Discovery of a Potent, $S1P_3$-Sparing Benzothiazole Agonist of Sphingosine-1-Phosphate Receptor 1 ($S1P_1$)

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Supporting Information

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Table SI-1. Statistical analysis of $hS1P_1$ and $hS1P_3$ data

Cmpd	$hS1P_1$ RI (n)	$hS1P_1$ RI EC_{50} (μM)	$hS1P_1$ RI EC_{50} SD (for n=2) or SE (for n>2)	$hS1P_1$ RI %efficacy	$hS1P_1$ RI %efficacy SD (for n=2) or SE (for n>2)	$hS1P_3$ Ca^{2+} (n)	$hS1P_3$ Ca^{2+} EC_{50} (μM)	$hS1P_3$ Ca^{2+} EC_{50} SD (for n=2) or SE (for n>2)	$hS1P_3$ Ca^{2+} %efficacy (n)	$hS1P_3$ Ca^{2+} %efficacy	$hS1P_3$ Ca^{2+} %efficacy SD (for n=2) or SE (for n>2)
3	18	0.057	0.018	96	6	28	1.725	0.186	28	42	2
4	2	0.325	0.186	105	16	2	0.354	0.013	2	77	9
5	2	0.221	0.124	84	9	2	3.466	0.418	2	50	8
6	2	>6.25				2	>25				
7	2	6.723	2.992	115	55	8	>25				
8	8	2.892	0.805	53	10	2	1.112	0.011	2	43	2
9	303	0.013	0.001	110	1	197	0.679	0.025	197	42	1
10	4	0.094	0.029	108	9	4	0.542	0.063	4	87	3
11	300	0.042	0.003	102	1	15	1.214	0.185	15	24	2
12	6	0.741	0.117	110	7	6	6.091	1.313	6	50	7
13	2	1.808	0.420	100	7	2	2.711	0.180	2	48	6
14	10	0.042	0.010	115	11	6	3.469	0.402	6	14	1
15	2	0.021	0.016	138	38	2	0.687	0.163	2	22	0
16	10	0.033	0.005	129	12	8	1.910	0.284	8	37	3
17	2	0.042	0.008	186	11	2	1.201	0.226	2	24	2
18	2	0.126	0.004	126	21	2	>25				
19	2	0.030	0.015	151	28	2	0.967	0.017	2	19	3
20	10	0.026	0.003	118	7	8	2.438	0.260	8	40	5
21	8	0.054	0.020	92	5	2	>2.5				
22	14	0.077	0.013	132	14	8	1.799	0.261	8	17	2
23	10	0.042	0.006	121	8	7	4.881	0.771	7	41	6
24	10	0.049	0.006	141	13	8	3.883	0.536	8	35	2
25	2	0.159	0.035	117	21	2	>25				

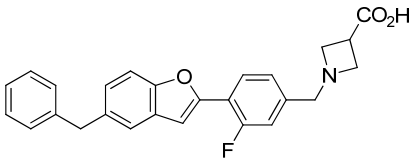
Synthesis of 3–25

Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Anhydrous solvents were obtained from EMD or Aldrich and used directly. All reactions involving air- or moisture-sensitive reagents were performed under a nitrogen or argon atmosphere. Microwave-assisted reactions were conducted with a Smith synthesizer from Personal Chemistry (Uppsala, Sweden). Silica gel chromatography was performed using medium pressure liquid chromatography (MPLC) on a CombiFlash® Companion® (Teledyne Isco) with RediSep® normal-phase silica gel (35–60 micron) columns and UV detection at 254 nm. Preparative reversed-phase HPLC was performed using a Shimadzu Prominence system and Phenomenex Gemini C18 column (30 μm, 150 × 30 mm I.D.), eluting with a binary solvent system (A: H_2O with 0.1% TFA; B: CH_3CN with 0.1% TFA; gradient elution) with UV detection at 254 nm. All final compounds were purified to ≥95% purity as determined by Agilent 1100 Series high performance liquid chromatography (HPLC) with UV detection at 254 nm using one of the following methods: Method **A**: Zorbax SB-C8 column (150 × 4.6 mm, 3.5 μm), mobile phase: A = H_2O with 0.1% TFA, B = CH_3CN with 0.1% TFA; gradient: 5–95% B (0.0–15.0 min); flow rate: 1.5 mL/min. Method **B**: Zorbax analytical C18 column (50 × 3 mm, 3.5 μm, 40 °C); mobile phase: A = H_2O with 0.1% TFA, B = CH_3CN with 0.1% TFA; gradient: 5–95% B (0.0–3.6 min); flow rate: 1.5 mL/min. Method **C**: YMCODS-AM (100 × 2.1 mm, 5 μm, 40 °C); mobile phase: A = H_2O with 0.1% HCO_2H, B = CH_3CN with 0.1% HCO_2H; gradient: 10% B (0.0–0.5 min), 10–100% B (0.5–7.0 min), 100% B (7.0–10 min); flow rate: 0.5 mL/min. Method **D**: Phenomenex Synergi MAX-RP (50 × 2.0 mm, 4.0 μm, 40 °C); mobile phase: A = H_2O with 0.1% TFA, B = CH_3CN with 0.1% TFA; gradient: 10% B (0.0–0.2 min), 10–100% B (0.2–3.0 min), 100% B (3.0–4.5 min), 100–10% B (4.5–5.0 min); flow rate: 0.8 mL/min. Low-resolution mass spectral (MS) data were obtained using an Agilent G1956B mass spectrometer operated in electrospray ionization (ESI) mode (positive or negative). NMR spectra were obtained at ambient temperature with a Bruker Avance II spectrometer operating at 300 or 400 MHz. Chemical shifts are reported in ppm downfield of an internal standard, tetramethylsilane (δ 0.00 ppm). Data are reported as follows: chemical shift, number of protons, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), and coupling constants.

D-*erythro*-Sphingosine-1-phosphate (S1P; **1**) (CAS# 26993-30-6, Catalog #567727; >95%) was purchased from Calbiochem (San Diego, CA) and used as received.

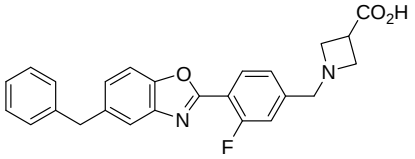
Fingolimod (FTY-720; **2**) (Catalog #344597; >98%) was purchased from Calbiochem (San Diego, CA) and used as received.

Synthesis of **3**



1-(4-(5-Benzylbenzofuran-2-yl)-3-fluorobenzyl)azetidine-3-carboxylic acid (**3**) was prepared according to the procedure reported in PCT Int. Appl. WO 2007/109334 (Compound 15).

Synthesis of **4**



1-(4-(5-Benzylbenzo[d]oxazol-2-yl)-3-fluorobenzyl)azetidine-3-carboxylic acid (**4**)

Step 1:

To a solution of 4-benzylphenol (10.0 g, 54.3 mmol) in AcOH (300 mL) at ambient temperature was slowly added (dropwise) a solution of red fuming nitric acid (2.28 mL, 54.3 mmol) in AcOH (100 mL) over 2 h. The resulting mixture was allowed to stir for 3 h and was judged complete by TLC. The reaction was then poured onto ice, and the resulting solid was collected by vacuum filtration, rinsing with water to give 4-benzyl-2-nitrophenol (10.8 g, 86.8% yield) as a yellow solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.74 (1H, s), 7.74 (1H, d, J = 1.5 Hz), 7.14–7.52 (6H, m), 7.06 (1H, d, J = 8.5 Hz), 3.92 (2H, s). MS (ESI) *m/z*: calculated: 229.1; observed: 227.8 $(M-1)^-$.

Step 2:

10% Palladium on carbon (50% wet with water; 2.3 g, 2.2 mmol) and 4-benzyl-2-nitrophenol (5.00 g, 22 mmol) were combined under nitrogen and diluted with MeOH (80 mL) in a pressure vessel. The vessel was pressurized with hydrogen gas (40 psi) and shaken in a Parr shaker for approximately 24 h. The resulting mixture was filtered through Celite®, rinsing with MeOH. The filtrate was concentrated in vacuo to give 2-amino-4-benzylphenol (3.0 g, 69% yield) as a brown solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.74 (1H, br s), 7.07–7.33 (5H, m), 6.53 (1H, d, J = 8.0 Hz), 6.41 (1H, d, J = 2.0 Hz), 6.25 (1H, dd, J = 7.8, 1.8 Hz), 4.41 (2H, br s), 3.69 (2H, s). MS (ESI) *m/z*: calculated: 199.1; observed: 200.0 $(M+1)^+$.

Step 3:

To a mixture of 2-fluoro-4-formylbenzoic acid (**31**; 0.600 g, 3.57 mmol) in DCM (15 mL) was added oxalyl chloride (0.374 mL, 4.28 mmol) and *N*,*N*-dimethylformamide (0.00261 g, 0.0357 mmol) (a few drops). The resulting mixture was allowed to stir for several hours. After TLC analysis of a MeOH-quenched aliquot, additional oxalyl

chloride (0.100 mL) was added. The reaction mixture was then stirred for 1 h and then concentrated in vacuo. The residual semi-solid was suspended in THF (15 mL). *N*,*N*-diisopropylethylamine (0.808 mL, 4.64 mmol) and 2-amino-4-benzylphenol (0.711 g, 3.57 mmol) were sequentially added, and the resulting mixture was stirred at 25 °C for 4 h. EtOAc, water, and 1N aqueous HCl were added to the resulting mixture, and the organic layer was washed with brine, dried over sodium sulfate, filtered, and concentrated in vacuo. Chromatographic purification of the residue (silica gel) gave *N*-(5-benzyl-2-hydroxyphenyl)-2-fluoro-4-formylbenzamide (0.551 g, 44.2% yield) as a solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.07 (1H, s), 9.79–9.88 (1H, m), 9.62 (1H, d, J = 6.0 Hz), 8.01 (1H, dd, J = 7.3, 7.3 Hz), 7.77–7.92 (3H, m), 7.08–7.38 (5H, m), 6.79–6.93 (2H, m), 3.86 (2H, s). MS (ESI) *m/z*: calculated: 349.1; observed: 347.7 $(M-1)^-$.

Step 4:

A mixture of *N*-(5-benzyl-2-hydroxyphenyl)-2-fluoro-4-formylbenzamide (0.500 g, 1.43 mmol) and *p*-toluenesulfonic acid monohydrate (0.817 g, 4.29 mmol) in toluene (14 mL) was heated under nitrogen at 115 °C in a flask fitted with a water-cooled reflux condenser. After 3 h, the reaction mixture was cooled to 25 °C and diluted with DCM and MeOH. SiliaBond® Carbonate (derivatized silica gel, Silicycle®; 15 g) was added, and the resulting mixture was concentrated in vacuo, then loaded onto a silica gel chromatography column and eluted with 0–50% EtOAc/hexanes to provide 5-benzyl-2-(4-(dimethoxymethyl)-2-fluorophenyl)benzo[d]oxazole (0.160 g, 29.6% yield) as an off-white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.22 (1H, dd, J = 7.8, 7.8 Hz), 7.68–7.76 (2H, m), 7.38–7.50 (2H, m), 7.26–7.37 (5H, m), 7.15–7.23 (1H, m), 5.50 (1H, s), 4.10 (2H, s), 3.30 (6H, s). MS (ESI) *m/z*: calculated: 377.1; observed: 378.1 $(M+1)^+$.

Step 5:

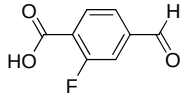
5-Benzyl-2-(4-(dimethoxymethyl)-2-fluorophenyl)benzo[d]oxazole (0.160 g, 0.424 mmol) was dissolved in THF (2 mL) and 5N aqueous HCl (1 mL) was added. The resulting mixture was stirred at ambient temperature for 1 h and then was diluted with DCM. 10N aqueous NaOH was added to adjust the pH > 7. The reaction mixture was then extracted with DCM (2×). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated to give 4-(5-benzylbenzo[d]oxazol-2-yl)-3-fluorobenzaldehyde (0.134 g, 95.4% yield) as an orange solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.09 (1H, s), 8.43 (1H, dd, J = 7.3, 7.3 Hz), 7.90–8.04 (2H, m), 7.72–7.83 (2H, m), 7.38 (1H, d, J = 8.5 Hz), 7.26–7.33 (4H, m), 7.17–7.24 (1H, m), 4.11 (2H, s).

Step 6:

To a slightly cloudy mixture of 4-(5-benzylbenzo[d]oxazol-2-yl)-3-fluorobenzaldehyde (0.133 g, 0.40 mmol) in 1:1 DCM/MeOH (4 mL) was added azetidine-3-carboxylic acid (0.061 g, 0.60 mmol) and acetic acid (0.046 mL, 0.80 mmol). The resulting mixture was stirred at 25 °C for 1 h. Sodium cyanoborohydride (0.013 g, 0.20 mmol) was then added,

and the resulting mixture stirred vigorously for 2 d. The reaction mixture was then vacuum filtered, and the collected solid was rinsed with DCM (2 × 1 mL). The washed solid was then suspended in 1M aqueous sodium phosphate buffer (2 mL; pH 6), sonicated, and collected by vacuum filtration, rinsing with water followed by EtOH, to give 1-(4-(5-benzylbenzo[d]oxazol-2-yl)-3-fluorobenzyl)azetidine-3-carboxylic acid (0.079 g, 47% yield) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.12 (1H, dd, J = 7.8, 7.8 Hz), 7.73 (1H, d, J = 8.0 Hz), 7.69 (1H, s), 7.26–7.40 (7H, m), 7.15–7.26 (1H, m), 4.11 (2H, s), 3.65 (2H, s), 3.40–3.49 (2H, m), 3.18–3.37 (3H, m). MS (ESI) *m/z*: calculated: 416.2; observed: 417.0 $(M+1)^+$.

