Accelerated Discovery of Potent Fusion Inhibitors for Respiratory Syncytial Virus

Supporting Information

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Contents

In Vitro Protocols for Antiviral Activity Determination

Cells and viruses

Human carcinoma (HEp-2, ATCC CCL-23) and human bronchial epithelial (BEAS-2B, ATCC CCL-9609) cells were maintained at 37°C and 5% CO2 in Dulbecco's modified Eagle's medium supplemented with 7.5% fetal bovine serum.

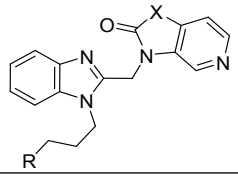
recRSV A2-L19F$_{D489E}$-fireSMASh was recovered as previously described,[1] and harbors a previously characterized pan-resistance mutation to RSV entry inhibitors in the F protein.[2] A recombinant RSV harboring a Nanoluciferase reporter, recRSV-A2-L19Fnanoluc, was constructed by exchanging the mKate reporter of previously described recRSV-A2-L19FmKate[3] with the Nanoluciferase ORF from pNL1.1.CMV[Nluc/CMV] (PROMEGA).

HEp-2 cells were infected at multiplicity of infection of 0.01 $TCID_{50}$ units/cell and incubated for 16 hours at 37°C, followed by 5 days at 32°C to generate RSV stocks. Infected cells were scrapped in DMEM and cell-associated progeny virions released through two freeze-thaw cycles. After clarification by centrifugation (4,000×g at 4°C for 20 mins), virus stocks were aliquoted and titers determined by $TCID_{50}$ titration on HEp-2 cells. For recRSV A2-L19F$_{D489E}$-fireSMASh, stocks were subsequently purified twice through ultracentrifugation (30,000×g in a SW41 rotor (Beckman Coulter); 2 hours at 4°C) through a 20–60% single-step sucrose gradient in TNE buffer (50 mM Tris-HCl (pH 7.2), 100 mM NaCl, 10 mM EDTA). All stocks were stored at −80°C.

Dose-response luciferase reporter assays

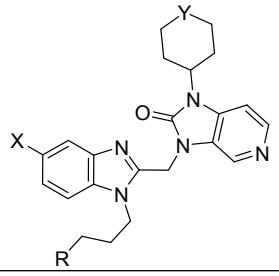
HEp-2 cells or BEAS-2B cells (compounds 49, 51, BMS-433771) were seeded on 96-well plates at 1.5 × 104 cells/well 6 hours prior to the assay. 3-fold dilutions of the compounds were prepared in three replicates in 96-well plates and transferred to the cells using a Nimbus liquid handler (Hamilton Robotics). Cells were infected with recRSV-A2-L19F$_{D489E}$-fireSMASh or recRSV-A2-L19F-nanoLuc at a multiplicity of infection of 0.1 $TCID_{50}$ units/cell. Each plate contained positive (1 mg/ml cycloheximide (Millipore Sigma), n=4) and negative (0.3% DMSO, n=4) control wells. Cell viability was assessed at 44 hours post-compound addition to uninfected cells using PrestoBlue substrate according to the manufacturer's instructions (10 µl/well (Life Technologies)). Luminescence activity of recRSV-A2-L19F$_{D489E}$-fireSMASh-infected plates were determined using ONE-Glo luciferase substrate (PROMEGA). recRSV-A2-L19F-nanoLuc-infected plates were washed twice with phosphate buffered saline and luminescence was determined using Nano-DLR substrate (PROMEGA). Fluorescence and luminescence reporter activity were recorded with an H1 synergy multimode plate reader (BioTek). Raw data were analyzed according to the formula: % effect (virus inhibition or cell viability) = $(X_{Sample} - X_{Min})/(X_{Max} - X_{Min}) \times 100$, with X_{Min}: mean of positive control wells and X_{Max}: mean of negative control wells. Four-parameter variable slope regression was applied to determine 50% or 90% maximal effective concentration (EC_{50} EC_{90}, respectively) or 50% cytotoxicity concentration (CC_{50}), using the nonlinear regression function in the Prism 8.3 (GraphPad) software package.

Table S1. Antiviral Activities of Compounds Identified by Machine Learning Algorithm against Wild-type RSV and the D489E Mutant Virus.



Compd	R	X	EC_{50} (nM)	EC_{90} (nM)	EC_{50} D489E (nM)	CC_{50} (nM)
1	CN	$NCH(CH_2)_2SO_2$	11	1180	>20000	>300000
2	CN	N-THP	5	470	>20000	>300000
3	CH_2OH	$NCH(CH_2)_2SO_2$	13	1010	>20000	>300000
4	CH_2OH	$C(CH_2)_2$	1940	>2000	>20000	>300000
5	CN	$C(CH_2)_2$	22	1530	>20000	>300000
BMS-433771	CH_2OH	N-*c*Pr	34.5	>2000	>20000	>300000

Table S2. Antiviral Activities of Compounds Featuring a Halogen on the Benzimidazole against Wild-type and the D489E Mutant Virus.



Compd	R	X	Y	EC_{50} (nM)	EC_{90} (nM)	EC_{50} D489E (nM)	CC_{50} (nM)
2	CN	H	O	5	470	>20000	>300000
44	CN	Br	O	0.5	2.0	>20000	>20000
45	CN	Cl	O	0.7	1.3	4071	>20000
46	CN	F	O	> 250	> 250	>20000	15,710
47	CN	Br	SO_2	3.1	10.4	>20000	> 20000
48	SO_2Me	Br	O	8.3	18.2	>20000	> 20000
49	CF_3	Br	O	2.5	15.3	10526	>20000
51	CH_2OH	Br	O	1.3	52.2	19459	>20000
BMS-433771	-	-	-	34.5	>2000	>20000	>300000
JNJ-53718678	-	-	-	0.9	1.1	6404	16056

In Vitro Protocols for Determination of Stability in Human Liver Microsomes

Experimental details

Test compounds were dissolved in 100% DMSO to make 10 mM stock solutions. Verapamil (human liver microsomes, Sigma Aldrich) aided as the positive control and was dissolved in 100% DMSO to make a 10 mM stock solution. The 10 mM stock solution of test and control compounds were further diluted in potassium phosphate buffer (100 mM, pH 7.4) to 500 µM to ensure the organic solvent content was < 0.2%.

Human liver microsomes were purchased from Xenotech at 20 mg/mL. NADPH (Sigma Aldrich) 10 mM stocks were prepared in deionized water.

The human liver microsome assay was prepared in a 1.5 mL Eppendorf tube with a final volume of 1100 µL for duplicate runs. Each reaction contained phosphate buffer (928.4 µL), liver microsomes (55 µL), and test compound resulting in a final concentration of 3 µM (6.6 µL of 500 µM). The reaction was initiated with 110 µL of 10 mM NADPH. At a temperature of 37°C, aliquots (100 µL) were removed in duplicate at 0, 5, 10, 15, 30 min time intervals and quenched in 100 µL of cold methanol, which contains internal standard (ISTD: d_5-7-ethoxy coumarin 4 mM). The aliquots were centrifuged at 12,000 g for 5 min and the supernatant removed and placed in an LCMS vial. Each time point was assessed on the LCMS and the area, based on the MRM transition, was integrated with respect to the ISTD. Positive controls were conducted at a final volume of 550 µL to give each time point in a single run. Negative controls without the presence of NADPH were used for the test compound and the control at the 30 min time point to account for degradation by other means than metabolism. Controls were processed and analyzed like test compounds. Each time point was run in duplicates followed by in-between blank washes to avoid the carryover and to equilibrate the column.

Instrumentation and method development

The analysis was performed using Agilent 1260 Infinity II HPLC, coupled with an Agilent G6460 triple quadrupole mass spectrometer (LC/MS/MS) (Agilent Technologies, USA). All the data were acquired employing Agilent 6460 Quantitative Analysis data processing software.

Separation was achieved using a Zorbax XDB C18 column (2.1 x 50 mm, 3.5 µm) that was maintained at 40°C. for all compounds. Mobile phases consisted of water (0.1% formic acid) and ACN (0.1% formic acid) at a flow rate of 0.5 mL/min. Instrumental analysis was done using Agilent JetStream electrospray ionization in the positive mode. All compounds were analyzed using multiple reaction monitoring (MRM) with quantifying and qualifying ions for increased reliability. Deuterated ethoxy coumarin was used as an internal standard (ISTD) as well. Table 1 shows the settings for each compound.

Table S3. Scan parameters for the tested compounds and the associated transitions

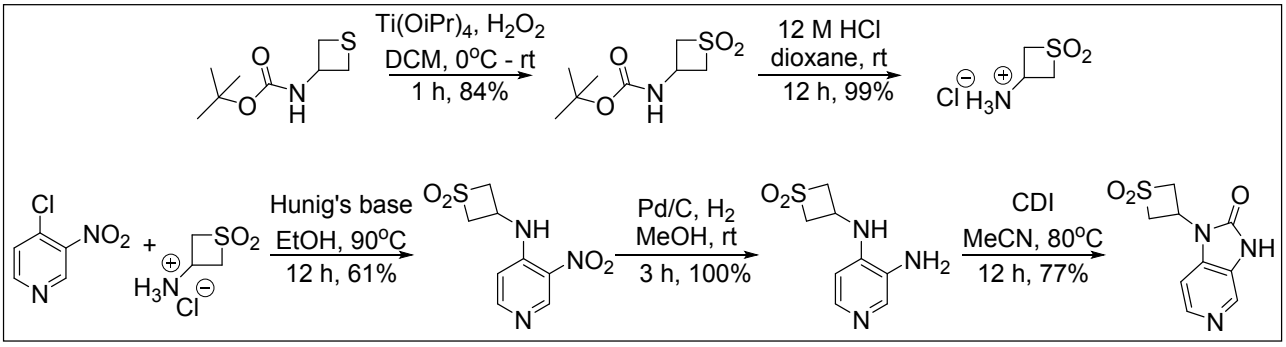
Compound	Precursor Ion MS1	Product ion MS2	Dwell (ms)	Fragmentor voltage (V)	Collision Energy (V)	Cell Accelerator (v)	Polarity
1	437.1	373.2 198.1 145.1	200	172	25 33 53	4	positive
3	442.2	203.1 176.1 133.1	200	162	25 41 41	4	positive
4	358.2	198.1 145.1	200	104	25 45	4	positive
5	363.2	291.1 161.1 131.1	200	164	21 29 37	4	positive
45	451.2	232.1 367.1	100	162	41 29	4	positive
46	435.2	351.1 216.1	200	162	29 41	4	positive
47	543.1	276	100	200	41	4	positive

		411			33		
49	538.1	454 319	200	110	33 45	4	positive
51	500.1	416 209 136	200	110	29 49 41	4	positive
d5-7-Ethoxy coumarin (ISTD)	196.1	164	100	116	17	4	positive

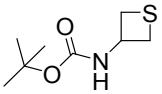
General Synthetic Procedures

Automated flash column chromatography was carried out using a Teledyne ISCO CombiFlash Companion system with silica gel-packed columns (SiliCycle Inc.) with one of or combinations of hexane, EtOAc, DCM or MeOH as the mobile phase. Analytical thin layer chromatography was performed using Merck silica gel 60 F254 coated on aluminium-supported plates. Visualization of compounds on TLC plates was accomplished UV light (254 nm) and/or using common stains such as *p*-anisaldehyde, ninhydrin (NIN) or a potassium permanganate ($KMnO_4$) solution followed by gentle heating. NMR spectra (^1H, ^{13}C and ^{19}F) were obtained using either a Varian INOVA 600 MHz spectrometer (150 MHz for ^{13}C), a Varian INOVA 500 MHz spectrometer (126 MHz for ^{13}C), a Varian INOVA 400 MHz spectrometer (101 MHz for ^{13}C), a Varian VNMR 400 MHz spectrometer (101 MHz for ^{13}C), a Bruker 600 MHz spectrometer (150 MHz for ^{13}C) or a Mercury 300 MHz spectrometer (75 MHz for ^{13}C). NMR samples were prepared in deuterated chloroform ($CDCl_3$), deuterated methanol (CD_3OD) or deuterated DMSO (DMSO-d_6) using the residual solvent peak ($CDCl_3$: ^1H = 7.26 ppm, ^{13}C = 77.16 ppm; CD_3OD: ^1H = 3.31 ppm, ^{13}C = 49.0 ppm; DMSO-d_6: ^1H = 2.50 ppm, ^{13}C = 39.5 ppm) as an internal reference. For ^{19}F NMR, the residual chloroform peak in ^1H NMR was used as an absolute reference unless otherwise specified. MestReNova software was used to process all NMR spectra. Chemical shifts (δ) are reported in ppm and *J* - values are given in Hz. Multiplicities are reported as a singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) or doublet of doublet (dd). All spectra were obtained at 25 °C unless otherwise reported. High resolution mass spectrometry (HRMS) was performed by the Emory University Mass Spectrometry Center, directed by Dr. Fred Strobel. Liquid chromatography-mass spectrometry (LC-MS) was performed on an Agilent 1200 HPLC equipped with a 6120 Quadrupole mass spectrometer (ESI-API) eluting with mixtures of HPLC grade MeOH and H_2O or MeCN and H_2O (all spiked with 0.1% formic acid) through an analytical, reverse-phase, Agilent C18 XDB eclipse column (50 mm x 4.6 mm, 3.5 µM). LC-MS samples were prepared in a solution of 75:25 MeOH/H_2O (spiked with 0.1% formic acid). All reactions requiring inert conditions were carried out under a positive atmosphere of argon. All glassware was flame-dried while under vacuum or oven dried overnight before purging with argon. Standard Schlenk techniques were employed when necessary. Solvents were removed using a rotary evaporator followed by the removal of trace amounts of solvent using a high vacuum pump at ca. 0.08 mm Hg. Final compound purity was assessed using ^1H NMR and LC-MS. All final compounds exhibited > 95% purity.

Synthetic Procedures and Characterization

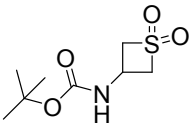


Tert-butyl thietan-3-ylcarbamate



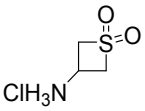
As in N. K. Thong *et al. Bioorg. Med. Chem. Lett.*, **2009**, *19,* 3832-5 and M. Muehle-Bach *et al.* WO 2007/080131; *tert*-butyl thietan-3-ylcarbamate (10.5 g, white solid) was synthesized from 2-aminopropane-1,3-diol in three steps and 48 % overall yield. **^{1}H NMR (400 MHz, $CDCl_3$) δ** 5.08 (s, 1H), 5.01 – 4.90 (m, 1H), 3.31 (d, *J* = 8.5 Hz, 4H), 1.42 (s, 9H).**^{13}C NMR (100 MHz, $CDCl_3$) δ** 153.9, 79.9, 48.5, 36.4, 28.3.

Tert-butyl (1,1-dioxidothietan-3-yl)carbamate



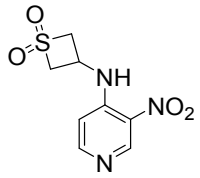
As in J. A. Burkhard *et al. Org. Lett.*, **2010**, *12*, 1944-1947; a 1 L RB flask equipped with a magnetic stir bar and rubber septum was charged with *tert*-butyl thietan-3-ylcarbamate (10.4 g, 54.7 mmol, 1 equiv.) and DCM (456 mL). Then $Ti(OiPr)_4$ (16.0 mL, 54.7 mmol, 1 equiv) was added dropwise, followed by H_2O_2 (22.4 mL, 219 mmol, 4 equiv.) at 0 °C. After stirring at 0 °C for 15 min, the reaction was allowed to warm to rt and the stirring was continued for 1 h. The reaction mixture was quenched by addition of water and the product was extracted with DCM (3x) and dried over Na_2SO_4. The organics were concentrated and the crude product was purified on silica gel column (40 g) using 0 to 20% EtOAc as eluent affording *tert*-butyl (1,1-dioxidothietan-3-yl)carbamate (10.2 g, 84%) as a white solid. **^{1}H NMR (400 MHz, $CDCl_3$) δ** 5.19 (s, 1H), 4.53 – 4.43 (m, 3H), 4.02 – 3.92 (m, 2H), 1.45 (s, 9H). **LC-MS (ESI-API, 254 nm)** 95 % MeOH in H_2O (0.1% HCO_2H), 3 min, 1.00 ml/min, C18 (Agilent Zorbax XDB-18, 50 mm x 4.6 mm, 3.5 µm), m/z = 244.0 (M + Na), t = 0.546 min (MS signal).

3-Aminothietane 1,1-dioxide hydrochloride



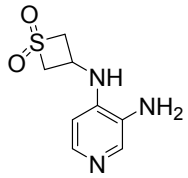
A 500 ml RB flask equipped with a stirrer bar was charged with *tert*-butyl (1,1-dioxidothietan-3-yl)carbamate (8.00 g, 36.2 mmol, 1 equiv.), 12 M HCl (12.1 mL, 145 mmol, 4 equiv.) and dioxane (181 mL). After stirring at rt for 12 h, the suspension was concentrated affording 3-aminothietane 1,1-dioxide hydrochloride (5.63 g, 99%) as a white solid. **^{1}H NMR (400 MHz, DMSO-d_6) δ** 8.85 (s, 3H), 4.56 – 4.48 (m, 2H), 4.44 – 4.36 (m, 2H), 4.09 (tt, *J* = 9.0, 6.3 Hz, 1H). **LC-MS (ESI-API, 254 nm)** 75-95 % MeOH in H_2O (0.1% HCO_2H), 3 min, 1.00 ml/min, C18 (Agilent Zorbax XDB-18, 50 mm x 4.6 mm, 3.5 µm), m/z = 122.0 (M + H), t = 0.499 min (MS signal).

3-((3-Nitropyridin-4-yl)amino)thietane 1,1-dioxide (7)



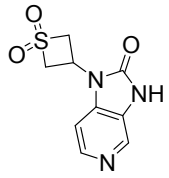
A 100 ml Schlenk tube equipped with a magnetic stirrer bar and cold finger condenser was charged with 3-aminothietane 1,1-dioxide hydrochloride (2.67 g, 16.9 mmol, 1.1 equiv.), DIPEA (5.7 mL, 32.3 mmol, 2.1 equiv.) and abs. EtOH (20 mL). The suspension was stirred at rt until the amine was dissolved. Then 4-chloro-3-nitropyridine (2.44 g, 15.4 mmol, 1 equiv.) was added and EtOH (5.0 mL) was used to wash in 4-chloro-3-nitropyridine. After heating at refluxing temperature for 5 h, NaOH (1.75 g, 32.3 mmol, 2.1 equiv.) dissolved in MeOH (5 mL) was added, stirred for 12 h and the product was filtered and washed with water. The product was dispensed in water and filtered again affording 3-((3-nitropyridin-4-yl)amino)thietane 1,1-dioxide (2.29 g, 61%) as a bright yellow solid. **^{1}H NMR (400 MHz, DMSO-d_6) δ** 9.07 (s, 1H), 8.45 (d, *J* = 5.9 Hz, 1H), 8.37 (d, *J* = 6.1 Hz, 1H), 7.00 (d, *J* = 6.1 Hz, 1H), 4.74 – 4.67 (m, 2H), 4.65 – 4.56 (m, 1H), 4.55 – 4.49 (m, 2H). **LC-MS (ESI-API, 254 nm)** 75-95 % MeOH in H_2O (0.1% HCO_2H), 3 min, 1.00 ml/min, C18 (Agilent Zorbax XDB-18, 50 mm x 4.6 mm, 3.5 µm), m/z = 244.0 (M + H), 266.0 (M + Na), t = 0.557 min.

