Supporting Information

Discovery of Disubstituted Imidazo[4,5-*b*]pyridines and Purines as Potent TrkA Inhibitors

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Structural Biology

Protein purification

Recombinant TrkA kinase domain (residues 473-796) was cloned into pFastbac Htb, yielding a construct coding for TrkA with an *N*-terminal 6 x His tag and a TEV protease cleavage site. Over-production of TrkA was carried in sf9 cells at 50 L scale. After harvest, cells were lysed using a Ultra Turrex 50. Clarified cell lysate was batch bound overnight with NiNTA Superflow resin (GE Healthcare). After elution of TrkA, the His tag was removed with TEV protease and uncleaved material was removed by subtractive IMAC using a NiNTA HiTrap (GE Healthcare) column. The sample was then concentrated using a Centricon MWCO 10K and further purified by gel filtration using Superdex 75 (GE Healthcare). The different phosphorylation forms were then separated on a Mono P HR (GE Healthcare) column. Briefly, the buffer was exchanged to 0.025 M Ethanolamine pH 9.4 and protein was loaded onto the column and eluted with Polybuffer^{TM} 96 (GE Healthcare) with a final pH of 6.0. Purified fractions of un-phosphorylated, mono-phosphorylated and di-phosphorylated TrkA were concentrated to 10 mg/mL in 10 mg/ml in 20 mM Tris pH 8.5, 0.1% CHAPS, 1mM TCEP, 8.7% glycerol and 100 mM NaCl and stored in a -80˚C freezer.

Crystallography

Compound **1** was added to mono-phosphorylated TrkA to a final concentration of 1 mM. 1 µL TrkA-compound sample was mixed with an equal volume of a well solution containing 35% w/v glycerol, 140 mM Na/K tartrate, 100 mM HEPES pH 8.0 supplemented with 40 mM zinc chloride. The drop was allowed to equilibrate over a reservoir containing well solution. Crystals were harvested by flash freezing into liquid nitrogen.

Data were collected at beam line ID14-4 at ESRF, Grenoble, France at a wavelength of 0.939 Å. The data were processed using MOSFLM[1], scaled, and further reduced using the CCP4 suite of program[2]; for statistics, see Table 3. Initial phasing was done by molecular replacement using the structure of the kinase domain of c-Met (PDB: 1R0P,[3]) as a starting model. Model rebuilding was performed within Coot[4] and refinement was performed using REFMAC5[1] and autobuster [5]. For statistics for the final models see Supporting Table 1.

Supporting Table 1. Crystallographic data collection and refinement statistics

Data collection	
Space group	I222
Unit cell parameters (Å)	a=130.73, b=158.42, c=152.58
Resolution range (Å)	42.06-2.75 (2.82-2.75)
No. Reflections (total/unique)	149812 / 41385
Redundancy	3.6 (3.6)
Data completeness (%)	99.9 (100.0)
Average I/σI	13.0 (3.0)
R_{merge} (%)	6.6 (38.5)
Statistics for the final model	
Number of nonhydrogen atoms	6412
R factor (%)	22.0
Free R factor (%)	24.2
Wilson B factor (Å^2)	71.3
Average B factors (Å^2):	
All atoms	55.7
Protein (chains A/B/C)	50.6 / 53.0 / 63.9
Inhibitor (A/B/C)	28.8 / 30.6 / 36.0
RMSD bond length (Å)	0.015
RMSD bond angles (°)	1.57
PDB code	4AOJ

Docking Studies

Structure of TrkA-**1** was prepared for docking using the Protein Preparation wizard in Maestro version 9.2 (Schrodinger, LLC, New York, NY 2011.). The protein Chain A was used. Water molecules and ions further than 5 Angstroms from the ligand were first removed, and hydrogen atoms were placed and optimized in 'fast mode' with water sampling at neutral pH. The remaining water molecules were removed, and the system was minimized using default conditions. Docking grids

were generated for the receptor using Glide (version 5.7, Schrödinger, LLC, New York, NY, 2011). Two-dimensional representations for **2d** and **3a** were read into Maestro, and the pyrazole tautomers (matching that of the binding mode for **1**) and the correct chiral flags were set manually. These settings were kept constant while 3-D conformers were generated with LigPrep version 2.5 and the OPLS 2005 force field.

