SUPPORTING INFORMATION

Fragment-Based Discovery of 7-Azabenzimidazoles as Potent, Highly Selective and Orally Active CDK4/6 Inhibitors.

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CDK Enzyme Assays. Human CDK4/cyclin D1 was expressed in Sf21 cells via baculovirus infection. An assay for monitoring CDK4/cyclin D1-catalyzed phosphorylation of pRb at the Ser780 site was performed using TR-FRET in a 384-well format, and was used for IC_{50} determination and kinetic analysis. The reaction was carried out in a 30 μL volume containing 0.25 nM CDK4/cyclin D1, 150 nM biotin-pRb (773-924), 3 μM ATP, and 1.3% DMSO (or compound in DMSO) in the assay buffer (50 mM HEPES-Na, pH 7.5; 5 mM $MgCl_2$, 1 mM DTT, 0.02% Tween-20 and 0.05% BSA). 3 μM ATP was added last to initiate the reaction. The reaction was quenched with 10 μL of 240 mM EDTA-Na (pH 8.0) after 60 min incubation at 22 °C. The signal was developed by the addition of 40 μL detection solution containing 40 nM SA-APC, 143 ng/mL anti-phospho-pRb (S780) antibody and 2 nM Eu-W1024 anti-rabbit IgG antibody in the detection buffer (50 mM HEPES-Na, pH 7.5, 60 mM EDTA-Na, pH 8.0, 0.05% BSA, and 0.1% Triton X-100). After 60 min incubation in the dark, the plate was read on Envision (Perkin Elmer 2102-0010).

Human CDK6/cyclin D3 was purchased from Carna Biosciences Inc. (catalog no. 04-107). An assay for monitoring CDK6/cyclin D3-catalyzed phosphorylation of pRb at the Ser780 site was performed using TR-FRET in a 384-well format, and was used for IC_{50} determination and kinetic analysis. The reaction was carried out in a 30 μL volume containing 0.20 nM CDK6/cyclin D3, 200 nM biotin-pRb (773-924), 3 μM ATP and 1.3% DMSO (or compound in DMSO) in the assay buffer (50 mM HEPES-Na, pH 7.5; 5 mM $MgCl_2$, 1 mM DTT, 0.02% Tween-20, and 0.05% BSA). 3 uM ATP was added last to initiate the reaction. The reaction was quenched with 10 uL of 120 mM EDTA-Na (pH 8.0) after 120 min incubation at 22 °C. The signal was developed by the addition of 40 uL detection solution containing 40 nM SA-APC, 143 ng/mL anti-phospho-pRb (S780) antibody, and 2 nM Eu-W1024 anti-rabbit IgG antibody in the detection buffer (50 mM HEPES-Na, pH 7.5, 60 mM EDTA-Na, pH 8.0, 0.05% BSA, and 0.1% Triton X-100). After 60 min incubation in the dark, the plate was read on Envision (Perkin Elmer 2102-0010). In the case of Compounds **A**, **B** and **1**, CDK6/cyclin D3 activity was measured using an ELISA format. Compounds were incubated with 50 mM HEPES, pH 7.4, 15 mM $MgCl_2$, 1 mM DTT, 1 mM EGTA, pH 8.0, 0.02% Triton X-100, 2 nM CDK6/cyclin D3 and 10 μM ATP for 30 minutes at room temperature in Maxisorp assay plates (pre-coated overnight with 300 ng/well Rb protein and blocked with 300 μl Superblock (Pierce)). Phosphorylated Rb was detected using (1:1000 dilution) rabbit polyclonal p-Rb S780 antibody (Cell Signalling Technology) and (1:3000 dilution) alkaline phosphatase linked Goat anti-rabbit secondary antibody (Cell Signalling Technology).

The signal was developed with attophos substrate (Promega) and read using a SpectraMax Gemini reader (450 nm excitation / 580 nm emission). IC_{50} values were determined using Prism GraphPad Software.

The inhibition of human CDK1/cyclin B and human CDK2/cyclin A (catalog no. 14-450 and 14-448, respectively, Millipore) was monitored using IMAP-FP assays (Molecular Devices, CA) by following the phosphorylation of Tamra-Histone H1-derived peptide (catalog no. R7385, Molecular Devices). The final reaction volume was 20 μL, and contained 0.25 nM CDK1/cyclin B or 0.3 nM CDK2/cyclin A, 100 nM Tamra-H1 and 20 μM ATP in 1x Reaction Buffer (R8139, Molecular Devices). The reactions were run for 2 h at 22 °C. Quenching and detection was carried out following the protocols for the peptide substrate provided by the vendor.

Selected inhibitory activity of compound 6 against a panel of 35 kinases (IC_{50} μM).[a]

Protein Kinase	IC50 (μM)
Alk	**>10**
AKT1	**>10**
BTK	**>10**
GSK3β	**5**
JAK1	**>10**
JAK2	**7.3**
JAK3	**>10**
MAP3K8	**>10**
MAPK1	**>10**
MAPK14	**>10**
MAPKAPK2	**>10**
MAPKAPK5	**>10**
MKNK2	**>10**
PDPK1	**>10**
PKN1	**4.9**
PRKACA	**>10**
PRKCA	**>10**
PRKCQ	**>10**
ROCK2	**>10**
TYK2	**9.5**
EPhA4	**>10**
EPhB4	**>10**
FGFR-4	**>10**

FGFR3K	**9.3**
HER1	**>10**
HER2	**>10**
IGF1R	**>10**
INS1R	**>10**
KDR	**8.6**
LCK	**5.6**
PDGFRa	**>10**
RET	**7.5**
SYK	**>10**
cKIT	**>10**
cMET	**>10**

[a]Results are from single IC_{50} determinations. For each determination, concentration–inhibition curves were obtained in triplicate and then averaged to afford a single IC_{50} curve with a ≥95% confidence interval.

CDK4 Cellular Assays. The pRb expressing Jeko-1 mantle cell lymphoma cell line was grown in complete media consisting of RPMI1640 (Gibco catalog no. 22400-071), 20% FBS (Gibco catalog no. 10082-131), 2 mM L-glutamine (Gibco catalog no. 25030-081) and 1% Penicillin/Streptomycin (Gibco catalog no. 15140-133). Jeko-1 cells were seeded in Biocoat Cell Environment Poly-D-Lysine 96-well tissue culture plates (Becton Dickinson catalog no. 356461) at 20,000 cells/well in 100 µL final volume of complete media. Cells were allowed to adhere overnight. Compounds were prepared as 10 mM stock solution in DMSO and diluted to a concentration of 110 µM in complete media in a 96 well tissue culture plate, and then serially diluted four fold, allowing a titration curve of 7 points with a final concentration of 26 nM. 10 µL of the dilution were then transferred to the cell culture plate, resulting in a final concentration range of 10 µM to 2 nM. The incubation was carried out at 37 °C with 5% CO_2. All compounds were tested in triplicates at each concentration. Following compound incubation, the media was removed and the cells were lysed in 35 µL of lysis buffer, consisting of 50 mM Tris.Cl, pH 7.2, 120 mM NaCl, 1 mM EDTA, 6 mM EGTA, 1% NP-40, complete protease inhibitor cocktail (Roche, catalog no. 11836170001) and a protease inhibitor cocktail from Calbiochem (catalog no. 524525). The plates were placed at 4 °C with vigorous shaking for 5 min to lyse the cells. The resulting lysates contained approximately 1 µg/µL of protein.

The 4H1 total pRb antibody from Cell signaling technology (catalog no. 9309) was added to clear MaxiSorp plates (Nunc catalog no. 442404) at a concentration of 50 ng per well in 50 µL Dulbecco's Phosphate Buffered Saline (DPBS) (Gibco catalog no. 14190-144). Plates were incubated overnight at 4 ºC with rocking. After a 250 µL wash with TBST (Teknova catalog no. T9501) and blot-drying, 250 µL Superblock (Pierce catalog no. 37535) was added to each well. After shaking for 10 minutes, the Superblock solution was replaced with fresh Superblock and plates were incubated on a shaker for an additional 50 min. After blocking, 30 µL of Jeko-1 cell lysate, containing approximately 10 µg total protein, were added to wells in triplicate. 20 µL PBS (Gibco catalog no. 10010-023) containing 10% Superblock (Pierce catalog no. 37535) were added to each well for a final reaction volume of 50 µL. Plates were then sealed with Uniseal plate sealers (Whatman catalog no. 7704-0007) and incubated for 2 h at room temperature on a shaker. Plates were washed with 3 x 250 µL TBST. 50 µL of a 1:1000 dilution of anti-phospho Rb Ser^{780} from Cell Signaling (catalog no. 9307) in PBS/10% Superblock were added and the plate was incubated on a shaker for 1 h at room temperature. For all incubation steps, plates were covered with Uniseal plate sealers. Following incubation, plates were washed with 3 x 250 µL TBST. Next, 50 µL of a 1:2500 dilution of donkey-anti-rabbit HRP (Promega catalog no. W401B) in PBS/10% Superblock were added and plates were incubated for 30 min at room temperature on a shaker. Plates were again washed as described above. Finally, 50 µL Ultra TMB ELISA (Pierce catalog no. 34028) were added and plates incubated, unsealed, 5-15 min in the dark, until blue color developed. After incubation, 50 µL 2 M sulfuric acid were added to plates to top the reaction and absorbance was determined on a SpectraMax (Molecular Devices, Sunnydale, CA) within 15 minutes at 450 nm. All washes were performed using a Bio-Tek plate washer.