3-((3-Aminopyridin-4-yl)amino)thietane 1,1-dioxide (10)

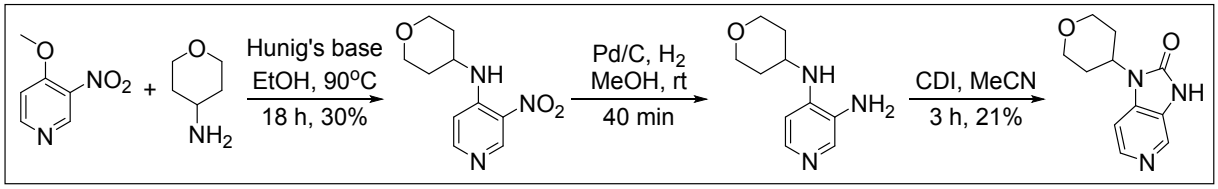


A 500 ml Parr shaker bomb was charged with 3-((3-nitropyridin-4-yl)amino)thietane 1,1-dioxide (2.24 g, 9.21 mmol, 1 equiv.), 10 w% palladium on carbon (98.0 mg, 0.0920 mmol, 0.01 equiv.) and MeOH (23 mL) and the bottle was installed on the Parr shaker. After shaking at rt and 40 psi H_2 pressure for 3 h, white precipitate was formed. More MeOH was added to dissolve the product and the suspension was filtered through a celite plug. The plug was slowly washed with MeOH, water and ether. The organics were concentrated and the product was dissolved in dioxane and toluene. After concentration of the organics, the product was dried under high vacuum for 12 h affording 3-((3-aminopyridin-4-yl)amino)thietane 1,1-dioxide (1.97 g, 100%) as a beige solid. **^{1}H NMR (400 MHz, DMSO-d_6) δ** 7.71 (s, 1H), 7.62 (d, *J* = 5.2 Hz, 1H), 6.29 (d, *J* = 5.3 Hz, 1H), 5.99 (d, *J* = 5.5 Hz, 1H), 4.74 – 4.66 (m, 4H), 4.24 (td, *J* = 8.4, 4.3 Hz, 1H), 4.11 – 4.04 (m, 2H). **LC-MS (ESI-API, 254 nm)** 75-95 % MeOH in H_2O (0.1% HCO_2H), 3 min, 1.00 ml/min, C18 (Agilent Zorbax XDB-18, 50 mm x 4.6 mm, 3.5 µm), m/z = 214.0 (M + H), t = 0.490 min.

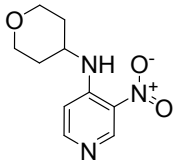
1-(1,1-Dioxidothietan-3-yl)-1,3-dihydro-2*H*-imidazo[4,5-c]pyridin-2-one (13)



A 100 ml Schlenk tube equipped with a magnetic stirrer bar and cold finger condenser was charged with 3-((3-aminopyridin-4-yl)amino)thietane 1,1-dioxide (400 mg, 1.88 mmol, 1 equiv.) and acetonitrile (18.8 mL) and heated to 80°C to dissolve the starting material. The heating was removed and CDI (608 mg, 3.75 mmol, 2 equiv.) dissolved in acetonitrile (18.8 mL) was added. After heating at 80°C for 12 h, the organics were concentrated, filtered and washed with DCM affording 1-(1,1-dioxidothietan-3-yl)-1,3-dihydro-2*H*-imidazo[4,5-c]pyridin-2-one (345 mg, 77%) as a brownish orange solid. **^{1}H NMR (400 MHz, DMSO-d_6) δ** 11.41 (s, 1H), 8.24 (d, *J* = 5.0 Hz, 1H), 8.23 (s, 1H), 7.43 (d, *J* = 5.4 Hz, 1H), 5.35 (tt, *J* = 9.8, 7.4 Hz, 1H), 5.02 – 4.94 (m, 2H), 4.67 – 4.58 (m, 2H). **^{13}C NMR (151 MHz, DMSO-d_6) δ** 153.2, 142.2, 135.2, 129.8, 125.8, 104.2, 68.2, 32.9. **LC-MS (ESI-API, 254 nm)** 75-95 % MeOH in H_2O (0.1% HCO_2H), 3 min, 1.00 ml/min, C18 (Agilent Zorbax XDB-18, 50 mm x 4.6 mm, 3.5 µm), m/z = 240.0 (M + H), 262.0 (M + Na), t = 0.495 min. **HRMS (ESI+)** m/z: $[M + H]^+$ Calcd for $C_9H_{10}N_3O_3S$ 240.0437; Found 240.0432, error -2.1 ppm. **Mp**= >250°C.

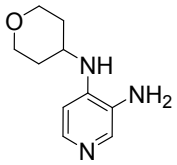


3-Nitro-*N*-tetrahydropyran-4-yl-pyridin-4-amine (8)



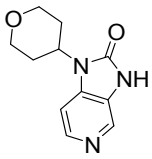
To a 75 ml sealed flask with teflon screw cap was added 4-methoxy-3-nitropyridine (6.86 g, 44.5 mmol), tetrahydro-2H-pyran-4-amine (3.1 ml, 30 mmol), absolute EtOH (49 ml) and Hunig's Base (7.8 ml, 45 mmol) then the vessel was tightly sealed and heated in a 90 °C oil bath for 18 h. The reaction was cooled to 0 °C and the yellow precipitate was collected and washed with hexanes. The solid was dried in vacuo to yield the product (2.0 g, 9.0 mmol, 30% yield) as a yellow solid. **^{1}H NMR (600 MHz, DMSO-*d*$_6$) δ** 9.03 (s, 1H), 8.27 (dd, J = 6.2, 0.8 Hz, 1H), 8.02 (d, *J* = 7.9 Hz, 1H), 7.15 (d, *J* = 6.3 Hz, 1H), 3.96 – 3.90 (m, 1H), 3.87 (ddd, *J* = 12.1, 4.2, 2.7 Hz, 2H), 3.47 (td, *J* = 11.6, 2.2 Hz, 2H), 1.89 (ddd, *J* = 12.6, 4.5, 2.2 Hz, 2H), 1.71 – 1.59 (m, 2H).**^{13}C NMR (126 MHz, DMSO- *d*$_6$) δ** 152.8, 148.3, 147.0, 109.5, 108.8, 65.6, 48.1, 31.8. **HRMS** (APCI) *m/z* calc. for $C_{10}H_{14}O_3N_3$ $[M+H]^+$, 224.10297 found, 224.10274.

N^4-Tetrahydropyran-4-ylpyridine-3,4-diamine (11)

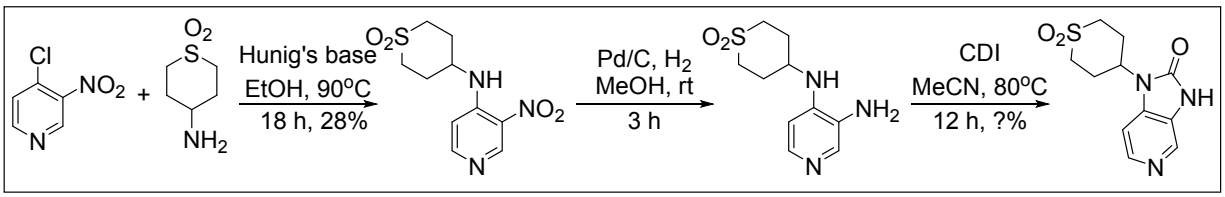


3-Nitro-N-(tetrahydro-2H-pyran-4-yl)pyridin-4-amine (1.14 g, 5.11 mmol) was hydrogenated in anhydrous MeOH (100 ml) in the presence of 10% palladium on carbon (0.8 g, 7.52 mmol) at 40 psi for 40 minutes. The mixture was filtered through celite and washed with MeOH then concentrated to afford N4-(tetrahydro-2H-pyran-4-yl)pyridine-3,4-diamine as a light orange solid. The light orange solid was used in the next step without purification. **^{1}H NMR (400 MHz, CD_3OD) δ** 7.68 (s, 1H), 7.65 (d, *J* = 5.6 Hz, 1H), 6.55 (d, *J* = 5.7 Hz, 1H), 4.01 – 3.92 (m, 2H), 3.63 (tt, *J* = 10.6, 4.1 Hz, 1H), 3.55 (td, *J* = 11.7, 2.1 Hz, 2H), 2.00 (ddd, *J* = 12.8, 4.4, 2.2 Hz, 2H), 1.63 – 1.48 (m, 2H). **^{13}C NMR (101 MHz, DMSO- *d*$_6$) δ** 139.8, 134.8, 130.9, 104.4, 88.2, 66.0, 47.6, 32.7. **HRMS** (APCI) *m/z* calc. for $C_{10}H_{16}ON_3$ $[M+H]^+$, 194.12879 found, 194.12861.

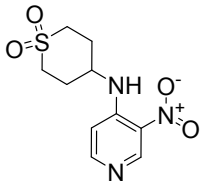
3-Tetrahydropyran-4-yl-1*H*-benzimidazol-2-one (14)



To a 50 ml RB flask was added N4-(tetrahydro-2H-pyran-4-yl)pyridine-3,4-diamine (595 mg, 3.08 mmol), MeCN (20 ml) and CDI (549 mg, 3.39 mmol) at rt. The reaction was heated to reflux for 3 h, cooled to rt and concentrated in vacuo. The residue was dissolved in DCM, washed with water, dried with Na_2SO_4, filtered and concentrated. The residue was purified via combiflash (5:95 to 30:70 MeOH:EtOAc) to afford 1-(tetrahydro-2H-pyran-4-yl)-1H-imidazo[4,5-c]pyridin-2(3H)-one (140 mg, 0.639 mmol, 21% yield). **^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ** 8.19 (s, 1H), 8.14 (d, *J* = 5.3 Hz, 1H), 7.32 (d, *J* = 5.3 Hz, 1H), 4.41 (tt, *J* = 12.3, 4.2 Hz, 1H), 3.98 (dd, *J* = 11.5, 4.5 Hz, 2H), 3.46 (td, *J* = 12.0, 1.9 Hz, 2H), 2.32 (qd, *J* = 12.4, 4.6 Hz, 2H), 1.70 – 1.60 (m, 2H). **^{13}C NMR (126 MHz, DMSO- *d*$_6$) δ** 153.3, 141.9, 135.2, 129.5, 125.9, 104.2, 66.5, 49.3, 29.5. **HRMS** (APCI) *m/z* calc. for $C_{11}H_{14}O_2N_3$ $[M+H]^+$, 220.10805 found, 220.1077. **LC/MS** 10-95% MeOH in H_2O over 6 minutes, r_t = 0.757 at 254 nM, MS (+) 220.2.

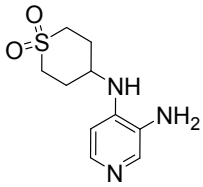


N-(1,1-Dioxothian-4-yl)-3-nitro-pyridin-4-amine (9)



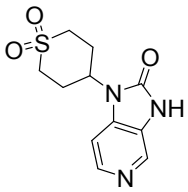
4-Chloro-3-nitro-pyridine (5.20 g, 32.8 mmol), tetrahydropyran-4-amine (5.1 mL, 49 mmol) and *N*,*N*-diisopropylethylamine (8.6 mL, 49 mmol) in ethanol (20 mL) was heated under reflux for 1.5 hours. Once TLC confirmed full consumption of the 4-chloro-3-nitro-pyridine, the reaction was cooled to room temperature and concentrated in vacuo. The crude yellow solid was subsequently recrystallized from hot methanol to yield the product 3-nitro-*N*-tetrahydropyran-4-yl-pyridin-4-amine (4.16 g, 18.6 mmol, 57% yield) as a bright yellow solid. **^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ** 9.03 (s, 1H), 8.32 (dd, J = 6.2, 0.8 Hz, 1H), 8.08 (d, J = 8.3 Hz, 1H), 7.12 (d, J = 6.3 Hz, 1H), 4.15 – 4.04 (m, 1H), 3.39 – 3.30 (m, 2H), 3.19 – 3.11 (m, 2H), 2.26 – 2.14 (m, 4H). **^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ** 153.0, 148.3, 147.0, 129.7, 108.6, 49.1, 47.7, 28.8. **HRMS** (APCI) *m/z* calc. for $C_{10}H_{14}O_4N_3{}^{32}S$ $[M+H]^+$, 272.06995 found, 272.06959.

N^4-(1,1-Dioxothian-4-yl)pyridine-3,4-diamine (12)

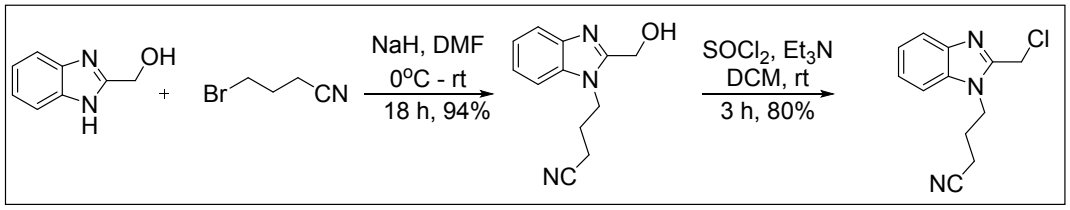


N-(1,1-Dioxothian-4-yl)-3-nitro-pyridin-4-amine (250 mg, 0.921 mmol, 1 eq) was hydrogenated in EtOH (10 ml) in the presence of 10% palladium on carbon (10 mg, 0.0092 mmol, 0.01 eq) under an atmosphere of hydrogen (balloon). The mixture was filtered through celite and washed with EtOH and then concentrated to afford the product (213 mg, 0.883 mmol) as an off-white solid. The product was used in the next step without purification. **^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ** 7.66 (s, 1H), 7.59 (d, J = 5.3 Hz, 1H), 6.43 (d, J = 5.5 Hz, 1H), 5.30 (d, J = 7.9 Hz, 1H), 4.69 (s, 2H), 3.80 – 3.70 (m, 1H), 3.32 – 3.22 (m, 2H), 3.19 – 3.11 (m, 2H), 2.24 – 2.14 (m, 2H), 2.01 – 1.90 (m, 2H). **^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ** 139.7, 139.5, 134.9, 131.2, 104.5, 48.8, 46.4, 29.1. **HRMS** (APCI) *m/z* calc. for $C_{10}H_{16}O_2N_3{}^{32}S$ $[M+H]^+$, 242.09577 found, 242.09553.

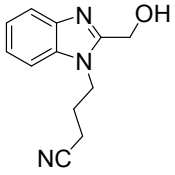
1-(1,1-Dioxothian-4-yl)-3*H*-imidazo[4,5-c]pyridin-2-one (15)



To a 25 mL flask was added *N*4-(1,1-dioxothian-4-yl)pyridine-3,4-diamine (381 mg, 1.58 mmol, 1 eq), MeCN (10 ml) and CDI (307 g, 1.90 mmol, 1.2 eq) at rt. The reaction was allowed to stir vigorously at room temperature for 18 hours after which the resulting white precipitate was collected by filtration and dried under vacuum to give the desired product (146 mg, 0.515 mmol, 33%) as white solid. **^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ** 11.27 (s, 1H), 8.22 – 8.18 (m, 2H), 7.19 (d, J = 5.4 Hz, 1H), 4.69 – 4.58 (m, 1H), 3.51 – 3.42 (m, 2H), 3.20 – 3.12 (m, 2H), 2.83 – 2.71 (m, 2H), 2.10 – 2.03 (m, 2H). **^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ** 153.1, 142.0, 135.2, 129.6, 126.0, 103.7, 49.4, 48.4, 26.9. **HRMS** (APCI) *m/z* calc. for $C_{11}H_{14}O_3N_3{}^{32}S$ $[M+H]^+$, 268.07504 found, 268.07478.

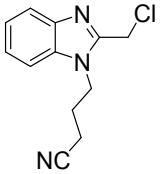


4-(2-(Hydroxymethyl)-1*H*-benzo[d]imidazol-1-yl)butanenitrile (18)

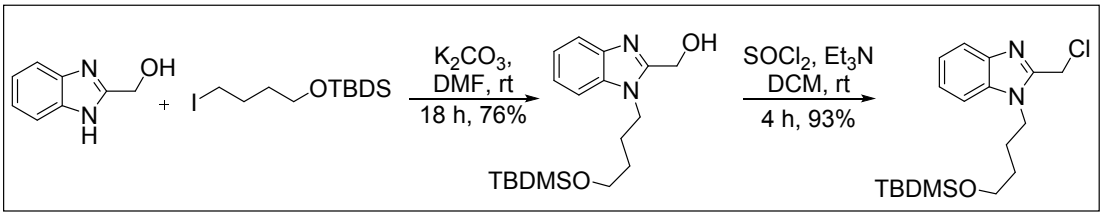


Into a 100 mL three neck RB flask containing Ar(g) was placed DMF (40 mL), followed by (1*H*-benzo[d]imidazol-2-yl)methanol (2.20 g, 14.9 mmol), thus forming a pale orange solution. This was cooled to 0 °C and 60% sodium hydride (0.772 g, 19.3 mmol) was added in one portion. After stirring for 10 min under Ar(g) at 0 °C, 4-bromobutanenitrile (2.64 g, 17.8 mmol) was added in one portion. The reaction was allowed to warm to rt and left to proceed for 18 h. The reaction mixture was diluted with DCM and then poured onto a brine solution. The organic layer was separated and the aqueous phase was extracted three times with DCM. The combined organic fractions were dried over anhydrous sodium sulfate and then concentrated in vacuo. Purification by column chromatography (100% DCM to 3% MeOH/DCM) afforded the desired product as a viscous oil which solidified to a white solid over a period of hours (2.99 g, 13.9 mmol, 94%). **^{1}H NMR (600 MHz, $CDCl_3$) δ** 7.68 – 7.63 (m, 1H), 7.37 – 7.35 (m, 1H), 7.31 – 7.24 (m, 2H), 6.50 (s, 1H), 4.87 (s, 2H), 4.40 (t, *J* = 7.0 Hz, 2H), 2.41 – 2.38 (m, 2H), 2.30 – 2.24 (m, 2H). **^{13}C NMR (151 MHz, $CDCl_3$) δ** 153.6, 141.4, 134.8, 123.5, 122.8, 119.5, 118.7, 109.5, 56.6, 42.3, 25.7, 14.8. **HRMS** calc. for $C_{12}H_{14}N_3O$ $[M+H]^+$, 216.11314 found, 216.11285.

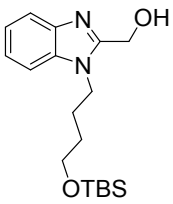
4-(2-(Chloromethyl)-1*H*-benzo[d]imidazol-1-yl)butanenitrile (20)



Into a three neck RB flask containing Ar(g) was placed DCM (10 mL), followed by 4-(2-(hydroxymethyl)-1*H*-benzo[d]imidazol-1-yl)butanenitrile (500 mg, 2.32 mmol), thus forming a clear solution. This was cooled to 0 °C using an ice bath and then $SOCl_2$ (359 mg, 3.02 mmol) was added in one portion against a flow of Ar(g), followed by triethylamine. The ice bath was removed after 5 min and the reaction was left to proceed at rt for 3 h. The reaction mixture was then concentrated in vacuo to afford a white solid. This was then taken up into DCM and washed with a saturated $NaHCO_3$ solution. After separation, the aqueous phase was extracted three times with DCM. The combined organic fractions were concentrated in vacuo. Purification by column chromatography (3% MeOH/DCM) afforded a clear viscous oil, which solidified to a white solid under vacuum for several hours (436 mg, 1.87 mmol, 80%). **^{1}H NMR (400 MHz, $CDCl_3$) δ** 7.83 – 7.72 (m, 1H), 7.46 – 7.25 (m, 3H), 4.86 (s, 2H), 4.43 – 4.35 (m, 2H), 2.47 – 2.43 (m, 2H), 2.34 – 2.25 (m, 2H). **^{13}C NMR (101 MHz, $CDCl_3$) δ** 148.6, 142.2, 135.0, 124.1, 123.0, 120.6, 118.3, 109.5, 42.5, 36.7, 25.3, 14.8. **HRMS** calc. for $C_{12}H_{13}ClN_3$ $[M+H]^+$, 234.07925, found 234.07906.