Glide SP was used for the docking calculations, with the rigid receptor approximation. Epik state penalties were not calculated as part of the docking score. Exceptions to default settings included a requirement that the ligands make at least one hydrogen bond to any of the backbone atoms of residues Glu 590 (carbonyl oxygen) or Met 592 (amide NH or carbonyl oxygen), no post-docking minimization was performed, and five poses per ligand were retained. The best-scoring poses for each molecule were refined first with GlideXP and then minimized further using the Prime MM-GBSA protocol (Prime, version 3.0, Schrödinger, LLC, New York, NY, 2011). Ligand strain energies were computed and protein residues within 4 Å were allowed to move, with harmonic constraints to their starting positions. Figure 3 was generated with the resulting structures using MOE (*Molecular Operating Environment* (MOE), 2011.10; Chemical Computing Group Inc., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2011).

Rat Hepatocyte Clearance Assay

Compounds were incubated at initial concentration of 1 µM in rat cryopreserved hepatocytes of 1 million viable cells/mL. The hepatocyte incubations were carried out at 37°C in Williams E media containing Pen/Strep, GlutaMAX, HEPES, supplemental ITS mixture (consisting of insulin, transferrin, and selenium) and Dexamethasone. The total assay volume was 500 µL. At each time point (e.g., 0, 5, 15, 30, 60, 90, and 120 minute), a 50 µL aliquot of cell suspension was transferred to 150 µl of ice-cold stop solution that was an acetonitrile solution containing 0.05% formic acid and a bioanalytical internal standard compound. After vortex and centrifugation, aliquots of 125 µL of clear supernatant were transferred to a 96-well plate. Then, 50 uL water was added to each well. Samples were analyzed by LC/MS/MS.

Efficacy studies in 3T3-TrkA-Δ allograft mouse model:

In this engineered TrkA-driven allograft model, 3T3-TrkA-Δ cells[6] were subcutaneously implanted ($1x10^6$ per mouse) into 6-7 week old athymic female mice CrTac:Ncr-Foxn1nu (Taconic, Hudson, NY, USA). Once tumors reached an average size of approximately 200 mm^3, mice were randomized into treatment groups and given dose orally twice (BID) daily, for seven days at 1, 5 mg/kg doses for **2d** and 1, 5, 25 mg/kg doses for **3a**. The vehicle treatment group was oral dosed twice daily with 0.5% HPMC/0.1%Tween 80 (W/V). Tumor growth inhibition (measured by T/C, as percent change in tumor size of treatment/control groups): $0 < T/C < 40$. Tumor stasis: $-49 < T/C \leq 0$). Regressions: $T/C \leq -50$.

Synthesis

General Chemical Methods. All commercially available solvents and reagents were used without further purification. All moisture sensitive reactions were carried out under a nitrogen atmosphere in commercially available anhydrous solvents. Column chromatography was performed on ISCO MPLC Combi-flash systems (4700 Superior Street, Lincoln, NE, USA) or Biotage system (Biotage Inc. 1725 Discovery Drive Charlotteville, Virginia, 22911, USA.) unless otherwise mentioned using silica gel cartridges.. Aluminum-backed sheets of silica gel 60 F254 (EM Science) were used for TLC. ^{1}H NMR spectra were recorded on either Bruker 300 MHz or 400 MHz NMR spectrometers using deuterated DMSO (DMSO-*d*6) unless otherwise stated. Chemical shifts are expressed in part per million (ppm, δ units). Splitting patterns describe apparent multiplicities and are designated as s (singlet), d (doublet), dd (doublet-doublet), t (triplet), q (quartet), m (multiplet), br s (broad singlet). The purity determination of all reported compounds was performed with an Agilent 1100 equipped with Waters columns (Atlantis T3, 2.1x50 mm, 3 μm) eluted for > 10 minutes with a gradient mixture of H_2O-acetonitrile with formic or trifluoroacetic acid at wavelengths of 220, 254 and 280 nm. All compounds analyzed were ≥ 95% pure. Reverse phase chromatography was performed with Gilson systems using using YMC Pack ODS-AQ reverse phase HPLC Column (100 mm × 20 mm id., S-5μ particle size, 12 nm pore size) on Agilent instruments. Mass spectrometry was performed using a Micromass Quattro Micro mass spectrometer (for ESP) and an Agilent 1100 MSD instrument (for APCI). SFC

(super critical fluid chromatography) refers to Analytical SFC (ASC-1000 Analytical SFC System with Diode Array Detector) and/or Preparative SFC (APS-1000 AutoPrep Preparative SFC), obtained from SFC Mettler Toledo AutoChem, Inc. 7075 Samuel Morse Drive Columbia, MD 21046, U.S.A.) and used according to the manufacturer's instructions

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6-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-3-nitropyridin-2-amine (6a).