The Total Rb ELISA kit (Invitrogen catalog no. KHO0011) was used to determine the levels of total pRb. This kit uses wells precoated with a proprietary total pRb antibody for capture. All reagents listed, with the exception of cell lysate, were included in the kit. The nature of the antibodies used for capture and detection was labeled as proprietary and not disclosed. 10 µg of cell lysate was loaded into the wells and volume adjusted to 50 µL with standard dilution buffer. Plates were sealed with film included in the kit and incubated for 2 h at room temperature on a shaker. Plates are then manually washed three times with 250 µL wash buffer. 50 µL of proprietary primary antibody (pre-conjugated to biotin) was added to wells and incubated for 1 h at room temperature on a shaker. Then plates were again washed as noted above. The secondary antibody (HRP pre-conjugated to Streptavidin) was diluted 1:100 in Streptavidin-HRP

diluent buffer and 50 µL was added to each well. Plates were then incubated for 30 min. Afterwards, plates were washed four times with buffer as outlined above. Finally, 50 µL stabilized Chromogen was added per well and plates were incubated for 15 min, at which point 50 µL of stop solution was added. Plates were then read on a Spectramax at 450nm.

Upon quantification of the pRb phosphorylation (p-pRb) levels, % inhibition values were derived for each concentration tested and used to determine 50% inhibitory concentrations (IC_{50}) for a particular compound (non-normalized). The total pRb levels were then used to adjust the p-pRb % inhibition values to account for any loss of signal due to the absence of the pRb protein itself, and the IC_{50} values obtained from the adjusted % inhibitions represent normalized cellular p-pRb IC_{50}.

Cellular IC_{50} data in a Jeko-1 cell line measuring inhibition of pRb phosphorylation, normalized with respect to pRb

Compound number	IC_{50} (µM)
2	**0.16**
3	**0.95**
4	**0.069**
5	**2.7***
6	**0.26**

*unnormalized with respect to Rb

Fluorescence Activated Cell Sorting (FACS). Analysis of cellular DNA content by Propidium Iodide (PI) staining was used to determine cells that were in G_0/G_1, S, or G_2/M phase. The media containing Jeko-1 cells was transferred to a fresh v-bottom 96-well polypropylene plate (Nunc catalog no. 249944). Next, cells were spun at 1000x g for 10 min and the media was gently removed using pipette. 100 µL of a hypotonic lysis buffer (0.1% sodium citrate (Sigma catalog no. S-4641)), 0.1% Triton X-100 (MP Biomedicals catalog no. 807423), 25 µg/mL PI (MP Biomedicals catalog no. 195458), and 10 µg/mL RNAse (Roche catalog no. 1 579 681) were added to the cells and the whole cocktail was incubated at room temperature in the dark for 1 h. If necessary, cells were stored at this step overnight at 4 ^{o}C. Finally, the cells were subjected to DNA content analysis using the BD LSR II System and FACSDiva, version 5.0.1 (BD biosciences, Franklin Lakes, NJ, USA). The data was analysed using the ModFit LT 3.1.

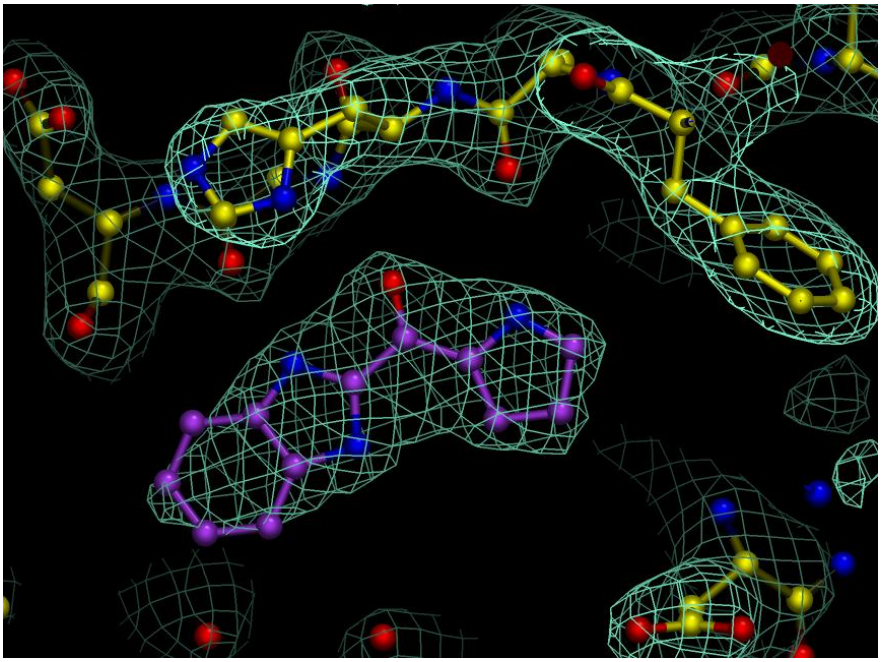
Docking Methods. The dockings of compounds **1** and **4** (shown in figures 1(b) and 2(b) respectively) were performed using an in-house version of the GOLD docking package, based around GOLD v5.0. Initial conformations for the compounds were generated from SMILES strings using Corina version 3.48 (Molecular Networks GmbH). In both cases the benzimidazole tautomer was specified manually, and in both cases the dimethyl amine was protonated. The protein structure used for both models was an in-house structure of compound 2 in CDK6, with the cognate ligand and all water molecules removed. Dockings were performed with the ChemPLP scoring function, using the "Default 3" settings (30,000 operations) and with the "diverse solutions" option turned on (1.5 Angstrom cluster RMSD, 3 solutions per cluster). Poses for the figures were selected manually based on overlays with x-ray structures of close analogues.

Crystallography for compound 3: An equimolar complex of a compound and CDK6 at 5 mg/mL in buffer (25mM Tris, 300mM NaCl, 1mM TCEP, pH 7.5, 20% glycerol) was crystallised using the hanging-drop vapour diffusion method. The well contained 100mM MES pH 6.0, 25–50mM NH_4NO_3, and 6–16% PEG3350. Crystals were frozen using glycerol as a cryoprotectant and data were collected at beamline 17ID of the Argonne Photo Source (Chicago, IL) using an ADSC-Q210 detector. Data were processed using the HKL200 package. Using CDK2 as the start model, structures were solved employing the software suites from CCP4 and CNX.

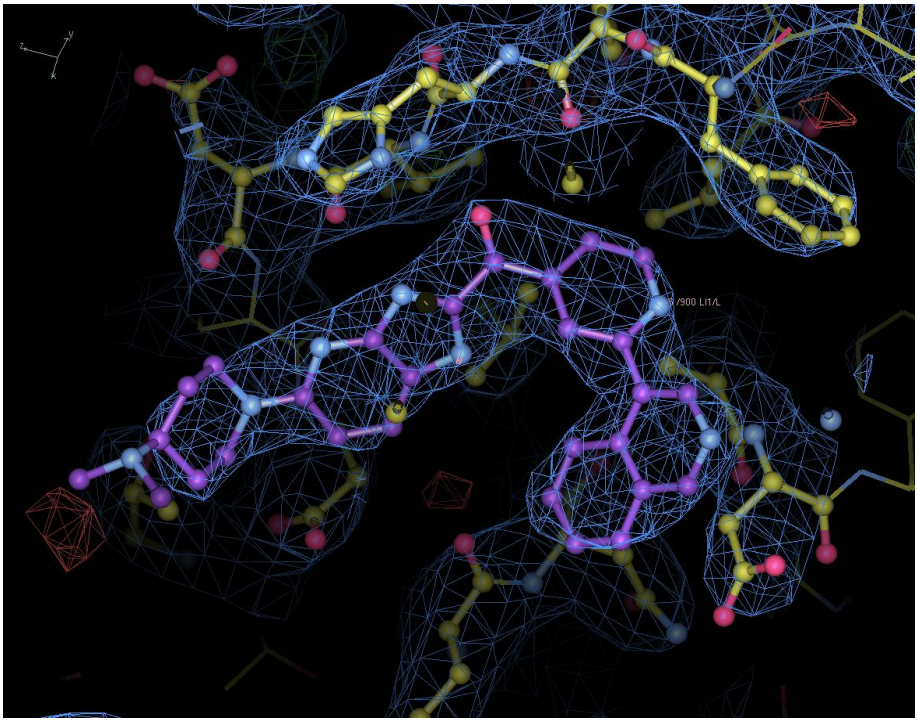
Crystallography for Fragment A: His-tagged CDK6 was expressed in insect cells and purified using NiNTA agarose and size exclusion chromatography. CDK6 was concentrated to 5 mg/mL and incubated with compound **12** from Wyatt *et al*, **J. Med. Chem**, 2008, 51(16), pp4986-4999, prior to crystallisation using hanging drop vapour diffusion. CDK6 and the reservoir solution, 0.1 MES pH=5.7, 4.5%w/v PEG 3350, 25mM sodium nitrate, 10.0%(v/v) glycerol, were combined in equal volumes. Crystals were back soaked to remove the co-crystallisation ligand **12** and then soaked in a solution of the compound of interest (fragment **A**). Crystals were cryo-protected using MPD. Data were collected at ESRF ID23.1. The structure was solved by molecular replacement using CDK6 from 1JOW. All data processing, molecular replacement and structure refinement were carried out using the CCP4 suite of programmes.

Coordinates: Coordinates for the CDK6 complexes with fragment **A** and compound **3** have been deposited in the Protein Data Bank (PDB) under accession codes 4aua and 4EZ5 respectively.

X-ray crystal structure of benzimidazole fragment **A** bound to CDK6, with the $2f_o$-f_c electron density map shown in green, contoured at 1σ.