Synthesis of **31**



2-Fluoro-4-formylbenzoic acid (**31**)

Step 1:

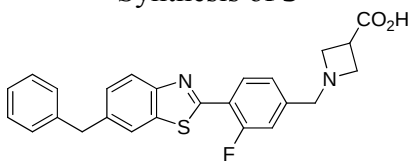
Acetyl chloride (2 mL, 28.3 mmol) was added to a solution of 4-bromo-3-fluorobenzaldehyde (3B Scientific Corporation, Libertyville, IL; 14.2 g, 69.9 mmol) in EtOH (80 mL), and the resulting mixture was heated at reflux for 5 h. The reaction mixture was then concentrated in vacuo, and additional EtOH (50 mL), acetyl chloride (1.0 mL, 14.1 mmol), and triethylorthoformate (5 mL, 30.1 mmol) were sequentially added. The resulting mixture was heated at 70°C for 30 min, then cooled to 25 °C and diluted with EtOAc (500 mL). The resulting mixture was sequentially washed with saturated aqueous $NaHCO_3$ (250 mL) and brine (200 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to provide 1-bromo-4-(diethoxymethyl)-2-fluorobenzene (17.97 g, 93% yield) as a yellow oil: ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 7.52 (1H, dd, J = 8.2, 7.0 Hz), 7.25–7.29 (1H, m), 7.14 (1H, dd, J = 8.2, 2.0 Hz), 5.46 (1H, s), 3.48–3.63 (4H, m), 1.23 (6H, t, J = 7.0 Hz).

Step 2:

n-Butyllithium (2.5M in hexanes; 13.2 mL, 33.0 mmol) was added to a solution of 1-bromo-4-(diethoxymethyl)-2-fluorobenzene (7.63 g, 27.5 mmol) in THF (100 mL) at –78 °C, and the resulting mixture was stirred under argon at –78 °C for 25 min. Solid carbon dioxide (finely chopped, ca. 5 g) was then added, the cooling bath was removed, and the resulting mixture was stirred for 30 min. 1N aqueous NaOH (30 mL) was then added, and the resulting mixture was washed with EtOAc (2 × 20 mL). The aqueous layer was acidified with 1N aqueous HCl (final pH ~2), and the resulting mixture was extracted with EtOAc (2 × 60 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated in vacuo. Ethyl ether (15 mL), water (1 mL), and TFA (1 mL) were sequentially added to the residue, and the resulting mixture was stirred at 25 °C for 12 h. The resulting mixture was then concentrated in vacuo, and the residue was azeotropically dried by concentration from toluene (2 × 10 mL) to provide 2-fluoro-4-

formylbenzoic acid (3.17 g, 68%) as an off-white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 13.68 (1H, br s), 10.06 (1H, d, J = 1.4 Hz), 8.06 (1H, t, J = 7.4 Hz), 7.77–7.86 (2H, m). MS (ESI) *m/z*: calculated: 168.0; observed: 169.0 $(M+1)^+$.

Synthesis of **5**



1-(4-(6-Benzylbenzo[d]thiazol-2-yl)-3-fluorobenzyl)azetidine-3-carboxylic acid (**5**)

Step 1:

Bromine (2.93 mL, 57.2 mmol) was added dropwise to a mixture of 4-benzylbenzenamine (Alfa Aesar, Ward Hill, MA; 10.37 g, 56.6 mmol) and ammonium thiocyanate (8.63 g, 113.4 mmol) in AcOH (100 mL) at 12 °C (temperature kept below 18 °C during addition), and the resulting mixture was stirred at 25 °C for 2 h. The reaction mixture was then partially concentrated in vacuo, and the precipitated solid was collected by vacuum filtration. The collected solid was partially dissolved in EtOAc (400 mL) at reflux temperature, and the resulting mixture was cooled to 50 °C. The undissolved solid was collected by vacuum filtration, washed with cold EtOAc (100 mL), and dried in vacuo. The collected light-yellow solid was then suspended in acetone (300 mL), warmed to 40 °C, and stirred for 5 min. The undissolved solid was collected by vacuum filtration, washed with cold acetone (50 mL), and dried in vacuo to provide 6-benzylbenzo[d]thiazol-2-amine (5.00 g, 37% yield) as a beige solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.96 (2H, br s), 7.68 (1H, s), 7.34–7.38 (1H, m), 7.26–7.32 (3H, m), 7.22–7.26 (2H, m), 7.16–7.21 (1H, m), 3.98 (2H, s). MS (ESI) *m/z*: calculated: 240.1; observed: 241.1 $(M+1)^+$.

Step 2:

A solution of potassium hydroxide (12.3 g, 219.2 mmol) in water (15 mL) and 6-benzylbenzo[d]thiazol-2-amine (3.00 g, 12.5 mmol) were sequentially added to ethylene glycol (8 mL), and the resulting mixture was stirred under nitrogen at 135 °C for 6 h. Ice (100 mL) was then added, and the resulting mixture was acidified with 5.0N aq. HCl to a final pH of 6. The resulting suspension was extracted with DCM (2 × 200 mL), and the combined organic extracts were washed with brine (150 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to afford a 2:1 mixture of 2,2'-disulfanediylbis(4-benzylaniline) and 2-amino-5-benzylbenzenethiol as a beige solid (1.81 g). MS (ESI) *m/z*: 2,2'-disulfanediylbis(4-benzylaniline): calculated: 428.1; observed: 429.2 $(M+1)^+$; 2-amino-5-benzylbenzenethiol: calculated: 215.1; observed: 216.1 $(M+1)^+$.

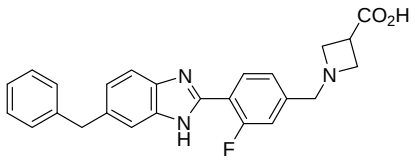
Step 3:

Oxalyl dichloride (286 μL, 3.23 mmol) and *N*,*N*-dimethylformamide (30 μL) were sequentially added to a suspension of 2-fluoro-4-formylbenzoic acid (**31**; 0.543 g, 3.23 mmol) in DCM (10 mL) at 25 °C, and the resulting mixture was stirred at 25 °C for 2 h. The reaction mixture was then concentrated in vacuo, and the residue was taken up in THF (5 mL). *N*,*N*-Diisopropylethylamine (0.844 mL, 4.84 mmol) and a solution of (2:1) 2,2'-disulfanediylbis(4-benzylaniline) / 2-amino-5-benzylbenzenethiol (0.695 g, 3.23 mmol; from Step 2) in THF (5 mL) were then sequentially added, and the resulting mixture was stirred at 25 °C for 17 h. The reaction mixture was then diluted with EtOAc (100 mL), and the resulting mixture was sequentially washed with sat. aq. $NaHCO_3$ (80 mL) and brine (50 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to provide crude *N*,*N*'-(2,2'-disulfanediylbis(4-benzyl-2,1-phenylene))bis(2-fluoro-4-formylbenzamide) (0.916 g) as a red foam. EtOH (16 mL), water (2 mL), conc. HCl (8 mL), and tin(II) chloride (1.67 g, 8.80 mmol) were sequentially added to this material, and the resulting mixture was heated at reflux for 6.5 h. The reaction mixture was then cooled to 0 °C and 10N aq. NaOH was added to adjust the pH of the reaction mixture to pH 10. The resulting mixture was partitioned between water (50 mL) and DCM (120 mL), and the organic layer was separated. The aqueous layer was then extracted with DCM (3 × 50 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. Chromatographic purification of the residue (silica gel, 0–20% EtOAc/hexanes) furnished 4-(6-benzylbenzo[d]thiazol-2-yl)-3-fluorobenzaldehyde (200 mg, 23% yield) as a yellow solid: ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 10.06 (1H, d, *J* = 1.8 Hz), 8.64–8.69 (1H, m), 8.09 (1H, d, *J* = 8.4 Hz), 7.83 (1H, dd, *J* = 8.0, 1.6 Hz), 7.72–7.77 (2H, m), 7.41 (1H, dd, *J* = 8.4, 1.6 Hz), 7.29–7.36 (2H, m), 7.23 (3H, d, *J* = 7.8 Hz), 4.16 (2H, s). MS (ESI) *m/z*: calculated: 347.1; observed: 348.1 $(M+1)^+$.

Step 4:

A mixture of 4-(6-benzylbenzo[d]thiazol-2-yl)-3-fluorobenzaldehyde (190 mg, 547 μmol), azetidine-3-carboxylic acid (166 mg, 1641 μmol), and acetic acid (0.095 mL, 1641 μmol) in a mixture of DCM (2.0 mL) and MeOH (2.0 mL) was stirred at 25 °C for 1 h. Sodium cyanoborohydride (34 mg, 547 μmol) was then added, and the resulting mixture was stirred at 25 °C for 12 h. The reaction mixture was then filtered through a glass frit, and the collected solids were sequentially washed with DCM (3 × 8 mL), suspended in aqueous phosphate buffer solution (pH = 6), and sonicated for 10 min. The resulting mixture was vacuum filtered, and the collected solid was washed with water (10 mL) and dried in vacuo to provide 1-((4-(6-benzylbenzo[d]thiazol-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylic acid (25.0 mg, 11% yield) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.27 (1H, t, *J* = 8.0 Hz), 8.04 (1H, s), 8.01 (1H, d, *J* = 8.4 Hz), 7.44 (1H, dd, *J* = 8.1, 1.5 Hz), 7.32–7.37 (2H, m), 7.27–7.32 (4H, m), 7.17–7.23 (1H, m), 4.11 (2H, s), 3.82 (1H, m), 3.63 (2H, s), 3.41 (2H, t, *J* = 7.1 Hz), 3.21 (2H, t, *J* = 6.6 Hz). MS (ESI) *m/z*: calculated: 432.1; observed: 433.2 $(M+1)^+$.

Synthesis of **6**



1-(4-(6-Benzyl-1H-benzo[d]imidazol-2-yl)-3-fluorobenzyl)azetidine-3-carboxylic acid (**6**)

Step 1:

4-Aminodiphenylmethane (5.00 g, 27 mmol) was added in portions to acetic anhydride (26 mL, 273 mmol) with rapid stirring. A solid mass formed which was dissolved by the addition of additional acetic anhydride (~15 mL). The reaction mixture was allowed to cool to ambient temperature, and nitric acid (2.0 mL, 41 mmol) was added slowly (dropwise, via addition funnel) over 30 min. The resulting homogeneous reaction was allowed to stir for 12 h. The reaction was then poured into a rapidly stirred solution of water (30 mL), conc. HCl (7 mL), and EtOH (24 mL) (CAUTION - EXOTHERM), and the flask was fitted with a reflux condenser. The reaction was subsequently heated at reflux for 4 h. The reaction was then cooled to ambient temperature, poured onto ice, and treated with 10N aqueous NaOH to a final pH of 8–9. The resulting mixture was extracted with DCM (3×), and the combined extracts were dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. Chromatographic purification of the resulting dark red oil (silica gel, 0–20% EtOAc/hexanes) provided 2.3 g of an impure red oil containing 4-benzyl-2-nitrobenzenamine, which was used without further purification: MS (ESI) *m/z*: calculated: 228.1; observed: 229.0 $(M+1)^+$.

Step 2:

2-Fluoro-4-formylbenzoic acid (**31**; 0.300 g, 1.78 mmol) was slurried in DCM (10 mL) and DMF (ca. 20–50 μL; 1-2 drops) was added, followed by oxalyl chloride (0.317 mL, 3.57 mmol). The resulting mixture was allowed to stir for 3 h, and the reaction was then concentrated in vacuo and diluted with THF (7 mL). A portion of impure 4-benzyl-2-nitrobenzenamine (0.611 g; from Step 1) and diisopropylethylamine (0.622 mL, 3.57 mmol) were then added as a solution in THF (5 mL), and the resulting mixture was stirred at ambient temperature for 12 h. The reaction was subsequently partitioned between EtOAc and saturated aqueous sodium bicarbonate. The organic layer was separated, dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was treated with *p*-toluenesulfonic acid monohydrate (0.170 g, 0.892 mmol) and MeOH (10 mL). After 1 h, the reaction was adsorbed onto silica gel and purified by silica gel chromatography to give *N*-(4-benzyl-2-nitrophenyl)-4-(dimethoxymethyl)-2-fluorobenzamide (0.466 g, 62% yield): ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.81 (1H, d, J = 3.0 Hz), 7.92 (1H, s), 7.76–7.84 (2H, m), 7.64 (1H, dd, J = 8.5, 1.5 Hz), 7.14–7.42 (7H, m), 5.48 (1H, s), 4.06 (2H, s), 3.28 (6H, s). MS (ESI) *m/z*: calculated: 424.1; observed: 425.2 $(M+1)^+$.