A mixture of 2,6-dichloro-3-nitropyridine (**4**, 5 g, 25.8 mmol) and 5-cyclopropyl-1*H*-pyrazol-3-amine (**5b**, 5 g, 40.6 mmol) in acetonitrile (40 mL) with diisopropylethylamine (6 mL) was stirred at room temperature for 24 hours, the resulting mixture was concentrated, and the resulting residue was purified by column chromatography (Hexane/Ethyl acetate) to afford **6a** (5g, 69%). LC/MS calcd, 279; found, $[M+H]^+$ 280. ^{1}H NMR (300 MHz, CD_3OD) 8.5 (m, 1 H), 6.9 (m, 1 H), 6.5 (s, 1 H), 1.9 (m, 1 H), 1.0 (m, 2 H), 0.70 (m, 2 H).

6-Chloro-*N*-(5-methyl-1*H*-pyrazol-3-yl)-3-nitropyridin-2-amine (6b).

6b was synthesized in a similar way to compound **6a**, using 2,6-dichloro-3-nitropyridine and 5-methyl-1*H*-pyrazol-3-amine (**5c**) as starting materials. LC/MS calcd, 253; found, $[M+H]^+$ 254.

6-Chloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)-3-nitropyridin-2-amine (6c).

A mixture of 2,6-dichloro-3-nitropyridine (**4**, 500 mg, 1.59 mmol), 5-isopropoxy-1*H*-pyrazol-3-amine (**5a**, 350 mg, 2.48 mmol) and triethylamine (2 mL) in acetonitrile (10 mL) was stirred at room temperature for 24 hours. The reaction mixture was concentrated and the residue was purified by column chromatography to afford **6c** (450 mg, 95%). LC/MS calcd, 297; found, $[M+H]^+$ 298.

2-Chloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)-5-nitropyrimidin-4-amine (13).

13 was synthesized in a similar way to compound **6a**, using 2,4-dichloro-5-nitropyrimidine and 5-isopropoxy-1*H*-pyrazol-3-amine (**5a**) as starting materials. LC/MS calcd, 298; found, $[M+H]^+$ 299.

N^2-(5-cyclopropyl-1*H*-pyrazol-3-yl)-N^6-[(1*S*)-1-(5-fluoropyrimidin-2-yl)ethyl]-3-nitropyridine-2,6-diamine (8a).

A mixture of 6-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-3-nitropyridin-2-amine (**6a**, 0.5 g, 1.78 mmol) and [(1*S*)-1-(5-fluoropyrimidin-2-yl)ethyl]amine (**7b**, 0.35 g, 2.48 mmol) in *n*-BuOH (10 mL) with diisopropylethylamine (1 mL) was stirred at 70 ^{o}C for 4 hours. The resulting mixture was diluted with ethyl acetate (20 mL), and washed with brine (10 mL × 3). The organic layer was dried and concentrated under reduced pressure. The resulting residue was purified by column chromatography (Hexane/Ethyl acetate) to yield **8a** (0.5g, 73%). LC/MS calcd, 384; found, $[M+H]^+$ 385. ^{1}H NMR (300 MHz, CD_3OD) 8.70 (s, 2 H), 8.20 (d, 1 H), 6.40 (d, 1 H), 6.20 (s, 1 H), 5.45-5.43 (m, 1 H), 1.90-1.89 (m, 1 H), 1.70 (d, 3 H), 1.05-1.03 (m, 2 H), 0.90-0.89 (m, 2 H).

N^6-[(1*S*)-1-(5-fluoropyrimidin-2-yl)ethyl]-N^2-(5-methyl-1*H*-pyrazol-3-yl)-3-nitropyridine-2,6-diamine (8b).

8b was synthesized in a similar way to compound **8a**, using 6-chloro-*N*-(5-methyl-1*H*-pyrazol-3-yl)-3-nitropyridin-2-amine **(6b)** and [(1*S*)-1-(5-fluoropyrimidin-2-yl)ethyl]amine (**7b**) as starting materials. LC/MS calcd, 358; found, $[M+H]^+$ 359.