X-ray crystal structure of benzimidazole compound **3** bound to CDK6, with the $2f_o$-f_c electron density map shown in cyan, contoured at 1σ.



PK data in male Sprague Dawley rats

	IV	**PO**
Compound 2	Dose = 2 mg/kg n = 2 Cl_p = 144 mL/min/kg V_{ss} = 21.3 L/kg AUC = 461 nM*h $t_{1/2}$ = 2.9 h	Dose = 5 mg/kg n = 3 AUC(0-8) = 250 nM*h F = 28% C_{max} = 54 nM t_{max} = 4 h
Dosed as a solution of the hydrochloride salt in: 4% 0.1 N HCl; 96% WFI		
Compound 5	Dose = 2 mg/kg n = 2 Cl_p = 27 mL/min/kg V_{ss} = 4.4 L/kg AUC = 2381 nM*h $t_{1/2}$ = 2.2 h	Dose = 5 mg/kg n = 3 AUC(0-8) = 2526 nM*h F = 44% C_{max} = 696 nM t_{max} = 1.3 h
Dosed as a solution of the hydrochloride salt in: 91% WFI,8% cremophor-EL, 1%0.1N NaOH		
Compound 6	Dose = 1 mg/kg n = 2 Cl_p = 37 mL/min/kg V_{ss} = 3.5 L/kg AUC = 908 nM*h $t_{1/2}$ = 2.1 h	Dose = 5 mg/kg n = 3 AUC(0-8) = 1147 nM*h F = 26% C_{max} = 245 nM t_{max} = 1.7 h
Dosed as a solution of the free base in: 2.5% 0.1 N HCl; 97.5% WFI		

Data for compound 6 in male C57BL/6 Mice

IV	PO
Dose = 1 mg/kg n = 3 Cl_p = 11 mL/min/kg V_{ss} = 0.6 L/kg AUC = 3246 nM*h $t_{1/2}$ = 0.9 h	Dose = 5 mg/kg n = 3 AUC(0-8) = 4062 nM*h F = 25% C_{max} = 1241 nM t_{max} = 2 h
Dosed as a solution of the free base in: 2.5% 0.1 N HCl; 97.5% WFI	

Liver microsome data for compound

Species	Extraction ratio (%)	Cl_{int} (µL/min/mg)	$t_{1/2}$ (min)
rat	74	88	16
mouse	48	22	65
human	72	64	22

Plasma protein binding data for Compound 6 (using equilibrium dialysis method)

Rat: % bound = 91.1

Mouse: % bound = 95.2

Human: % bound = 84.8

In vivo Experiments Method. Mice were maintained and handled in accordance with Novartis Biomedical Research Animal Care and Use Committee protocols and regulations. 6-8 week old female SCID-beige mice were purchased from Charles River Labs (Wilmington MA) and housed in temperature and humidity-controlled vivarium with a 12 h light cycle and provided food and water and libitum.

Mice were implanted subcutaneously with $20x10^6$ Jeko-1 cells in 50% matrigel (BD Biosciences, #354234) in the right dorsal axillary region. Evaluation of PK and PD relationship was conducted when established tumor averaged 600 mm^3 in size. Compound **6** was dosed orally at 250-500 mg/kg/day twice daily for 3 days, and blood and tumor samples were harvested at select timepoints after final dose. Drug concentrations in plasma and tumors were analyzed by mass spectrometry. Modulation of pRb phosphorylation was measured by ppRb and total pRb ELISA as previously described.

For evaluation of anti-tumor efficacy, drug treatment was initiated when tumor volume reached an average of 143 mm^3 (22 days post tumor implantation). Compound **6** solution was prepared weekly and dosed orally at 75-250 mg/kg/day twice daily (n=8 mice per dose level). Tumor volume was determined by twice weekly digital calipering and calculated as Length x $Width^2$ /2. Data were expressed as mean $\pm$ SEM, and differences were considered statistically significant at $p < 0.05$ by Student t-test.

Exposure levels in xenograft-bearing SCID mice (p.o.)

		250 mg/kg/day BID			500 mg/kg/day BID		
		Plasma	**Tumor**	**Tumor/plasma**	**Plasma**	**Tumor**	**Tumor/plasma**
AUC0-t	nM*Hours	365259	427929	1.2	370159	469487	1.3
AUC Extrap	nM*Hours	439196	619867	1.4	653084	953453	1.5
% AUC Extrap	%	17	31		43	51	
Cmax	nM	36582	27047	0.74	83163	81944.8	0.99
Tmax	Hours	1	1		1		

t= 24 h for 125 mg/kg dose
t=7 h for 250 mg/kg dose

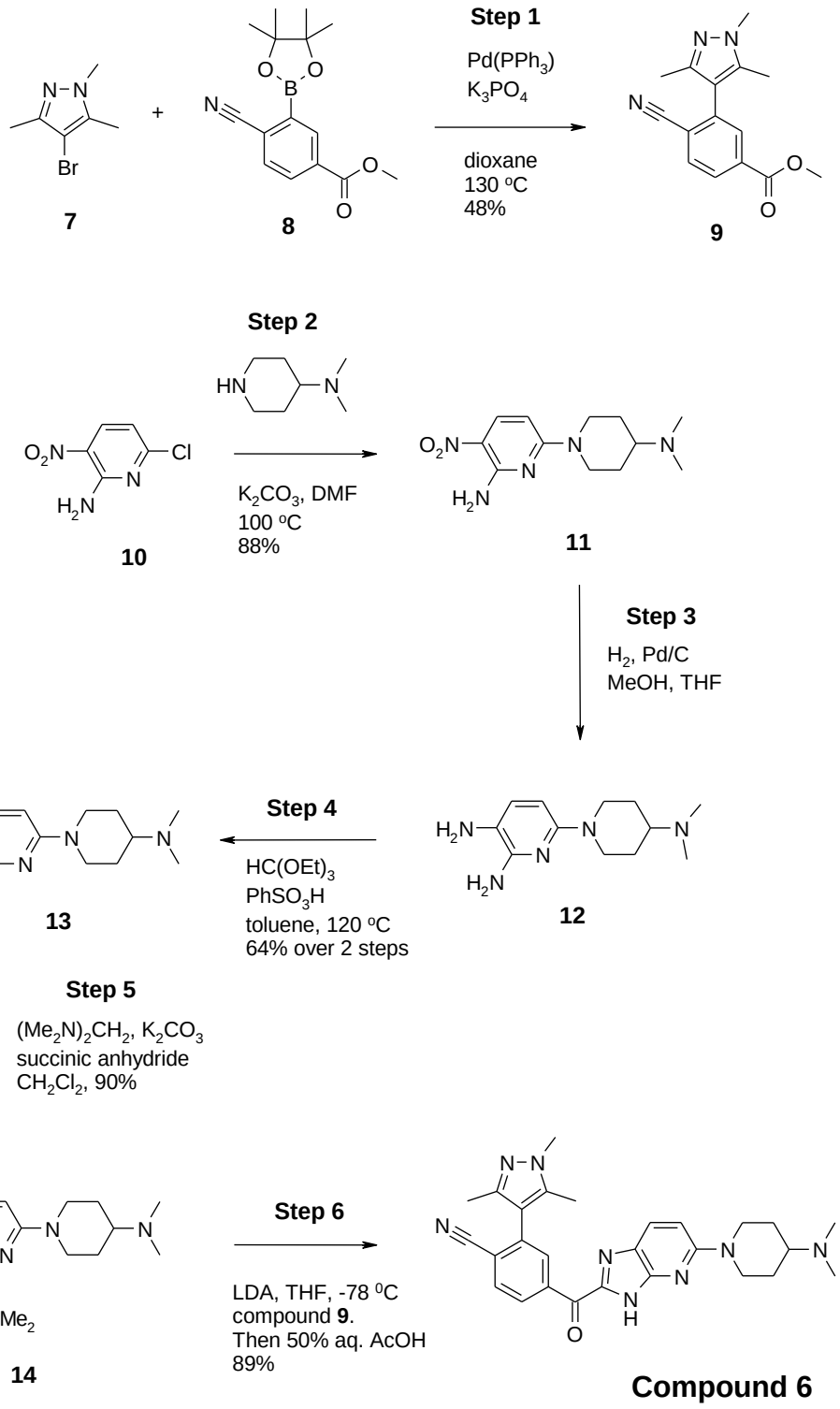
Chemistry. All solvents employed were commercially available “anhydrous” grade, and reagents were used as received unless otherwise noted. A Biotage Initiator™ Sixty system was used for microwave heating. Flash column chromatography was performed on either an Analogix Intelliflash 280 using Si 50 columns (32–63 µm, 230–400 mesh, 60Å) or on a Biotage SP1 system (32–63 µm particle size, KP-Sil, 60 Å pore size). Preparative high pressure liquid chromatography (HPLC) was performed using a Waters 2525 pump with 2487 dual wavelength detector and 2767 Sample manager. Columns were Waters C18 OBD 5µm, either 50x100 mm Xbridge or 30x100 mm Sunfire. NMR spectra were recorded on a Bruker AV400 (Avance 400 MHz) or AV600 (Avance 600 MHz) instruments. Analytical LC–MS was conducted using an Agilent 1100 series with UV detection at 214 nm and 254 nm, and an electrospray mode (ESI) coupled with a Waters ZQ single quad mass detector. One of two methods was used: Method A) 5–95% acetonitrile/H_2O with 5 mM ammonium formate with a 2 min run, 3 µL injection through an inertisil C8 3 cm x 5 mm x 3µm; Method B) 20–95% acetonitrile/H_2O with 10 mM ammonium formate with a 2 min run, 3 µL injection through an inertisil C8 3 cm x 5 mm x 3µm.