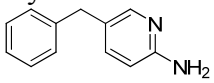
Step 3:

To a solution of *N*-(4-benzyl-2-nitrophenyl)-4-(dimethoxymethyl)-2-fluorobenzamide (0.466 g, 1.1 mmol) in 3:2 AcOH/EtOH (5 mL) was added iron powder (325 mesh; Aldrich, St. Louis, MO; 0.078 mL, 11 mmol). The reaction flask was fitted with a reflux condenser, and the reaction was stirred rapidly and heated to 120 °C. After 10 min, additional 2:1 AcOH/EtOH (3 mL) was added to promote stirring. After 3 h, the mixture was diluted with water, EtOAc, and brine, and the organic layer was separated. The aqueous layer was extracted with EtOAc (2×), and the combined organic extracts were sequentially washed with 1N aqueous NaOH (1×) and brine (1×), dried over sodium sulfate, filtered, and concentrated in vacuo. Chromatographic purification of the residue (silica gel, 0–4% MeOH/DCM) gave 0.064 g of impure material. To this material was added triethylamine (0.12 mL, 0.83 mmol) and 1:1 DCM/DMSO (1.5 mL), and the resulting mixture was cooled to 0 °C. A solution of sulfur trioxide–pyridine complex (0.13 g, 0.83 mmol) in DMSO (0.75 mL) was then added, and the resulting mixture was stirred under nitrogen atmosphere for 2 h. The reaction mixture was then diluted with EtOAc and water. The organic layer was separated and sequentially washed with water (1×) and brine (1×), dried over sodium sulfate, filtered, and concentrated in vacuo. Chromatographic purification of the residue (silica gel 0–40% EtOAc/hexanes) provided 4-(5-benzyl-1H-benzo[d]imidazol-2-yl)-3-fluorobenzaldehyde (0.048 g, 14% yield) as a light yellow oil: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.68 (1H, br s), 10.06 (1H, s), 8.45 (1H, dd, J = 7.5, 7.5 Hz), 7.84–8.02 (2H, m), 7.59 (1H, d, J = 7.0 Hz), 7.48 (1H, br s), 7.23–7.34 (4H, m), 7.12–7.22 (2H, m), 4.08 (2H, s). MS (ESI) *m/z*: calculated: 330.1; observed: 331.0 $(M+1)^+$.

Step 4:

A slurry of 4-(5-benzyl-1H-benzo[d]imidazol-2-yl)-3-fluorobenzaldehyde (0.048 g, 0.15 mmol), azetidine-3-carboxylic acid (0.044 g, 0.44 mmol), and glacial acetic acid (0.025 mL, 0.44 mmol) in 1:1 MeOH/DCM (2 mL) was rapidly stirred at 25 °C for 1 h. Sodium cyanoborohydride (0.0076 mL, 0.15 mmol) was then added, and the reaction was allowed to stir for 12 h. The resulting mixture was vacuum filtered, and the collected solid was rinsed with DCM. The filtrate was concentrated in vacuo and the residue was sonicated in DCM. The resulting suspension was vacuum filtered, and the collected solid was rinsed with DCM. The collected solids were subsequently combined and suspended in a small volume of 1M aqueous sodium phosphate buffer (pH 6). The resulting suspension was filtered, and the collected solid was rinsed with water and dried in vacuo to give 1-(4-(5-benzyl-1H-benzo[d]imidazol-2-yl)-3-fluorobenzyl)azetidine-3-carboxylic acid (0.011 g, 18% yield) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.41 (1H, d, J = 19.1 Hz), 8.12 (1H, dd, J = 7.8, 7.8 Hz), 7.42–7.61 (2H, m), 7.22–7.38 (6H, m), 7.06–7.22 (2H, m), 4.05 (2H, m), 3.62 (2H, br s), 3.17–3.43 (5H, m). MS (ESI) *m/z*: calculated: 415.2; observed: 416.2 $(M+1)^+$.

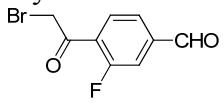
Synthesis of **32**



5-Benzylpyridin-2-amine (**32**)

9-Benzyl-9-bora-bicyclo[3.3.1]nonane (12.73 mL, 6.36 mmol) was added to a suspension of 5-iodopyridin-2-amine (700 mg, 3.18 mmol), potassium phosphate (2.03 g, 9.55 mmol), Pd_2dba_3 (58 mg, 64 μmol), and 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (XPhos; 61 mg, 127 μmol) in water (1 mL) in a microwave process vial. The tube was flushed with argon gas and then sealed and heated (microwave) at 120 °C for 30 min. After cooling to 25 °C, the reaction mixture was diluted with EtOAc (200 mL) and sequentially washed with 1M aqueous NaOH (100 mL) and brine (80 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated in vacuo. Chromatographic purification of the residue (silica gel, 0–10% MeOH/CH_2Cl_2) provided 5-benzylpyridin-2-amine•9-BBN adduct as a yellow oil, which was taken up in MeOH (5 mL). Conc. HCl (300 μL) was added, and the resulting solution was stirred at 25 °C for 10 min. NaOH (120 mg) was then added to neutralize HCl, and the resulting solution was concentrated silica gel. Chromatographic purification (silica gel, 0–10% MeOH/CH_2Cl_2) furnished 5-benzylpyridin-2-amine (347.7 mg, 59% yield) as a yellow solid: ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 7.95 (1H, s), 7.25–7.30 (2H, m), 7.20–7.24 (1H, m), 7.14–7.20 (3H, m), 6.44 (1H, d, *J* = 8.5 Hz), 4.34 (2H, br s), 3.83 (2H, s)

Synthesis of **33**



4-(2-Bromoacetyl)-3-fluorobenzaldehyde (**33**)

Step 1:

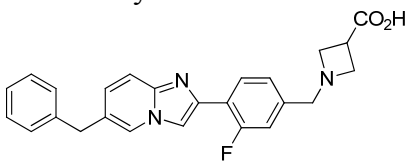
To a solution of 4-bromo-3-fluorobenzaldehyde (3B Scientific Corporation, Libertyville, IL; 1.00 g, 4.9 mmol) in dioxane (10.0 mL) was added tributyl(1-ethoxyvinyl)stannane (1.7 mL, 5.2 mmol) and $Pd(PPh_3)_2Cl_2$ (0.069 g, 0.099 mmol). The resulting solution was purged with argon for 2 min, then sealed and heated (microwave) at 130 °C for 30 min. After cooling to 25 °C, the reaction mixture was filtered through a plug of silica gel, eluting with EtOAc (80 mL). The filtrate was then concentrated in vacuo and chromatographically purified (silica gel, 0–30% EtOAc/hexanes) to provide 4-(1-ethoxyvinyl)-3-fluorobenzaldehyde (0.881 g, 92% yield) as a light-yellow oil: ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 9.98 (1H, d, *J* = 1.0 Hz), 7.81 (1H, t, *J* = 7.5 Hz), 7.66 (1H, s), 7.58 (1H, s), 4.90 (1H, d, *J* = 2.0 Hz), 4.58 (1H, br s), 3.94 (2H, q, *J* = 7.0 Hz), 1.42 (3H, t, *J* = 6.8 Hz).

Step 2:

N-Bromosuccinimide (505.0 mg, 2.84 mmol) was added in one portion to a solution of 4-(1-ethoxyvinyl)-3-fluorobenzaldehyde (551.0 mg, 2.84 mmol) in 3:1 THF:H_2O (6.0 mL) at 25 °C, and the resulting solution was stirred at 25 °C for 10 min. The reaction solution was then partitioned between ethyl acetate (50 mL) and brine (8 mL). The organic layer

was separated, dried over sodium sulfate, and concentrated in vacuo. Chromatographic purification of the residue (silica gel, 0–100% EtOAc/hexanes) furnished 4-(2-bromoacetyl)-3-fluorobenzaldehyde (453.4 mg, 65% yield) as a white solid: ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 10.08 (1H, d, *J* = 1.8 Hz), 8.09 (1H, s), 7.79 (1H, dd, *J* = 8.0, 1.4 Hz), 7.69 (1H, dd, *J* = 10.6, 1.4 Hz), 4.52 (2H, d, *J* = 2.3 Hz).

Synthesis of **7**



1-(4-(6-Benzylimidazo[1,2-a]pyridin-2-yl)-3-fluorobenzyl)azetidine-3-carboxylic acid (**7**)

Step 1:

A solution of 5-benzylpyridin-2-amine (**32**; 340.9 mg, 1.85 mmol) and 4-(2-bromoacetyl)-3-fluorobenzaldehyde (**33**; 453.4 mg, 1.85 mmol) in ethanol (6.0 mL) was heated at reflux for 5 hr. The reaction mixture was then cooled to 25 °C, triethylamine (260 μL) was added, and the resulting solution was concentrated onto silica gel. Chromatographic purification (silica gel, 0–100% EtOAc/hexanes (+2% triethylamine, both solvents)) furnished 6-benzyl-2-(4-(diethoxymethyl)-2-fluorophenyl)H-imidazo[1,2-a]pyridine (201.2 mg, 27% yield) as an orange oil: MS (ESI) *m/z*: calculated: 404.2; observed: 405.2 $(M+1)^+$.

Step 2:

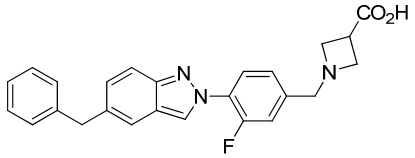
2.0N aqueous HCl (1.24 mL, 2.48 mmol) was added to a solution of 6-benzyl-2-(4-(diethoxymethyl)-2-fluorophenyl)H-imidazo[1,2-a]pyridine (201.2 mg, 497 μmol) in THF (10.0 mL) at 25 °C, and the resulting mixture was stirred at 25 °C for 20 min. 1N aqueous NaOH (2.58 mL, 2.58 mmol) was then added, and the resulting mixture was partitioned between EtOAc (40 mL) and brine (5 mL). The organic layer separated, dried over sodium sulfate, filtered, and concentrated in vacuo. Chromatographic purification of the residue (silica gel, 0–100% EtOAc/hexanes (+2% triethylamine, both solvents)) furnished 4-(6-benzylH-imidazo[1,2-a]pyridin-2-yl)-3-fluorobenzaldehyde (100.5 mg, 61% yield) as a yellow oil: ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 10.00 (1H, s), 8.54 (1H, t, *J* = 7.5 Hz), 8.08 (1H, d, *J* = 4.0 Hz), 7.89 (1H, s), 7.77 (1H, d, *J* = 8.0 Hz), 7.65 (1H, d, *J* = 11.5 Hz), 7.57 (1H, d, *J* = 9.0 Hz), 7.31–7.38 (2H, m), 7.28 (1H, d, *J* = 7.0 Hz), 7.23 (2H, d, *J* = 7.5 Hz), 7.09 (1H, d, *J* = 9.0 Hz), 3.97 (2H, s). MS (ESI) *m/z*: calculated: 330.1; observed: 331.2 $(M+1)^+$.

Step 3:

A mixture of 4-(6-benzylH-imidazo[1,2-a]pyridin-2-yl)-3-fluorobenzaldehyde (100.5 mg, 304 μmol), azetidine-3-carboxylic acid (92.3 mg, 913 μmol), and acetic acid (52.7 μl,

913 μmol) in a mixture of DCM (2.0 mL) and MeOH (2.0 mL) was stirred at 25 °C for 1 h. Sodium cyanoborohydride (19.1 mg, 304 μmol) was then added, and the resulting mixture was stirred at 25 °C for 2.5 d. The reaction mixture was then diluted DCM (2 mL), and the resulting slurry was filtered on a glass frit, rinsing with DCM (5 mL). The filtrate was concentrated in vacuo, and the residue was taken up in MeOH (3.0 mL), filtered through a cotton plug, and purified by rpHPLC (Phenomenex C18, 5–100% CH_3CN/H_2O (+0.1% TFA, both solvents), 120 mL/min) to afford 1-(4-(6-benzylH-imidazo[1,2-a]pyridin-2-yl)-3-fluorobenzyl)azetidine-3-carboxylic acid • 2,2,2-trifluoroacetic acid (100 mg, 62% yield) as a colorless oil: ^{1}H NMR (400 MHz, MeOH-d_4) δ ppm 8.66 (1H, s), 8.59 (1H, s), 8.03–8.11 (1H, m), 7.87 (2H, s), 7.53–7.62 (2H, m), 7.31–7.40 (4H, m), 7.24–7.30 (1H, m), 4.55 (2H, s), 4.35–4.48 (4H, m), 4.18 (2H, s), 3.76 (1H, quin, J = 8.3 Hz). MS (ESI) *m/z*: calculated: 415.2; observed: 416.4 $(M+1)^+$.