N^6-[(1*S*)-1-(5-fluoropyridin-2-yl)ethyl]-3-nitro-N^2-[5-(isopropoxy)-1*H*-pyrazol-3-yl]pyridine-2,6-diamine (8c).

8c was synthesized in a similar way to compound **8a**, using 6-chloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)-3-nitropyridin-2-amine **(6c)** and (1*S*)-1-(5-fluoropyridin-2-yl)ethanamine (**7c**) as starting materials. LC/MS calcd, 401; found, $[M+H]^+$ 402. ^{1}H NMR (300 MHz, DMSO-*d6*) 12.3 (s, 1 H), 11.0 (s, 1 H), 8.80 (d, 1 H), 8.50 (d, 1 H), 8.15-8.13 (m, 1 H), 7.80 (d, 1 H), 7.30 (d, 1 H), 6.20 (br. s, 1 H), 5.80-5.79 (m, 1 H), 5.35-5.34 (m, 1 H), 4.60-4.59 (m, 1 H), 1.50 (d, 3 H), 1.20 (d, 6 H).

(*R*)-2-(5-fluoropyridin-2-yl)-2-(6-(5-isopropoxy-1*H*-pyrazol-3-ylamino)-5-nitropyridin-2-ylamino)ethanol (8d).

8d was synthesized in a similar way to compound **8a**, using 6-chloro-3-nitro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)pyridin-2-amine **(6c)** and (*R*)-2-amino-2-(5-fluoropyridin-2-yl)ethanol (**7a**) as starting materials. LC/MS calcd, 418; found, $[M+H]^+$ 419. ^{1}H NMR (300 MHz, CD_3OD) 8.50 (s, 1 H), 8.20 (d, 1 H), 7.60-7.59 (m, 1 H), 7.45-7.44 (m, 1 H), 6.30-6.29 (m, 1 H), 5.70 (s, 1 H), 5.45-5.43 (m, 1 H), 4.60-4.58 (m, 1 H), 3.90-3.89 (m, 2 H), 1.30 (d, 6 H).

N^2-[(1*S*)-1-(5-fluoropyrimidin-2-yl)ethyl]-5-nitro-N^4-[5-(isopropoxy)-1*H*-pyrazol-3-yl]pyrimidine-2,4-diamine (14).

14 was synthesized in a similar way to compound **8a**, using 2-chloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)-5-nitropyrimidin-4-amine (**13**) and [(1*S*)-1-(5-fluoropyrimidin-2-yl)ethyl]amine (**7b**) as starting materials. LC/MS calcd, 403; found, $[M+H]^+$ 404.

(*S*)-3-(5-cyclopropyl-1*H*-pyrazol-3-yl)-*N*-(1-(5-fluoropyrimidin-2-yl)ethyl)-3*H*-imidazo[4,5-b]pyridin-5-amine (2a).

N^2-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-N^6-[(1*S*)-1-(5-fluoropyrimidin-2-yl)ethyl]-3-nitropyridine-2,6-diamine (**8a**, 0.45 g, 1.17 mmol) was dissolved in ethanol (20 mL) and palladium on charcoal (60 mg) was added in a hydrogen atmosphere. The mixture was stirred at room temperature until no starting material was detected with TLC or LCMS. The mixture was filtered and formamidine acetate (0.5 g, 4.81 mmol) was added to the filtrate. The resulting mixture was heated at 95 ^oC for 4 hours. Ethyl acetate (40 mL) was added into the resulting mixture, and brine (10 mL × 3) was used to wash the organic layer. The organic layer was dried and concentrated. The resulting residue was separated by silica gel column (Ethyl acetate/ MeOH) to afford **2a** (0.08 g, 19% yield for two steps). LC/MS calcd, 364; found, $[M+H]^+$ 365. ^1H NMR (300 MHz, CD_3OD) 8.70 (s, 2 H), 8.30 (s, 1 H), 7.70 (d, 1 H), 6.70 (d,1 H), 6.50 (s, 1 H), 5.30-5.27 (m, 1 H), 2.00-1.98 (m, 1 H), 1.60 (d, 3 H), 1.10-1.07 (m, 2 H), 0.90-0.88 (m, 2 H).

(*S*)-*N*-(1-(5-fluoropyrimidin-2-yl)ethyl)-3-(5-methyl-1*H*-pyrazol-3-yl)-3*H*-imidazo[4,5-b]pyridin-5-amine (2b).