Purity of all tested compounds was determined by an Agilent 1100 HPLC system and one of the following methods. Method 1 (at both 214 and 254 nm): Used an Inertsil ODS3 3µm 3.0 x 100 mm C18 column at the flow rate of 1.0 mL/min, with a gradient of 5–95% acetonitrile/water 5 mM ammonia formate over 7.75 min. Method 2 (at both 214 and 254 nm): Used an Inertsil ODS3 100 x 3 mm C18 column at the flow rate of 1.0 mL/min, with a gradient of 5–95% acetonitrile/water with 0.1% formic acid over 7.75 min. Method 3 (at both 214 and 254 nm): Used an Inertsil ODS3 3µm 3.0 x 100 mm C18 column at the flow rate of 0.5 mL/min, with a gradient of 5–95% acetonitrile/water 5 mM ammonia formate over 4.50 min. LC/ESI-MS data were recorded using a Waters LCT Premier mass spectrometer with dual electrospray ionization source and Agilent 1100 liquid chromatograph. The resolution of the MS system was approximately 12000 (FWHM definition). HPLC separation was performed at 1.0 mL/min flow rate with the gradient from 10% to 95% in 2.5min. 10 mM ammonia formate was used as the modifier additive in the Aqueous Phase. Sulfadimethoxine (Sigma; protonated molecule m/z 311.0814) was used as a reference and acquired through the LockSpray™ channel every third scan.

Synthesis of Compound 6

Scheme 1 describes a synthetic route which generated several grams of compound **6** for the in vivo animal studies. Suzuki reaction between **7** and **8**[1] provided 4.55 g of **9**. Treatment of the chloride **10** with 4-(dimethylamino)piperidine, followed by reduction of the nitro group and subsequent ring closure to give 8.2 g of **13** in 56% yield over three steps. We chose dimethylaminomethyl as a protecting group of the azabenzimidazole **13** because of the stability in basic reaction conditions and lability under mild acidic conditions.[2] Following coupling reaction between **14** and **9** and deprotection provided 3.89 g of compound **6** in 81% yield over two steps.

Scheme 1. Synthesis of 6.



Step 1: A mixture of 4-bromo-1,3,5-trimethyl-1*H*-pyrazole (6.63 g, 35 mmol) **7**, 4-cyano-3-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-benzoic acid methyl ester **8** (12.08 g, 42 mmol, 1.2 equiv) (Boropharm 02-0009), $Pd(PPh_3)_4$ (4.0 g, 3.5 mmol, 0.10 equiv) and K_3PO_4 (18.57 g, 87.5 mmol, 2.5 equiv) in dioxane (200 mL) was prepared in a sealed tube, degassed and heated at 130 °C for 3 h. After cooling, the reaction mixture was filtered through a pad of Celite and concentrated. The residue was purified by silica gel column chromatography (EtOAc/heptane 0 to 80%) to afford 4-cyano-3-(1,3,5-trimethyl-1*H*-pyrazol-4-yl)-benzoic acid methyl ester **9** (4.55 g) in 48% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (dd, *J* = 8.1, 1.6 Hz, 1 H), 8.01 (d, *J* = 1.6 Hz, 1 H), 7.84 (d, *J* = 8.1 Hz, 1 H), 3.98 (s, 3 H), 3.83 (s, 3 H), 2.22 (s, 3 H), 2.21 (s, 3 H); MS *m/z* 270.1 $[M+H]^+$.

Step 2: A solution of 6-chloro-3-nitro-pyridine-2-yl-amine **10** (10 g, 58 mmol), dimethylpiperidine-4-yl-amine (8.13 g, 63 mmol, 1.1 equiv) and K_2CO_3 (9.55 g, 69 mmol, 1.2 equiv) in DMF (150 mL) was heated at 100 °C for 3 h. After cooling, water was added to the reaction mixture and the resulting precipitate was collected by filtration, washed with water and dried to give 6-(4-dimethylamino-piperidin-1-yl)-3-nitro-pyridin-2-yl-amine **11** (13.4 g) in 88% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, *J* = 9.4 Hz,1 H), 6.14 (d, *J* = 9.4 Hz, 1 H), 4.54 (br s, 2 H), 3.04 – 2.96 (m, 2 H), 3.53 – 3.44 (m, 1 H), 2.34 (s, 6 H), 1.97 – 1.94 (m, 2 H), 1.51 (qd, *J* = 12, 4.2 Hz, 2 H); MS *m/z* 266.3 $[M+H]^+$.

Step 3: A solution of 6-(4-dimethylamino-piperidin-1-yl)-3-nitro-pyridin-2-yl-amine **11** (13.7 g, 52 mmol) in MeOH/THF (200 mL/50 mL) was treated with 10% Pd/C (1.37 g). The reaction bottle was evacuated and flushed with H_2 three time and was shaken under 50 psi in a Parr hydrogenator for 5 d. The solution was filtered through a pad of Celite and concentrated to give the crude product 6-(4-dimethylamino-piperidin-1-yl)-pyridin-2,4-yl-diamine **12** which was used for the next reaction without purification. ^{1}H NMR (400 MHz, CD_3OD) δ 6.90 (d, *J* = 8.2 Hz,1 H), 6.01 (br d, *J* = 8.1 Hz, 1 H), 4.02 (br d, *J* = 13 Hz, 2 H) 2.59 (t, *J* = 12 Hz, 2 H), 2.36 – 2.32 (m, 1 H), 2.30 (s, 6 H), 1.93 – 1.89 (m, 2 H), 1.52 (qd, *J* = 12, 4.1 Hz, 2 H); MS *m/z* 236.4 $[M+H]^+$.

Step 4: A mixture of crude 6-(4-dimethylamino-piperidin-1-yl)-pyridin-2,4-yl-diamine, triethylorthoformate **12** (9.0 mL, 54 mmol, 1.0 equiv) and benzenesulfonic acid (41 mg, 0.26 mmol, 0.005 equiv) in toluene (100 mL) was heated at 120 °C overnight. After cooling, the reaction mixture was concentrated and purified by silica gel column chromatography (7N NH_3 in MeOH/CH_2Cl_2 0 to 10%) to afford [1-(3*H*-imidazo[4,5-b]pyridin-5-yl)-piperidin-4-yl]-dimethyl-

amine **13** (8.2 g) in 64% yield over two steps. 1H NMR (400MHz, $CDCl_3$) δ 10.26 (br s, 1 H), 7.87 (s, 1 H), 7.88 (d, *J* = 9.0 Hz,1 H), 6.73 (d, *J* = 9.0 Hz, 1 H), 4.37 (br d, *J* = 13 Hz, 2 H), 2.93 (td, *J* = 13, 2.4 Hz, 2 H), 2.44 (tt, *J* = 11, 3.7 Hz, 1 H), 2.35 (s, 6 H), 1.99 – 1.96 (m, 2 H), 1.67 – 1.57 (m, 2 H); MS *m/z* 246.4 $[M+H]^+$.

Step 5: To a mixture of [1-(3*H*-imidazo[4,5-b]pyridin-5-yl)-piperidin-4-yl]-dimethyl-amine **13** (3.72 g, 15 mmol), tetramethyldiaminomethane (2.28 mL, 16.7 mmol, 1.1 equiv) and K_2CO_3 (2.3 g, 17 mmol, 1.1 equiv) in CH_2Cl_2 (100 mL) was added succinic anhydride (1.67 g, 17 mmol, 1.1 equiv) and the resulting mixture was stirred at room temperature overnight. The reaction mixture was treated with 6N aqueous NaOH (100 mL). After separation, the organic layer was washed with saturated aqueous NaCl (2 x 150 mL), dried over Na_2SO_4 and concentrated to give crude [1-(3-dimethylaminomethyl-3*H*-imidazo[4,5-b]pyridin-5-yl)-piperidin-4-yl]-dimethyl-amine **14** (4.1 g) in 90% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, *J* = 8.8 Hz, 1 H), 7.82 (s, 1 H), 6.71 (d, *J* = 8.8 Hz, 1 H), 5.02 (s, 2 H), 4.41 (br d, *J* = 13 Hz, 2 H), 2.90 (td, *J* = 13, 2.5 Hz, 2 H), 2.42 – 2.40 (m, 1 H), 2.39 (s, 6 H), 2.33 (s, 6 H), 1.95 (br d, *J* = 13 Hz, 2 H), 1.58 (qd, *J* = 12, 4.2 Hz, 2 H).