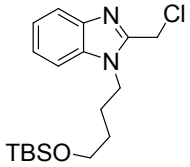


(1-(4-((tert-Butyldimethylsilyl)oxy)butyl)-1*H*-benzo[d]imidazol-2-yl)methanol (19)

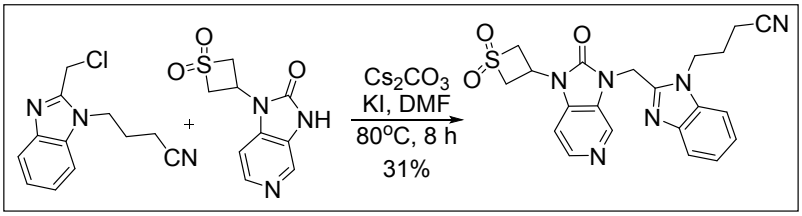


An oven-dried 100 mL three neck RB flask was charged with Ar(g), (1H-benzo[d]imidazol-2-yl)methanol (300 mg, 2.03 mmol), and anhydrous DMF (10 mL) to give a pale orange solution. To this was added potassium carbonate (420 mg, 3.04 mmol) in one portion, followed by *tert*-butyl(4-iodobutoxy)dimethylsilane (0.63 mL, 2.43 mmol). The reaction was left to stir under Ar(g) at rt for 18 h. The reaction was diluted with DCM and washed with brine solution before being extracted with DCM (2x50 mL). The organic extract was dried over sodium sulfate, filtered and concentrated to 0.865 g of lightly yellow oil, which was purified via silica gel flash column chromatography (0-10% MeOH in DCM). The fractions of interest were pooled, and the desired product was isolated as well as unreacted starting material (0.515 g, 76% yield). **^{1}H NMR (500 MHz, $CDCl_3$)** δ 7.65 (d, *J* = 8.0 Hz, 1H), 7.30 (d, *J* = 7.0 Hz, 1H), 7.24-7.19 (m, 2H), 4.86 (s, 2H), 4.25 (t, *J* = 7.5 Hz, 2H), 3.62 (t, *J* = 6.0 Hz, 2H), 1.92 (p, *J* = 7.5 Hz, 2H), 1.57 (p, *J* = 6.0 Hz, 2H), 0.88 (s, 9H), 0.03 (s, 6H) ppm. **^{13}C NMR (125 MHz, $CDCl_3$)** δ 154.0, 141.6, 135.1, 122.9, 122.2, 119.3, 110.0, 62.5, 56.6, 43.9, 30.0, 26.7, 26.0, 18.4, -5.22, -5.25, ppm. **HRMS (ESI+)** m/z: $[M + H]^+$ Calcd for $C_{18}H_{31}N_2O_2Si$ 335.2149; Found 335.2144.

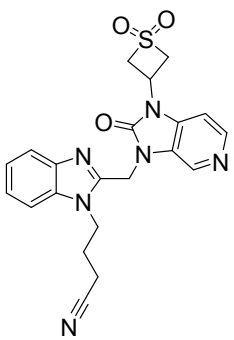
1-(4-((tert-butyldimethylsilyl)oxy)butyl)-2-(chloromethyl)-1*H*-benzo[d]imidazole (21)



An oven-dried three neck 100 mL RB flask was charged with Ar(g), (1-(4-((*tert*-butyldimethylsilyl)oxy)butyl)-1H-benzo[d]imidazol-2-yl)methanol (0.718 g, 2.146 mmol), and benzene (10.7 mL) to give a light green solution. Triethylamine (0.45 mL, 3.22 mmol) was then added, which turned the solution completely colorless, and the solution was stirred briefly before thionyl chloride (0.28 mL, 3.86 mmol) was added dropwise under Ar(g), immediately releasing a gas and turning the solution light red. The reaction was allowed to stir at ambient temperature for 3.5 hours when monitoring by TLC (5% methanol in DCM) showed complete conversion of starting material to the desired less polar spot. The reaction was diluted with DCM before being quenched with saturated sodium bicarbonate solution, and the product was extracted with DCM (2x50 mL). The organic extract was dried over sodium sulfate, filtered and concentrated to give the desired product as a red oil (0.702 g, 93% yield). **^{1}H NMR (600 MHz, $CDCl_3$)** δ 7.78-7.75 (m, 1H), 7.39 (dd, *J* = 6.6, 0.6 Hz, 1H), 7.33-7.27 (m, 2H), 4.86 (s, 2H), 4.30 (t, *J* = 7.8 Hz, 2H), 3.68 (t, *J* = 6.0 Hz, 2H), 2.02-1.97 (m, 2H), 1.65-1.60 (m, 2H), 0.89 (s, 9H), 0.05 (s, 6H) ppm. **^{13}C NMR (150 MHz, $CDCl_3$)** δ 148.8, 135.5, 128.5, 123.7, 122.8, 120.5, 110.2, 62.5, 44.5, 36.9, 30.1, 26.8, 26.1, 18.4, -5.2 ppm. **HRMS (ESI+)** m/z: $[M + H]^+$ Calcd for $C_{18}H_{30}ClN_2OSi$ 353.1810; Found 353.1811.

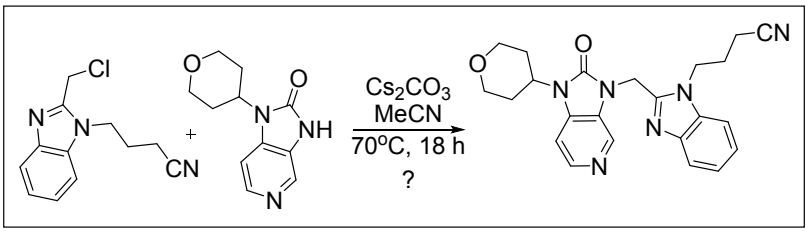


4-(2-((1-(1,1-dioxidothietan-3-yl)-2-oxo-1,2-dihydro-3*H*-imidazo[4,5-c]pyridin-3-yl)methyl)-1*H*-benzo[d]imidazol-1-yl)butanenitrile (1)

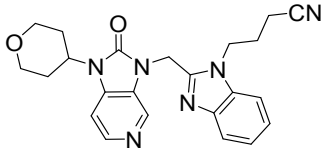


A 50 mL Schlenk tube equipped with a magnetic stirrer bar and cold finger condenser was charged with 1-(1,1-dioxidothietan-3-yl)-1,3-dihydro-2*H*-imidazo[4,5-c]pyridin-2-one (160 mg, 0.669 mmol, 1 equiv.), Cs_2CO_3 (327 mg, 1.00 mmol, 1.5 equiv.), KI (11.0 mg, 0.0670 mmol, 0.1 equiv.), 4-(2-(chloromethyl)-1*H*-benzo[d]imidazol-1-yl)butanenitrile (188 mg, 0.803 mmol, 1.2 equiv.) and DMF (6.7 mL). After heating at

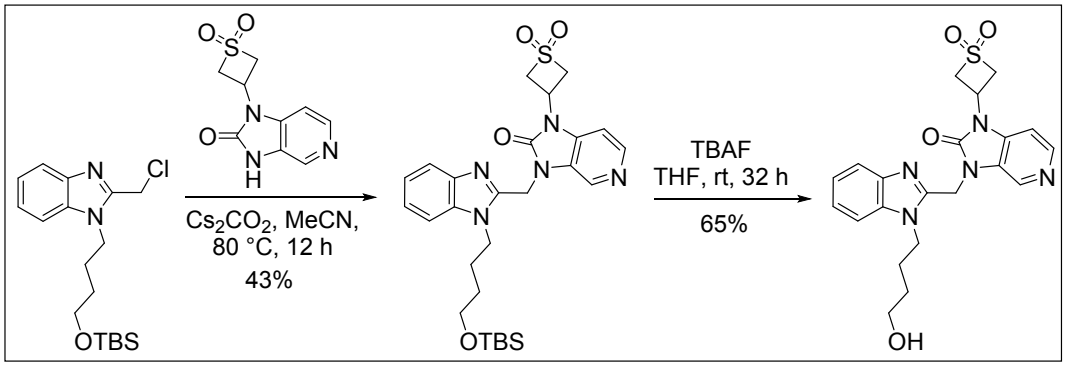
80°C for 8 h, the reaction mixture was quenched by addition of water, extracted with DCM (3x) and dried over Na_2SO_4. The crude product was purified on silica gel column (120 g) using 0 to 10% MeOH in DCM as eluent affording 4-(2-((1-(1,1-dioxidothietan-3-yl)-2-oxo-1,2-dihydro-3*H*-imidazo[4,5-c]pyridin-3-yl)methyl)-1*H*-benzo[d]imidazol-1-yl)butanenitrile (89.0 mg, 31%). The product was recrystallized from EtOAc and MeOH affording slightly brown crystals which were submitted for X-ray crystal structure determination. **^{1}H NMR (400 MHz, $CDCl_3$) δ** 8.87 (d, *J* = 0.8 Hz, 1H), 8.45 (d, *J* = 5.4 Hz, 1H), 7.80 – 7.75 (m, 1H), 7.41 (dd, *J* = 5.4, 0.8 Hz, 1H), 7.39 – 7.28 (m, 3H), 5.50 – 5.37 (m, 1H), 5.40 (s, 2H), 4.93 – 4.83 (m, 2H), 4.69 – 4.58 (m, 2H), 4.49 (dd, *J* = 8.1, 6.9 Hz, 1H), 3.49 (d, *J* = 5.4 Hz, 1.3H, MeOH), 2.45 (t, *J* = 7.0 Hz, 2H), 2.15 (p, *J* = 7.1 Hz, 2H), 0.97 (q, *J* = 5.5 Hz,0.43H, MeOH). **^{13}C NMR (100 MHz, $CDCl_3$) δ** 152.4, 146.9, 144.5, 142.4, 134.9, 132.9, 131.8, 126.3, 124.0, 123.1, 120.6, 118.4, 109.5, 103.7, 68.7, 42.4, 38.7, 33.5, 25.9, 14.7. **HRMS (ESI+)** calcd for $C_{21}H_{21}N_6O_3S$ ($[M+H]^+$): 437.1390. Found: 437.1386, error 0.5 ppm; calcd for $C_{21}H_{20}N_6O_3SNa$ ($[M+Na]^+$): 4591210. Found: 459.1216, error 0.6 pp; **LC-MS (ESI-API, 254 nm)** 50-95 % MeOH in H_2O (0.1% HCO_2H), 6 min, 1.00 mL/min, C18 (Agilent Zorbax XDB-18, 50 mm x 4.6 mm, 3.5 µm), m/z = 437.0 (M + H), 459.0 (M + Na), t = 0.569 min. **Mp** = 132.5-133.5°C.



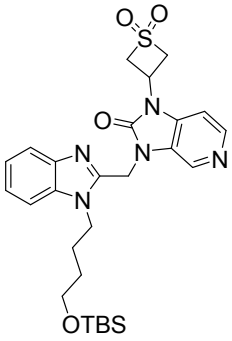
4-(2-((2-oxo-1-(tetrahydro-2H-pyran-4-yl)-1,2-dihydro-3*H*-imidazo[4,5-c]pyridin-3-yl)methyl)-1*H*-benzo[d]imidazol-1-yl)butanenitrile (2)



To a 15 mL tube was added cesium carbonate (0.250 g, 0.766 mmol), 1-(tetrahydro-2H-pyran-4-yl)-1H-imidazo[4,5-c]pyridin-2(3H)-one (0.140 g, 0.639 mmol) and 4-(2-(chloromethyl)-1H-benzo[d]imidazol-1-yl)butanenitrile (0.157 g, 0.670 mmol) dissolved in MeCN (8 mL) then the vessel was sealed with a teflon screw cap and heated in a 70 °C oil bath overnight. The reaction was cooled to rt, filtered over celite with MeOH and concentrated in vacuo. The residue was purified by column (0-10% MeOH in DCM). The fractions were concentrated in vacuo, triturated with $CHCl_3$ then PhMe and dried in vacuo overnight. The solid (168 mg) was recrystallized using MeOH (7 mL) to obtain white needles (88 mg, 0.21 mmol, 33%) co-crystallized with MeOH (X-Ray). **^{1}H NMR (600 MHz, $CDCl_3$) δ** 8.83 (s, 1H), 8.31 (d, J= 5.4 Hz, 1H), 7.79 (dd, J= 7.0, 1.6 Hz, 1H), 7.36 (dd, J= 6.8, 1.4 Hz, 1H), 7.31 (m, 2H), 7.13 (dd, J= 5.4, 0.6 Hz, 1H), 5.39 (s, 2H),4.59 (m, 1H), 4.48 (dd, J= 7.5, 7.6 Hz, 2H), 4.15 (dd, J= 11.5, 4.5 Hz, 2H), 3.56 (dt, J= 12.2, 1.7 Hz, 2H), 3.48 (d, J= 5.2, 2H, MeOH), 2.47 – 2.40 (m, 4H), 2.06 (p, J= 6.9 Hz, 2H), 1.78 (dd, J= 12.4, 2.6 Hz, 2H), 1.08 (q, J= 5.5 Hz, 0.66H, MeOH); **^{13}C NMR (150 MHz, $CDCl_3$) δ** 152.8, 147.6, 143.7, 142.5, 135.1, 134.3, 131.4, 126.4, 124.0, 123.0, 120.7, 118.4, 109.5, 104.5, 67.4, 50.9, 50.8, 42.5, 38.7, 30.1, 26.2, 14.8; **LC/MS** 10-95% MeOH in H_2O over 6 minutes, r_t = 3.523 at 254 nM, MS (+) 417.2, 20-95% MeOH in H_2O over 8 minutes, r_t = 2.459 at 254 nM, MS (+) 417.0. **HRMS** (APCI) *m/z* calc. for $C_{23}H_{25}O_2N_6$ $[M+H]^+$, 417.20335 found, 417.20261.

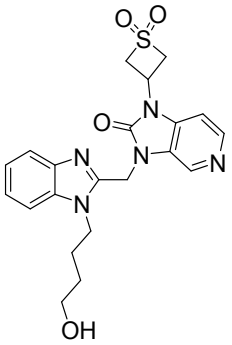


3-((1-(4-((Tert-butyldimethylsilyl)oxy)butyl)-1*H*-benzo[d]imidazol-2-yl)methyl)-1-(1,1-dioxidothietan-3-yl)-1,3-dihydro-2*H*-imidazo[4,5-c]pyridin-2-one (16)



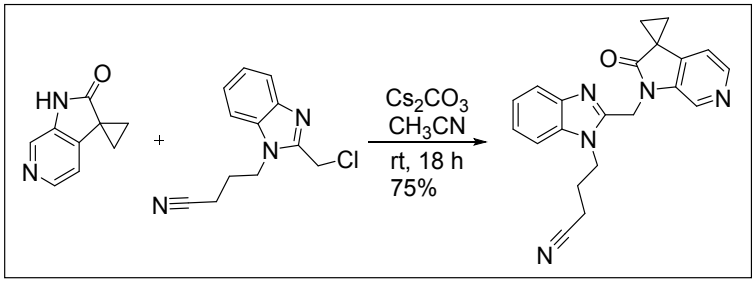
A 100 mL Schlenk tube equipped with a magnetic stirrer bar and cold finger condenser was charged with 1-(1,1-dioxidothietan-3-yl)-1,3-dihydro-2*H*-imidazo[4,5-c]pyridin-2-one (150 mg, 0.627 mmol, 1 equiv.), Cs_2CO_3 (306 mg, 0.940 mmol, 1.5 equiv.) and acetonitrile (8.0 mL) and warmed up to 80°C to dissolve the starting material. The heating was removed and 1-(4-((*tert*-butyldimethylsilyl)oxy)butyl)-2-(chloromethyl)-1H-benzo[d]imidazole (266 mg, 0.752 mmol, 1.2 equiv.) dissolved in 4.5 mL of acetonitrile was added. After heating at 85°C for 12 h, MeOH and DCM was added and the suspension was filtered and concentrated. The crude product was purified on silica gel column (80 g) using 5 to 30% MeOH in DCM affording 3-((1-(4-((*tert*-butyldimethylsilyl)oxy)butyl)-1*H*-benzo[d]imidazol-2-yl)methyl)-1-(1,1-dioxidothietan-3-yl)-1,3-dihydro-2*H*-imidazo[4,5-c]pyridin-2-one (150 mg, 43%) as a beige solid. **^{1}H NMR (400 MHz, $CDCl_3$) δ** 8.81 (d, *J* = 0.8 Hz, 1H), 8.42 (d, *J* = 5.4 Hz, 1H), 7.77 – 7.72 (m, 1H), 7.39 – 7.34 (m, 2H), 7.31 – 7.24 (m, 2H), 5.45 – 5.37 (m, 1H), 5.38 (s, 2H), 4.95 – 4.88 (m, 2H), 4.64 – 4.56 (m, 2H), 4.34 (t, *J* = 7.5 Hz, 2H), 3.61 (t, *J* = 6.0 Hz, 2H), 1.86 – 1.76 (m, 2H), 1.58 – 1.49 (m, 2H), 0.86 (s, 9H), 0.02 (s, 6H). **^{13}C NMR (151 MHz, $CDCl_3$) δ** 152.4, 147.1, 144.4, 142.4, 135.3, 133.0, 131.9, 126.5, 123.6, 122.7, 120.3, 110.2, 103.7, 68.8, 62.4, 44.1, 38.8, 33.5, 29.9, 26.9, 26.0, 18.4, -5.2. **LC-MS (ESI-API, 254 nm)** 75-95 % MeOH in H_2O (0.1% HCO_2H), 3 min, 1.00 mL/min, C18 (Agilent Zorbax XDB-18, 50 mm x 4.6 mm, 3.5 µm), m/z = 556.2 (M + H), 278.6 (M/2 + H), t = 1.523 min, 93 % purity. **HRMS (ESI+)** m/z: $[M + H]^+$ Calcd for $C_{27}H_{38}N_5O_4SSi$ 556.2408; Found 556.2400, error -1.5 ppm. **Mp** = 157-159.5°C.

1-(1,1-Dioxidothietan-3-yl)-3-((1-(4-hydroxybutyl)-1*H*-benzo[d]imidazol-2-yl)methyl)-1,3-dihydro-2*H*-imidazo[4,5-c]pyridin-2-one (3)

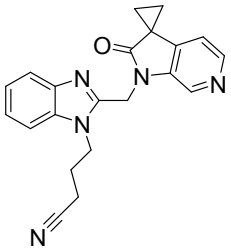


A 20 mL vial equipped with magnetic stir bar was charged with 3-((1-(4-((*tert*-butyldimethylsilyl)oxy)butyl)-1*H*-benzo[d]imidazol-2-yl)methyl)-1-(1,1-dioxidothietan-3-yl)-1,3-dihydro-2*H*-imidazo[4,5-c]pyridin-2-one (110 mg, 0.198 mmol, 1 equiv.) dissolved in THF (2.0 mL). Then 1 M TBAF in THF (0.59 mL, 0.594 mmol, 3 equiv.) was added and the mixture was stirred at rt for 32 h. Then reaction mixture was quenched by addition of water and the product was extracted with chloroform (4x), washed with water and dried over Na_2SO_4. The organics were concentrated and the crude product (105 mg) was recrystallized to afford 1-(1,1-dioxidothietan-3-yl)-3-((1-(4-hydroxybutyl)-1*H*-benzo[d]imidazol-2-yl)methyl)-1,3-dihydro-2*H*-imidazo[4,5-c]pyridin-2-one (57.0 mg, 65%) as light brown crystals. **^{1}H NMR (400 MHz, DMSO-d_6) δ** 8.50 (s, 1H), 8.30 (dd, *J* = 5.4, 0.8 Hz, 1H), 7.58 (d, *J* = 8.6 Hz, 2H), 7.53 (d, *J* =

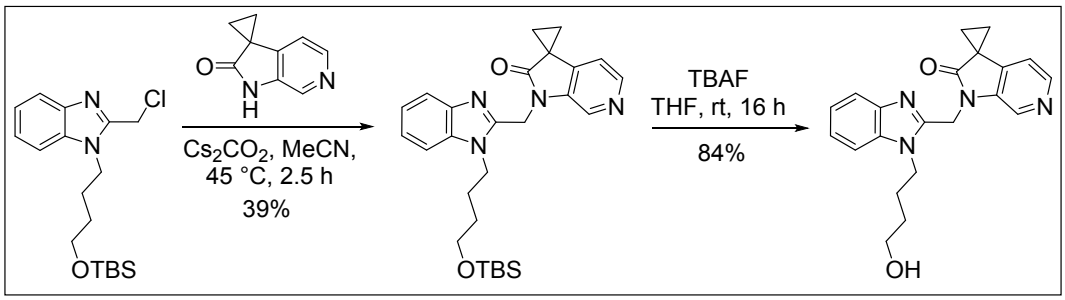
5.5 Hz, 1H), 7.29 – 7.20 (m, 1H), 7.22 – 7.12 (m, 1H), 5.51 – 5.39 (m, 1H), 5.44 (s, 2H), 5.00 (dd, *J* = 14.9, 7.1 Hz, 2H), 4.68 (dd, *J* = 14.3, 10.5 Hz, 2H), 4.48 (t, *J* = 5.2 Hz, 1H), 4.36 (t, *J* = 7.5 Hz, 2H), 3.40 (q, *J* = 6.4 Hz, 1H), 1.70 (p, *J* = 7.7 Hz, 2H), 1.45 (p, *J* = 6.4 Hz, 2H). **^{13}C NMR (100 MHz, DMSO-d_6) δ** 152.01, 148.54, 142.85, 141.86, 135.24, 134.19, 130.07, 126.43, 122.48, 121.71, 119.08, 110.54, 104.39, 68.25, 60.24, 43.23, 37.79, 33.60, 29.55, 26.36. **HRMS (ESI+)** calcd. for $C_{21}H_{24}N_5O_4S$ ([M+H]$^+$): 442.1544. Found: 442.1537, error 0.7 ppm. **LC-MS (ESI-API, 254 nm)** 50-95 % MeOH in H_2O (0.1% HCO_2H), 6 min, 1.00 mL/min, C18 (Agilent Zorbax XDB-18, 50 mm x 4.6 mm, 3.5 µm), m/z = 442.0 (M + H), t = 0.579 min. **Mp** = 211-214°C.