2b was synthesized in a similar way to compound **2a**, from N^6-[(1*S*)-1-(5-fluoropyrimidin-2-yl)ethyl]-N^2-(5-methyl-1*H*-pyrazol-3-yl)-3-nitropyridine-2,6-diamine (**8b**). LC/MS calcd, 338; found, $[M+H]^+$ 339. ^1H NMR 8.77 (s, 2 H), 7.71 (s, 1 H), 6.65 (s, 1 H), 6.33 (s, 1 H), 5.08-5.06 (m, 1 H), 2.28 (s, 3 H), 1.46 (d, 3 H). HRMS (ESI) *m/z* calcd for $[C_{16}H_{15}FN_8 + H]^+$: 339.1482, found 339.1479.

(*S*)-*N*-(1-(5-fluoropyridin-2-yl)ethyl)-3-(5-isopropoxy-1*H*-pyrazol-3-yl)-3*H*-imidazo[4,5-b]pyridin-5-amine (2c).

2c was synthesized in a similar way to compound **2a** from N^6-[(1*S*)-1-(5-fluoropyridin-2-yl)ethyl]-3-nitro-N^2-[5-(isopropoxy)-1*H*-pyrazol-3-yl]pyridine-2,6-diamine (**8c**). LC/MS calcd, 381; found, $[M+H]^+$ 382. ^{1}H NMR (300 MHz, CD_3OD) 8.45 (s, 1 H), 8.35 (s, 1 H), 7.75 (d, 1 H), 7.50-7.48 (m, 2 H), 6.70 (d, 1 H), 6.00 (s, 1 H), 5.10-5.08 (m, 1 H), 4.50-4.48 (m, 1 H), 1.55 (d, 3 H), 1.35 (d, 6 H). HRMS (ESI) *m/z* calcd for $[C_{19}H_{20}FN_7O + H]^+$: 382.1792, found 382.1786.

(*R*)-2-(5-fluoropyridin-2-yl)-2-(3-(5-isopropoxy-1*H*-pyrazol-3-yl)-3*H*-imidazo[4,5-b]pyridin-5-ylamino)ethanol (2d).

2d was synthesized in a similar way to compound **2a**, from (*R*)-2-(5-fluoropyridin-2-yl)-2-(6-(5-isopropoxy-1*H*-pyrazol-3-ylamino)-5-nitropyridin-2-ylamino)ethanol (**8d**). LC/MS calcd, 397; found, $[M+H]^+$ 398. ^{1}H NMR (300 MHz, CD_3OD) 8.50 (d, 1 H), 8.30 (s, 1 H), 7.80 (d, 1 H), 7.50 (dd, 2 H), 6.70 (d, 1 H), 6.05 (s, 1 H), 5.20-5.18 (m, 1 H), 4.65-4.63 (m, 1 H), 4.00-3.99 (m, 2 H), 1.55 - 1.35 (m, 6 H). HRMS (ESI) *m/z* calcd for $[C_{19}H_{20}FN_7O_2 + H]^+$: 398.1741, found 398.1748.

(*S*)-*N*-(1-(5-fluoropyrimidin-2-yl)ethyl)-9-(5-isopropoxy-1*H*-pyrazol-3-yl)-9*H*-purin-2-amine (2e).

2e was synthesized in a similar way to compound **2a**, from N^2-[(1*S*)-1-(5-fluoropyrimidin-2-yl)ethyl]-5-nitro-N^4-[5-(isopropoxy)-1*H*-pyrazol-3-yl]pyrimidine-2,4-diamine (**14**). LC/MS calcd, 383; found, $[M+H]^+$ 384. ^{1}H NMR (300 MHz, CD_3OD) 8.70 (s, 2 H), 8.60 (s, 1 H), 8.40 (s, 1 H), 6.20 (s, 1 H), 5.30 (m, 1 H), 4.60 (m, 1 H), 1.60 (d, 3 H), 1.50 (d, 6 H). HRMS (ESI) *m/z* calcd for $[C_{17}H_{18}FN_9O + H]^+$: 384.1697, found 384.1705.

2,3,6-Trifluoro-5-nitropyridine (10).