Synthesis of **8**



1-(4-(5-Benzyl-2H-indazol-2-yl)-3-fluorobenzyl)azetidine-3-carboxylic acid (**8**)

Step 1:

3-Bromobenzaldehyde (11.71 mL, 100 mmol) was added dropwise to mixture of concentrated nitric acid (10 mL) and concentrated sulfuric acid (120 mL) at 5 °C. The resulting mixture was allowed to warm to 25 °C and stir for 20 hours. The reaction mixture was then poured onto ice, and the precipitated solid was collected by vacuum filtration. The collected solid was dissolved in dichloromethane (150 mL), dried over sodium sulfate, and concentrated in vacuo. Chromatographic purification of the residue (silica gel, 0–70% ethyl acetate/hexanes) furnished 5-bromo-2-nitrobenzaldehyde (10.81 g, 47% yield) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.42 (s, 1H), 8.07 (d, J = 2.0 Hz, 1H), 8.03 (d, J = 8.6 Hz, 1H), 7.88 (dd, J = 2.0, 8.6 Hz, 1H).

Step 2:

A mixture of 5-bromo-2-nitrobenzaldehyde (2.79 g, 12.13 mmol) and ethyl 4-amino-3-fluorobenzoate (Oakwood Products, West Columbia, SC; 2.22 g, 12.12 mmol) in ethanol (60 mL) was stirred at reflux for 2 hours. The reaction mixture was then concentrated in vacuo, and the residue was purified by column chromatography (silica gel, 0–80% ethyl acetate/hexanes) to provide (*E*)-ethyl 4-(5-bromo-2-nitrobenzylideneamino)-3-fluorobenzoate (1.63 g, 34% yield) as a pale yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.01 (s, 1H), 8.47 (d, J = 2.0 Hz, 1H), 8.02 (d, J = 8.6 Hz, 1H), 7.91 (dd, J = 1.6, 2.3 Hz, 1H), 7.85 (dd, J = 1.6, 11.0 Hz, 1H), 7.80 (dd, J = 2.3, 8.6 Hz, 1H), 7.24 (t, J = 8.0 Hz, 1H), 4.40 (q, J = 7.0 Hz, 2H), 1.42 (t, J = 7.0 Hz, 3H). ^{19}F NMR (376 MHz, $CDCl_3$) δ –125.6.

Step 3:

A mixture of (*E*)-ethyl 4-(5-bromo-2-nitrobenzylideneamino)-3-fluorobenzoate (432 mg, 1.09 mmol) and triethyl phosphate (1.5 mL, 9.0 mmol) was heated (microwave) at 150 °C for 1.5 hours. The cooled reaction mixture was then purified by column chromatography (silica gel, 0–75% ethyl acetate/hexanes) to furnish ethyl 4-(5-bromo-2H-indazol-2-yl)-3-fluorobenzoate (185 mg, 0.51 mmol, 47% yield) as a pale yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (d, *J* = 2.0 Hz, 1H), 8.25 (t, *J* = 7.8 Hz, 1H), 8.02–7.90 (m, 2H), 7.89 (d, *J* = 0.8 Hz, 1H), 7.66 (d, *J* = 9.2 Hz, 1H), 7.40 (dd, *J* = 1.6, 9.2 Hz, 1H), 4.43 (q, *J* = 7.2 Hz, 2H), 1.44 (t, *J* = 7.2 Hz, 3H); ^{19}F NMR (376 MHz, $CDCl_3$) δ –123.4. MS (ESI) *m/z*: calculated: 362.0; observed: 363.2 $(M+1)^+$.

Step 4:

A mixture of ethyl 4-(5-bromo-2H-indazol-2-yl)-3-fluorobenzoate (135 mg, 0372 mmol), benzylzinc(II) bromide (0.5 M in THF; 2.16 mL, 1.08 mmol) and Pd(*t*-$Bu_3P)_2$ (9.5 mg, 19 μmol) in a microwave reaction tube was sparged with N_2(g) for 3 minutes and then heated (microwave) at 100 °C for 30 minutes. The reaction mixture was then diluted with ethyl acetate (100 mL), sequentially washed with 1N aqueous HCl (10 mL) and brine (20 mL), and filtered through Celite. The filtrate was dried over Na_2SO_4 and concentrated in vacuo. Chromatographic purification of the residue (silica gel, 0–80% ethyl acetate/hexanes) furnished a 3:1 mixture (171 mg) of ethyl 4-(5-benzyl-2H-indazol-2-yl)-3-fluorobenzoate and benzyl 4-(5-benzyl-2H-indazol-2-yl)-3-fluorobenzoate ethyl 4-(5-bromo-2H-indazol-2-yl)-3-fluorobenzoate (135 mg, 0372 mmol): Ethyl 4-(5-benzyl-2H-indazol-2-yl)-3-fluorobenzoate: MS (ESI) *m/z*: calculated: 374.1; observed: 375.3 $(M+1)^+$. Benzyl 4-(5-benzyl-2H-indazol-2-yl)-3-fluorobenzoate: MS (ESI) *m/z*: calculated: 436.2; observed: 437.3 $(M+1)^+$.

Step 5:

DIBAL-H (1.0M in dichloromethane; 1.37 mL, 1.37 mmol) was slowly added to a 3:1 mixture (171 mg) of ethyl 4-(5-benzyl-2H-indazol-2-yl)-3-fluorobenzoate and benzyl 4-(5-benzyl-2H-indazol-2-yl)-3-fluorobenzoate in dichloromethane (10 mL) at –78 °C, and the resulting mixture was stirred at –78 °C for 1 hour. Saturated aqueous ammonium chloride solution (0.5 mL) was then added at –78 °C, followed by hydrochloric acid (2N, aqueous; 0.6 mL). The cooling bath was then removed, and the mixture was stirred at 25 °C for 1 hour. The resulting mixture was subsequently extracted with dichloromethane (100 mL), and the extracts were dried over sodium sulfate and concentrated in vacuo to give (4-(5-benzyl-2H-indazol-2-yl)-3-fluorophenyl)methanol (150 mg) as a white solid: MS (ESI) *m/z*: calculated: 332.1; observed: 333.3 $(M+1)^+$.

Step 6:

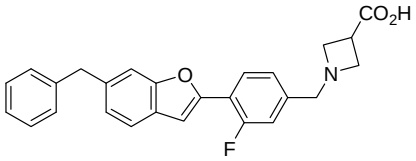
Tetrapropylammonium perruthenate (TPAP) (10.6 mg, 0.03 mmol) was added to a mixture of (4-(5-benzyl-2H-indazol-2-yl)-3-fluorophenyl)methanol (100 mg, 0.30 mmol), 4-methylmorpholine *N*-oxide (43 mg, 0.36 mmol), and activated molecular sieves (200

mg) in dichloromethane (10 mL) at 25 °C, and the resulting mixture was stirred at 25 °C for 16 h. The reaction mixture was then vacuum filtered, and the filtrate was concentrated in vacuo. Chromatographic purification of the residue (silica gel, 0–80% ethyl acetate/hexanes) furnished 4-(5-benzyl-2H-indazol-2-yl)-3-fluorobenzaldehyde (36.4 mg, 0.11 mmol, 37% yield) as white solid: ^{1}H NMR (400 MHz, CD_3OD) δ 10.0 (d, J = 1.6 Hz,1H), 8.55 (m, 1H), 8.41 (t, J = 7.4 Hz, 1H), 7.84 (m, 2H), 7.68 (d, J = 9.0 Hz, 1H), 7.46 (s, 1H), 7.34–7.30 (m, 2H), 7.27–7.19 (m, 4H), 4.06 (s, 2H); ^{19}F NMR (376 MHz, CD_3OD) δ –122.3. MS (ESI) *m/z*: calculated: 330.1; observed: 331.2 $(M+1)^+$.

Step 7:

A mixture of 4-(5-benzyl-2H-indazol-2-yl)-3-fluorobenzaldehyde (108 mg, 0.328 mmol), azetidine-3-carboxylic acid (66 mg, 0.657 mmol), and acetic acid (45 µL) in a mixture of methanol (7.5 mL) and dichloromethane (4.5 mL) was stirred at 25 °C for 1 h. Sodium cyanoborohydride (41 mg, 0.66 mmol) was then added, and the resulting mixture was stirred at 25 °C for 2 h. The reaction mixture was then concentrated in vacuo, and the residue was dissolved in DMSO (2 mL) and purified by reverse-phase preparative HPLC (Phenomenex Luna 5µm C18 column, 60 × 21.2 mm ID, mobile phase: A = 0.05% TFA in water; B = 0.05% TFA in acetonitrile, 5–80% B, flow rate: 12 mL/min) to provide 1-(4-(5-benzyl-2H-indazol-2-yl)-3-fluorobenzyl)azetidine-3-carboxylic acid • 2,2,2-trifluoroacetate (56.1 mg, 0.106 mmol, 32% yield) as a white foam: ^{1}H NMR (400 MHz, CD_3OD) δ 8.61 (d, J = 2.0 Hz, 1H), 8.09 (t, J = 8.2 Hz, 1H), 7.61–7.49 (m, 4H), 7.30–7.18 (m, 6H), 4.52 (s, 2H), 4.43–4.35 (m, 4H), 4.05 (s, 2H), 3.76–3.67 (m, 1H); ^{19}F NMR (376 MHz, CD_3OD) δ –77.5 (TFA), –124.3. MS (ESI) *m/z*: calculated (without TFA): 415.2; observed: 416.2 $(M+1)^+$.

Synthesis of **9**



1-(4-(6-Benzylbenzofuran-2-yl)-3-fluorobenzyl)azetidine-3-carboxylic acid (**9**)

Step 1:

To a mixture of benzofuran-6-ol (1.85 g, 13.8 mmol) and *N*,*N*-diisopropylethylamine (7.21 mL, 41.4 mmol) in DCM (50 mL) was added 1,1,1-trifluoro-*N*-phenyl-*N*-((trifluoromethyl)sulfonyl)methanesulfonamide (4.93 g, 13.8 mmol), and the resulting mixture was stirred at ambient temperature for 2 h. The reaction mixture was then partitioned between saturated aqueous sodium bicarbonate and DCM. The organic layer was separated, and the aqueous layer was extracted with DCM. The combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. Chromatographic purification of the residue (silica gel, 0–15% EtOAc/hexanes) gave 3.07 g of a semi-solid containing impure benzofuran-6-yl trifluoromethanesulfonate. A

portion of this material was carried on as follows: A mixture of 9-benzyl-9-bora-bicyclo[3.3.1]nonane (0.5M in THF; 23 mL, 11 mmol), benzofuran-6-yl trifluoromethanesulfonate (1.50 g, 5.6 mmol), potassium phosphate (3.6 g, 17 mmol), benzofuran-6-yl trifluoromethanesulfonate (1.50 g, 5.6 mmol), dicyclohexyl(2',6'-dimethoxybiphenyl-2-yl)phosphine (S-Phos, 0.19 g, 0.45 mmol), and $Pd(OAc)_2$ (0.051 g, 0.23 mmol) in a sealable pressure vial was sparged with argon, sealed, and heated at 60 °C overnight. The resulting grayish-green mixture was allowed to cool to ambient temperature and was filtered through Celite®, rinsing with Et_2O. The filtrate was concentrated and adsorbed onto silica gel (15 g). Chromatographic purification (silica gel, 0–10% EtOAc/hexanes) gave impure 6-benzylbenzofuran (0.66 g). This material was taken up in THF (30 mL), cooled to –78 °C, and *n*-butyllithium (2.5M in hexanes; 1.53 mL, 3.82 mmol) was added (dropwise). The resulting mixture was stirred at –78 °C for 25 min, at which point triisopropyl borate (1.08 mL, 4.68 mmol) was slowly added (dropwise). After 30 min, the bath was removed, and the reaction was allowed to warm to ambient temperature. After 10 min, 2N aqueous HCl (50 mL) was added. The resulting mixture was diluted with MTBE, and the organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo to give an oil. The oil was triturated with hexanes to give a solid, which was isolated via vacuum filtration, rinsed with hexanes, and dried in vacuo to give 6-benzylbenzofuran-2-ylboronic acid (0.369 g) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.47 (2H, s), 7.57 (1H, d, J = 8.0 Hz), 7.42 (1H, s), 7.39 (1H, s), 7.24–7.33 (4H, m), 7.15–7.22 (1H, m), 7.10 (1H, d, J = 8.0 Hz), 4.06 (2H, s).

Step 2:

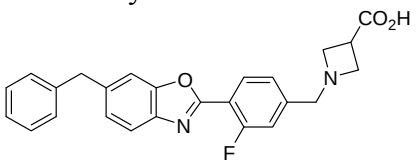
A slurry of bis(4-(di-*tert*-butylphosphino)-*N*,*N*-dimethylbenzenamine) palladium dichloride (0.0223 g, 0.0314 mmol), methyl 1-(4-bromo-3-fluorobenzyl)azetidine-3-carboxylate (0.190 g, 0.629 mmol), 6-benzylbenzofuran-2-ylboronic acid (0.206 g, 0.818 mmol), and potassium acetate (0.123 g, 1.26 mmol) in MeOH (4 mL) was heated in a sealed tube at 60 °C overnight. The reaction mixture was then partitioned between saturated aqueous sodium bicarbonate and DCM, and the aqueous layer was extracted with DCM (2×). The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting material was purified by silica gel chromatography to give methyl 1-((4-(6-benzylbenzofuran-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylate (0.217 g, 80.3% yield): ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 7.92 (1H, dd, J = 8.0, 8.0 Hz), 7.52 (1H, s), 7.29–7.36 (3H, m), 7.19–7.26 (3H, m), 7.07–7.19 (4H, m), 4.12 (2H, s), 3.73 (3H, s), 3.56–3.68 (3H, m), 3.38 (2H, br s). MS (ESI) *m/z*: calculated: 429.2; observed: 430.2 $(M+1)^+$.