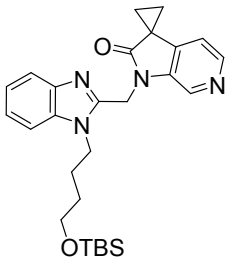
4-(2-((2'-oxospiro[cyclopropane-1,3'-pyrrolo[2,3-c]pyridin]-1'(2'*H*)-yl)methyl)-1*H*-benzo[d]imidazol-1-yl)butanenitrile (4)



To a solution of spiro[cyclopropane-1,3'-pyrrolo[2,3-c]pyridin]-2'(1'H)-one (100 mg, 0.624 mmol) and 4-(2-(chloromethyl)-1H-benzo[d]imidazol-1-yl)butanenitrile (0.146 g, 0.624 mmol) in CH_3CN (4.2 mL) was added Cs_2CO_3 at rt. The reaction was stirred at rt and followed by TLC (18 h to completion). The reaction mixture was filtered and washed with MeOH (~50 mL). The filtrate was concentrated in vacuo. The residue was purified by combiflash (0 to 10% MeOH in DCM) to give the desired compound as a beige solid (0.167 g, 75% yield). **^{1}H NMR (400 MHz, $CDCl_3$) δ** 8.82 (s, 1H), 8.34 (d, *J* = 4.8 Hz, 1H), 7.81 – 7.76 (m, 1H), 7.38 – 7.27 (m, 3H), 6.82 (d, *J* = 5.2 Hz, 1H), 5.33 (s, 2H), 4.41 (t, *J* = 7.6 Hz, 2H), 2.43 (t, *J* = 7.2 Hz, 2H), 2.06 – 1.99 (m, 2H), 1.94 – 1.91 (m, 2H), 1.74 – 1.71 (m, 2H). **^{13}C NMR (100 MHz, $CDCl_3$) δ** 175.6, 147.6, 144.8, 142.3, 139.5, 135.0, 131.2, 123.8, 122.9, 120.4, 118.5, 113.5, 109.5, 42.4, 38.1, 27.2, 6.0, 20.8, 14.7. **HRMS (ESI+)** m/z calculated for $C_{21}H_{20}N_5O$ [M+H$^+$]$^+$: 358.16624, found: 358.16575. **LCMS** (ESI) 10–95% MeOH in H_2O (0.1 % HCO_2H), 10 min, rt = 4.338, *m/z* = 358.2 [M + H]$^+$.



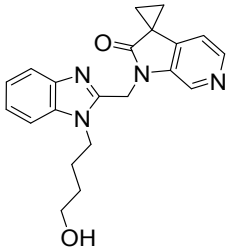
1'-((1-(4-((tert-butyldimethylsilyl)oxy)butyl)-1*H*-benzo[d]imidazol-2-yl)methyl)spiro[cyclopropane-1,3'-pyrrolo[2,3-c]pyridin]-2'(1'*H*)-one (22)



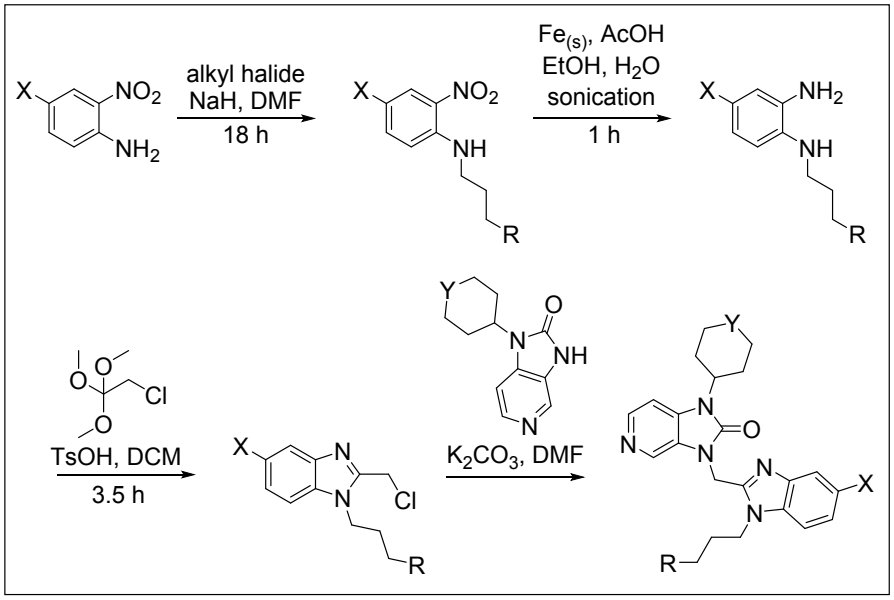
An oven-dried 50 mL RB flask was charged with Ar(g), spiro[cyclopropane-1,3'-pyrrolo[2,3-c]pyridin]-2'(1'H)-one (0.1 g, 0.62 mmol) and anhydrous acetonitrile (2.5 mL) to give a brown suspension; 1-(4-((*tert*-butyldimethylsilyl)oxy)butyl)-2-(chloromethyl)-1H-benzo[d]imidazole (1.4 mL, 0.56 mmol) was then added as a

0.45 M solution in anhydrous acetonitrile, followed by cesium carbonate (0.2 g, 0.61 mmol). The reaction stirred at 45 °C for 2.5 hours when TLC (5% methanol in DCM) showed complete conversion of the starting chloride to a more polar fluorescent spot with some pyrrolidinone remaining. The reaction mixture was filtered and rinsed with MeOH before the filtrate was adsorbed onto 3 g of Celite and purified via silica gel flash column chromatography (0-10% MeOH in DCM) to afford the desired product (0.103 g, 39% yield). **^{1}H NMR (600 MHz, $CDCl_3$)** δ 8.73 (s, 1H), 8.28 (d, *J* = 4.8 Hz, 1H), 7.76 – 7.73 (m, 1H), 7.33 – 7.30 (m, 1H), 7.25 – 7.22 (m, 2H), 6.75 (dd, *J* = 4.8, 0.6 Hz, 1H), 5.31, (s, 2H), 4.26 (t, *J* = 7.8 Hz, 2H), 3.56 (t, *J* = 6.6 Hz), 1.88 (dd, *J* = 7.8, 4.2 Hz, 2H), 1.72 – 1.67 (m, 2H), 1.65 (dd, *J* = 7.8, 4.2 Hz, 2H), 1.54 – 1.49 (m, 2H), 0.84 (s, 9H), – 0.02 (s, 6H) ppm; **^{13}C NMR (150 MHz, $CDCl_3$)** δ 175.4, 147.8, 144.7, 142.5, 139.6, 139.5, 135.5, 131.4, 123.3, 122.4, 120.3, 113.4, 110.1, 62.5, 44.0, 38.3, 29.9, 27.2, 26.7, 26.0, 20.7, 18.4, -5.2 ppm; **HRMS (ESI+)** m/z: $[M + Na]^+$ Calcd for $C_{27}H_{36}N_4O_2SiNa$ 499.2500; Found 499.2497.

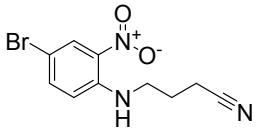
1'-((1-(4-hydroxybutyl)-1*H*-benzo[d]imidazol-2-yl)methyl)spiro[cyclopropane-1,3'-pyrrolo[2,3-c]pyridin]-2'(1'*H*)-one (5)



To a stirring solution of 1'-((1-(4-((*tert*-butyldimethylsilyl)oxy)butyl)-1H-benzo[d]imidazol-2-yl)methyl)spiro[cyclopropane-1,3' -pyrrolo[2,3-c]pyridin]-2'(1'H)-one (0.15 g, 0.315 mmol) in anhydrous THF (1.6 mL) was dropwise added tetra-n-butylammonium fluoride (0.50 mL, 0.50 mmol) as a 1.0 M solution in THF under Ar(g) , and the reaction stirred at ambient temperature overnight. The following morning, the remaining beige solid was dissolved in MeOH, adsorbed onto 1.7 g Celite and purified via silica gel flash column chromatograph (0-20% MeOH in DCM). The relevant fractions were combined and concentrated to 0.1 g of white solid which was subsequently washed with deionized H_2O, collected and dried *in vacuo* to afford the desired final compound (96 mg, 84% yield). **^{1}H NMR (500 MHz, DMSO-d_6)** δ 8.42 (s, 1H), 8.25 (d, *J* = 5.0 Hz, 1H), 7.60 (d, *J* = 8.0 Hz, 1H), 7.56 (d, *J* = 8.0 Hz, 1H), 7.26 – 7.22 (m, 1H), 7.19 – 7.16 (m, 2H), 5.35 (s, 2H), 4.47 (t, *J* = 5.0 Hz, 1H), 4.31 (t, *J* = 8.0 Hz, 2H), 3.39 (dd, *J* = 11.5, 6.0 Hz, 2H) 1.85 (dd, *J* = 8.5, 4.5 Hz, 2H), 1.72 (dd, *J* = 7.5, 3.0 Hz, 2H), 1.68 – 1.62 (m, 2H), 1.48 – 1.42 (m, 2H) ppm; **^{13}C NMR (125 MHz, DMSO-d_6)** δ 174.8, 148.6, 143.7, 141.9, 139.4, 139.1, 135.2, 130.0, 122.4, 121.7, 119.1, 114.5,110.5, 60.3, 43.2, 37.1, 29.5, 26.8, 26.3, 20.0 (x2) ppm. **HRMS (ESI+)** m/z: $[M + Na]^+$ Calcd for $C_{21}H_{21}DN_4O_2Na$ 386.1698; Found 386.1692. **LCMS** (ESI) 25–95% MeOH in H_2O (0.1 % HCO_2H), 6 min, rt = 2.412, *m/z* = 363.2 $[M + H]^+$.

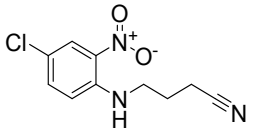


4-(4-Bromo-2-nitro-anilino)butanenitrile (26)



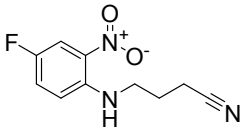
In a three-necked 100 mL round-bottomed flask, sodium hydride (60% dispersion in mineral oil, 277 mg, 6.91 mmol, 1.5 eq) was added to a solution of 4-bromo-2-nitroaniline (1.0 g, 4.6 mmol, 1.0 eq) in DMF (20 mL) at 0°C. After approximately 30 minutes at 0°C, the appropriate 4-bromobutyronitrile (1.02 g, 6.91 mmol, 1.5 eq) was added to the reaction mixture dropwise and the reaction was allowed to warm to room temperature overnight. For the workup, the reaction was quenched with a solution of saturated ammonium chloride and then extracted three times with EtOAc. The organic phases were subsequently combined, dried over $MgSO_4$ and concentrated in vacuo. The resulting crude material was then purified by column chromatography to yield the product (399 mg, 1.40 mmol, 31%) as an orange solid. **^{1}H NMR (500 MHz, CDCl3) δ** 8.33 (d, *J* = 2.4 Hz, 1H), 8.01 (s, 1H), 7.54 (dd, *J* = 9.1, 2.3 Hz, 1H), 6.79 (d, *J* = 9.1 Hz, 1H), 3.54 – 3.47 (m, 2H), 2.53 (t, *J* = 6.9 Hz, 2H), 2.13 – 2.04 (m, 2H). **^{13}C NMR (151 MHz, $CDCl_3$) δ** 144.0, 139.3, 132.9, 129.3, 118.6, 115.3, 107.4, 41.6, 24.9, 15.1. **HRMS** calc. for $C_{10}H_{11}BrN_3O_2$ $[M+H]^+$, 284.00292 found, 284.00322.

4-(4-Chloro-2-nitro-anilino)butanenitrile (27)



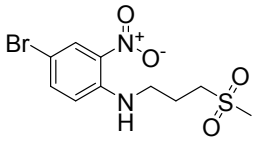
In a three-necked 100 mL round-bottomed flask, sodium hydride (60% dispersion in mineral oil, 209 mg, 5.22 mmol, 1.5 eq) was added to a solution of 4-chloro-2-nitroaniline (600 mg, 3.48 mmol, 1.0 eq) in DMF (20 mL) at 0°C. After approximately 30 minutes at 0°C, the appropriate 4-bromobutyronitrile (772 mg, 5.22 mmol, 1.5 eq) was added to the reaction mixture dropwise and the reaction was allowed to warm to room temperature overnight. For the workup, the reaction was quenched with a solution of saturated ammonium chloride and then extracted three times with EtOAc. The organic phases were subsequently combined, dried over $MgSO_4$ and concentrated in vacuo. The resulting crude material was then purified by column chromatography to yield the product (218 mg, 0.910 mmol, 26%) as an orange solid. **^{1}H NMR (500 MHz, $CDCl_3$) δ** 8.16 (d, *J* = 2.5 Hz, 1H), 8.00 (s, 1H), 7.43 – 7.40 (m, 1H), 6.84 (d, *J* = 9.2 Hz, 1H), 3.53 – 3.47 (m, 2H), 2.53 (t, *J* = 7.0 Hz, 2H), 2.08 (p, *J* = 6.9 Hz, 2H). **^{13}C NMR (151 MHz, $CDCl_3$) δ** 143.6, 136.7, 132.2, 126.3, 121.0, 118.7, 115.0, 41.5, 24.9, 15.1. **HRMS** (APCI) *m/z* calc. for $C_{10}H_{11}O_2N_3{}^{35}Cl$ $[M+H]^+$, 240.05343 found, 240.05321.

4-(4-Fluoro-2-nitro-anilino)butanenitrile (28)



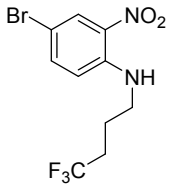
In a three-necked 100 mL round-bottomed flask, sodium hydride (60% dispersion in mineral oil, 384 mg, 9.61 mmol, 1.5 eq) was added to a solution of 4-fluoro-2-nitroaniline (1.0 g, 6.4mmol, 1.0 eq) in DMF (20 mL) at 0°C. After approximately 30 minutes at 0°C, the appropriate 4-bromobutyronitrile (0.96 mL, 9.61 mmol, 1.5 eq) was added to the reaction mixture dropwise and the reaction was allowed to warm to room temperature overnight. For the workup, the reaction was quenched with a solution of saturated ammonium chloride and then extracted three times with EtOAc. The organic phases were subsequently combined, dried over $MgSO_4$ and concentrated in vacuo. The resulting crude material was then purified by column chromatography to yield the product (210 mg, 0.94 mmol, 15%) as an orange solid. **^{1}H NMR (600 MHz, $CDCl_3$) δ** 7.92 – 7.87 (m, 2H), 7.31 – 7.27 (m, 1H), 6.86 (dd, J = 9.4, 4.5 Hz, 1H), 3.53 – 3.49 (m, 2H), 2.53 (t, J = 7.0 Hz, 2H), 2.09 (p, J = 6.9 Hz, 2H). **^{13}C NMR (151 MHz, $CDCl_3$) δ** 152.8 (d, J_{CF} = 239.2 Hz), 142.1, 131.3 (d, J_{CF} = 8.3 Hz), 125.3 (d, J_{CF} = 23.7 Hz), 118.8, 114.9 (d, J_{CF} = 7.2 Hz), 112.5 (d, J_{CF} = 26.2 Hz), 41.6, 24.9, 15.0. **HRMS** (APCI) *m/z* calc. for $C_{10}H_{11}O_2N_3F$ $[M+H]^+$, 224.08298 found, 224.08275.

4-Bromo-N-(3-methylsulfonylpropyl)-2-nitroaniline (29)



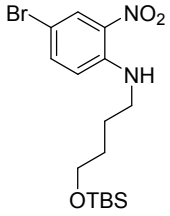
In a three-necked 100 mL round-bottomed flask, sodium hydride (60% dispersion in mineral oil, 111 mg, 2.77 mmol, 1.5 eq) was added to a solution of 4-bromo-2-nitroaniline (400 mg, 1.84 mmol ,1.0 eq) in DMF (10 mL) at 0°C. After approximately 30 minutes at 0°C, 1-bromo-3-methylsulfonylpropane (556 mg, 2.77 mmol) was added to the reaction mixture dropwise and the reaction was allowed to warm to room temperature overnight. For the workup, the reaction was quenched with a solution of saturated ammonium chloride and then extracted three times with EtOAc. The organic phases were subsequently combined, dried over $MgSO_4$ and concentrated in vacuo. The resulting crude material was then purified by column chromatography to yield the product (316 mg, 0.937 mmol, 51%) as an orange solid. **^{1}H NMR (500 MHz, $CDCl_3$) δ** 8.31 (d, *J* = 2.4 Hz, 1H), 8.07 (s, 1H), 7.53 (dd, *J* = 9.2, 2.2 Hz, 1H), 6.81 (d, *J* = 9.1 Hz, 1H), 3.59 – 3.53 (m, 2H), 3.15 (t, *J* = 7.3 Hz, 2H), 2.97 (s, 3H), 2.31 – 2.23 (m, 2H). **^{13}C NMR (151 MHz, $CDCl_3$) δ** 144.0, 139.4, 132.8, 129.3, 115.4, 107.3, 51.8, 41.4, 21.9. **HRMS** (APCI) *m/z* calc. for $C_{10}H_{14}O_4N_2{}^{79}Br^{32}S$ $[M+H]^+$, 336.98522 found, 336.98471.