Step 6 (Compound 6): To a cooled (-78 °C) solution of [1-(3-dimethylaminomethyl-3*H*-imidazo[4,5-b]pyridin-5-yl)-piperidin-4-yl]-dimethyl-amine **14** (1.09 g, 3.61 mmol, 1.2 equiv) in tetrahydrofuran (10 mL) was added a freshly prepared solution of lithium diisopropylamide in THF (1N, 4.5 mL, 4.5 mmol, 1.5 equiv) slowly. After 3 min, the resulting mixture was treated with a solution of 4-cyano-3-(1,3,5-trimethyl-1H-pyrazol-4-yl)-benzoic acid methyl ester (810 mg, 3.01 mmol) in THF (10 mL) slowly and stirred at -78 °C for 5 min. The reaction mixture was treated with 50% aqueous AcOH (4.0 mL), warmed to room temperature and basified with aqueous NH_4OH. The above procedure was repeated two more times at the same scale. The combined resulting mixture was diluted with EtOAc and washed with saturated aqueous NaCl. After separation, the organic layer was dried over Na_2SO_4, filtered, concentrated. Silica gel chromatography (7% NH_3 in MeOH/CH_2Cl_2 0 to 15%) provided 4-[5-(4-dimethylamino-piperidin-1-yl)-3*H*-imidazo[4,5-b]pyridine-2-carbonyl]-2-(1,3,5-trimethyl-1*H*-pyrazol-4-yl)-benzonitrile (**compound 6**) (3.89 g) in 89% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.48 (br s, 1 H), 8.43 – 8.42 (m, 2 H), 8.13 – 8.11 (m, 2 H), 7.94 (d, *J* = 9.2 Hz, 1 H), 7.04 (d, *J* = 9.2 Hz, 1 H), 4.43 (br d, *J* = 13 Hz, 2 H), 3.77 (s, 3 H), 2.95 (t, *J* = 12 Hz, 2 H), 2.40 – 2.33 (m, 1 H), 2.23 (s, 3 H), 2.18 (s, 6 H), 2.14 (s, 3 H), 1.84 (br d, *J* = 11 Hz, 2 H), 1.42 – 1.32 (m, 2 H); Anal. RP-HPLC t_R = 3.75 min (method 2, purity 100%/100%); HR-MS *m/z* M^+: measured 482.2544, calcd 482.2543;

Elemental analysis: found C = 67.20%, H = 6.21%, N = 23.14%, calcd C = 67.20%, H = 6.27%, N = 23.22%.

References

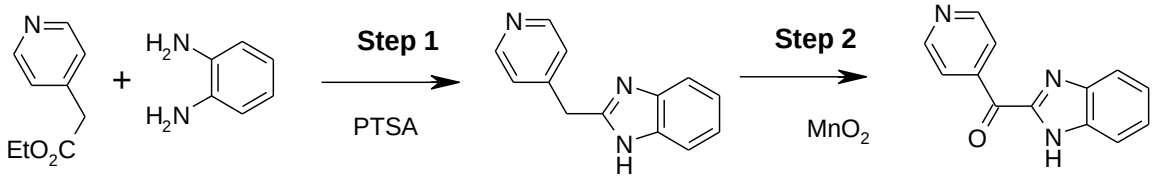
(1) Chotana, G. A.; Rak, M. A.; Smith, M. R., III. Sterically directed functionalization of aromatic C-H bonds: selective borylation ortho to cyano groups in arenes and heterocycles. J. Am. Chem. Soc. 2005, 127, 10539–10544.

(2) Love, B. E. Facile synthesis of N-dialkylaminomethyl-substituted heterocycles. J. Org. Chem. 2007, 72, 630–632.

Fragment A

Fragment **A** was prepared from benzimidazole and N-Boc-pyrrole-2-carboxylic acid ethyl ester by following methods analogous to those described in Asakawa *et. al*., *Tet. Lett*, 2005, 46, 5081. H NMR (400 MHz, Me-*d*3-OD): 7.86 (br, 1 H), 7.68 (d, *J* = 4.0 Hz, 1 H), 7.64 (br, 1 H), 7.39 (br, 2 H), 7.30-7.29 (m, 1 H), 6.41 (dd, *J* = 2.8, 2.4 Hz, 1 H) (mixture of rotamers). MS *m/z* 212 $[M+H]^+$. Anal. RP-HPLC (purity: 95%)

Compound B



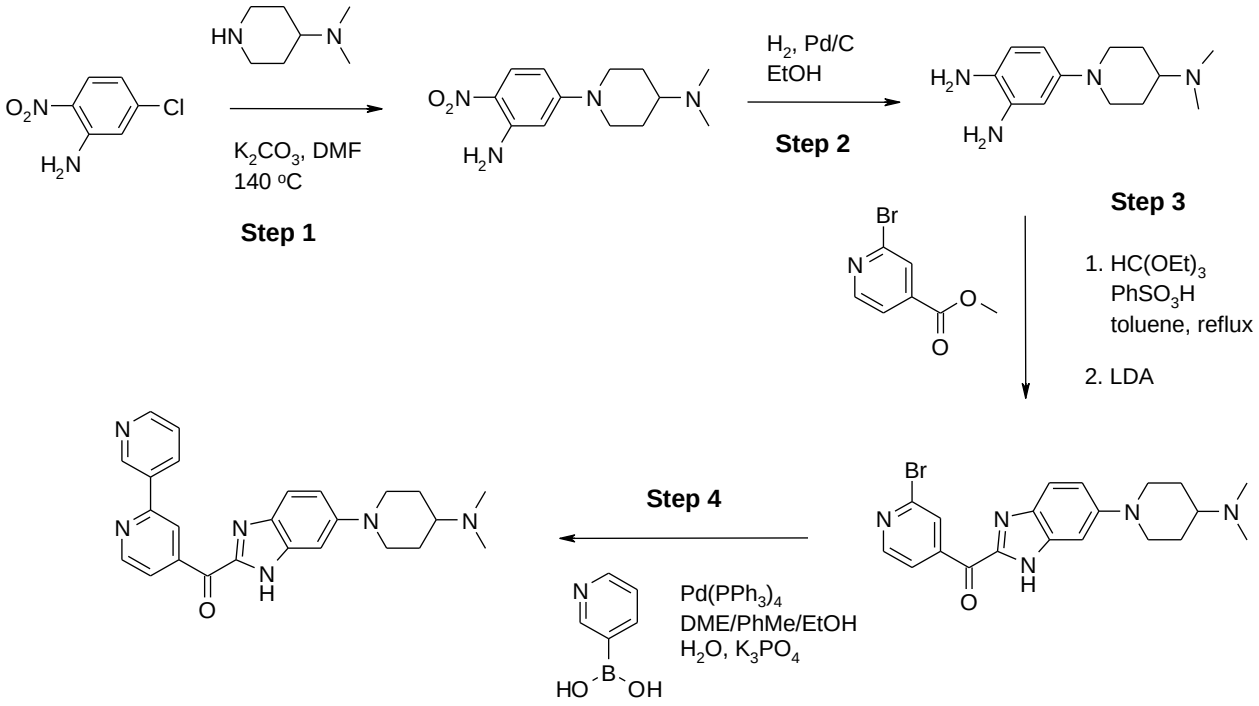
Compound **B**

Step 1: o-Phenylenediamine (1.0 g, 9.5 mmol), ethyl-4-pyridylacetate (1.6 mL, 10.4 mmol) and PTSA (17 mg) were combined and heated at 195 ºC for 4 hours. The mixture was allowed to cool and then purified by SiO_2 chromatography (eluting with 10% MeOH/CH_2Cl_2) to give the 2-pyridin-4-ylmethyl-1H-benzoimidazole (0.96 g, 50%). H NMR (400 MHz, CD_3OD): 8.50 (m, 2 H), 7.53 (m, 2 H), 7.40 (d, *J* = 4.0 Hz, 2 H), 4.33 (s, 2 H). MS *m/z* 210 $[M+H]^+$

Step 2: To 2-pyridin-4-ylmethyl-1H-benzoimidazole (0.94 g, 4.5 mmol) in 1,4-dioxane (45 mL) was added MnO_2 (8.7 g, 100 mmol) and the mixture stirred for 3 days at room temperature. The

mixture was then filtered through Celite and the Celite plug was washed with CH_2Cl_2. The combined organic fractions were evaporated and the mixture then purified by SiO_2 chromatography (eluting with 7% MeOH/CH_2Cl_2) to give the compound **B** (0.16 g, 16%). ^{1}H NMR(400 MHz, $CDCl_3$) δ 10.49 (br s, 1 H), 8.96 – 8.94 (m, 2 H), 8.54 – 8.53 (m, 2 H), 8.00 (br s, 1 H), 7.64 (br s, 1 H), 7.48 (br s, 2 H); Anal. RP-HPLC t_R = 2.13 min (method 3, purity 96.4%/84.2%); HR-MS *m/z* $(M+H)^+$: measured 224.0831, calcd 224.0824.

Compound 1



Step 1: To a solution of 5-chloro-2-nitroaniline (2.9 g, 16.9 mmol) in DMF (56 mL) was added 4-(dimethylamino)piperidine (2.3g, 18.5 mmol) and K_2CO_3 (2.5 g, 18.5 mmol). The reaction mixture was stirred at 140 °C overnight after which another further 9.2 mmol of the amine was added. Stirring at 140 °C was continued for a further 5 h. The mixture was allowed to cool and then the precipitate was collected by filtration. The precipitate was washed with EtOAc and then the combined filtrates were evaporated to dryness. The residue was suspended in water and the

resulting precipitate collected by filtration. The product was washed with water and then dried to give a [1-(3-amino-4-nitro-phenyl)-piperidin-4-yl]-dimethyl-amine as a brown/orange solid (3.9 g, 88%). 1H NMR (400 MHz, DMSO-*d*6): 7.80 (d, 1 H), 7.23 (s, 2 H), 6.39 (dd, 1H), 6.22 (d, 1 H), 3.87 (d, 2 H), 2.97-2.84 (m, 2 H), 2.38-2.26 (m, 1 H), 2.17 (s, 6 H), 1.81 (d, 2 H), 1.45-1.30 (m, 2 H); MS *m/z* 265 $[M+H]^+$

Step 2: To [1-(3-amino-4-nitro-phenyl)-piperidin-4-yl]-dimethyl-amine (3.8 g, 14.4 mmol) was added Pd/C (300 mg) in EtOH (205 mL). The flask was shaken under H_2 atmosphere for 6 h. The catalyst was removed by filtration and the filtrate was evaporated to dryness to give 4-(4-dimethylamino-piperidin-1-yl)-benzene-1,2-diamine. MS *m/z* 235 $[M+H]^+$. The material was used directly in the next step without any further purification.