Step 3:

To a solution of methyl 1-((4-(6-benzylbenzofuran-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylate (0.217 g, 0.505 mmol) in THF (3 mL) was added 1.0M aqueous sodium hydroxide (1.52 mL, 1.52 mmol). The slightly cloudy yellow reaction mixture was then stirred for 1 h at ambient temperature, at which point THF was removed under a stream of nitrogen. The resulting mixture was treated with 1N

aqueous HCl (1.5 mL) to give a thick white slurry, which was diluted with water and 1M aqueous sodium phosphate buffer (pH 6; 2 mL). The resulting suspension was stirred rapidly for 5 min and then was vacuum filtered; the collected solid was sequentially rinsed with water and MeOH (2×); and the solid was then dried in vacuo to give 1-((4-(6-benzylbenzofuran-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylic acid (0.161 g, 76.7% yield) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.89 (1H, dd, J = 8.0, 8.0 Hz), 7.60 (1H, d, J = 8.0 Hz), 7.50 (1H, s), 7.21–7.34 (7H, m), 7.15–7.21 (2H, m), 4.07 (2H, s), 3.60 (2H, s), 3.37–3.49 (2H, m), 3.15–3.30 (3H, m). MS (ESI) *m/z*: calculated: 415.2; observed: 416.1 $(M+1)^+$.

Synthesis of **10**



1-(4-(6-Benzylbenzo[d]oxazol-2-yl)-3-fluorobenzyl)azetidine-3-carboxylic acid (**10**)

Step 1:

To a solution of 4-benzylbenzenamine (3.00 g, 16.4 mmol) in MeOH (16 mL) was sequentially added iodine (2.49 g, 9.82 mmol) and hydrogen peroxide (30% in water; 1.67 mL, 16.4 mmol). The resulting dark solution was stirred at ambient temperature overnight. The reaction was then partitioned between half-saturated brine and EtOAc. The organic layer was separated, washed with brine, dried over $MgSO_4$, filtered, and concentrated. The resulting oil was adsorbed onto silica gel and chromatographically purified (silica gel, 0–10% EtOAc/hexanes) to provide 4-benzyl-2-iodobenzenamine (3.25 g, 64% yield) as a red oil which slowly solidified to a red solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.40 (1H, s), 7.23–7.31 (2H, m), 7.12–7.21 (3H, m), 6.94 (1H, d, J = 8.0 Hz), 6.68 (1H, d, J = 8.0 Hz), 5.03 (2H, s), 3.74 (2H, s). MS (ESI) *m/z*: calculated: 309.0; observed: 310.0 $(M+1)^+$.

Step 2:

To a slurry of 2-fluoro-4-formylbenzoic acid (**31**; 0.250 g, 1.5 mmol) in DCM (5 mL) was added *N*,*N*-dimethylformamide (2 drops, ca. 30 μL) and oxalyl dichloride (0.16 mL, 1.8 mmol). The reaction mixture was stirred rapidly at 25 °C for 1 h and then was concentrated in vacuo. The residual oil was taken up in THF (5 mL), and *N*-ethyl-*N*-isopropylpropan-2-amine (0.39 mL, 2.2 mmol) and 4-benzyl-2-iodobenzenamine (0.41 g, 1.3 mmol) were sequentially added to the resulting slurry. After 30 min, the resulting mixture was partitioned between ethyl ether and 1N aqueous HCl. The organic layer separated, sequentially washed with 1N aqueous HCl, saturated aqueous $NaHCO_3$ (2×), and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. Chromatographic purification of the residue (silica gel, 0–20% EtOAc/hexanes) furnished *N*-(4-benzyl-2-iodophenyl)-2-fluoro-4-formylbenzamide (0.36 g, 52% yield) as a white solid: ^{1}H NMR

(400 MHz, DMSO-d_6) δ ppm 10.13 (1H, s), 10.07 (1H, s), 7.78–8.00 (4H, m), 7.43 (1H, d, J = 8.0 Hz), 7.18–7.35 (6H, m), 3.95 (2H, s). MS (ESI) *m/z*: calculated: 459.0; observed: 457.9 $(M-1)^-$.

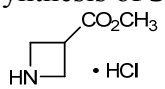
Step 3:

1,10-Phenanthroline (0.016 g, 0.087 mmol), cuprous iodide (0.0015 mL, 0.044 mmol), cesium carbonate (0.21 g, 0.65 mmol), and *N*-(4-benzyl-2-iodophenyl)-2-fluoro-4-formylbenzamide (0.200 g, 0.44 mmol) were combined under argon in a sealable pressure tube. Dioxane (2 mL) was added and the reaction tube was sealed and heated at 90 °C for 24 h. The reaction mixture was then cooled to ambient temperature and partitioned between EtOAc and water. The organic layer was separated, washed with brine, dried over sodium sulfate, filtered, and concentrated. Chromatographic purification of the residue (silica gel, 0–30% EtOAc/hexanes) gave 4-(6-benzylbenzo[d]oxazol-2-yl)-3-fluorobenzaldehyde (0.090 g, 62% yield) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.09 (1H, s), 8.43 (1H, dd, J = 7.5, 7.5 Hz), 7.96 (2H, d, J = 9.5 Hz), 7.80 (1H, d, J = 8.0 Hz), 7.75 (1H, s), 7.36 (1H, d, J = 8.0 Hz), 7.26–7.33 (4H, m), 7.15–7.26 (1H, m), 4.13 (2H, s). MS (ESI) *m/z*: calculated: 331.1; observed: 332.1 $(M+1)^+$.

Step 4:

4-(6-Benzylbenzo[d]oxazol-2-yl)-3-fluorobenzaldehyde (0.090 g, 0.27 mmol) was dissolved in DCM (1 mL), and MeOH (1 mL), azetidine-3-carboxylic acid (0.082 g, 0.81 mmol), and glacial acetic acid (0.047 mL, 0.81 mmol) were sequentially added. The resulting mixture was stirred rapidly at 25 °C for 1 h, at which point sodium cyanoborohydride (0.017 g, 0.27 mmol) was added in one portion. After 3 h, the reaction mixture was diluted with DCM (1 mL) and the resulting slurry was vacuum filtered, rinsing the collected material with DCM to give a white solid. This material was suspended in 1M aqueous sodium phosphate buffer (pH 6; 5 mL) and sonicated for 10 min. The slurry was filtered and the collected solid was sequentially rinsed with water (3×) and EtOH (3×) and dried in vacuo to give 1-(4-(6-benzylbenzo[d]oxazol-2-yl)-3-fluorobenzyl)azetidine-3-carboxylic acid (0.047 g, 42% yield): ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.12 (1H, dd, J = 7.8 Hz), 7.73 (1H, d, J = 8.0 Hz), 7.69 (1H, s), 7.26–7.38 (7H, m), 7.16–7.25 (1H, m), 4.11 (2H, s), 3.65 (2H, s), 3.19–3.48 (5H, m). MS (ESI) *m/z*: calculated: 416.2; observed: 417.2 $(M+1)^+$.

Synthesis of **35**

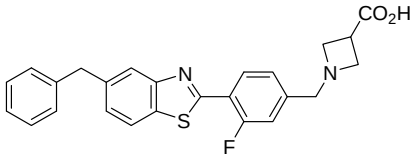


Methyl azetidine-3-carboxylate hydrochloride (**35**)

Thionyl chloride (36 mL, 495 mmol) was added (dropwise, over 4 h) to a slurry of azetidine-3-carboxylic acid (20 g, 198 mmol) in anhydrous MeOH (500 mL) at 0 °C.

The resulting solution was concentrated in vacuo to provide an oil, which was triturated with benzene (100 mL) and concentrated in vacuo give a solid. This solid was then twice suspended in benzene (50 mL) and concentrated in vacuo to give methyl azetidine-3-carboxylate hydrochloride (29.0 g, 97% yield) as a light-yellow solid: MS (ESI) *m/z*: calculated: 115.1; observed: 116.1 $(M+1)^+$.

Synthesis of **11**



1-(4-(5-Benzylbenzo[d]thiazol-2-yl)-3-fluorobenzyl)azetidine-3-carboxylic acid (**11**)

Step 1:

Benzylzinc bromide (0.5M in THF; 100 ml, 50 mmol) was added via a pressure-equalizing dropping funnel to a mixture of 2-(4-(1,3-dioxan-2-yl)-2-fluorophenyl)-5-bromobenzo[d]thiazole (**28**; 18.0 g, 46 mmol) and bis(di-*tert*-butyl(4-dimethylaminophenyl)phosphine)dichloropalladium(II) (1.6 g, 2.3 mmol) in THF (300 mL) at 25 °C, and the resulting mixture was stirred at 25 °C for 2.5 h. Saturated aqueous sodium bicarbonate was added, and the resulting mixture was stirred at 25 °C for 15 min. The resulting mixture was extracted with EtOAc (3×), and the combined extracts were dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was suspended in ethyl ether and then vacuum filtered. The collected solid was washed with ethyl ether and dried in vacuo to provide 2-(4-(1,3-dioxan-2-yl)-2-fluorophenyl)-5-benzylbenzo[d]thiazole (17.8 g, 96% yield) as a beige solid: MS (ESI) *m/z*: calculated: 405.1; observed: 406.0 $(M+1)^+$.

Step 2:

A solution of 2-(4-(1,3-dioxan-2-yl)-2-fluorophenyl)-5-benzylbenzo[d]thiazole (25.00 g, 61.65 mmol) in a mixture of THF (1 L) and 5N aqueous HCl (250 mL) was heated at 55 ^oC for 2 h. The resulting mixture was transferred to a 4-L Erlenmeyer flask and neutralized with saturated aqueous $NaHCO_3$. The resulting mixture was extracted with EtOAc (2×), and the combined extracts were dried over $MgSO_4$, filtered, and concentrated in vacuo to furnish 4-(5-benzylbenzo[d]thiazol-2-yl)-3-fluorobenzaldehyde (21.35 g, 99% yield) as a red solid: MS (ESI) *m/z*: calculated: 347.1; observed: 348.0 $(M+1)^+$.

Step 3:

N-Ethyl-*N*-isopropylpropan-2-amine (11 ml, 65 mmol) was added to a solution of methyl azetidine-3-carboxylate hydrochloride (**35**; 9.3 g, 61 mmol) in MeOH (105 ml), and a

solution of 4-(5-benzylbenzo[d]thiazol-2-yl)-3-fluorobenzaldehyde (21.35 g, 61 mmol) in 1:1 MeOH/DCM (210 ml) was then added, followed by acetic acid (5.3 ml, 92 mmol). The resulting mixture was stirred at 25 °C for 1 h, and sodium cyanoborohydride (1.9 g, 31 mmol) was then added in five portions over 40 min. The resulting mixture was stirred at 25 °C for 5 h, saturated aqueous sodium bicarbonate (210 ml) was added, and the resulting mixture was extracted with DCM (3 × 250 mL). The combined organic extracts were dried over magnesium sulfate, filtered, concentrated in vacuo. Chromatographic purification of the residue (silica gel, 0–100% EtOAc/hexanes) provided methyl 1-((4-(5-benzylbenzo[d]thiazol-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylate (18.78 g, 68% yield) as a light-yellow solid: MS (ESI) *m/z*: calculated: 446.1; observed: 447.1 $(M+1)^+$.

Step 4:

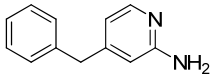
A mixture of methyl 1-((4-(5-benzylbenzo[d]thiazol-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylate (14.28 g, 32.0 mmol) in THF (143 mL) and 5M aqueous sodium hydroxide (12.8 mL, 64.0 mmol) was heated at 60 °C in a flask fitted with an air-cooled reflux condenser. After 1 h, the reaction mixture was allowed to cool to ambient temperature and THF (143 mL) was added, resulting in the formation of a precipitate. The resulting mixture was cooled to 0 °C and then filtered through a ZAPCAP® CR 0.45 micron filter (Whatman, Florham Park, NJ), rinsing the reaction flask with cold THF (ca. 50 mL) to complete the transfer. The collected solid was washed with cold THF (70 mL) and then dried in vacuo to provide an off-white solid (15.7 g). This material was taken up in THF (90 mL) and water (11 mL) and heated at 60 °C until a homogeneous solution resulted. The heating bath was removed and THF (135 mL) was added. The resulting mixture was allowed to cool slowly to ambient temperature and then was cooled to 0 °C. The precipitated solid was collected by vacuum filtration, rinsed with cold THF (75 mL), and dried in vacuo to give sodium 1-((4-(5-benzylbenzo[d]thiazol-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylate (14.2 g, 97.7% yield) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.26 (1H, dd, J = 8.0, 8.0 Hz), 8.06 (1H, d, J = 8.0 Hz), 7.95 (1H, s), 7.37 (1H, dd, J = 8.5, 1.5 Hz), 7.26–7.35 (6H, m), 7.16–7.24 (1H, m), 4.12 (2H, s), 3.58 (2H, s), 3.26–3.33 (2H, m), 3.09–3.17 (2H, m), 2.78 (1H, m). MS (ESI) *m/z*: calculated: 432.1; observed: 433.0 $(M+1)^+$.