4-Bromo-2-nitro-*N*-(4,4,4-trifluorobutyl)aniline (30)



Into a 2 neck round bottom flask was placed the 4-bromo-2-nitroaniline (2.00 g, 9.22 mmol) followed by DMF (50 ml), thus forming a bright orange solution. This was cooled to 0 °C using an ice bath and then sodium hydride (553 mg, 13.8 mmol) was added in portions against a flow of argon. The reaction mixture turned dark purple upon the addition of sodium hydride. The reaction was left to proceed under argon for 30 min at 0 °C. After this time the 1,1,1-trifluoro-4-iodo-butane (3.29 g, 13.8 mmol, 1.83 ml) was added in one portion and the reaction was left for 18 h, over which time it warmed to room temperature. After this time, the reaction mixture was diluted with ethyl acetate (200 ml) and saturated sodium bicarbonate solution (150 ml). After thoroughly mixing the phases the organic phase was separated and the aqueous phase was extracted twice more with EtOAc (2 x 100 ml). The combined organic fractions were washed with brine, and then dried over anhydrous magnesium sulfate. After concentration in vacuo, the crude material was purified by column chromatography (EtOAc/Hex) to afford the title compound as an orange oil, which became a waxy solid after 24 h (2.71 g, 8.29 mmol, 90%). **^{1}H NMR (600 MHz, $CDCl_3$) δ** 8.32 (d, *J* = 2.4 Hz, 1H), 8.02 (s, 1H), 7.52 (dd, *J* = 9.2, 2.4 Hz, 1H), 6.75 (d, *J* = 9.1 Hz, 1H), 3.41 (q, *J* = 6.7 Hz, 2H), 2.50 – 2.13 (m, 2H), 2.00 (p, *J* = 7.3 Hz, 2H). **^{13}C NMR (151 MHz, $CDCl_3$) δ** 144.0, 139.1, 132.5, 129.1, 126.8 (q, J_{C-F} = 276.4 Hz) 115.2, 106.9, 41.8, 31.3 (q, J_{C-F} = 29.4 Hz), 21.7. **^{19}F NMR (376 MHz, $CDCl_3$) δ** -66.00 (t, *J* = 10.7 Hz). **HRMS** calc. for $C_{10}H_{11}{}^{79}BrF_3N_2O_2$ $[M+H]^+$, 326.99505 found, 326.99481

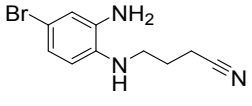
4-Bromo-*N*-(4-((*tert*-butyldimethylsilyl)oxy)butyl)-2-nitroaniline (31)



Into a 2 neck round bottom flask was placed the 4-bromo-2-nitroaniline (3.00 g, 13.8 mmol) followed by DMF (50 ml), thus forming a bright orange solution. This was cooled to 0 °C using an ice bath and then sodium hydride (0.829 g, 20.7 mmol) was added in one portion against a flow of argon. The reaction mixture turned dark purple upon the addition of sodium hydride. The reaction was left to proceed under argon for 30 min at 0 °C. After this time the *tert*-butyl(4-iodobutoxy)dimethylsilane (6.52 g, 20.7 mmol, 5.37 ml) was added in one portion and the reaction was left for 18 h, over which time it warmed to room temperature. After this time, the reaction mixture was diluted with ethyl acetate (150 ml) and saturated sodium bicarbonate solution (150 ml). After thoroughly mixing the phases the organic phase was separated and the aqueous phase was extracted twice more with EtOAc (2 x 100 ml). The combined organic fractions were washed with brine, and then dried over anhydrous magnesium sulfate. After concentration

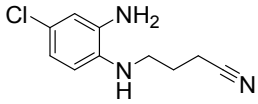
in vacuo, the crude material was purified by column chromatography (EtOAc/Hex) to afford the title compound as an orange oil (4.86 g, 12.1 mmol, 87%). **^{1}H NMR (600 MHz, $CDCl_3$) δ** 8.28 (d, *J* = 2.4 Hz, 1H), 8.07 – 7.99 (m, 1H), 7.47 – 7.44 (m, 1H), 6.76 (d, *J* = 9.2 Hz, 1H), 3.67 (t, *J* = 6.1 Hz, 2H), 3.32 (td, *J* = 7.1, 5.2 Hz, 2H), 1.83 – 1.75 (m, 2H), 1.68 – 1.61 (m, 2H), 0.89 (s, 9H), 0.05 (s, 6H). **^{13}C NMR (151 MHz, $CDCl_3$) δ** 144.4, 138.8, 132.0, 128.8, 115.5, 106.1, 62.4, 43.0, 30.0, 25.9, 25.5, 18.2, -5.4. **HRMS** calc. for $C_{16}H_{28}{}^{79}BrN_2O_3Si$ $[M+H]^+$, 403.10471 found, 403.10452.

4-(2-Amino-4-bromo-anilino)butanenitrile (32)



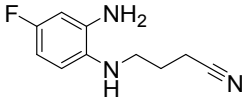
Iron powder (392 mg, 7.02 mmol, 5 eq) was suspended in a solution of 4-(4-bromo-2-nitro-anilino)butanenitrile (399 mg, 1.40 mmol, 1 eq) in ethanol (3 mL), acetic acid (3 mL) and water (1.5 mL) in a single-necked 25 mL round bottomed flask. The flask was subsequently placed in a sonicator and irradiated at room temperature for 1 hour. Once TLC confirmed that all starting material had been consumed the iron powder was filtered off over celite and the resulting filtrate was basified with a 1M solution of NaOH. The crude product was then extracted from the basic solution into DCM three times. The organic phases were combined, dried over $MgSO_4$ and concentrated in vacuo. The resulting crude material was then purified by column chromatography to yield the product (240 mg, 0.944 mmol, 67%) as a pale orange solid. **^{1}H NMR (500 MHz, $CDCl_3$) δ** 6.90 (dd, *J* = 8.3, 2.2 Hz, 1H), 6.84 (d, *J* = 2.2 Hz, 1H), 6.50 (d, *J* = 8.4 Hz, 1H), 3.39 (s, 3H), 3.26 (t, *J* = 6.6 Hz, 2H), 2.49 (t, *J* = 7.0 Hz, 2H), 2.03 – 1.95 (m, 2H). **^{13}C NMR (126 MHz, $CDCl_3$) δ** 136.4, 136.0, 123.1, 119.7, 119.2, 113.3, 111.4, 42.8, 25.2, 15.2. **HRMS** calc. for $C_{10}H_{13}BrN_3$ $[M+H]^+$, 254.02874 found, 254.02899.

4-(2-Amino-4-chloro-anilino)butanenitrile (33)



Iron powder (303 mg, 5.42 mmol, 5 eq) was suspended in a solution of 4-(4-chloro-2-nitro-anilino)butanenitrile (260 mg, 1.09 mmol, 1 eq) in ethanol (3 mL), acetic acid (3 mL) and water (1.5 mL) in a single-necked 25 mL round bottomed flask. The flask was subsequently placed in a sonicator and irradiated at room temperature for 1 hour. Once TLC confirmed that all starting material had been consumed the iron powder was filtered off over celite and the resulting filtrate was basified with a 1M solution of NaOH. The crude product was then extracted from the basic solution into DCM three times. The organic phases were combined, dried over $MgSO_4$ and concentrated in vacuo. The resulting crude material was then employed for the subsequent step without further purification. **^{1}H NMR (400 MHz, $CDCl_3$) δ** 6.76 (dd, *J* = 8.3, 2.3 Hz, 1H), 6.70 (d, *J* = 2.3 Hz, 1H), 6.54 (d, *J* = 8.4 Hz, 1H), 3.40 (s, 3H), 3.25 (t, *J* = 6.6 Hz, 2H), 2.49 (t, *J* = 7.0 Hz, 2H), 1.98 (t, *J* = 6.8 Hz, 2H). **HRMS** (APCI) *m/z* calc. for $C_{10}H_{13}N_3{}^{35}Cl$ $[M+H]^+$, 210.07925 found, 210.07923.

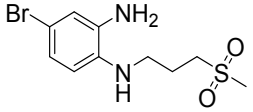
4-(2-Amino-4-fluoro-anilino)butanenitrile (34)



Iron powder (213 mg, 3.81 mmol, 5 eq) was suspended in a solution of 4-(4-fluoro-2-nitro-anilino)butanenitrile (170 mg, 0.762 mmol, 1 eq) in ethanol (2 mL), acetic acid (2 mL) and water (1 mL) in a single-necked 25 mL round bottomed flask. The flask was subsequently placed in a sonicator and irradiated at room temperature for 1 hour. Once TLC confirmed that all starting material had been consumed the iron powder was filtered off over celite and the resulting filtrate was basified with a 1M solution of NaOH. The crude product was then extracted from the basic solution into DCM three times. The organic phases were combined, dried over $MgSO_4$ and concentrated in vacuo. The resulting crude material was then purified by column chromatography to yield the product (70 mg, 0.36 mmol, 48%) as a pale orange solid. **^{1}H NMR (500 MHz, $CDCl_3$) δ** 6.59 – 6.55 (m, 1H), 6.49 – 6.43 (m, 2H), 3.33 (s, 3H), 3.21 (t, *J* = 6.5 Hz, 2H), 2.49 (t, *J* = 7.0 Hz, 2H), 1.97 (p, *J* = 6.8 Hz, 2H).

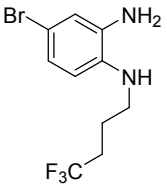
^{13}C NMR (126 MHz, $CDCl_3$) δ 157.6 (d, J_{CF} = 236.5 Hz), 137.6 (d, J_{CF} = 10.3 Hz), 132.1 (d, J_{CF} = 2.0 Hz), 119.8, 113.9 (d, J_{CF} = 9.6 Hz), 105.3 (d, J_{CF} = 21.7 Hz), 103.3 (d, J_{CF} = 25.7 Hz), 43.5, 25.3, 15.1. **HRMS** (APCI) *m/z* calc. for $C_{10}H_{13}N_3F$ $[M+H]^+$, 194.1088 found, 194.10861.

4-bromo-*N*^{1}-(3-methylsulfonylpropyl)benzene-1,2-diamine (35)



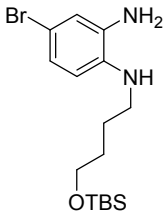
Iron powder (207 mg, 3.71 mmol, 5 eq) was suspended in a solution of 4-bromo-N-(3-methylsulfonylpropyl)-2-nitroaniline (250 mg, 0.741 mmol, 1 eq) in ethanol (2.0 mL), acetic acid (2.0 mL) and water (1.0 mL) in a single-necked 25 mL round bottomed flask. The flask was subsequently placed in a sonicator and irradiated at room temperature for 1 hour. Once TLC confirmed that all starting material had been consumed the iron powder was filtered off over celite and the resulting filtrate was basified with a 1M solution of NaOH. The crude product was then extracted from the basic solution into DCM three times. The organic phases were combined, dried over $MgSO_4$ and concentrated in vacuo. This resulted in the product (205 mg, 0.667 mmol) as a tan solid. No further purification was carried out. **^{1}H NMR (500 MHz, CDCl3) δ** 6.89 (dd, *J* = 8.3, 1.8 Hz, 1H), 6.84 (d, *J* = 1.7 Hz, 1H), 6.48 (d, *J* = 8.4 Hz, 1H), 3.45 (s, 2H), 3.27 (t, *J* = 6.5 Hz, 2H), 3.18 (t, *J* = 7.4 Hz, 2H), 2.93 (s, 3H), 2.24 – 2.16 (m, 2H). **^{13}C NMR (126 MHz, $CDCl_3$) δ** 136.28, 135.95, 123.06, 119.16, 113.23, 111.26, 52.63, 42.57, 41.03, 22.26. **HRMS** (APCI) *m/z* calc. for $C_{10}H_{16}O_2N_2{}^{79}Br^{32}S$ $[M+H]^+$, 307.01104 found, 307.01079.

4-Bromo-*N*^{1}-(4,4,4-trifluorobutyl)benzene-1,2-diamine (36)



Into a one neck 250 ml round bottom flask was placed the 4-bromo-2-nitro-*N*-(4,4,4-trifluorobutyl)aniline (2.65 g, 8.09 mmol) followed by the ethanol (40 ml), acetic acid (40 ml) and water (20 ml), thus forming a light brown solution. To this was added the Iron (3.16 g, 56.6 mmol) and the reaction was carried out in a sonicator for 3 h. After this time, almost complete consumption of the starting material was observed by TLC analysis. The reaction mixture was then filtered through celite and diluted with Ethyl acetate (200 ml) and 1 N NaOH solution (200 ml). After thoroughly mixing the phases the organic phase was separated and the aqueous phase was further extracted with ethyl acetate (2 x 200 ml). The combined organic fractions were dried over anhydrous magnesium sulfate and then after concentration in vacuo, the crude material was purified by column chromatography to afford the title compound as a dark oil (1.99 g, 6.70 mmol, 83%). **^{1}H NMR (500 MHz, $CDCl_3$) δ** 6.90 (dd, *J* = 8.4, 2.3 Hz, 1H), 6.83 (d, *J* = 2.2 Hz, 1H), 6.48 (d, *J* = 8.4 Hz, 1H), 3.38 – 3.28 (m, 3H), 3.15 (t, *J* = 7.0 Hz, 2H), 2.31 – 2.11 (m, 2H), 1.97 – 1.82 (m, 2H). **^{13}C NMR (151 MHz, $CDCl_3$) δ** 136.2, 135.9, 127.0 (q, J_{C-F} = 276.3 Hz), 123.0, 118.9, 113.0, 110.8, 42.9, 31.4 (q, J_{C-F} = 29.0 Hz), 22.0 (q, J_{C-F} = 2.9 Hz). **^{19}F NMR (376 MHz, $CDCl_3$) δ** -66.14 (t, *J* = 10.9 Hz). **HRMS** calc. for $C_{10}H_{13}N_2{}^{79}BrF_3$ $[M+H]^+$, 297.0209 found, 297.0204

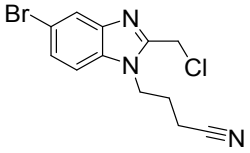
4-Bromo-*N*^{1}-(4-((*tert*-butyldimethylsilyl)oxy)butyl)benzene-1,2-diamine (37)



Into a 2 neck round bottom flask containing argon was placed 4-bromo-*N*-[4-[*tert*-butyl(dimethyl)silyl]oxybutyl]-2-nitro-aniline (4.91 g, 12.2 mmol) followed by ethyl acetate (150 mL) thus forming an orange solution. To this solution was added 10% platinum on carbon (2.37 g, 1.22 mmol) in one portion and a balloon filled with hydrogen gas was fitted to the flask. After purging the flask with hydrogen the reaction was left to stir for 18 h, after which time analysis by TLC indicated that all of the starting material had been consumed and a new product had formed. The reaction mixture was then filtered through celite, concentrated in vacuo and then purified by column chromatography (EtOAc/Hex) to afford 4-bromo-*N*^{1}-(4-

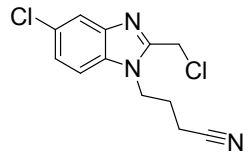
((*tert*-butyldimethylsilyl)oxy)butyl)benzene-1,2-diamine (3.18 g, 8.51 mmol, 70 % yield) as a purple oil which solidified into a waxy solid after 24 h. **^{1}H NMR (600 MHz, $CDCl_3$) δ** 6.90 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.81 (d, *J* = 2.2 Hz, 1H), 6.49 (d, *J* = 8.4 Hz, 1H), 3.68 (t, *J* = 6.1 Hz, 2H), 3.43 – 3.22 (m, 3H), 3.08 (t, *J* = 7.0 Hz, 2H), 1.75 – 1.69 (m, 2H), 1.68 – 1.62 (m, 2H), 0.92 (s, 9H), 0.08 (s, 6H). **^{13}C NMR (151 MHz, $CDCl_3$) δ** 136.9, 135.6, 122.9, 118.7, 112.7, 110.0, 62.8, 44.0, 30.4, 25.9, -5.3. **HRMS** calc. for $C_{16}H_{30}{}^{79}BrN_2OSi$ $[M+H]^+$, 373.13053, found 373.13018.

4-[5-Bromo-2-(chloromethyl)benzimidazole-1-yl]butanenitrile (38)



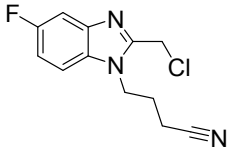
A solution of 4-(2-amino-4-bromo-anilino)butanenitrile (200 mg, 0.787 mmol, 1.0 eq), 2-chloro-1,1,1-trimethoxy-ethane (0.32 mL, 2.36 mmol, 3.0 eq) and *p*-toluenesulfonyl chloride monohydrate (15 mg, 0.079 mmol, 0.1 eq) in DCM (4 mL) was stirred vigorously at room temperature, under an atmosphere of argon for approximately 4 hours, until TLC confirmed full consumption of the starting material. The reaction was then concentrated *in vacuo* and the resulting crude product was dry-loaded onto silica gel. Purification was undertaken with column chromatography to yield the product (215 mg, 0.688 mmol, 87%) as a pale-yellow solid. **^{1}H NMR (400 MHz, $CDCl_3$) δ** 7.89 (d, *J* = 1.7 Hz, 1H), 7.44 (dd, *J* = 8.6, 1.8 Hz, 1H), 7.30 – 7.23 (m, 1H), 4.83 (s, 2H), 4.38 (t, *J* = 7.4 Hz, 2H), 2.45 (t, *J* = 6.9 Hz, 2H), 2.31 – 2.23 (m, 2H). **^{13}C NMR (101 MHz, $CDCl_3$) δ** 149.8, 143.6, 134.2, 127.4, 123.6, 118.4, 116.2, 110.9, 42.8, 36.6, 25.5, 15.0. **HRMS** calc. for $C_{12}H_{12}BrClN_3$ $[M+H]^+$, 311.98976 found, 311.99105.

4-[5-Chloro-2-(chloromethyl)benzimidazole-1-yl]butanenitrile (39)



A solution of 4-(2-amino-4-chloro-anilino)butanenitrile (150 mg, 0.715 mmol, 1.0 eq), 2-chloro-1,1,1-trimethoxy-ethane (0.29 mL, 2.15 mmol, 3.0 eq) and *p*-toluenesulfonyl chloride monohydrate (14 mg, 0.072 mmol, 0.1 eq) in DCM (4 mL) was stirred vigorously at room temperature, under an atmosphere of argon for approximately 4 hours, until TLC confirmed full consumption of the starting material. The reaction was then concentrated *in vacuo* and the resulting crude product was dry-loaded onto silica gel. Purification was undertaken with column chromatography to yield the product (150 mg, 0.559 mmol, 78%) as a pale-yellow solid. **^{1}H NMR (500 MHz, $CDCl_3$) δ** 7.74 – 7.65 (m, 1H), 7.29 – 7.27 (m, 2H), 4.81 (s, 2H), 4.37 – 4.32 (m, 2H), 2.43 (t, J = 6.9 Hz, 2H), 2.29 – 2.21 (m, 2H). **^{13}C NMR (126 MHz, $CDCl_3$) δ** 150.0, 143.1, 133.8, 128.7, 124.6, 120.3, 118.4, 110.4, 42.8, 36.5, 25.4, 14.9. **HRMS** (APCI) *m/z* calc. for $C_{12}H_{12}N_3{}^{35}Cl_2$ $[M+H]^+$, 268.04028 found, 268.04027.

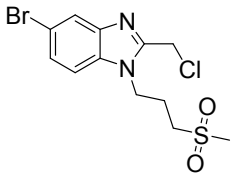
4-[5-Fluoro-2-(chloromethyl)benzimidazole-1-yl]butanenitrile (40)



A solution of 4-(2-amino-4-fluoro-anilino)butanenitrile (240 mg, 1.24 mmol, 1.0 eq), 2-chloro-1,1,1-trimethoxy-ethane (0.50 mL, 3.7 mmol, 3.0 eq) and *p*-toluenesulfonyl chloride monohydrate (24 mg, 0.12 mmol, 0.1 eq) in DCM (6 mL) was stirred vigorously at room temperature, under an atmosphere of argon for approximately 4 hours, until TLC confirmed full consumption of the starting material. The reaction was then concentrated *in vacuo* and the resulting crude product was dry-loaded onto silica gel. Purification was undertaken with column chromatography to yield the product (80 mg, 0.32 mmol, 26%) as a pale-yellow solid. **^{1}H NMR (500 MHz, $CDCl_3$) δ** 7.44 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.34 (dd, *J* = 8.9, 4.4 Hz, 1H), 7.12 (td, *J* = 9.0, 2.4 Hz, 1H), 4.85 (s, 2H), 4.40 (t, *J* = 7.4 Hz, 2H), 2.47 (t, *J* = 6.8 Hz, 2H), 2.35 – 2.25 (m, 2H). **^{13}C NMR (151 MHz, $CDCl_3$) δ** 159.9 (d, J_{CF} = 239.4 Hz), 150.2, 142.7 (d, J_{CF} = 12.6 Hz), 131.8, 118.4, 112.9 (d, J_{CF} = 26.5

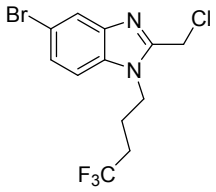
Hz), 110.1 (d, J_{CF} = 10.0 Hz), 106.5 (d, J_{CF} = 24.1 Hz), 42.9, 36.6, 25.6, 15.0. **HRMS** (APCI) *m/z* calc. for $C_{12}H_{12}N_3{}^{35}ClF$ $[M+H]^+$, 252.06983 found, 252.06965.