To a 3-neck, round-bottomed flask was added 2,3,6-trifluoropyridine (9, 25 g, 0.19 mol) followed by the addition of fuming nitric acid (210 mL, 4.7 mol). Sulfuric acid (150 mL, 2.8 mol) was added slowly *via* an addition funnel, maintaining internal temperature below 40^{o}C. The resulting solution was heated to 60 ^{o}C for 30 minutes and allowed to cool to room temperature after heating. This solution was then further cooled in an ice-water bath and inversely quenched into a 2L Erlenmeyer flask containing a mixture of ice and water (700 mL, 1:1 ratio). The quenched solution was then transferred to a 2L separatory funnel and partitioned with hexanes (600 mL). The aqueous layer was subsequently washed with hexanes (600 mL) and methylene chloride (600 mL). The combined

organic layers were then dried over Na_2SO_4, filtered, and concentrated to provide **10** (19.2 g, 57% yield). ^{1}H NMR (300 MHz, $CDCl_3$) 8.74 (s, 1 H).

(*R*)-2-(5,6-difluoro-3-nitropyridin-2-ylamino)-2-(5-fluoropyridin-2-yl)ethanol (11).

To a cold (0 ^{o}C) solution of 2,3,6-trifluoro-5-nitropyridine (**10**, 180 mg, 1.01 mmol) and triethylamine (0.35 mL, 2.53 mmol) in ethanol (5 mL) was added the (*R*)-2-amino-2-(5-fluoropyridin-2-yl)ethanol hydrochloride (**7a**, 214 mg, 1.11 mmol) in portions. The reaction was stirred at this temperature for 1 hour and was then allowed to warm up to room temperature. Brine and EtOAc were added to the mixture and separated. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by column chromatography (Biotage, 50% EtOAc/hexanes) gave **11** (227 mg, 71%). LC/MS calcd, 314; found, $[M+H]^+$ 315. ^{1}H NMR (300 MHz, $CDCl_3$) 8.42 (s, 1 H), 8.09 - 8.00 (m, 1 H), 7.55 - 7.41 (m, 2 H), 6.97 (d, 1 H), 5.37 - 5.30 (m, 1 H), 3.93 - 4.22 (m, 2 H), 3.40 (dd, 1 H).

(*R*)-2-(5-fluoro-6-(5-isopropoxy-1*H*-pyrazol-3-ylamino)-3-nitropyridin-2-ylamino)-2-(5-fluoropyridin-2-yl)ethanol (12a).

A mixture of (*R*)-2-(5,6-difluoro-3-nitropyridin-2-ylamino)-2-(5-fluoropyridin-2-yl)ethanol (**11**, 5.2 g, 16.55 mmol), 5-isopropoxy-1*H*-pyrazol-3-amine (**5a**, 2.7 g, 19.13 mmol), and diisopropylethylamine (5.78 mL, 33.10 mmol) in propan-2-ol (90 mL) was heated with stirring at 75 °C for 16 hours. The volatiles were removed under reduced pressure and water was added, followed by EtOAc. The two layers were separated and the organic layer was concentrated under reduced pressure. Purification by column chromatography (70-100% EtOAc in hexanes) gave **12a** (4.8 g, 67% yield). LC/MS calcd, 435; found, $[M+H]^+$ 436. ^{1}H NMR (300 MHz, $CDCl_3$) 10.90 (s, 1 H), 8.48 (s, 1 H), 8.03 (d, 1 H), 7.43 (t, 2 H), 7.19 (d, 1 H), 5.48- 5.36 (m, 2 H), 4.74-4.63 (m, 1 H), 4.17-3.97 (m, 2 H), 1.40-1.31 (m, 6 H).

(*R*)-2-(6-(5-ethoxy-1*H*-pyrazol-3-ylamino)-5-fluoro-3-nitropyridin-2-ylamino)-2-(5-fluoropyridin-2-yl)ethanol (12b).

12b was prepared via a procedure similar to that described for the synthesis of **12a** using (*R*)-2-(5,6-difluoro-3-nitropyridin-2-ylamino)-2-(5-fluoropyridin-2-yl)ethanol (**11**) and 5-ethoxy-1*H*-pyrazol-3-amine

(**5d**) as the starting materials. LC/MS calcd, 421; found, $[M+H]^+$ 422. ^{1}H NMR (300 MHz, $CDCl_3$) 10.89 (s, 1 H), 8.49 (s, 1 H), 8.02 (d, 1 H), 7.48-7.39 (m, 2 H), 7.18 (d, 1 H), 5.50-5.41 (m, 1 H), 5.39 (s, 1 H), 4.27-3.95 (m, 5 H), 1.39 (t, 3 H).