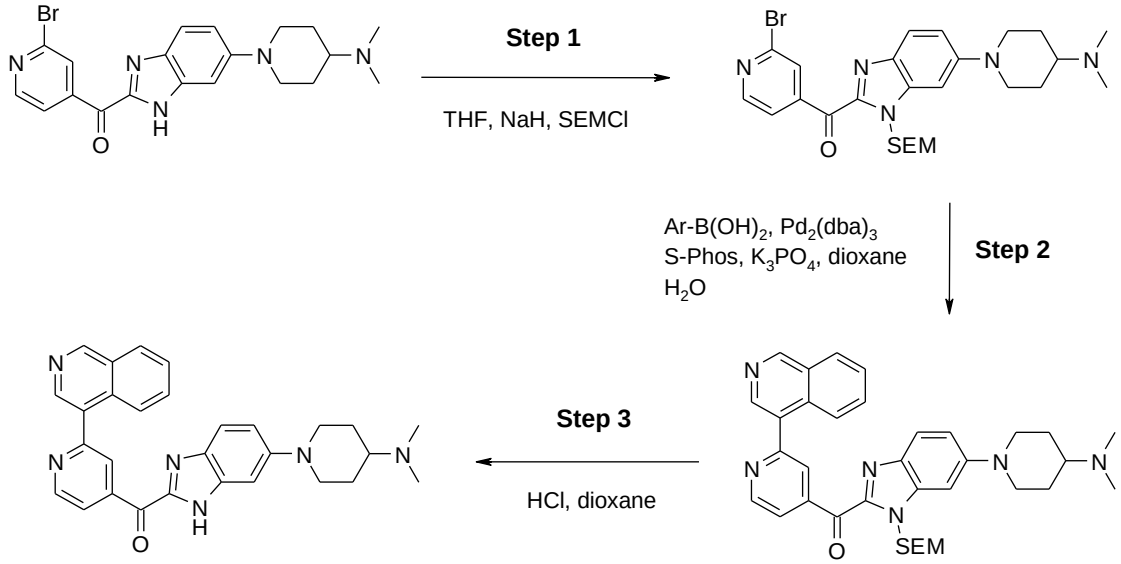
Step 3: A mixture of 4-(4-dimethylamino-piperidin-1-yl)-benzene-1,2-diamine (3.5 g, 14.9 mmol), triethyl orthoformate (10.0 mL, 59.7 mmol), benzenesulfonic acid (83 mg, 0.52 mmol) in toluene (30 mL) was heated at reflux, under N_2, overnight. Approximately half the volume of the toluene added was then distilled off. The same quantity of anhydrous toluene was re-added. Again this quantity of solvent was distilled off. The mixture was allowed to cool to room temperature.

To the mixture was then added diisopropylethylamine (3.1 mL, 1.2 mmol), THF (~ 20 mL) and 2-bromo-isonicotinic acid methyl ester (4.2 mmol, 19.4 mmol) under N_2. The reaction was cooled to -78 °C and LDA (9.7 mL, 19.4 mmol) was added slowly. The mixture was stirred for another 2 h at -78 °C and quenched at this temperature with water. The reaction was allowed to warm up to room temperature. The reaction was concentrated *in vacuo*. The aqueous mixture was then acidified with aqueous HCl (2 M; approx 1/3 volume), left for 1 hour and then basified with saturated aqueous $NaHCO_3$. The product was extracted with EtOAc (x4). The combined organic layers were washed with brine and dried over $MgSO_4$. The product was filtered and evaporated to dryness *in vacuo*. The residue was stirred with Et_2O and the resulting precipitate was collected by filtration and washed Et_2O. The product was dried *in vacuo* to give the product (6 g). H NMR (400 MHz, DMSO-*d*6): 13.31 (s, 1 H), 8.67 (d, 1 H), 8.53 (s, 1 H), 8.29 (dd, 1 H), 7.71 (d, 1 H), 7.20 (d, 1 H), 6.85 (s, 1 H), 3.77 (s, 2 H), 2.85-2.64 (m, 2 H), 2.24 (s, 7 H), 1.88 (d, 2 H), 1.53 (q, 2 H). MS *m/z* 428 $[M+H]^+$.

Step 4: A mixture of (2-bromo-pyridin-4-yl)-[6-(4-dimethylamino-piperidin-1-yl)-1*H*-benzoimidazol-2-yl]-methanone (100 mg, 0.233 mmol), pyridin-3-ylboronic acid (57.4 mg, 0.466 mmol, 2.0 equiv) and Pd $(PPh_3)_4$ (27 mg, 0.023 mmol, 0.10 equiv) in DME/toluene/EtOH (0.8 mL/0.8 mL/0.2 mL) was treated with 2 M aqueous K_3PO_4 (0.47 mL, 0.94 mmol, 4.0 equiv) and

degassed. The resulting mixture was heated in a microwave reactor at 120 °C for 2 h. After cooling, the mixture was treated with saturated aqueous NaCl and extracted with EtOAc. The combined organic fractions were dried over Na_2SO_4, filtered, and concentrated. The residue was then purified by silica gel column chromatography (7N NH_3 in MeOH/CH_2Cl_2 0 to 10%) to give [2,3'-bipyridin]-4-yl(6-(4-(dimethylamino)piperidin-1-yl)-1*H*-benzo[d]imidazol-2-yl)methanone (28 mg) in 29% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.32 (br s, 1 H), 9.32 (d, *J* = 2.3 Hz, 1 H), 8.99 (d, *J* = 5.1 Hz, 1 H), 8.78 (s, 1 H), 8.70 (dd, *J* = 4.7, 1.6 Hz, 1 H), 8.50 (dt, *J* = 8.0, 1.9 Hz, 1 H), 8.32 (d, *J* = 5.1 Hz, 1 H), 7.70 (br d, *J* = 8.1 Hz, 1 H), 7.59 (dd, *J* = 8.0, 4.8 Hz, 1 H), 7.18 (br d, *J* = 7.8 Hz, 1 H), 6.87 (br s, 1 H), 3.77 (br d, *J* = 10 Hz, 2 H), 2.77 (br t, *J* = 11 Hz, 2 H), 2.28 – 2.22 (br m, 1 H), 2.21 (s, 6 H), 1.88 (br d, *J* = 12, 2 H), 1.57 – 1.47 (m, 2 H); Anal. RP-HPLC t_R = 3.54 min (method 2, purity 100%/100%); HR-MS *m/z* $(M+H)^+$: measured 427.2243, calcd 427.2246.

Compound 2



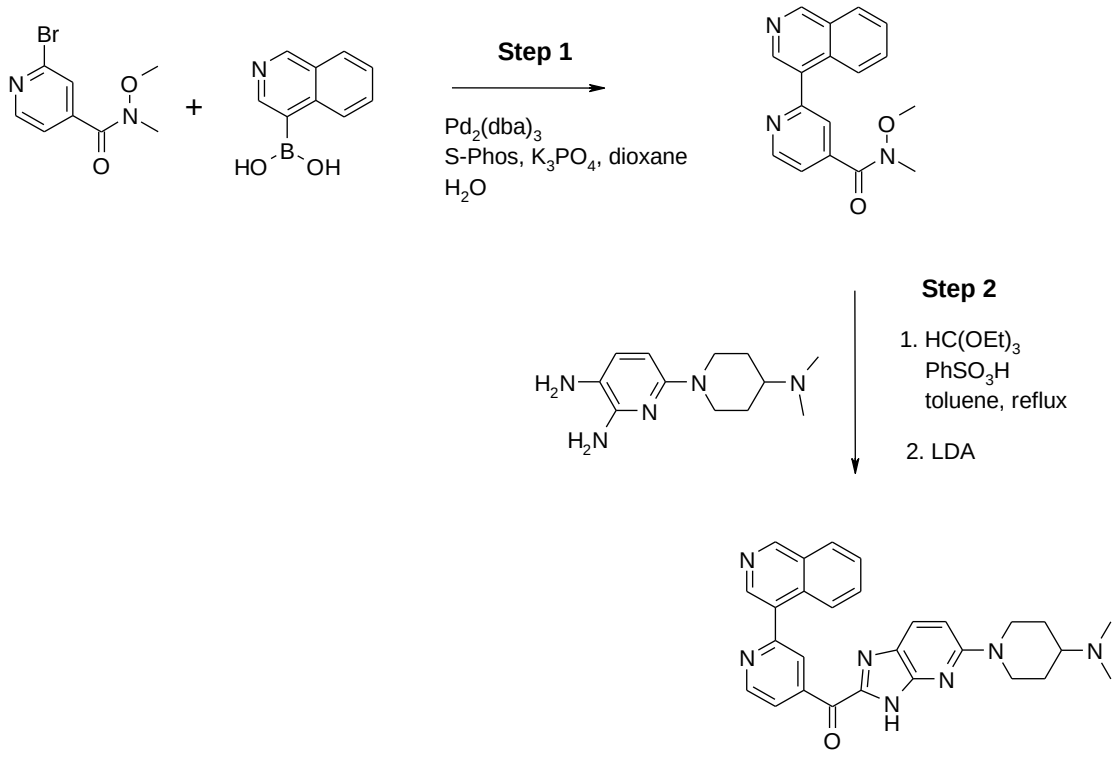
Compound 2

Step 1: (2-Bromo-pyridin-4-yl)-[5-(4-dimethylamino-piperidin-1-yl)-1*H*-benzoimidazol-2-yl]-methanone (11.9 g, 27.8 mmol) was dissolved in 200 mL of dry THF. This solution was cooled at 0°C and NaH 60% dispersion (1.34 g, 33.3 mmol) was added in portions. The mixture was stirred at 0°C for 30 min and then the 2-(trimethylsilyl)ethoxymethyl chloride (5.56 g, 33.3 mmol) was added dropwise. The mixture was allowed to warm at room temperature while stirring overnight. 2 M