5-Bromo-2-(chloromethyl)-1-(3-methylsulfonylpropyl)benzimidazole (41)



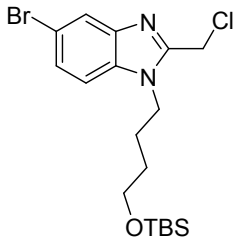
A solution of 4-bromo-*N*[1]-(3-methylsulfonylpropyl)benzene-1,2-diamine (150 mg, 0.488 mmol, 1.0 eq), 2-chloro-1,1,1-trimethoxy-ethane (0.20 mL, 1.47 mmol, 3.0 eq) and *p*-toluenesulfonyl chloride monohydrate (9.0 mg, 0.049 mmol, 0.1 eq) in DCM (3 mL) was stirred vigorously at room temperature, under an atmosphere of argon for approximately 4 hours, until TLC confirmed full consumption of the starting material. The reaction was then concentrated *in vacuo* and the resulting crude product was dry-loaded onto silica gel. Purification was undertaken with column chromatography to yield the product (138 mg, 0.377 mmol, 77%). **^{1}H NMR (500 MHz, $CDCl_3$) δ** 7.90 (d, *J* = 1.6 Hz, 1H), 7.44 (dd, *J* = 8.6, 1.8 Hz, 1H), 7.32 (d, *J* = 8.6 Hz, 1H), 4.85 (s, 2H), 4.53 – 4.40 (m, 2H), 3.09 (t, *J* = 6.9 Hz, 2H), 2.96 (s, 3H), 2.55 – 2.35 (m, 2H). **^{13}C NMR (126 MHz, $CDCl_3$) δ** 149.9, 143.7, 134.2, 127.3, 123.6, 116.2, 111.2, 51.2, 42.8, 41.4, 36.6, 22.5. **HRMS** (APCI) *m/z* calc. for $C_{12}H_{15}O_2N_2{}^{79}Br{}^{35}Cl{}^{32}S$ $[M+H]^+$, 364.97207 found, 363.97192.

5-bromo-2-(chloromethyl)-1-(4,4,4-trifluorobutyl)-1*H*-benzo[*d*]imidazole (42)



Into a 2-neck round bottom flask was added 4-bromo-*N*[1]-(4,4,4-trifluorobutyl)benzene-1,2-diamine (2.00 g, 6.73 mmol) followed by DCM , thus forming a grey solution. To this, against a flow of argon was added the 2-chloro-1,1,1-trimethoxyethane (3.12 g, 20.2 mmol, 2.72 ml) followed by the toluene sulfonic acid monohydrate (0.12 g, 0.67 mmol). The reaction was left to proceed under argon for 18 h after which time analysis by TLC confirmed that all of the starting material had reacted and that a new product had formed at slightly lower Rf. The reaction mixture was diluted with ethyl acetate (200 ml) and saturated sodium bicarbonate solution (200 ml). After thoroughly mixing the phases, the organic phase was separated and the aqueous phase was extracted twice more with ethyl acetate (2 x 100 ml). The combined organic fractions were dried over anhydrous magnesium sulfate after concentration in vacuo. The crude material was purified by column chromatography to afford the title compound as a white solid (1.94 g, 5.46 mmol, 81%). **^{1}H NMR (600 MHz, $CDCl_3$) δ** 7.91 (s, 1H), 7.45 (d, *J* = 8.6 Hz, 1H), 7.22 (d, *J* = 8.6 Hz, 1H), 4.82 (s, 2H), 4.31 (t, *J* = 7.3 Hz, 2H), 2.31 – 2.11 (m, 4H). **^{13}C NMR (151 MHz, $CDCl_3$) δ** 149.6, 143.6, 134.1, 127.1, 126.5 (q, J_{C-F} = 276.5 Hz), 123.5, 116.0, 110.8, 43.2, 36.5, 31.2 (q, J_{C-F} = 29.7 Hz), 22.5. **^{19}F NMR (376 MHz, $CDCl_3$) δ** -65.79 (t, *J* = 10.1 Hz). **HRMS** calc. for $C_{12}H_{12}{}^{79}BrClF_3N_2$ $[M+H]^+$, 354.9891 found, 354.9819

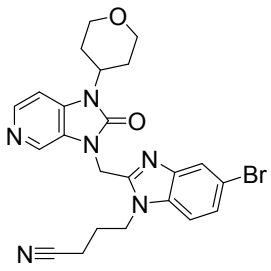
5-bromo-1-(4-((*tert*-butyldimethylsilyl)oxy)butyl)-2-(chloromethyl)-1*H*-benzo[*d*]imidazole (43)



Into a 2 neck round bottom flask was added the 4-bromo-*N*[1]-[4-[*tert*-butyl(dimethyl)silyl]oxybutyl]benzene-1,2-diamine (3.60 g, 9.64 mmol) followed by DCM (100 ml), thus forming a pale grey solution. To this, against a flow of argon was added the 2-chloro-1,1,1-trimethoxy-ethane (4.47 g, 28.9 mmol, 3.90 ml) followed by the toluene sulfonic acid monohydrate (0.18 g, 0.96 mmol). The reaction was left to proceed under argon for 18 h after which time analysis by TLC confirmed that all of the starting material had reacted and that a new product had formed at slightly higher Rf. The reaction mixture was diluted with dichloromethane (200 ml) and saturated sodium bicarbonate solution (200 ml). After thoroughly mixing the phases, the organic phase was separated, and the aqueous phase was extracted once more with dichloromethane (100 ml). The

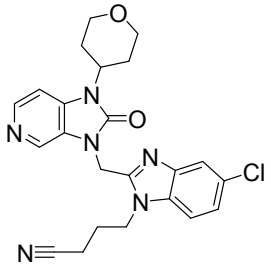
combined organic fractions were dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The crude material was purified by column chromatography (EtOAc/Hex) to afford the title compound as a white solid (3.69 g, 8.54 mmol, 89%). **^{1}H NMR (600 MHz, $CDCl_3$) δ** 7.84 (s, 1H), 7.34 (d, *J* = 8.5 Hz, 1H), 7.20 (d, *J* = 8.6 Hz, 1H), 4.77 (s, 2H), 4.20 (t, *J* = 7.7 Hz, 2H), 3.63 (t, *J* = 5.8 Hz, 2H), 1.96 – 1.88 (m, 2H), 1.61 – 1.51 (m, 2H), 0.85 (s, 9H), 0.01 (s, 6H). **^{13}C NMR (151 MHz, $CDCl_3$) δ** 149.7, 143.4, 134.2, 126.4, 122.9, 115.3, 111.1, 62.12, 44.3, 36.4, 29.6, 26.5, 25.8, 18.1, -5.48. **HRMS** calc. for $C_{18}H_{29}{}^{79}BrClN_2OSi$ $[M+H]^+$, 431.09156 found, 431.09160

4-[5-Bromo-2-[(2-oxo-1-tetrahydropyran-4-yl-imidazo[4,5-*c*]pyridin-3-yl)methyl]benzimidazol-1-yl]butanenitrile (44)



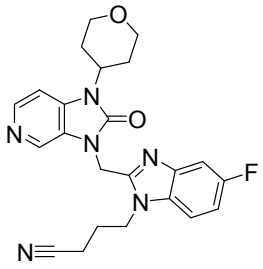
Potassium carbonate (76 mg, 0.55 mmol, 1.2 eq) and 4-[5-bromo-2-(chloromethyl)benzimidazole-1-yl]butanenitrile (171 mg, 0.547 mmol, 1.2 eq) were added to a solution of 3-tetrahydropyran-4-yl-1*H*-benzimidazol-2-one (100 mg, 0.456 mmol, 1 eq) in DMF (3.0 mL) and the reaction was heated to 50 °C. After approximately 18 hours the reaction was cooled to room temperature, quenched with a saturated solution of NH_4Cl and extracted into DCM three times. The organic phases were combined, dried over $MgSO_4$ and concentrated *in vacuo*. The resulting crude material was then purified by column chromatography and then recrystallized from hot MeOH to yield the product (139 mg, 0.281 mmol, 62%) as a yellow solid. **^{1}H NMR (500 MHz, DMSO) δ** 8.46 (s, 1H), 8.24 (d, *J* = 5.4 Hz, 1H), 7.80 (d, *J* = 1.7 Hz, 1H), 7.62 (d, *J* = 8.7 Hz, 1H), 7.45 (d, *J* = 5.4 Hz, 1H), 7.41 (dd, *J* = 8.6, 1.8 Hz, 1H), 5.46 (s, 2H), 4.54 – 4.46 (m, 1H), 4.45 – 4.38 (m, 2H), 3.99 (dd, *J* = 11.2, 4.0 Hz, 2H), 3.48 (t, *J* = 11.3 Hz, 2H), 2.62 (t, *J* = 7.4 Hz, 2H), 2.42 – 2.30 (m, 2H), 2.10 – 2.00 (m, 2H), 1.73 – 1.66 (m, 2H). **^{13}C NMR (101 MHz, DMSO)** δ 152.3, 150.5, 143.3, 142.6, 134.4, 134.3, 129.9, 126.5, 125.3, 121.7, 120.0, 114.3, 112.3, 104.5, 66.5, 50.2, 42.4, 37.6, 29.5, 25.3, 13.9. **HRMS** (APCI) *m/z* calc. for $C_{23}H_{24}BrN_6O_2$ $[M+H]^+$, 495.11386 found, 495.11472. **LCMS** (ESI) 10–95% MeOH in H_2O (0.1 % HCO_2H), 6 min, rt = 4.285, *m/z* = 496.2 $[M + H]^+$; 50-95% MeOH in H_2O (0.1 % HCO_2H), 6 min, rt = 0.959, *m/z* = 496.2 $[M + H]^+$.

4-[5-Chloro-2-[(2-oxo-1-tetrahydropyran-4-yl-imidazo[4,5-*c*]pyridin-3-yl)methyl]benzimidazol-1-yl]butanenitrile (45)



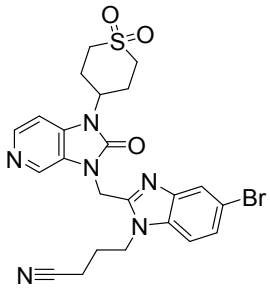
Potassium carbonate (78 mg, 0.56 mmol, 1.5 eq) and 4-[5-chloro-2-(chloromethyl)benzimidazole-1-yl]butanenitrile (150 mg, 0.561 mmol, 1.5 eq) were added to a solution of 3-tetrahydropyran-4-yl-1*H*-benzimidazol-2-one (82 mg, 0.437 mmol, 1 eq) in DMF (3.0 mL) and the reaction was heated to 50 °C. After approximately 18 hours the reaction was cooled to room temperature, quenched with a saturated solution of NH_4Cl and extracted into DCM three times. The organic phases were combined, dried over $MgSO_4$ and concentrated *in vacuo*. The resulting crude material was then purified by column chromatography and then recrystallized from hot MeOH to yield the product (145 mg, 0.322 mmol, 86%) as a yellow solid. **^{1}H NMR (500 MHz, DMSO-d_6) δ** 8.46 (s, 1H), 8.24 (d, *J* = 5.4 Hz, 1H), 7.69 – 7.63 (m, 2H), 7.46 (dd, *J* = 5.5, 0.8 Hz, 1H), 7.30 (dd, *J* = 8.7, 1.9 Hz, 1H), 5.46 (s, 2H), 4.55 – 4.46 (m, 1H), 4.45 – 4.38 (m, 2H), 3.99 (dd, *J* = 11.4, 4.5 Hz, 2H), 3.54 – 3.43 (m, 2H), 2.63 (t, *J* = 7.4 Hz, 2H), 2.43 – 2.32 (m, 2H), 2.05 (p, *J* = 7.5 Hz, 2H), 1.74 – 1.66 (m, 2H). **^{13}C NMR (126 MHz, DMSO-d_6) δ** 152.3, 150.7, 142.8, 142.6, 134.3, 134.1, 129.9, 126.5, 126.4, 122.7, 119.9, 118.7, 111.8, 104.5, 66.4, 50.2, 42.3, 37.6, 29.5, 25.3, 13.9. **HRMS** (APCI) *m/z* calc. for $C_{23}H_{24}ClN_6O_2$ $[M+H]^+$, 451.16438 found, 451.16400. **LCMS** (ESI) 25–95% MeOH in H_2O (0.1 % HCO_2H), 3 min, rt = 2.682, *m/z* = 451.0 $[M + H]^+$; 50-95% MeOH in H_2O (0.1 % HCO_2H), 3 min, rt = 0.872, *m/z* = 451.0 $[M + H]^+$.

4-[5-Fluoro-2-[(2-oxo-1-tetrahydropyran-4-yl-imidazo[4,5-*c*]pyridin-3-yl)methyl]benzimidazol-1-yl]butanenitrile (46)



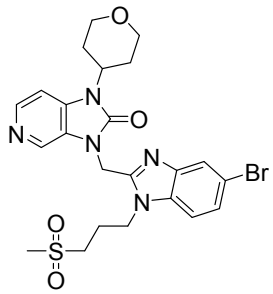
Potassium carbonate (42 mg, 0.30 mmol, 1.2 eq) and 4-[5-fluoro-2-(chloromethyl)benzimidazole-1-yl]butanenitrile (76 mg, 0.30 mmol, 1.2 eq) were added to a solution of 3-tetrahydropyran-4-yl-1*H*-benzimidazol-2-one (55 mg, 0.25 mmol, 1 eq) in DMF (1.0 mL) and the reaction was heated to 50 °C. After approximately 18 hours the reaction was cooled to room temperature, quenched with a saturated solution of NH_4Cl and extracted into DCM three times. The organic phases were combined, dried over $MgSO_4$ and concentrated *in vacuo*. The resulting crude material was then purified by column chromatography and then recrystallized from hot MeOH to yield the product (20 mg, 0.046 mmol, 18%) as a yellow solid. **^{1}H NMR (500 MHz, DMSO-d_6) δ** 8.47 (d, *J* = 0.7 Hz, 1H), 8.24 (d, *J* = 5.4 Hz, 1H), 7.64 (dd, *J* = 8.9, 4.7 Hz, 1H), 7.46 (dd, *J* = 5.5, 0.8 Hz, 1H), 7.41 (dd, *J* = 9.9, 2.5 Hz, 1H), 7.18 – 7.12 (m, 1H), 5.44 (s, 2H), 4.50 (tt, *J* = 12.2, 4.2 Hz, 1H), 4.45 – 4.39 (m, 2H), 3.99 (dd, *J* = 11.4, 4.5 Hz, 2H), 3.48 (t, *J* = 11.0 Hz, 2H), 2.63 (t, *J* = 7.4 Hz, 2H), 2.43 – 2.31 (m, 2H), 2.05 (p, *J* = 7.5 Hz, 2H), 1.75 – 1.67 (m, 2H). **^{13}C NMR (151 MHz, DMSO-d_6) δ** 158.6 (d, J_{CF} = 234.8 Hz), 152.2, 150.7, 142.6, 142.2 (d, J_{CF} = 12.9 Hz), 134.3, 130.9 (d, J_{CF} = 296.5 Hz), 126.5, 119.9, 111.2 (d, J_{CF} = 10.4 Hz), 110.7 (d, J_{CF} = 25.9 Hz), 104.8 (d, J_{CF} = 24.1 Hz), 104.5, 66.4, 50.2, 42.3, 37.6, 29.5, 25.3, 13.9. **HRMS** (APCI) *m/z* calc. for $C_{23}H_{24}FN_6O_2$ $[M+H]^+$, 435.19393 found, 435.19355. **LCMS** (ESI) 25–95% MeOH in H_2O (0.1 % HCO_2H), 3 min, rt = 2.367, *m/z* = 435.2 $[M + H]^+$; 50-95% MeOH in H_2O (0.1 % HCO_2H), 3 min, rt = 0.667, *m/z* = 435.2 $[M + H]^+$.

4-[5-Bromo-2-[[1-(1,1-dioxothian-4-yl)-2-oxo-imidazo[4,5-*c*]pyridin-3-yl]methyl]benzimidazol-1-yl]butanenitrile (47)



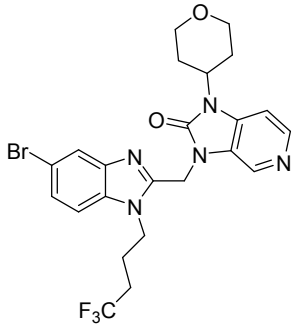
Potassium carbonate (78 mg, 0.56 mmol, 1.5 eq) and 4-[5-bromo-2-(chloromethyl)benzimidazole-1-yl]butanenitrile (175 mg, 0.561 mmol, 1.5 eq) were added to a solution of 1-(1,1-dioxothian-4-yl)-3H-imidazo[4,5-c]pyridin-2-one (100 mg, 0.374 mmol, 1 eq) in DMF (3.0 mL) and the reaction was heated to 50 °C. After approximately 18 hours the reaction was cooled to room temperature, quenched with a saturated solution of NH_4Cl and extracted into DCM three times. The organic phases were combined, dried over $MgSO_4$ and concentrated *in vacuo*. The resulting crude material was then purified by column chromatography and then recrystallized from hot MeOH to yield the product (159 mg, 0.293 mmol, 78%) as a yellow solid. **^{1}H NMR (500 MHz, DMSO-d_6) δ** 8.48 (s, 1H), 8.30 (d, *J* = 5.4 Hz, 1H), 7.80 (d, *J* = 1.8 Hz, 1H), 7.63 (d, *J* = 8.7 Hz, 1H), 7.42 (dd, *J* = 8.6, 1.9 Hz, 1H), 7.30 (dd, *J* = 5.4, 0.8 Hz, 1H), 5.45 (s, 2H), 4.76 – 4.66 (m, 1H), 4.47 – 4.37 (m, 2H), 3.53 – 3.42 (m, 2H), 3.23 – 3.15 (m, 2H), 2.88 – 2.75 (m, 2H), 2.63 (t, *J* = 7.4 Hz, 2H), 2.17 – 2.04 (m, 4H). **^{13}C NMR (126 MHz, DMSO-d_6) δ** 152.1, 150.4, 143.3, 142.6, 134.4, 134.3, 130.1, 126.6, 125.3, 121.6, 119.9, 114.2, 112.3, 104.0, 49.4, 49.3, 42.3, 37.6, 26.9, 25.2, 13.9. **HRMS** (APCI) *m/z* calc. for $C_{23}H_{24}BrN_6O_3S$ $[M+H]^+$, 543.08085 found, 543.08067. **LCMS** (ESI) 25–95% MeOH in H_2O (0.1 % HCO_2H), 3 min, rt = 2.588, *m/z* = 543.0 $[M + H]^+$; 50-95% MeOH in H_2O (0.1 % HCO_2H), 3 min, rt = 0.789, *m/z* = 453.0 $[M + H]^+$.