Step 5:

A slurry of sodium 1-((4-(5-benzylbenzo[d]thiazol-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylate (15.2 g, 33.4 mmol) in MeOH (300 mL) was heated at 60 °C for 45 min, resulting in a slightly cloudy suspension. This suspension was allowed to partially cool, and then (while still slightly warm) was filtered through a ZAPCAP® CR 0.45 micron filter (Whatman, Florham Park, NJ), rinsing with MeOH (50 mL). The resulting clear, slightly yellow solution was then cooled to ambient temperature in a water bath. 1M aqueous HCl (33.4 mL, 33.4 mmol) was added (dropwise over 5 min) with rapid stirring, resulting in the formation of a precipitate. The resulting mixture was stirred rapidly for 1.5 h and then was filtered through a ZAPCAP® CR 0.45 micron filter (Whatman, Florham Park, NJ). The collected solid was washed

with cold 1:1 MeOH/H_2O (100 mL) and then was dried in vacuo to give 1-((4-(5-benzylbenzo[d]thiazol-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylic acid (12.6 g, 87% yield) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.27 (1H, dd, J = 8.0, 8.0 Hz), 8.07 (1H, d, J = 8.5 Hz), 7.95 (1H, s), 7.27–7.44 (7H, m), 7.16–7.25 (1H, m), 4.13 (2H, s), 3.65 (2H, s), 3.40–3.50 (2H, m), 3.20–3.30 (3H, m). MS (ESI) *m/z*: calculated: 432.1; observed: 433.0 $(M+1)^+$.

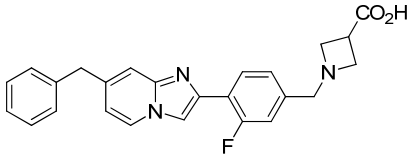
Synthesis of **34**



4-Benzylpyridin-2-amine (**34**)

A mixture of 4-benzylpyridine (14.1 mL, 88.6 mmol), sodium amide (5.71 g, 146 mmol), and p-cymene (105 mL) was heated with stirring at 165 °C for 1 d. The reaction mixture was then allowed to cool to 25 °C, and water (30 mL) was slowly added, followed by concentrated hydrochloric acid (30 mL). The aqueous layer was separated, and the organic layer was extracted with 2N aq. HCl (60 mL). The aqueous layers were then combined, washed once with ether (50 mL), and then made strongly basic (pH > 10) by slowly adding portions of solid potassium hydroxide, resulting in the separation of a brown oil. The oil was extracted into dichloromethane (2 × 150 mL), and the combined organic extracts were then dried over sodium sulfate, filtered, and concentrated in vacuo. Chromatographic purification of the resulting oil (silica gel, 50–100% EtOAc/hexanes) afforded 4-benzylpyridin-2-amine (3.35 g, 21% yield) as a tan solid: ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 7.93 (1H, d, J = 5.5 Hz), 7.28–7.34 (2H, m), 7.21–7.25 (1H, m), 7.18 (2H, d, J = 7.5 Hz), 6.51 (1H, d, J = 5.0 Hz), 6.30 (1H, s), 4.54 (2H, br s), 3.86 (2H, s). MS (ESI) *m/z*: calculated: 184.1; observed: 185.2 $(M+l)^+$.

Synthesis of **12**



1-(4-(7-Benzylimidazo[1,2-a]pyridin-2-yl)-3-fluorobenzyl)azetidine-3-carboxylic acid (**12**)

Step 1:

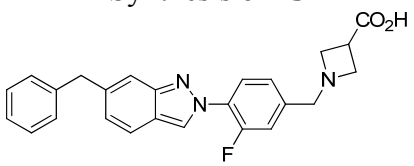
A solution of 4-benzylpyridin-2-amine (**34**; 175 mg, 949 μmol) and 4-(2-bromoacetyl)-3-fluorobenzaldehyde (**33**; 232.5 mg, 949 μmol) in ethanol (3.0 mL) was heated at reflux for 2 h. The reaction mixture was then cooled to 25 °C and concentrated in vacuo. The residue was taken up in DCM (20 mL) and shaken with 2N aqueous HCl (10 mL). The organic layer was separated, dried over sodium sulfate, filtered, and concentrated in vacuo. Chromatographic purification of the residue (silica gel, 0–100% EtOAc/hexanes (+2% triethylamine, both solvents)) furnished a 3:1 mixture of 4-(7-benzylH-

imidazo[1,2-a]pyridin-2-yl)-3-fluorobenzaldehyde and the corresponding ethyl acetal as an orange solid (128 mg). This mixture was taken up in THF (8.0 mL), 2.0N aqueous HCl (788 µl, 1.58 mmol) was added, and the resulting mixture was stirred at 25 °C for 20 min. 1.0N aqueous NaOH (1.64 mL, 1.64 mmol) was then added, and the resulting mixture was partitioned between EtOAc (20 mL) and brine (3 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated in vacuo. Chromatographic purification of the residue (silica gel, 0–100% EtOAc/hexanes (+2% triethylamine, both solvents)) furnished 4-(7-benzylH-imidazo[1,2-a]pyridin-2-yl)-3-fluorobenzaldehyde (47.3 mg, 45% yield) as a white solid: ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 10.01 (1H, s), 8.59 (1H, t, *J* = 7.3 Hz), 8.11 (1H, br s), 8.06 (1H, d, *J* = 7.0 Hz), 7.79 (1H, d, *J* = 8.0 Hz), 7.67 (1H, d, *J* = 11.0 Hz), 7.52 (1H, br s), 7.30–7.36 (2H, m), 7.28 (1H, br s), 7.20–7.25 (2H, m), 6.72 (1H, d, *J* = 6.5 Hz), 4.04 (2H, s). MS (ESI) *m/z*: calculated: 330.1; observed: 331.2 $(M+l)^+$.

Step 2:

A mixture of 4-(7-benzylH-imidazo[1,2-a]pyridin-2-yl)-3-fluorobenzaldehyde (47.3 mg, 143 µmol), azetidine-3-carboxylic acid (43 mg, 430 µmol), and acetic acid (25 µl, 430 µmol) in a mixture of DCM (1.0 mL) and MeOH (1.0 mL) was stirred at 25 °C for 1 h. Sodium cyanoborohydride (9.0 mg, 143 µmol) was then added, and the resulting mixture was stirred at 25 °C for 15 h. The reaction mixture was then diluted with DCM (1 mL), and the resulting slurry was filtered through a glass frit, rinsing with DCM (10 mL). The filtrate was concentrated in vacuo, and the residue was taken up in MeOH (3.0 mL), filtered through a cotton plug, and purified by rpHPLC (Phenomenex C18, 5–100% CH_3CN/H_2O (+0.1% TFA, both solvents), 120 mL/min) to afford 1-(4-(7-benzylH-imidazo[1,2-a]pyridin-2-yl)-3-fluorobenzyl)azetidine-3-carboxylic acid • 2,2,2-trifluoroacetic acid (12.5 mg, 16% yield) as a colorless oil: ^{1}H NMR (400 MHz, MeOH-d_4) δ ppm 8.71 (1H, d, *J* = 6.8 Hz), 8.59 (1H, d, *J* = 2.0 Hz), 8.05 (1H, t, *J* = 7.9 Hz), 7.69 (1H, s), 7.53–7.62 (2H, m), 7.38–7.43 (2H, m), 7.36 (2H, d, *J* = 6.1 Hz), 7.32–7.35 (1H, m), 7.28–7.32 (1H, m), 4.55 (2H, s), 4.36–4.47 (4H, m), 4.28 (2H, s), 3.76 (1H, quin, *J* = 8.3 Hz). MS (ESI) *m/z*: calculated: 415.2; observed: 416.4 $(M+1)^+$.

Synthesis of **13**



1-(4-(6-Benzyl-2H-indazol-2-yl)-3-fluorobenzyl)azetidine-3-carboxylic acid (**13**)

Step 1:

A mixture of 4-bromo-2-nitrobenzaldehyde (Carbocore, The Woodlands, TX; 3.77 g, 16.4 mmol) and ethyl 4-amino-3-fluorobenzoate (Oakwood Products, West Columbia, SC; 3.0 g, 16.4 mmol) in ethanol (45 mL) was stirred at reflux for 2 hours. The reaction mixture was then concentrated in vacuo, and the residue was purified by column

chromatography (silica gel, 0–80% ethyl acetate/hexanes) to provide (*E*)-ethyl-4-(4-bromo-2-nitrobenzylideneamino)-3-fluorobenzoate (1.35 g, 21% yield) as a pale yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.97 (s, 1H), 8.27–8.24 (m, 2H), 7.95–7.82 (m, 3H), 7.23 (t, *J* = 8.0 Hz, 1H), 4.41 (q, *J* = 7.0 Hz, 2H), 1.41 (t, *J* = 7.0 Hz, 3H).

Step 2:

A mixture of (*E*)-ethyl-4-(4-bromo-2-nitrobenzylideneamino)-3-fluorobenzoate (1.35 g, 3.41 mmol) and triethyl phosphate (3.0 mL, 18.0 mmol) was heated at 150 °C for 1.5 hours. The cooled reaction mixture was then purified by column chromatography (silica gel, 0–75% ethyl acetate/hexanes) to furnish ethyl-4-(6-bromo-2*H*-indazol-2-yl)-3-fluorobenzoate (1.01 g, 82% yield) as a pale yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (s, 1H), 8.26 (t, *J* = 8.2 Hz, 1H), 8.02 (d, *J* = 10.2 Hz, 2H), 7.97 (s, 1H), 7.61 (d, *J* = 9.0 Hz, 1H), 7.21 (d, *J* = 9.0 Hz, 1H), 4.43 (q, *J* = 7.0 Hz, 2H), 1.44 (t, *J* = 7.0 Hz, 3H). MS (ESI) *m/z*: calculated: 362.0; observed: 363.0 $(M+1)^+$.

Step 3:

A mixture of ethyl 4-(6-bromo-2H-indazol-2-yl)-3-fluorobenzoate (181 mg, 0.50 mmol), benzylzinc(II) bromide (0.5 M solution in THF, 2.0 mL, 1.0 mmol), and $Pd(t\text{-}Bu_3P)_2$ (12.7 mg, 0.025 mmol) in a microwave reaction tube was sparged with N_2(g) for 3 minutes and then heated (microwave) at 100 °C for 30 minutes. The reaction mixture was then diluted with ethyl acetate (100 mL), sequentially washed with 1N aqueous HCl (10 mL) and brine (20 mL), and filtered through Celite. The filtrate was dried over sodium sulfate and concentrated in vacuo. Chromatographic purification of the residue (silica gel, 0–80% ethyl acetate/hexanes) furnished a 3:1 mixture (123 mg) of ethyl 4-(6-benzyl-2H-indazol-2-yl)-3-fluorobenzoate and benzyl 4-(6-benzyl-2H-indazol-2-yl)-3-fluorobenzoate. Ethyl 4-(5-benzyl-2H-indazol-2-yl)-3-fluorobenzoate: MS (ESI) *m/z*: calculated: 374.1; observed: 375.2 $(M+1)^+$; Benzyl 4-(5-benzyl-2H-indazol-2-yl)-3-fluorobenzoate: MS (ESI) *m/z*: calculated: 436.2; observed: 437.2 $(M+1)^+$.

Step 4:

DIBAL-H (1.0 M in dichloromethane; 1.0 mL, 1.0 mmol) was slowly added to a 3:1 mixture (123 mg) of ethyl 4-(6-benzyl-2H-indazol-2-yl)-3-fluorobenzoate and benzyl 4-(6-benzyl-2Hindazol-2-yl)-3-fluorobenzoate in dichloromethane (7 mL) at –78 °C, and the resulting mixture was stirred at –78 °C for 1 hour. Saturated aqueous ammonium chloride solution (0.4 mL) was then added at –78 °C, followed by hydrochloric acid (2N, aqueous; 0.5 mL). The cooling bath was then removed, and the mixture was stirred at 25 °C for 1 hour. The resulting mixture was subsequently extracted with dichloromethane (80 mL), and the extracts were dried over sodium sulfate and concentrated in vacuo to give (4-(6-benzyl-2H-indazol-2-yl)-3-fluorophenyl)methanol (109 mg) as a white solid: MS (ESI) *m/z*: calculated: 332.1; observed: 333.2 $(M+1)^+$.