aqueous HCl (300 mL) was carefully added and then the aqueous mixture was washed with EtOAc (3 x 100 mL). The aqueous phase was then neutralized with saturated aqueous $NaHCO_3$ and then extracted with $CHCl_3$/^{i}PrOH (3:1; 3 x 250 mL). The combined $CHCl_3$/i-PrOH fractions were dried (Na_2SO_4) and then evaporated to dryness. Purification by SiO_2 chromatography (dichloromethane/NH_3 2.0M in MeOH; 98:2 to 90:10) gave the product as a single regioisomer (5.02g, 32%). 1H NMR (400 MHz, $CDCl_3$): 8.58 (d, 1 H), 8.32 (s, 1 H), 8.13-8.05 (m, 1 H), 7.78 (d, 1 H), 7.19 (dd, 1 H), 6.97-6.90 (m, 1 H), 6.06 (s, 2 H), 3.90 (d, 2 H), 3.66 (dd, 2 H), 2.90 (t, 2 H), 2.46-2.31 (m, 7 H), 1.99 (d, 2 H), 1.82-1.64 (m, 2 H), 0.95 (dd, 2 H), 0.09-0.09 (m, 9 H). MS *m/z* 558 $[M+H]^+$.

Step 2 & 3: The bromopyridine (100 mg, 0.179 mmol), isoquinoline-4-boronic acid (47 mg, 0.27 mmol), $Pd_2(dba)_3$ (3.3 mg, 0.004 mmol) and S-Phos (14 mg, 0.014 mmol) were added to a microwave reaction tube equipped with a stir bar in air. The flask was evacuated and refilled with nitrogen twice. 1,4-dioxane (2 mL) and aqueous K_3PO_4 (0.18 mL, 0.36 mmol) were added by syringe. The tube was sealed and heated in a CEM Discovery microwave at 120 ºC for 40 min. The mixture was then diluted with H_2O / $CHCl_3$, filtered and the aqueous layer was extracted three times with $CHCl_3$ (or $CHCl_3$ / ^{i}PrOH, 2:1). The combined extracts were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was then purified by preparative HPLC to give the SEM-protected isoquinoline analogue (47 mg). This material was treated directly with HCl (4 M in 1,4-dioxane; 2 mL), H_2O (0.2 mL) and the mixture stirred at room temperature for 2 h then concentrated to dryness *in vacuo* to give [6-(4-dimethylamino-piperidin-1-yl)-1*H*-benzoimidazol-2-yl]-(2-isoquinolin-4-yl-pyridin-4-yl)-methanone (47 mg, 48%) as the di-hydrochloride salt. ^{1}H NMR (400 MHz, MeOD): 9.93 (s, 1 H), 8.99 (d, *J* = 5.2 Hz, 1 H), 8.82 (s, 1 H), 8.68 (d, *J* = 8.4 Hz, 1 H), 8.45 (d, *J* = 8.8 Hz, 1 H), 8.35 (m, 1 H), 8.30 (t, *J* = 8.0 Hz, 1 H), 8.15 (t, *J* = 7.8 Hz, 1 H), 8.05 (dd, *J* = 5.2, 1.6 Hz, 1 H), 7.76 (d, *J* = 9.2 Hz, 1 H), 7.57 (m, 2 H), 3.98 (m, 2 H), 3.55 (m, 1 H), 3.21 (m, 2 H), 2.95 (m, 6 H), 2.31 (m, 2 H), 2.10 (m, 2 H); Anal. RP-HPLC t_R = 2.25 min (method 3, purity 93.3%/93.5%); HR-MS *m/z* $(M+H)^+$: measured 477.2419, calcd 477.2403.

Compound 3

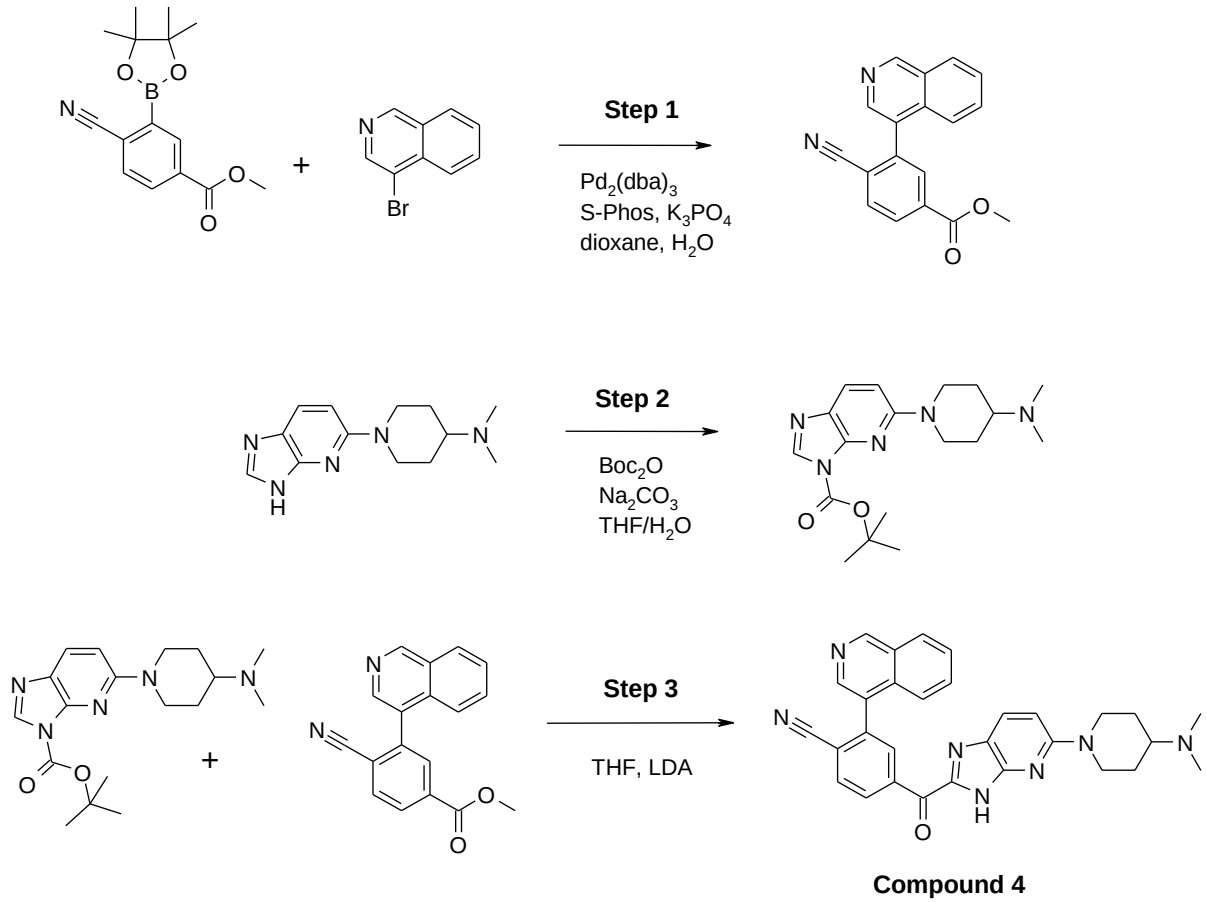


Compound 3

Step 1: To a mixture of 2-bromo-*N*-methoxy-*N*-methyl-isonicotinamide (550 mg, 2.24 mmol), isoquinone-3-boromic acid (579 mg, 3.36 mmol, 1.5 equiv), $Pd_2(dba)_3$ (6 mg, 0.03 equiv.) and S-Phos (2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl; 165 mg, 0.4 mmol, 0.18 equiv) were added dioxane (30 mL), water (0.3 mL) and K_3PO_4 (951 mg, 4.50 mmol, 2.0 equiv). The vessel was evacuated and flushed with N_2 several times to remove oxygen. The resulting mixture was heated at 120 °C for 40 min in a microwave reactor. The reaction mixture was then cooled and concentrated. The residue was purified by silica gel column chromatography (EtOAc/heptane 0 to 100%) to give 2-isoquinolin-4-yl-*N*-methoxy-*N*-methyl-isonicotinamide (605 mg) in 92% yield. ^{1}H NMR (400 MHz, CD_2Cl_2) δ 9.22 (s, 1 H), 8.80 (s, 1 H), 8.56 (s, 1 H), 8.16 (d, *J* = 8.5 Hz, 1 H), 8.00 (d, *J* = 8.0 Hz, 1 H), 7.76 (s, 1 H), 7.71 – 7.55 (m, 2 H), 7.50 (d, *J* = 5.0 Hz, 1 H), 3.53 (s, 3 H), 3.28 (s, 3 H); MS *m/z* 294.1 $[M+H]^+$.