3-[[5-Bromo-1-(3-methylsulfonylpropyl)benzimidazol-2-yl]methyl]-1-tetrahydropyran-4-yl-imidazo[4,5-*c*]pyridin-2-one (48)



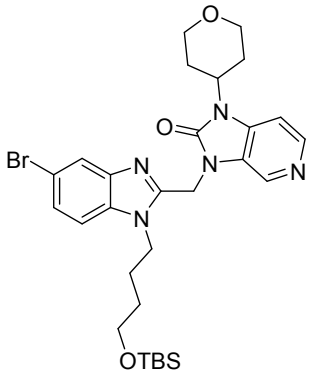
Potassium carbonate (36 mg, 0.26 mmol, 1.2 eq) and 5-bromo-2-(chloromethyl)-1-(3-methylsulfonylpropyl)benzimidazole (96 mg, 0.26 mmol, 1.2 eq) were added to a solution of 3-tetrahydropyran-4-yl-1*H*-benzimidazol-2-one (48 mg, 0.22 mmol, 1 eq) in DMF (1.0 mL) and the reaction was heated to 50 °C. After approximately 18 hours the reaction was cooled to room temperature, quenched with a saturated solution of NH_4Cl and extracted into DCM three times. The organic phases were combined, dried over $MgSO_4$ and concentrated *in vacuo*. The resulting crude material was then purified by column chromatography and then recrystallized from hot MeOH to yield the product (19 mg, 0.035 mmol, 16%) as a white solid. **^{1}H NMR (500 MHz, $CDCl_3$) δ** 8.72 (s, 1H), 8.28 (d, *J* = 5.3 Hz, 1H), 7.86 (s, 1H), 7.34 (d, *J* = 10.0 Hz, 1H), 7.23 (d, *J* = 8.6 Hz, 1H), 7.12 (d, *J* = 5.3 Hz, 1H), 5.34 (s, 2H), 5.27 (s, 1H), 4.55 – 4.46 (m, 2H), 4.11 (dd, *J* = 11.5, 3.9 Hz, 2H), 3.52 (t, *J* = 11.6 Hz, 2H), 3.07 (t, *J* = 7.2 Hz, 2H), 2.90 (s, 3H), 2.47 – 2.34 (m, 2H), 2.29 – 2.18 (m, 2H), 1.81 – 1.73 (m, 2H). **^{13}C NMR (126 MHz, $CDCl_3$) δ** 152.7, 148.9, 143.6, 134.4, 134.0, 131.0, 126.9, 126.4, 123.3, 115.9, 111.1, 110.1, 104.6, 67.4, 51.2, 50.8, 42.4, 41.2, 38.4, 30.1, 22.7. **HRMS** (APCI) *m/z* calc. for $C_{23}H_{27}BrN_5O_4S$ $[M+H]^+$, 548.09616 found, 548.09723. **LCMS** (ESI) 10–95% MeOH in H_2O (0.1 % HCO_2H), 6 min, rt = 4.071, *m/z* = 549.2 $[M + H]^+$; 50-95% MeOH in H_2O (0.1 % HCO_2H), 6 min, rt = 0.802, *m/z* = 549.0 $[M + H]^+$.

3-((5-bromo-1-(4,4,4-trifluorobutyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)-1-(tetrahydro-2*H*-pyran-4-yl)-1,3-dihydro-2*H*-imidazo[4,5-*c*]pyridin-2-one (49)



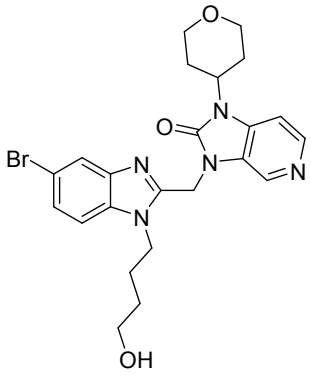
Into a two neck round bottom flask was added the 5-bromo-2-(chloromethyl)-1-(4,4,4-trifluorobutyl)-1*H*-benzo[*d*]imidazole (500 mg, 1.41 mmol) followed by the 1-(tetrahydro-2*H*-pyran-4-yl)-1,3-dihydro-2*H*-benzo[*d*]imidazol-2-one (308 mg, 1.41 mmol). To this was added DMF (15 ml) thus forming a light brown solution. Potassium carbonate (194 mg, 1.41 mmol) was then added and the reaction was carried out at 60 °C for 18 h. After this time the reaction mixture was diluted with ethyl acetate (100 ml) and water (100 ml). The phases were thoroughly mixed and after separation, the aqueous phase was extracted with ethyl acetate (2 x 100 ml). The combined organic fractions were then washed with brine (100 ml) and then dried over anhydrous magnesium sulfate. After concentrating in vacuo, the crude material was purified by column chromatography (DCM/MeOH) and then recrystallized from ethyl acetate/ethanol mixture, thereby affording the title compound as a white solid (641 mg, 1.19 mmol, 85%). **^{1}H NMR (600 MHz, DMSO-d_6) δ** 8.47 (s, 1H), 8.23 (d, *J* = 5.3 Hz, 1H), 7.81 (s, 1H), 7.65 (d, *J* = 8.6 Hz, 1H), 7.45 (d, *J* = 5.4 Hz, 1H), 7.42 (d, *J* = 8.7 Hz, 1H), 5.45 (s, 2H), 4.54 – 4.46 (m, 1H), 4.44 (t, *J* = 7.7 Hz, 2H), 3.99 (dd, *J* = 11.6, 4.4 Hz, 2H), 3.48 (t, *J* = 11.9 Hz, 2H), 2.42 – 2.31 (m, 4H), 1.96 – 1.84 (m, 2H), 1.69 (d, *J* = 11.7 Hz, 2H). **^{13}C NMR (151 MHz, DMSO-d_6) δ** 152.7, 151.0, 143.8, 143.1, 134.8, 130.3, 127.7 (q, $J_{C\text{-}F}$ = 276.2 Hz), 127.0, 125.9, 122.1, 114.7, 112.8, 105.0, 66.89, 50.7, 42.6, 38.1, 30.5 (q, $J_{C\text{-}F}$ = 28.4 Hz), 30.0, 22.7. **HRMS** calc. for $C_{23}H_{24}{}^{79}BrF_3N_5O_2$ $[M+H]^+$, 538.1060 found, 538.1057. **LCMS** (ESI) 25–95% MeOH in H_2O (0.1 % HCO_2H), 6 min, rt = 4.645, *m/z* = 540.0 $C_{23}H_{24}{}^{81}BrF_3N_5O_2$ $[M+H]^+$.

3-((5-Bromo-1-(4-((*tert*-butyldimethylsilyl)oxy)butyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)-1-(tetrahydro-2*H*-pyran-4-yl)-1,3-dihydro-2*H*-imidazo[4,5-*c*]pyridin-2-one (50)



Into a two neck round bottom flask containing argon was added 4-[5-bromo-2-(chloromethyl)benzimidazol-1-yl]butoxy-*tert*-butyl-dimethyl-silane (1.00 g, 2.32 mmol) followed by 1-tetrahydropyran-4-yl-3*H*-imidazo[4,5-*c*]pyridin-2-one (508 mg, 2.32 mmol). To this was added DMF (15 ml) thus forming a light brown solution. Potassium carbonate (320 mg, 2.32 mmol) was then added and the reaction was carried out at 60 °C for 18 h. After this time the reaction mixture was diluted with ethyl acetate (200 ml) and water (200 ml). The phases were thoroughly mixed and after separation, the aqueous phase was extracted with ethyl acetate (2 x 100 ml). The combined organic fractions were then washed with brine (100 ml) and then dried over anhydrous magnesium sulfate. After filtering and concentrating in vacuo, the crude material was purified by column chromatography (EtOAc/MeOH), to afford the title compound (1.08 g, 1.76 mmol, 76%) as a pale orange solid. **^{1}H NMR (600 MHz, $CDCl_3$) δ** 8.67 (s, 1H), 8.27 (d, *J* = 4.9 Hz, 1H), 7.87 (s, 1H), 7.32 (d, *J* = 8.5 Hz, 1H), 7.19 (d, *J* = 8.5 Hz, 1H), 7.10 (d, *J* = 5.0 Hz, 1H), 5.34 (s, 2H), 4.54 (t, *J* = 12.3 Hz, 1H), 4.30 (t, *J* = 7.3 Hz, 2H), 4.15 – 4.05 (m, 2H), 3.60 – 3.47 (m, 4H), 2.49 – 2.33 (m, 2H), 1.81 – 1.63 (m, 4H), 1.55 – 1.42 (m, 2H), 0.82 (s, 9H), -0.02 (s, 6H). **^{13}C NMR (151 MHz, $CDCl_3$) δ** 152.5, 148.7, 143.5, 143.3, 134.2, 134.2, 130.9, 126.3, 126.2, 122.9, 115.3, 111.2, 104.3, 67.2, 62.1, 50.6, 44.0, 38.5, 29.9, 29.6, 26.6, 25.8, 18.1, -5.6. **HRMS** calc. for $C_{29}H_{41}{}^{79}BrN_5O_3Si$ $[M+H]^+$, 614.21566 found, 614.21573

3-((5-bromo-1-(4-hydroxybutyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)-1-(tetrahydro-2*H*-pyran-4-yl)-1,3-dihydro-2*H*-imidazo[4,5-*c*]pyridin-2-one (51)



Into a 2 neck round bottom flask containing argon was added 3-[[5-bromo-1-[4-[*tert*-butyl(dimethyl)silyl]oxybutyl]benzimidazol-2-yl]methyl]-1-tetrahydropyran-4-yl-imidazo[4,5-*c*]pyridin-2-one (500 mg, 0.810 mmol) followed by THF (50 mL), thus forming a clear solution. To this, at room temperature, was added HF-pyridine (1.0 mL, 38 mmol) and the reaction was left to proceed for 3 h, after which time analysis by TLC showed partial conversion of the starting material to a new product. At this point the reaction was quenched by the addition of saturated sodium bicarbonate solution (150 ml) and was further diluted with EtOAc (150 ml). After thoroughly mixing, the phases were separated, and the aqueous phase was extracted with EtOAc (2 x 150 ml). The organic fractions were combined and dried over anhydrous magnesium sulfate, filtered, and then concentrated in vacuo. The crude material was purified by column chromatography (EtOAc/Hex) to afford the title compound (235 mg, 0.470 mmol, 58%) as well as 216 mg of the starting material. **^{1}H NMR (600 MHz, DMSO-d_6) δ** 8.43 (s, 1H), 8.23 (d, *J* = 5.4 Hz, 1H), 7.81 (d, *J* = 1.9 Hz, 1H), 7.58 (d, *J* = 8.6 Hz, 1H), 7.46 (d, *J* = 5.4 Hz, 1H), 7.39 (dd, *J* = 8.6, 1.9 Hz, 1H), 5.43 (s, 2H), 4.55 – 4.47 (m, 1H), 4.45 (t, *J* = 5.1 Hz, 1H), 4.06 – 3.97 (m, 2H), 3.52 – 3.45 (m, 2H), 3.41 – 3.35 (m, 2H), 3.32 (s, 2H), 2.37 (qd, *J* = 12.4, 4.7 Hz, 2H), 1.74 – 1.62 (m, 4H), 1.47 – 1.39 (m, 2H). **^{13}C NMR (151 MHz, DMSO-d_6) δ** 152.1, 150.3, 143.2, 142.5, 134.4, 134.4, 129.6, 126.5, 125.2, 121.6, 114.0, 112.5, 104.5, 66.4, 60.2, 50.2, 43.4, 37.7, 29.5, 29.4, 26.3. **HRMS** calc. for $C_{23}H_{27}{}^{79}BrN_5O_3$ $[M+H]^+$, 500.1292 found, 500.1286. **LCMS** (ESI) 25–95% MeOH in H_2O (0.1 % HCO_2H), 6 min, rt = 3.069, *m/z* = 502.0 $C_{23}H_{27}{}^{81}BrN_5O_3$ $[M+H]^+$.

Appendix A

Protocols for Molecular Modeling

Protein preparation

PDB ID 5EA4 was prepared using the protein preparation wizard. Hydrogen atoms were added, protonation states assigned prior to being visually inspected and followed by energy minimization to relieve unfavorable energetic constraints (Schrödinger Release 2018-4: Protein Preparation Wizard; Epik, Schrödinger, LLC, New York, NY, 2016; Impact, Schrödinger, LLC, New York, NY, 2016; Prime, Schrödinger, LLC, New York, NY, 2019). Molecular minimization was first performed on only the hydrogen atoms, followed by an all atom restrained minimization with the heavy atoms set to converge at 0.3Å. The OPLS3 force field was used in all calculations.[4]

Molecular overlay

We used the flexible ligand alignment module within the Schrödinger Release 2018-4 to align compound **2**, JNJ-53718678 and BMS-433771 to JNJ-4193390 within the F protein (PDB ID 5EA4). We selected to use the maximum common substructure option as our alignment criteria.

Because of the shared scaffold between our compound series and the similar binding poses observed for resolved JNJ-49153390, JNJ-53718678 and BMS-433771 structures (PDB ID 5EA4, 5EA7 and 5KWW), we assumed a similar binding pose for our compound series. To illustrate the proposed shared binding pose, we performed a molecular overlay and superimposed our most potent compound, compound **2,** JNJ-53718678 and BMS-433771 onto JNJ-49153390 within the PDB ID 5EA4 crystal structure (**Figure S1**). **Figure S1** highlights the proposed binding mode of compound **2** and the expected shared interactions of these inhibitors along the trimeric axis of the central cavity of the F protein is illustrated in **Figure S1**.[5]

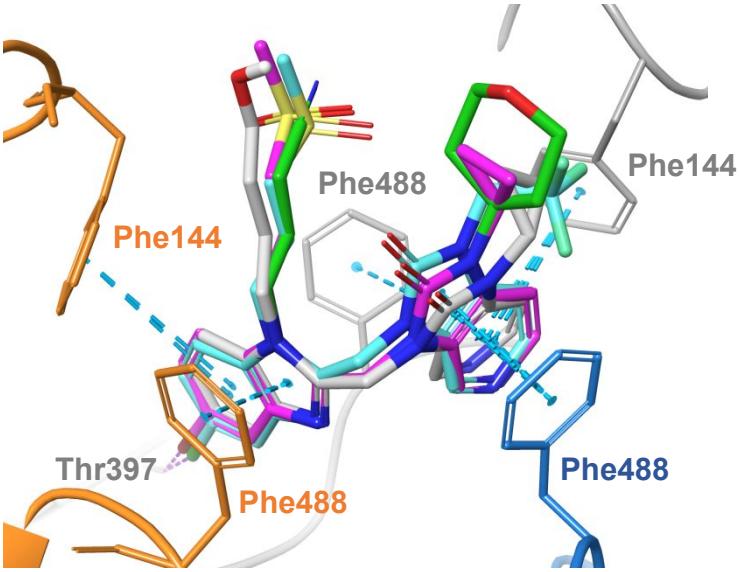
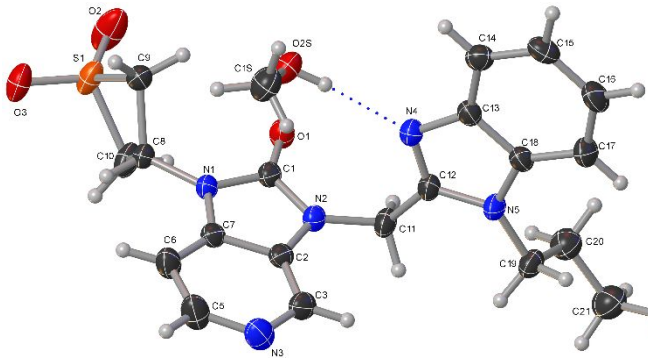


Figure S1 An illustration comparing the binding poses of compound **2** (green), BMS-433771 (white), JNJ-53718678 (cyan) and JNJ-49153390 (magenta). Blue dashed lines highlight the protein-ligand π-π interactions and purple dashed line shows the halogen bond formed with Thr397. The blue, orange and gray cartoons represent the monomer of the trimeric structures.

Appendix B

Crystal Data and Experimental for Compound 1

Compound	**1**
Formula	$C_{22}H_{24}N_6O_4S$
$D_{calc.}$/ g cm^{-3}	1.423
μ/mm^{-1}	1.668
Formula Weight	468.53
Colour	orange
Shape	plate
Size/mm^3	0.35×0.15×0.05
T/K	100(2)
Crystal System	monoclinic
Space Group	$P2_1/c$
a/Å	11.2813(5)
b/Å	11.5366(5)
c/Å	16.8254(6)
α/°	90
β/°	93.222(4)
γ/°	90
V/Å^3	2186.33(16)
Z	4
Z'	1
Wavelength/Å	1.54184
Radiation type	CuK_α



Experimental. Single orange plate-shaped crystals of (**1**) were crystallized from methanol and ethyl acetate by. A suitable crystal (0.35×0.15×0.05 mm^3) was selected and mounted on a loop with paratone oil on a SYNERGY diffractometer. The crystal was cooled to T = 100(2) K during data collection. The structure was solved with the **XT**[6] structure solution program using the Intrinsic Phasing solution method and by using **Olex2**[7] as the graphical interface. The model was refined with version 2014/7 of **XL**[8] using Least Squares minimisation.

Crystal Data. $C_{22}H_{24}N_6O_4S$, M_r = 468.53, monoclinic, $P2_1/c$ (No. 14), a = 11.2813(5) Å, b = 11.5366(5) Å, c =

16.8254(6) Å, β = 93.222(4)°, $\alpha = \gamma$ = 90°, V =

2186.33(16) Å^3, T = 100(2) K, Z = 4, Z' = 1, $\mu(CuK_\alpha)$ = 1.668 mm^{-1}, 16015 reflections measured, 4120 unique (R_{int} = 0.0439) which were used in all calculations. The final wR_2 was 0.1345

$\Theta_{min}/^{\circ}$	3.925
$\Theta_{max}/^{\circ}$	70.063
Measured Refl.	16015
Independent Refl.	4120
Reflections with I > 2σ (I)	3644
R_{int}	0.0439
Parameters	344
Restraints	92
Largest Peak	0.399
Deepest Hole	-0.505
GooF	1.047
wR_2 (all data)	0.1345
wR_2	0.1306
R_1 (all data)	0.0543
R_1	0.0495

(all data) and R_1 was 0.0495 (I > 2σ(I)).

Structure Quality Indicators

Reflections:	d min (Cu)	0.82	I/σ	18.9	Rint	4.39%	complete	100%
Refinement:	Shift	0.001	Max Peak	0.4	Min Peak	-0.5	GooF	1.047

A orange plate-shaped crystal with dimensions 0.35×0.15×0.05 mm^3 was mounted on a loop with paratone oil. Data were collected using a SYNERGY diffractometer diffractometer equipped with an Oxford Cryosystems low-temperature device, operating at T = 100(2) K.

Data were measured using ω scans with a narrow frame width of 0.5˚ per frame for 3 and 15 s using CuK_α radiation (micro-focus sealed X-ray tube, 50 kV, 1.0 mA). The total number of runs and images was based on the strategy calculation from the program CrysAlisPro (Agilent). The maximum resolution that was achieved was Θ = 70.063˚.

The diffraction patterns were indexed using CrysAlisPro (Agilent) and the unit cells were refined using CrysAlisPro (Agilent) on 2920 reflections. Data reduction, scaling and absorption corrections were performed using CrysAlisPro (Agilent) and CrysAlisPro 1.171.39.20a (Rigaku Oxford Diffraction, 2015). A numerical absorption correction based on gaussian integration over a multifaceted crystal model was carried out. An empirical absorption correction using spherical harmonics, as implemented in SCALE3 ABSPACK scaling algorithm was also carried out. The final completeness is 99.9% out to 70.063˚ in Θ. The absorption coefficient μ of this material is 1.668 mm^{-1} at this wavelength (λ = 1.54184 Å) and the minimum and maximum transmissions are 0.585 and 1.000.

The structure was solved and the space group $P2_1/c$ (# 14) determined by the **XT**[6] structure solution program using Intrinsic Phasing and refined by Least Squares using version 2014/7 of **XL**[8]. All non-hydrogen atoms were refined anisotropically. Hydrogen atom positions were calculated geometrically and refined using the riding model.

There is a single molecule in the asymmetric unit, which is represented by the reported sum formula. In other words: Z is 4 and Z' is 1.