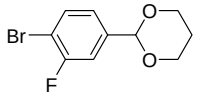
Step 5:

Tetrapropylammonium perruthenate (TPAP) (11.5 mg, 0.033 mmol) was added to a mixture of (4-(6-benzyl-2H-indazol-2-yl)-3-fluorophenyl)methanol (109 mg, 0.33 mmol), 4-methylmorpholine *N*-oxide (46.1 mg, 0.394 mmol), and activated molecular sieves (240 mg) in dichloromethane (10 mL) at 25 °C, and the resulting mixture was stirred at 25 °C for 16 hours. The reaction mixture was then vacuum filtered, and the filtrate was concentrated in vacuo. Chromatographic purification of the residue (silica gel, 0–80% ethyl acetate/hexanes) furnished 4-(6-benzyl-2H-indazol-2-yl)-3-fluorobenzaldehyde (36.4 mg, 37% yield) as white solid: ^{1}H NMR (400 MHz, CDCl3) δ 10.03 (s, 1H), 8.59 (s, 1H), 8.41 (t, *J* = 8.2 Hz, 1H), 7.84 (d, *J* = 8.2 Hz, 1H), 7.82 (d, *J* = 11.4 Hz, 1H), 7.61 (dd, *J* = 1.2, 8.6 Hz, 1H), 7.53 (s, 1H), 7.33–7.20 (m, 5H), 6.98 (d, *J* = 7.8 Hz, 1H), 4.01 (s, 2H); ^{19}F NMR (376 MHz, CDCl3) δ –122.3. MS (ESI) *m/z*: calculated: 330.1; observed: 331.0 $(M+1)^+$.

Step 6:

A mixture of 4-(6-benzyl-2H-indazol-2-yl)-3-fluorobenzaldehyde (82.5 mg, 0.250 mmol), azetidine-3-carboxylic acid (129 mg, 1.27 mmol) and acetic acid (80 μL) in a mixture of methanol (14 mL) and dichloromethane (7 mL) was stirred at 25 °C for 1 h. Sodium cyanoborohydride (80 mg, 1.27 mmol) was then added, and the resulting mixture was stirred at 25 °C for 2 h. The reaction mixture was then concentrated in vacuo, and the residue was dissolved in DMSO (2 mL) and purified by reverse-phase preparative HPLC (Phenomenex Luna 5 μm C18 column, 60×21.2 mm ID, mobile phase: A = 0.05% TFA in water; B = 0.05% TFA in acetonitrile, gradient: 5–80% B, flow rate: 12 mL/min) to provide 1-(4-(6-benzyl-2H-indazol-2-yl)-3-fluorobenzyl)azetidine-3-carboxylic acid•2,2,2-trifluoroacetate (43.3 mg, 33% yield) as a white foam: ^{1}H NMR (400 MHz, CD_3OD) δ 8.63 (d, *J* = 2.3 Hz, 1H), 8.10 (t, *J* = 8.2 Hz, 1H), 7.67 (d, *J* = 8.6 Hz, 1H), 7.58 (dd, *J* = 11.7, 1.6 Hz, 1H), 7.51 (dd, *J* = 8.2, 1.6 Hz, 1H), 7.45 (s, 1H), 7.31–7.17 (m, 5H), 7.02 (dd, *J* = 8.6, 1.2 Hz, 1H), 4.52 (s, 2H), 4.44–4.36 (m, 4H); 4.08 (s, 2H), 3.77–3.69 (m, 1H). ^{19}F NMR (376 MHz, CD_3OD) δ –77.5 (TFA), –124.3. MS (ESI) *m/z*: calculated (without TFA): 415.2; observed: 416.1 $(M+l)^+$.

Synthesis of **26**

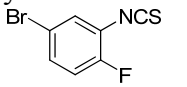


2-(4-Bromo-3-fluorophenyl)-1,3-dioxane (**26**)

In a round-bottomed flask equipped with a Dean–Stark trap was mixed 4-bromo-3-fluorobenzaldehyde (10.00 g, 49.2 mmol), 1,3-propanediol (4.26 mL, 59.0 mmol), and *p*-toluenesulfonic acid monohydrate (0.468 g, 2.46 mmol) in toluene (300 mL). The mixture was heated at reflux for 2 h, removing water via the Dean–Stark trap. The reaction mixture was subsequently allowed to cool to room temperature and then was washed with saturated aqueous $NaHCO_3$ (2x), dried over $MgSO_4$, filtered, and concentrated to afford 2-(4-bromo-3-fluorophenyl)-1,3-dioxane (12.70, 99% yield) as a white solid: ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 7.49–7.56 (1H, m), 7.25–

7.30 (1H, m), 7.12–7.17 (1H, m), 5.44 (1H, s), 4.25 (2H, dd, *J* = 12.1, 5.0 Hz), 3.97 (2H, t, *J* = 12.1 Hz), 2.14–2.26 (1H, m), 1.42–1.49 (1H, m). MS (ESI) *m/z*: calculated: 260.0; observed: 261.1 $(M+1)^+$.

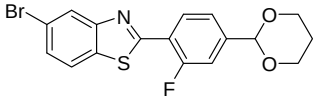
Synthesis of **27**



4-Bromo-1-fluoro-2-isothiocyanatobenzene (**27**)

Thiophosgene (4.8 mL, 63 mmol) was added to a stirred mixture of sodium carbonate (13 g, 126 mmol) and 5-bromo-2-fluorobenzenamine (11.44 g, 60.2 mmol) in chloroform (350 mL). The reaction mixture was stirred at room temperature for 16 h and then was vacuum filtered. The filtrate was concentrated in vacuo, and the residue was suspended in hexanes. The resulting suspension was vacuum filtered, and the filtrate was concentrated in vacuo to afford 4-bromo-1-fluoro-2-isothiocyanatobenzene (13.7 g, 98% yield) as a light orange oil: ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 7.31–7.36 (2H, m), 7.00–7.06 (1H, m).

Synthesis of **28**



2-(4-(1,3-Dioxan-2-yl)-2-fluorophenyl)-5-bromobenzo[d]thiazole (**28**)

Step 1:

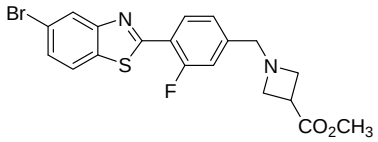
n-Butyllithium (2.5M in hexanes; 13.9 mL, 34.8 mmol,) was added (dropwise) to a stirred solution of 2-(4-bromo-3-fluorophenyl)-1,3-dioxane (**26**; 8.27 g, 31.7 mmol) in THF (120 mL) at –78 °C under a nitrogen atmosphere. The resulting mixture was stirred at –78 °C for 15 min, and 4-bromo-1-fluoro-2-isothiocyanatobenzene (**27**; 7.35 g, 31.7 mmol) in THF (30 mL) was then added (dropwise). The resulting mixture was stirred at –78 °C for an additional 45 min, saturated aqueous NH_4Cl was added, and the resulting mixture was allowed to warm to ambient temperature. The organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The resulting dark orange oil was dissolved in a minimal amount of DCM and then diluted with hexanes. The resulting solution was partially concentrated in vacuo, and the precipitated solid was collected by vacuum filtration to provide *N*-(5-bromo-2-fluorophenyl)-4-(1,3-dioxan-2-yl)-2-fluorobenzothioamide (3.40 g, 26% yield) as a yellow solid. The filtrate was subsequently concentrated in vacuo and the residue chromatographically purified (silica gel, 0–30% EtOAc/hexanes) to provide additional product (1.82 g, 14% yield): ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 9.39 (1H, br d, *J* = 9.2 Hz), 8.92 (1H, d, *J* =

5.9 Hz), 8.15 (1H, t, *J* = 8.1 Hz), 7.27–7.41 (3H, m), 7.04–7.13 (1H, m), 5.51 (1H, s), 4.28 (2H, dd, *J* = 11.1, 4.8 Hz), 3.95–4.04 (2H, m), 2.16–2.28 (1H, m), 1.45–1.51 (1H, m). MS (ESI) *m/z*: calculated: 413.0; observed: 414.0 $(M+1)^+$.

Step 2:

A mixture of *N*-(5-bromo-2-fluorophenyl)-4-(1,3-dioxan-2-yl)-2-fluorobenzothioamide (5.22 g, 12.6 mmol) and sodium carbonate (1.34 g, 12.6 mmol) in DMF (60 mL) was stirred at 110 °C for 16 h. The mixture was then allowed to cool to ambient temperature and was diluted with water. The resulting precipitate was collected by vacuum filtration, washed with water, and dried in vacuo to give 2-(4-(1,3-dioxan-2-yl)-2-fluorophenyl)-5-bromobenzo[d]thiazole (4.50 g, 91% yield) as a light-brown solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.37 (1H, t, *J* = 7.9 Hz), 8.35 (1H, d, *J* = 1.8 Hz), 8.19 (1H, d, *J* = 8.6 Hz), 7.68 (1H, dd, *J* = 8.6, 2.0 Hz), 7.43–7.51 (2H, m), 5.64 (1H, s), 4.19 (2H, dd, *J* = 11.0, 4.9 Hz), 3.99 (2H, td, *J* = 12.1, 2.2 Hz), 1.97–2.09 (1H, m), 1.46–1.52 (1H, m). MS (ESI) *m/z*: calculated: 393.0; observed: 394.1 $(M+1)^+$.

Synthesis of **29**



Methyl 1-((4-(5-bromobenzo[d]thiazol-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylate (**29**)

Step 1:

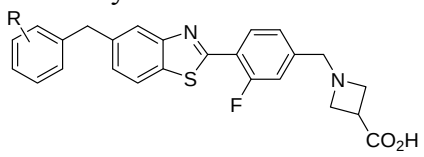
To a stirred suspension of 2-(4-(1,3-dioxan-2-yl)-2-fluorophenyl)-5-bromobenzo[d]thiazole (**28**; 3.00 g, 7.6 mmol) in acetone (120 mL) was added 5N aqueous hydrochloric acid (30 mL, 150 mmol), and the resulting mixture was stirred at 55 °C for 5 h. The reaction mixture was then cooled to room temperature and saturated aqueous $NaHCO_3$ was added. The precipitated solid was collected by vacuum filtration, washed with water, and dried in vacuo to afford 4-(5-bromobenzo[d]thiazol-2-yl)-3-fluorobenzaldehyde (2.6 g, 100% yield) as a light yellow solid: ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 10.08 (1H, d, *J* = 1.8 Hz), 8.63–8.67 (1H, m), 8.31 (1H, d, *J* = 1.8 Hz), 7.83–7.86 (2H, m), 7.76 (1H, dd, *J* = 10.8, 1.4 Hz), 7.58 (1H, dd, *J* = 8.5, 1.8 Hz). MS (ESI) *m/z*: calculated: 334.9; observed: 336.0 $(M+1)^+$.

Step 2:

Diisopropylethylamine (1.41 mL, 8.12 mmol) was added to a stirred solution of methyl azetidine-3-carboxylate hydrochloride (**35**; 1.17 g, 7.73 mmol) in MeOH (15 mL). 4-(5-bromobenzo[d]thiazol-2-yl)-3-fluorobenzaldehyde (2.60 g, 7.73 mmol) in 2:1 DCM/MeOH (30 mL) and acetic acid (0.670 mL, 11.6 mmol) were then sequentially

added, and the resulting mixture was stirred at room temperature for 1 h. Sodium cyanoborohydride (0.243 g, 3.87 mmol) was added, and the reaction mixture was stirred at room temperature for an additional 3 h. Saturated aqueous $NaHCO_3$ was added, and the resulting mixture was extracted with DCM (2×). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated in vacuo. Chromatographic purification of the residue (silica gel, 0–50% EtOAc/hexanes) gave methyl 1-((4-(5-bromobenzo[d]thiazol-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylate (2.03 g, 60% yield) as a yellow solid: ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 8.33 (1H, t, *J* = 7.9 Hz), 8.25 (1H, d, *J* = 1.8 Hz), 7.79 (1H, d, *J* = 8.5 Hz), 7.51 (1H, dd, *J* = 8.5, 1.9 Hz), 7.18–7.25 (2H, m), 3.73 (3H, s), 3.69 (2H, s), 3.54–3.62 (2H, m), 3.35–3.41 (3H, m). MS (ESI) *m/z*: calculated: 434.0; observed: 435.1 $(M+1)^+$.

Synthesis of **14-25**



General Method A (**14-25**)

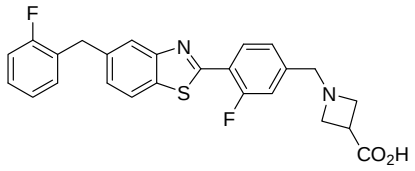
Step 1:

Methyl 1-((4-(5-bromobenzo[d]thiazol-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylate (**29**; 1 equiv.) and *trans*-dichlorobis(di-*tert*-butyl(phenyl)phosphine) palladium (II) (0.05 equiv.) were combined in a round-bottomed flask under an argon atmosphere. The appropriate benzylzinc chloride (0.5M solution in THF; 1.10 equiv.) was added via syringe, and the reaction mixture was stirred at room temperature for up to 3 h. Saturated aqueous $NaHCO_3$ was added, and the resulting mixture was extracted with EtOAc (2×). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. Chromatographic purification of the residue (silica gel, 0–50% EtOAc/hexanes) gave the desired product.

