48.6 mmol, 73% yield).

N,N-Dicyclopropyl-6-ethyl-4-(1-ethyl-5-methyl-1H-pyrazol-3-ylamino)-1-methyl-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (12):

Synthesized using modified procedure for BMS-911543 from **15** and Boc-ethylhydrazine with 57% yield

Retention time: 6.45 min

HPLC Purity: 99.2%

HRMS for C₂₄H₃₁N₈O- 447.26110 [M+H]⁺

Calculated- 447.26153

Found- 447.26110 [M+H]⁺

¹H NMR (400MHz, CDCl₃) δ 8.08 (s, 1H), 7.62 (s, 1H), 6.93 (s, 1H), 6.85 (s, 1H), 4.66 (q, J=7.0 Hz, 2H), 4.07 - 3.96 (m, 5H), 2.82 (tt, J=7.1, 3.7 Hz, 2H), 2.34 (s, 3H), 1.49 (t, J=7.0 Hz, 3H), 1.41 (t, J=7.3 Hz, 3H), 0.89 - 0.80 (m, 4H), 0.79 - 0.71 (m, 4H)

¹³C NMR (126MHz, CHLOROFORM-d) δ 167.3, 147.7, 144.5, 144.1, 139.1, 137.7, 133.5, 126.3, 124.8, 101.0, 98.2, 96.1, 77.3, 77.0, 76.7, 43.3, 38.9, 32.5, 31.3, 15.8, 15.4, 11.4, 9.0

N,N-Dicyclopropyl-10-ethyl-3-methyl-7-[(5-methyl-1H-pyrazol-3-yl)amino]-3,5,8,10-tetraazatricyclo[7.3.0.0²,6]dodeca-1(9),2(6),4,7,11-pentaene-11-carboxamide (14):

To a vial charged with (Z)-N,N-dicyclopropyl-6-ethyl-1-methyl-4-(1-(methylthio)-3-oxobut-1-enylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (31.5 mg, 0.070 mmol) and EtOH (348 μL) was added hydrazine (10.92 μL, 0.348 mmol). The vial was capped and placed in a reaction block at 60 °C for 90 min. The reaction mixture was concentrated in vacuo. The crude solid was triturated with 2:1 ether:methanol (3 x). The pale yellow solid was taken up in 1:1 MeCN:water and lyophilized for 16 h. N,N-Dicyclopropyl-6-ethyl-1-methyl-4-(5-methyl-1H-pyrazol-3-ylamino)-1,6-dihydroimidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (24.4 mg, 0.055 mmol, 79 % yield) was isolated as a yellow solid.

Retention time: 5.41 min

HPLC Purity: 94%

HRMS for C₂₂H₂₇N₈O-

Calculated- 419.2301

Found - 419.2302 (M+H⁺)

¹H NMR (500MHz, DMSO-d₆ with TFA-d) δ 8.75 (s, 1H), 7.29 (s, 1H), 6.60 (s, 1H), 4.59 (q, J=7.0 Hz, 2H), 4.12 (s, 3H), 2.99 - 2.84 (m, 2H), 2.35 (s, 3H), 1.32 (t, J=7.1 Hz, 3H), 0.81 - 0.71 (m, 4H), 0.70 - 0.60 (m, 4H).

^{13}C NMR (126MHz, DMSO- d_6 with TFA- d) δ 165.8, 146.0 and 145.9 (due to pyrazole rotomers), 142.8 and 142.7 (due to pyrazole rotomers), 141.5, 141.2, 140.2, 134.3, 128.9, 118.9, 101.6, 98.9, 94.9, 38.6, 33.5, 30.9, 15.6, 11.0 and 10.9 (due to pyrazole rotomers), 8.7.