(*R*)-2-(5-fluoro-6-(5-methoxy-1*H*-pyrazol-3-ylamino)-3-nitropyridin-2-ylamino)-2-(5-fluoropyridin-2-yl)ethanol (12c).

12c was prepared via a procedure similar to that described for the synthesis of **12a** using (*R*)-2-(5,6-difluoro-3-nitropyridin-2-ylamino)-2-(5-fluoropyridin-2-yl)ethanol (**11**) and 5-methoxy-1*H*-pyrazol-3-amine (**5e**) as the starting materials. LC/MS calcd, 407; found, $[M+H]^+$ 408. ^{1}H NMR (300 MHz, $CDCl_3$) 10.90 (s, 1 H), 8.49 (s, 1 H), 8.03 (d, 1 H), 7.44 (d, 2 H), 7.17 (s, 1 H), 5.48-5.38 (m, 2 H), 4.92 (d, 1 H), 4.19-4.05 (m, 2 H), 3.90 (s, 3 H).

(*R*)-2-(6-fluoro-5-(5-isopropoxy-1*H*-pyrazol-3-ylamino)-3*H*-imidazo[4,5-b]pyridin-3-yl)-2-(5-fluoropyridin-2-yl)ethanol (3a).

3a was prepared via a procedure similar to that described for the synthesis of compound **2a** using (*R*)-2-(5-fluoro-6-(5-isopropoxy-1*H*-pyrazol-3-ylamino)-3-nitropyridin-2-ylamino)-2-(5-fluoropyridin-2-yl)ethanol (**12a**) as the starting material. LC/MS calcd, 415; found, $[M+H]^+$ 416. ^{1}H NMR (300 MHz, CD_3OD) 8.43 (s, 1 H), 8.35 (s, 1 H), 7.68 (d, 1 H), 7.53-7.51 (m, 2 H), 5.99 (s, 1 H), 5.28-5.25 (m, 1 H), 4.59-4.53 (m, 1 H), 4.07-3.95 (m, 2 H), 1.43 (d, 3H), 1.39 (d, 3H). HRMS (ESI) *m/z* calcd for $[C_{19}H_{19}F_2N_7O_2 + H]^+$: 416.1647, found 416.1636.

(*R*)-2-(5-(5-ethoxy-1*H*-pyrazol-3-ylamino)-6-fluoro-3*H*-imidazo[4,5-b]pyridin-3-yl)-2-(5-fluoropyridin-2-yl)ethanol (3b).

3b was prepared via a procedure similar to that described for the synthesis of compound **2a** using (*R*)-2-(6-(5-ethoxy-1*H*-pyrazol-3-ylamino)-5-fluoro-3-nitropyridin-2-ylamino)-2-(5-fluoropyridin-2-yl)ethanol (**12b**) as the starting material. LC/MS calcd, 401; found, $[M+H]^+$ 402. ^{1}H NMR (300 MHz) 8.46 (s, 1 H), 8.37 (s, 1H), 7.90 (d, 1H), 7.62-7.61 (m, 1H), 7.49-7.48 (1H, d), 7.45 (d, 1H), 5.99 (s, 1H), 5.14-5.13 (m, 1H), 5.01-4.99 (m, 1H), 4.19-4.14 (m, 2H), 3.86-3.81 (m, 2H), 1.42 (t, 3H).HRMS (ESI) *m/z* calcd for $[C_{18}H_{17}F_2N_7O_2 + H]^+$: 402.1490, found 402.1501.

(*R*)-2-(6-fluoro-5-(5-methoxy-1*H*-pyrazol-3-ylamino)-3*H*-imidazo[4,5-b]pyridin-3-yl)-2-(5-fluoropyridin-2-yl)ethanol (3c).

3c was prepared via a procedure similar to that described for the synthesis of compound **2a** using (*R*)-2-(5-fluoro-6-(5-methoxy-1*H*-pyrazol-3-ylamino)-3-nitropyridin-2-ylamino)-2-(5-fluoropyridin-2-yl)ethanol (**12c**) as the starting material. LC/MS calcd, 387; found, $[M+H]^+$ 388. ^{1}H NMR (300 MHz, CD_3OD) 8.44 (s, 1 H), 8.35 (s, 1 H), 7.68 (d, 1 H), 7.54-7.51 (m, 2 H), 6.05 (s, 1 H), 5.29-5.26 (m, 1 H), 4.06-3.94 (m, 5 H). HRMS (ESI) *m/z* calcd for $[C_{17}H_{15}F_2N_7O_2 + H]^+$: 388.1334, found 388.1344.