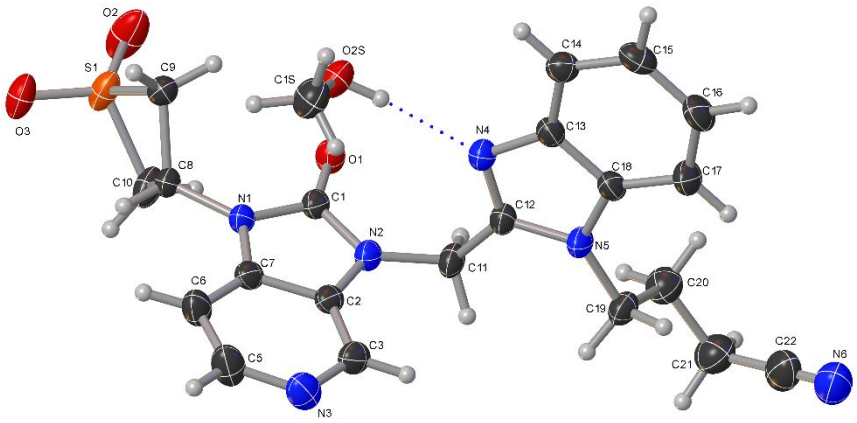


Figure S2:

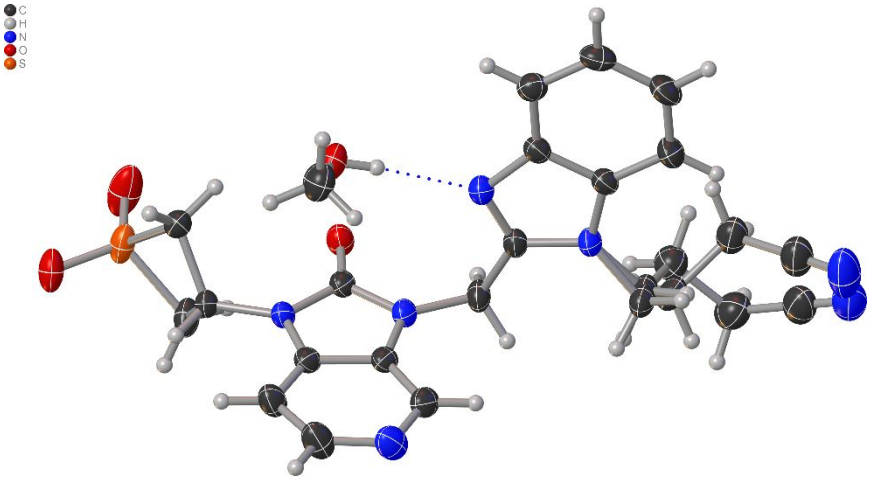
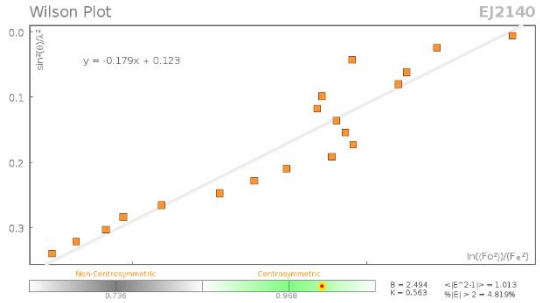
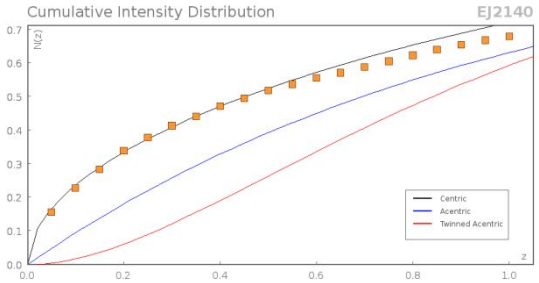
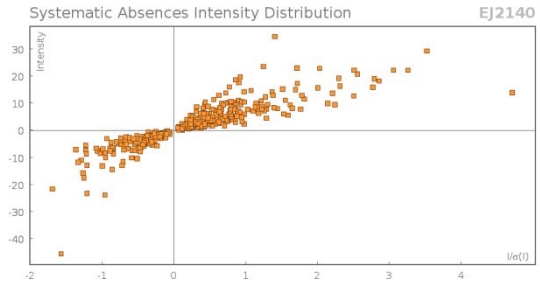


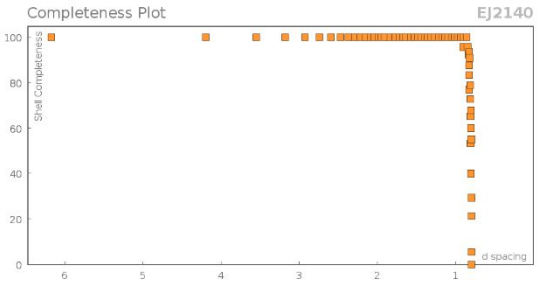
Figure S3:

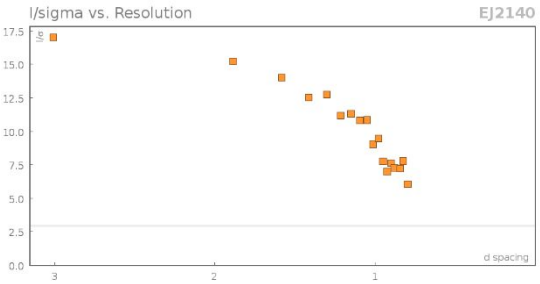
Data Plots: Diffraction Data



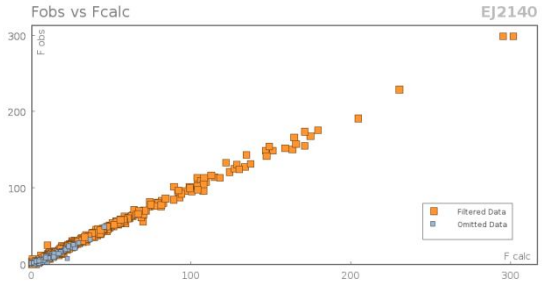


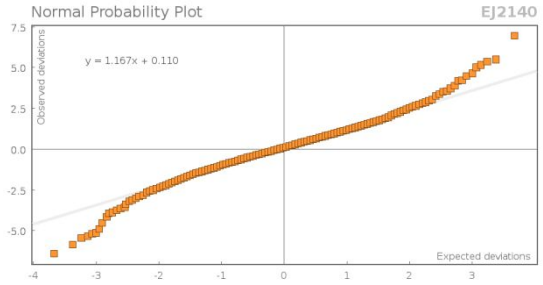






Data Plots: Refinement and Data





Reflection Statistics

Total reflections (after filtering)	16590	Unique reflections	4120
Completeness	0.996	Mean I/σ	18.85
hkl_{max} collected	(13, 13, 20)	hkl_{min} collected	(-14, -13, -21)
hkl_{max} used	(13, 13, 20)	hkl_{min} used	(-13, 0, 0)
Lim d_{max} collected	20.0	Lim d_{min} collected	0.82
d_{max} used	16.8	d_{min} used	0.82
Friedel pairs	2642	Friedel pairs merged	1
Inconsistent equivalents	12	R_{int}	0.0439
R_{sigma}	0.0338	Intensity transformed	0
Omitted reflections	0	Omitted by user (OMIT hkl)	0
Multiplicity	(5980, 2576, 1238, 445, 80, 2, 1)	Maximum multiplicity	20
Removed systematic absences	575	Filtered off (Shel/OMIT)	455

Images of the Crystal on the Diffractometer

Table S4: Fractional Atomic Coordinates (×10^4) and Equivalent Isotropic Displacement Parameters (Å^2×10^3) for **1**. U_{eq} is defined as 1/3 of the trace of the orthogonalised U_{ij}.

Atom	x	y	z	U_{eq}
S1	8216.5(4)	5688.7(5)	5628.0(3)	38.16(18)
O1	5503.7(12)	5247.1(13)	4056.7(8)	33.0(3)
N1	7155.4(14)	4064.4(14)	3884.3(9)	26.3(4)
O3	9358.2(13)	5398.3(16)	5998.4(9)	43.5(4)
O2S	7186.5(14)	6196.4(14)	2609.8(9)	40.8(4)
N5	3520.5(15)	4450.9(15)	1343.8(10)	30.2(4)
N2	5568.6(15)	3828.2(14)	3070.9(9)	28.0(4)
N4	5123.4(15)	5457.2(15)	1789.4(10)	28.8(4)
N3	7379.0(18)	1581.4(17)	2140.4(11)	42.2(5)
O2	7569.1(16)	6602.5(19)	5981.7(13)	63.7(6)
C13	4716.3(18)	5899.8(17)	1051.3(12)	28.5(4)
C7	7434.4(18)	3216.4(17)	3345.5(11)	28.5(4)
C18	3716.1(18)	5282.7(17)	771.4(12)	29.3(4)
C1	6009.8(18)	4477.4(17)	3711.2(11)	27.8(4)
C2	6419.3(18)	3055.5(17)	2836.5(11)	28.0(4)
C11	4383.3(18)	3966.1(19)	2708.5(12)	31.4(4)

Atom	x	y	z	U_{eq}
C12	4384.4(17)	4618.4(17)	1937.5(12)	28.6(4)
C17	3111(2)	5543(2)	47.8(13)	39.6(5)
C15	4557(2)	7078.2(19)	-103.0(13)	37.8(5)
C3	6415(2)	2222.1(19)	2251.0(12)	34.8(5)
C8	7929.0(17)	4440.7(17)	4552.0(11)	26.9(4)
C14	5161.5(19)	6811.0(18)	612.4(12)	32.6(4)
C5	8351(2)	1778(2)	2613.2(14)	45.3(6)
C6	8437(2)	2579(2)	3235.0(13)	37.9(5)
C9	8279.9(19)	5736.0(18)	4566.8(13)	33.3(5)
C10	7386(2)	4411(2)	5376.1(12)	37.9(5)
C1S	8100(2)	5791(2)	2150.3(16)	46.5(6)
C16	3554(2)	6448(2)	-380.2(13)	42.6(5)
C19	2524(3)	3635(4)	1335(4)	34.4(6)
C20	1426(4)	4130(4)	1706(3)	43.4(11)
C21	372(4)	3296(4)	1617(3)	52.0(11)
C22	-42(4)	3164(5)	775(3)	53.4(13)
N6	-307(5)	3057(5)	121(3)	59.4(12)
C19'	2734(4)	3445(3)	1233(4)	34.4(6)
C20'	1578(4)	3624(4)	1648(3)	39.2(11)
C21'	713(4)	4405(4)	1161(3)	44.9(11)
C22'	334(5)	3859(5)	396(3)	52.9(13)
N6'	108(5)	3433(6)	-200(3)	69.9(15)

Table S5: Anisotropic Displacement Parameters (×10^4) **1**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2} \times U_{11} + \ldots + 2hka^* \times b^* \times U_{12}]$

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
S1	26.0(3)	50.6(4)	37.4(3)	-18.3(2)	-2.5(2)	-0.1(2)
O1	31.6(7)	35.7(8)	31.0(7)	-3.0(6)	-3.0(6)	3.7(6)
N1	28.7(8)	25.7(8)	23.8(8)	-0.7(6)	-5.4(6)	1.0(7)
O3	28.3(8)	65.6(11)	35.6(8)	-11.0(7)	-5.5(6)	-2.6(7)
O2S	37.4(8)	47.1(9)	37.4(8)	-6.7(7)	-3.6(7)	-9.7(7)
N5	31.6(8)	31.5(9)	26.3(8)	3.4(6)	-8.3(6)	-4.5(7)
N2	29.0(8)	29.7(8)	24.8(8)	1.5(7)	-4.1(6)	-1.3(7)
N4	28.2(8)	31.4(9)	26.4(8)	-0.3(7)	-3.1(7)	-0.6(7)
N3	53.7(12)	40.4(10)	31.6(9)	-6.6(8)	-5.7(8)	7.7(9)
O2	38.4(9)	79.1(14)	73.5(13)	-46.5(11)	1.2(9)	6.7(9)
C13	30.7(10)	26.6(10)	27.8(10)	0.5(8)	-1.9(8)	3.3(8)
C7	35.6(11)	25.6(9)	23.7(9)	3.0(7)	-3.8(8)	-0.7(8)
C18	33.5(10)	26.0(9)	27.8(10)	1.5(8)	-3.5(8)	2.7(8)
C1	30.8(10)	27.4(10)	24.7(9)	2.7(8)	-3.6(8)	-1.8(8)
C2	35.2(11)	25.6(9)	22.7(9)	4.0(7)	-3.1(8)	-2.7(8)
C11	28.2(10)	38.8(11)	26.3(10)	2.1(8)	-5.7(8)	-5.1(8)
C12	27.2(10)	30.3(10)	27.5(10)	-1.4(8)	-4.7(8)	0.2(8)
C17	44.1(13)	38.8(12)	34.2(11)	6.6(9)	-13.3(10)	-2.2(10)
C15	50.0(13)	28.1(10)	35.6(11)	6.9(9)	5.5(10)	4.7(9)

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
C3	44.0(12)	33.6(11)	26.1(10)	-0.1(8)	-5.0(9)	-2.7(9)
C8	27.6(10)	27.8(10)	24.5(9)	-0.7(7)	-5.7(8)	-1.0(8)
C14	36.0(11)	28.7(10)	33.2(10)	-1.1(8)	2.0(9)	-0.1(8)
C5	50.3(14)	48.0(14)	36.6(12)	-8.9(10)	-5.8(10)	16.3(11)
C6	41.5(12)	38.8(12)	32.2(11)	-3.1(9)	-8.4(9)	8.1(10)
C9	34.9(11)	27.7(10)	36.4(11)	-1.8(8)	-6.1(9)	-0.9(8)
C10	32.4(11)	55.4(14)	25.4(10)	-1.5(9)	-3.4(8)	-9.3(10)
C1S	36.5(12)	53.0(15)	49.9(14)	-4.6(11)	-0.1(11)	-5.4(11)
C16	53.4(14)	38.5(12)	34.5(11)	9.4(9)	-9.7(10)	5.5(11)
C19	35.2(10)	37.4(12)	29.7(14)	-0.5(11)	-6.6(9)	-7.8(10)
C20	40.8(15)	44(2)	45(2)	2.0(19)	1.3(14)	-3.8(14)
C21	45.2(15)	50.1(18)	60.5(16)	-1.2(13)	1.4(12)	-6.2(13)
C22	42(2)	50(3)	66.7(17)	-9.6(16)	-8.4(15)	9(2)
N6	56(2)	55(2)	66.0(17)	-9.3(16)	-14.0(15)	4.9(19)
C19'	35.1(10)	37.4(12)	29.7(14)	-0.5(11)	-6.6(9)	-7.8(10)
C20'	36.0(16)	37(2)	44(2)	-8.9(17)	-2.4(13)	-7.0(15)
C21'	39.9(16)	46.0(17)	48.1(16)	-8.7(12)	-4.6(12)	0.7(13)
C22'	48.6(19)	54(2)	54.3(15)	-12.0(13)	-10.5(13)	4.7(15)
N6'	60(3)	81(3)	65.7(19)	-30(2)	-21(2)	18(2)

Table S6: Bond Lengths in Å for **1**.

Atom	Atom	Length/Å
S1	O3	1.4385(16)
S1	O2	1.4310(18)
S1	C9	1.792(2)
S1	C10	1.785(2)
O1	C1	1.221(2)
N1	C7	1.382(3)
N1	C1	1.393(3)
N1	C8	1.450(2)
O2S	C1S	1.403(3)
N5	C18	1.386(3)
N5	C12	1.369(2)
N5	C19	1.466(3)
N5	C19'	1.466(3)
N2	C1	1.382(3)
N2	C2	1.383(3)
N2	C11	1.447(2)

Atom	Atom	Length/Å
N4	C13	1.396(2)
N4	C12	1.310(3)
N3	C3	1.337(3)
N3	C5	1.337(3)
C13	C18	1.394(3)
C13	C14	1.394(3)
C7	C2	1.403(3)
C7	C6	1.371(3)
C18	C17	1.395(3)
C2	C3	1.376(3)
C11	C12	1.500(3)
C17	C16	1.379(3)
C15	C14	1.385(3)
C15	C16	1.402(3)
C8	C9	1.546(3)
C8	C10	1.547(3)
C5	C6	1.395(3)
C19	C20	1.529(5)
C20	C21	1.531(5)
C21	C22	1.474(5)
C22	N6	1.132(5)
C19'	C20'	1.528(5)
C20'	C21'	1.531(5)
C21'	C22'	1.474(5)
C22'	N6'	1.133(5)

Table S7: Bond Angles in ° for **1**.

Atom	Atom	Atom	Angle/°
O3	S1	C9	110.73(10)
O3	S1	C10	110.84(11)
O2	S1	O3	117.18(11)
O2	S1	C9	116.22(12)
O2	S1	C10	115.71(11)
C10	S1	C9	80.70(10)
C7	N1	C1	110.35(16)
C7	N1	C8	124.82(16)
C1	N1	C8	124.80(16)
C18	N5	C19	126.2(3)
C18	N5	C19'	125.4(3)
C12	N5	C18	105.93(16)
C12	N5	C19	127.7(3)
C12	N5	C19'	127.2(3)
C1	N2	C2	110.26(16)
C1	N2	C11	123.57(17)
C2	N2	C11	126.17(16)
C12	N4	C13	104.97(16)
C3	N3	C5	118.03(19)
C18	C13	N4	109.47(17)
C18	C13	C14	121.00(18)
C14	C13	N4	129.53(19)
N1	C7	C2	106.61(17)
C6	C7	N1	133.54(19)
C6	C7	C2	119.84(19)
N5	C18	C13	106.05(17)
N5	C18	C17	131.8(2)
C13	C18	C17	122.18(19)
O1	C1	N1	127.06(18)
O1	C1	N2	127.45(19)
N2	C1	N1	105.49(17)
N2	C2	C7	107.23(17)
C3	C2	N2	132.79(19)
C3	C2	C7	119.97(19)
N2	C11	C12	111.97(16)
N5	C12	C11	121.67(18)
N4	C12	N5	113.57(17)
N4	C12	C11	124.51(18)
C16	C17	C18	116.4(2)
C14	C15	C16	121.5(2)
N3	C3	C2	121.0(2)
N1	C8	C9	116.39(16)
N1	C8	C10	115.86(17)
C9	C8	C10	96.97(16)
C15	C14	C13	116.99(19)
N3	C5	C6	125.3(2)
C7	C6	C5	115.7(2)
C8	C9	S1	87.82(13)
C8	C10	S1	88.00(13)
C17	C16	C15	121.9(2)

Atom	Atom	Atom	Angle/°
N5	C19	C20	113.2(3)
C19	C20	C21	111.6(3)
C22	C21	C20	111.2(3)
N6	C22	C21	176.9(6)
N5	C19'	C20'	111.1(3)

Atom	Atom	Atom	Angle/°
C19'	C20'	C21'	111.7(3)
C22'	C21'	C20'	111.2(3)
N6'	C22'	C21'	176.1(6)

Table S8: Hydrogen Fractional Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($Å^2 \times 10^3$) for **1**. U_{eq} is defined as 1/3 of the trace of the orthogonalised U_{ij}.

Atom	x	y	z	U_{eq}
H2S	6	5426	0262390	62
H11A	3	8974	3783074	39
H11B	4	0323	2072614	39
H17	2	4425	128-136	48
H15	4	8237	688-408	46
H3	5	7312	1031927	42
H8	8	6513	9704574	33
H14	5	8327	222 792	40
H5	9	0251	3442519	55
H6	9	1312	6743554	46
H9A	7	6906	2424306	41
H9B	9	0635	8884386	41
H10A	7	6163	7405695	46
H10B	6	5324	5245355	46
H1SA	8	3306	3911797	71
H1SB	7	8285	1281848	71
H1SC	8	7715	5752497	71
H16	3	1766	649-866	52
H19A	2	7732	9411614	41
H19B	2	3123	428 784	41
H20A	1	6094	2612265	53
H20B	1	2094	8501455	53
H21A	5	97	25441833	58
H21B	-2	73	35931917	58
H19C	3	1432	7611459	41
H19D	2	5743	306 672	41
H20C	1	7543	9662159	58
H20D	1	2122	8761717	58
H21C		28	45451465	53
H21D	1	0955	1381064	53

Table S9: Atomic Occupancies for all atoms that are not fully occupied in **1**.

Atom	Occupancy
C19	0.511(4)
C20	0.511(4)
C21	0.511(4)
C22	0.511(4)
N6	0.511(4)
H19A	0.511(4)
H19B	0.511(4)
H20A	0.511(4)
H20B	0.511(4)
H21A	0.511(4)

Atom	Occupancy
H21B	0.511(4)
C19'	0.489(4)
C20'	0.489(4)
C21'	0.489(4)
C22'	0.489(4)
N6'	0.489(4)
H19C	0.489(4)
H19D	0.489(4)
H20C	0.489(4)
H20D	0.489(4)

Atom	Occupancy
H21C	0.489(4)
H21D	0.489(4)

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