Step 2: A solution of 6-(4-dimethylamino-piperidin-1-yl)-pyridin-2,4-yl-diamine **12** (see Example 6, Step 3) (150 mg, 0.64 mmol), triethylorthoformate (378 mg, 2.56 mmol, 4.0 equiv) and benzenesulfonic acid (0.5 M in toluene, 0.05 mL, 0.025 mmol, 0.040 equiv) in toluene (25 mL) was heated to reflux overnight. After cooling, approximately half the volume of the toluene was then removed and the same quantity of anhydrous toluene was added. This procedure was repeated several times to remove ethanol by-product. The resulting solution was treated with *N,N*-diisopropylethylamine (0.13 mL, 0.050 mmol, 0.080 equiv) to neutralize and concentrated. The residue was then treated with THF (12 mL) and 2-isoquinolin-4-yl-*N*-methoxy-*N*-methyl-isonicotinamide (243 mg, 0.83 mmol, 1.3 equiv) under N_2. The resulting mixture was cooled to -78 °C and treated with a solution of lithium diisopropylamide in heptane/THF/ethylbenzene (2.0 M, 0.41mL, 0.83 mmol, 1.3 equiv) slowly. The mixture was stirred at -78 °C for 2 h and quenched at this temperature with water. The reaction mixture was warmed room temperature, acidified with 2 N aqueous HCl until pH was ~3, stirred for 1 h and basified with saturated aqueous $NaHCO_3$ until pH was ~9. The product was extracted with EtOAc. The combined organic layers were washed with saturated aqueous NaCl, dried over $MgSO_4$, filtered and concentrated. Silica gel column chromatography ($MeOH/CH_2Cl_2$ 0 to 10%) provided [5-(4-dimethylamino-piperidin-1-yl)-3*H*-imidazo[4,5-b]pyridin-2-yl]-(2-isoquinolin-4-yl-pyridin-4-yl)-methanone (33 mg) in 21% yield. ^{1}H NMR(400 MHz, CD_3OD) δ 9.37 (s, 1 H), 9.00 (d, J = 5.0 Hz, 1 H), 8.68 (s, 1 H), 8.64 (s, 1 H), 8.35 (d, J = 5.0 Hz, 1 H), 8.24 (t, J = 8.5 Hz, 2 H), 7.91 – 7.68 (m, 2 H), 7.81 – 7.77 (m, 1 H), 7.03 (d, J = 9.0 Hz, 1 H), 4.61 (d, J = 14 Hz, 2 H), 2.96 (t, J = 13 Hz, 2 H), 2.58 – 2.50 (m, 1 H), 2.34 (s, 6 H), 2.00 (d, J = 12 Hz, 2 H), 1.50 (qd, J = 13, 3.8 Hz, 2 H); Anal. RP-HPLC t_R = 2.60 min (method 2, purity 100%/100%); HR-MS *m/z* $(M+H)^+$: measured 478.2350, calcd 478.2355.

Compound 4



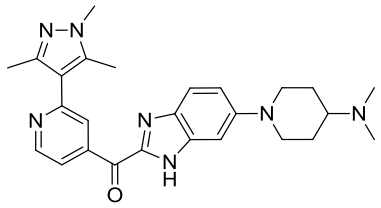
Compound 4

Step 1: A mixture of 4-cyano-3-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-benzoic acid methyl ester (100 mg, 0.348 mmol), 4-bromo-isoquinoline (80 mg, 0.38 mmol, 1.1 equiv), $Pd_2(dba)_3$ (32 mg, 0.035 mmol, 0.10 equiv), S-Phos (2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl, 29 mg, 0.070 mmol, 0.20 equiv) and 2 M aqueous K_3PO_4 (0.40 mL, 0.80 mmol, 2.0 equiv) in dioxane (5 mL) was degassed and heated at 120 °C for 40 min in a microwave reactor. After cooling, the reaction mixture was diluted with water and extracted with EtOAc. Combined organics were dried over $MgSO_4$, filtered and concentrated. The residue was purified by silica gel column chromatography (EtOAc/heptane 0 to 100%) to give 4-cyano-3-isoquinolin-4-yl-benzoic acid methyl ester (60 mg) in 60% yield. ^{1}H NMR (400 MHz, CD_2Cl_2) δ 9.41 (s, 1 H), 8.54 (s, 1 H), 8.29 – 8.25 (m, 2 H), 8.19 – 8.16 (m, 1 H), 8.04 – 8.00 (m, 1 H), 7.80 – 7.74 (m, 2 H), 7.58 – 7.56 (m, 1 H), 3.98 (s, 3 H); MS *m/z* 289.1 $[M+H]^+$.

Step 2: A solution of [1-(3*H*-imidazo[4,5-b]pyridin-5-yl)-piperidin-4-yl]-dimethyl-amine **13** (4 g, 16 mmol; compound **6**, Step 4), di-*tert*-butyl dicarbonate (4.3 g, 20 mmol) and Na_2CO_3 (5.1 g, 49 mmol) in THF/water (60 mL/20 mL) was stirred at room temperature overnight. The reaction solution was treated with water and extracted with EtOAc. Combined organic layers were dried over $MgSO_4$, filtered and concentrated. Silica gel column chromatography ($MeOH/CH_2Cl_2$ 5 to 10%) gave [5-(4-dimethylamino-piperidin-1yl)-imidazo[4,5-b]pyridine-3-carboxylic acid tert-butyl ester (4.2 g) in 66% yield over three steps. ^{1}H NMR (400 MHz, CD_2Cl_2) δ 8.45 (s, 1 H), 8.05 (d, *J* = 9.0 Hz, 1 H), 6.81 (d, *J* = 9.0 Hz, 1 H), 4.41 (br d, *J* = 14 Hz, 2 H), 2.96 – 2.89 (m, 2 H), 2.40 (dddd, *J* = 11, 7.3, 4.0, 3.8 Hz, 1 H), 2.31 (s, 6 H), 1.95 (br d, *J* = 13 Hz, 2 H), 1.70 (s, 9 H), 1.60 – 1.40 (m, 2 H); MS *m/z* 346.5 $[M+H]^+$.

Step 3: To a cooled (-78 °C) solution of 4-cyano-3-isoquinolin-4-yl-benzoic acid methyl ester (42 mg, 0.15 mmol) and 5-(4-dimethylamino-piperidin-1-yl)-imidazo[4,5-b]pyridine-3-carboxylic acid tert-butyl ester (50 mg, 0.15 mmol, 1.0 equiv) in THF (3 mL) was added a solution of lithium diisopropylamide in heptane/THF/ethylbenzene (2.0 M, 0.15 mL, 0.30 mmol, 2.0 equiv) slowly and the resulting mixture was stirred at -78 °C for 2 h and quenched at this temperature with water. The reaction mixture was warmed room temperature and extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated. Prep HPLC (MeCN/0.1% NH_4OH water 20 to 100%) provided 4-[5-(4-dimethylamino-piperidin-1-yl)-3*H*-imidazo[4,5-b]pyridine-2-carbonyl]-2-isoquinolin-4-yl-benzonitrile (2 mg) in 2.8% yield. ^{1}H NMR (400 MHz, CD_2Cl_2) δ 9.43 (s, 1 H), 8.86 (d, *J* = 8.0 Hz, 1 H), 8.81 (s, 1 H), 8.62 (s, 1 H), 8.19 – 8.17 (m, 1 H), 8.08 (d, *J* = 8.0 Hz, 1 H), 7.88 (d, *J* = 9.5 Hz, 1 H), 7.82 – 7.74 (m, 2 H), 7.71 – 7.69 (m, 1 H), 6.86 (d, *J* = 9.0 Hz, 1 H), 4.49 (br d, *J* = 14 Hz, 2 H), 3.02 (t, *J* = 13 Hz, 3 H), 2.48 – 2.43 (m, 1 H), 2.32 (s, 6 H), 1.97 (br d, *J* = 12 Hz, 2 H), 1.59 – 1.51 (m, 2 H); Anal. RP-HPLC t_R = 3.08 min (method 2, purity 91.0%/87.8%); HR-MS *m/z* $(M+H)^+$: measured 502.2353, calcd 502.2355.

Compound 5



[6-(4-Dimethylamino-piperidin-1-yl)-1*H*-benzoimidazol-2-yl]-[2-(1,3,5-trimethyl-1H-pyrazol-4-yl)-pyridin-4-yl]-methanone was prepared by following a similar method to that described for compound **1**, but using 1,3,5-trimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole. Product obtained as the di-hydrochloride salt (31 mg, 32%). ^{1}H NMR (400 MHz, DMSO-d_6): 8.91 (dd, *J* = 5.6 Hz, 0.2 H), 8.78 (d, *J* = 6.0 Hz, 0.8 H), 8.53 (s, 0.2 H), 8.12 (dd, *J* = 5.6, 1.2 Hz, 0.2 H), 7.88 (br s, 0.8 H), 7.84-7.77 (m, 0.8H), 7.75 (d, *J* = 8.9 Hz, 0.2 H), 7.63-7.60 (m, 0.8 H), 7.41-7.37 (m, 1 H), 7.15 (br s, 1 H), 3.92-3.79 (m, 2 H), 3.77 (s, 0.6 H), 3.75 (s, 2.4 H), 3.73-3.65 (m, 0.5 H), 3.54-3.44 (m, 0.5 H), 3.42-3.27 (m, 1 H), 2.92-2.80 (m, 1 H), 2.76 (d, *J* = 5.0 Hz, 1.4 H), 2.72 (d, *J* = 5.0 Hz, 4.6 H), 2.39 (s, 2.3 H), 2.37 (s, 0.7 H), 2.29 (s, 0.5 H), 2.28 (s, 2.5 H), 2.24-2.13 (m, 2 H), 2.04-1.77 (m, 2 H) (mixture of rotamers); Anal. RP-HPLC t_R = 1.99 min (method 3, purity 95.9%/96.4%); HR-MS *m/z* $(M+H)^+$: measured 458.2686, calcd 458.2668.