(*R*)-*N*-(2-{[*tert*-Butyl(dimethyl)silyl]oxy}ethylidene)-2-methylpropane-2-sulfinamide (15).

To a solution of (*R*)-2-methylpropane-2-sulfinamide (2.5 g, 20.6 mmol) and {[*tert*-butyl(dimethyl)silyl]oxy}acetaldehyde (4.32 mL, 22.7 mmol) in CH_2Cl_2 (30 mL) was added anhydrous $CuSO_4$ (7.23 g, 45.32 mmol). The reaction mixture was stirred at room temperature for 2 days. The mixture was filtered through Celite®, washed with CH_2Cl_2 and concentrated under reduced pressure. Purification by column chromatography (0-30% EtOAc in hexanes) gave **15**[7] in almost quantitative yield. ^{1}H NMR (300 MHz, $CDCl_3$) 8.24-7.86 (m, 1 H), 4.53 (d, 2 H), 1.23-1.15 (m, 9 H), 0.90 (s, 9 H), 0.08 (s, 6 H).

***N*-((*R*$_S$)-2-(*tert*-butyldimethylsilyloxy)-1-(5-fluoropyridin-2-yl)ethyl)-2-methylpropane-2-sulfinamide * (16).**

To a solution of 2-bromo-5-fluoropyridine (1.3 g, 7.2 mmol) in Et_2O (8 ml) at -68 ^{o}C was added a solution of *t*-BuLi (1.7 M in pentane, 8.5 mL, 14.4 mmol). The temperature of the mixture was kept below –65 ^{o}C during the addition and subsequently was allowed to stir for 15 minutes at approximately –70 ^{o}C. This mixture was cannulated, over a period of 15 minutes, to a solution of (*R*)-*N*-(2-{[*tert*-butyl(dimethyl)silyl]oxy}ethylidene)-2-methylpropane-2-sulfinamide (**15**, 1.0 g, 3.6 mmol) in Et_2O (24 mL) which was pre-cooled to –75 ^{o}C. The resulting mixture was stirred at –78 ^{o}C for 3 hours, whereupon it was quenched by the addition of aqueous saturated NH_4Cl solution. EtOAc was then added and the organic layer was washed with brine and concentrated under reduced pressure. Purification by column chromatography (20-40% EtOAc in hexanes) gave *N*-((*R*$_S$)-2-(*tert*-butyldimethylsilyloxy)-1-(5-fluoropyridin-2-yl)ethyl)-2-methylpropane-2-sulfinamide (**16**)* (higher *Rf* on TLC, 1.19 g) together with diastereoisomer (lower *Rf* on TLC, 166 mg). ^{1}H NMR (300 MHz, $CDCl_3$)

8.41 (s, 1 H), 7.35 (d, 2 H), 4.59 (t, 1 H), 4.43 (d, 1 H), 4.02-3.82 (m, 2 H), 1.23 (s, 9 H), 0.81 (s, 9 H), -0.06 (d, 6 H).

* "*Rs*" is intended to denote that the sulfur has an R configuration.

(*R*)-2-amino-2-(5-fluoropyridin-2-yl)ethanol, hydrochloride (7a).

To a solution *N*-((R_S)-2-(*tert*-butyldimethylsilyloxy)-1-(5-fluoropyridin-2-yl)ethyl)-2-methylpropane-2-sulfinamide (**16**, 1.13 g, 3.02 mmol) in MeOH (15 mL) was added hydrochloric acid (4 M in dioxane, ~3.0 mL, 12.08 mol) at 0 ^{o}C. The resulting mixture was stirred for 15 minutes at this temperature and was subsequently concentrated under reduced pressure. The mixture was triturated from hexanes to obtain the desired product **7a** (575 mg, almost quantitative). This product is highly hygroscopic. ^{1}H NMR (300 MHz) 8.62 (s, 1 H), 8.55 (s, 2 H), 7.93-7.76 (m, 1 H), 7.65 (dd, 1 H), 4.43 (d, 1 H), 3.77 (s, 2 H).

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