Step 2:

2M aqueous lithium hydroxide monohydrate (3 equiv.) was added to a stirred 0.13M solution of the product from step 1 (1 equiv.) in THF, and the resulting mixture was stirred at room temperature for up to 2 h. The reaction mixture was partially concentrated in vacuo and diluted with water. 1M aqueous HCl was added to adjust the aqueous mixture to pH ~ 2. 1M aqueous sodium phosphate buffer solution was then added to adjust the mixture to pH 6. The resulting precipitate collected by vacuum filtration, washed with water, and dried in vacuo to give the desired product.

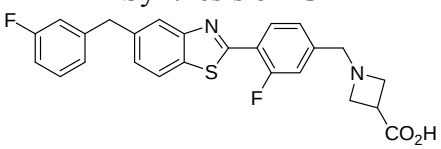
Synthesis of **14**



1-((4-(5-(2-Fluorobenzyl)benzo[d]thiazol-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylic acid (**14**)

Prepared via General Method A to give 1-((4-(5-(2-fluorobenzyl)benzo[d]thiazol-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylic acid (76% yield) as an off-white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.24 (1H, br s), 8.29 (1H, t, J = 7.9 Hz), 8.09 (1H, d, J = 8.2 Hz), 7.91 (1H, s), 7.27–7.42 (5H, m), 7.14–7.22 (2H, m), 4.17 (2H, s), 3.71 (2H, s), 3.46–3.55 (2H, m), 3.22–3.36 (3H, m). MS (ESI) *m/z*: calculated: 450.1; observed: 451.2 $(M+1)^+$.

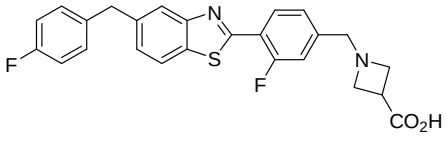
Synthesis of **15**



1-((4-(5-(3-Fluorobenzyl)benzo[d]thiazol-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylic acid (**15**)

Prepared via General Method A to give 1-((4-(5-(3-fluorobenzyl)benzo[d]thiazol-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylic acid (88% yield) as an off-white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.20 (1H, br s), 8.28 (1H, t, J = 7.9 Hz), 8.09 (1H, d, J = 8.2 Hz), 7.99 (1H, s), 7.31–7.42 (4H, m), 7.14–7.19 (2H, m), 6.99–7.06 (1H, m), 4.15 (2H, s), 3.68 (2H, s), 3.43–3.51 (2H, m), 3.21–3.31 (3H, m). MS (ESI) *m/z*: calculated: 450.1; observed: 451.2 $(M+1)^+$.

Synthesis of **16**

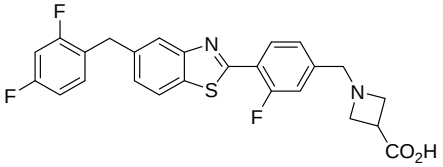


1-((4-(5-(4-Fluorobenzyl)benzo[d]thiazol-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylic acid (**16**)

Prepared via General Method A to give 1-((4-(5-(4-fluorobenzyl)benzo[d]thiazol-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylic acid (89% yield) as an off-white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.24 (1H, br s), 8.27 (1H, t, J = 8.0 Hz), 8.08 (1H, d, J = 8.2 Hz), 7.96 (1H, s), 7.31–7.39 (5H, m), 7.09–7.16 (2H, m), 4.12 (2H, s), 3.65

(2H, s), 3.42–3.47 (2H, m), 3.22–3.28 (3H, m). MS (ESI) *m/z*: calculated: 450.1; observed: 451.2 $(M+1)^+$.

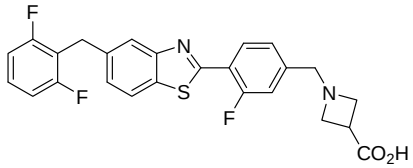
Synthesis of **17**



1-((4-(5-(2,4-Difluorobenzyl)benzo[d]thiazol-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylic acid (**17**)

Prepared via General Method A to give 1-((4-(5-(2,4-difluorobenzyl)benzo[d]thiazol-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylic acid (96% yield) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.16 (1H, br s), 8.31 (1H, t, *J* = 7.8 Hz), 8.10 (1H, d, *J* = 8.0 Hz), 7.91 (1H, s), 7.35–7.48 (4H, m), 7.19–7.26 (1H, m), 7.03–7.09 (1H, m), 4.15 (2H, s), 3.83 (2H, s), 3.48–3.67 (2H, m), 3.23–3.39 (3H, m). MS (ESI) *m/z*: calculated: 468.1; observed: 469.1 $(M+1)^+$.

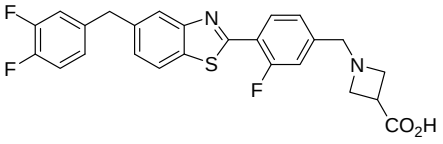
Synthesis of **18**



1-((4-(5-(2,6-Difluorobenzyl)benzo[d]thiazol-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylic acid (**18**)

Prepared via General Method A to give 1-((4-(5-(2,6-difluorobenzyl)benzo[d]thiazol-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylic acid (79% yield) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.21 (1H, br s), 8.28 (1H, t, *J* = 7.8 Hz), 8.10 (1H, d, *J* = 8.3 Hz), 7.84 (1H, s), 7.30–7.44 (4H, m), 7.11–7.19 (2H, m), 4.18 (2H, s), 3.65 (2H, s), 3.41–3.48 (2H, m), 3.21–3.28 (3H, m). MS (ESI) *m/z*: calculated: 468.1; observed: 469.1 $(M+1)^+$.

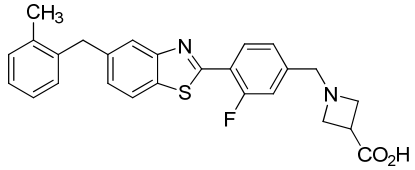
Synthesis of **19**



1-((4-(5-(3,4-Difluorobenzyl)benzo[d]thiazol-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylic acid (**19**)

Prepared via General Method A to give 1-((4-(5-(3,4-difluorobenzyl)benzo[d]thiazol-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylic acid (99% yield) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.11 (1H, br s), 8.33 (1H, t, J = 7.8 Hz), 8.10 (1H, d, J = 8.0 Hz), 8.01 (1H, s), 7.32–7.53 (5H, m), 7.14–7.20 (1H, m), 4.13 (2H, s), 4.02 (2H, br s), 3.60–3.86 (3H, m), 3.37–3.49 (2H, m). MS (ESI) *m/z*: calculated: 468.1; observed: 469.1 $(M+1)^+$.

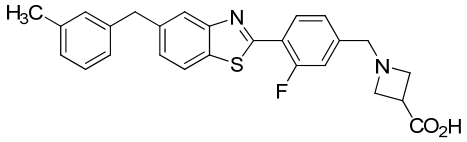
Synthesis of **20**



1-(3-Fluoro-4-(5-(2-methylbenzyl)benzo[d]thiazol-2-yl)benzyl)azetidine-3-carboxylic acid (**20**)

Prepared via General Method A to give 1-(3-fluoro-4-(5-(2-methylbenzyl)benzo[d]thiazol-2-yl)benzyl)azetidine-3-carboxylic acid (77% yield) as a light yellow solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.20 (1H, br s), 8.28 (1H, t, J = 7.9 Hz), 8.08 (1H, d, J = 8.4 Hz), 7.79 (1H, s), 7.30–7.40 (3H, m), 7.14–7.23 (4H, m), 4.15 (2H, s), 3.73 (2H, s), 3.48–3.57 (2H, m), 3.23–3.38 (3H, m), 2.24 (3H, s). MS (ESI) *m/z*: calculated: 446.2; observed: 447.2 $(M+1)^+$.

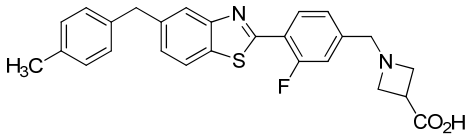
Synthesis of **21**



1-(3-Fluoro-4-(5-(3-methylbenzyl)benzo[d]thiazol-2-yl)benzyl)azetidine-3-carboxylic acid (**21**)

Prepared via General Method A to give 1-(3-fluoro-4-(5-(3-methylbenzyl)benzo[d]thiazol-2-yl)benzyl)azetidine-3-carboxylic acid (89% yield) as an off-white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.14 (1H, br s), 8.32 (1H, t, J = 7.9 Hz), 8.08 (1H, d, J = 8.2 Hz), 7.95 (1H, s), 7.49 (1H, d, J = 12.1 Hz), 7.42 (1H, d, J = 8.3 Hz), 7.38 (1H, dd, J = 8.3, 1.5 Hz), 7.19 (1H, t, J = 7.5 Hz), 7.08–7.13 (2H, m), 7.02 (1H, d, J = 7.5 Hz), 4.08 (2H, s), 3.98 (2H, br s), 3.57–3.83 (3H, m), 3.36–3.45 (2H, m), 2.27 (3H, s). MS (ESI) *m/z*: calculated: 446.2; observed: 447.2 $(M+1)^+$.

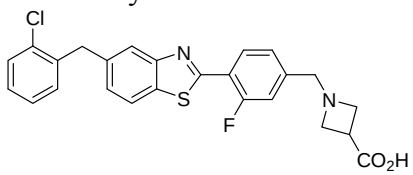
Synthesis of **22**



1-(3-Fluoro-4-(5-(4-methylbenzyl)benzo[d]thiazol-2-yl)benzyl)azetidine-3-carboxylic acid (**22**)

Prepared via General Method A to give 1-(3-fluoro-4-(5-(4-methylbenzyl)benzo[d]thiazol-2-yl)benzyl)azetidine-3-carboxylic acid (84% yield) as an off-white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.39 (1H, br s), 8.27 (1H, t, J = 8.0 Hz), 8.06 (1H, d, J = 8.2 Hz), 7.92 (1H, s), 7.30–7.37 (3H, m), 7.19 (2H, d, J = 8.0 Hz), 7.11 (2H, d, J = 8.0 Hz), 4.07 (2H, s), 3.65 (2H, s), 3.41–3.48 (2H, m), 3.21–3.28 (3H, m), 2.26 (3H, s). MS (ESI) *m/z*: calculated: 446.2; observed: 447.2 $(M+1)^+$.

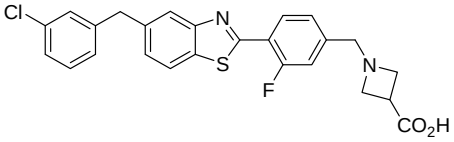
Synthesis of **23**



1-((4-(5-(2-Chlorobenzyl)benzo[d]thiazol-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylic acid (**23**)

Prepared via General Method A to give 1-((4-(5-(2-chlorobenzyl)benzo[d]thiazol-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylic acid (76% yield) as an off-white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.14 (1H, br s), 8.34 (1H, t, J = 8.0 Hz), 8.11 (1H, d, J = 8.4 Hz), 7.87 (1H, s), 7.28–7.54 (7H, m), 4.27 (2H, s), 4.06 (2H, s), 3.63–3.91 (4H, m), 3.38–3.50 (1H, m). MS (ESI) *m/z*: calculated: 466.1; observed: 467.2 $(M+1)^+$.

Synthesis of **24**

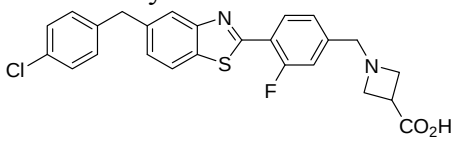


1-((4-(5-(3-Chlorobenzyl)benzo[d]thiazol-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylic acid (**24**)

Prepared via General Method A to give 1-((4-(5-(3-chlorobenzyl)benzo[d]thiazol-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylic acid (87% yield) as an off-white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.22 (1H, br s), 8.29 (1H, t, J = 7.8 Hz), 8.09 (1H,

d, J = 8.5 Hz), 8.00 (1H, s), 7.25–7.42 (7H, m), 4.14 (2H, s), 3.71 (2H, s), 3.47–3.56 (2H, m), 3.22–3.36 (3H, m). MS (ESI) *m/z*: calculated: 466.1; observed: 467.2 $(M+1)^+$.

Synthesis of **25**



1-((4-(5-(4-Chlorobenzyl)benzo[d]thiazol-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylic acid (**25**)

Prepared via General Method A to give 1-((4-(5-(4-chlorobenzyl)benzo[d]thiazol-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylic acid (81% yield) as an off-white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.26 (1H, br s), 8.28 (1H, t, J = 8.0 Hz), 8.08 (3H, d, J = 8.2 Hz), 7.96 (1H, s), 7.31–7.39 (7H, m), 4.13 (2H, s), 3.65 (2H, s), 3.42–3.47 (2H, m), 3.22–3.27 (3H, m). MS (ESI) *m/z*: calculated: 466.1; observed: 467.2 $(M+1)^+$.

hS1PR cellular assays

The $hS1P_1$ receptor internalization assay was performed using a U2OS cell line expressing $hS1P_1$-eGFP chimeric protein (Thermo Scientific (BioImage), Søborg, Denmark). Upon compound treatment, the $hS1P_1$ receptor was internalized into the cytoplasm, forming GFP-containing endosomes. This event was detected using an ArrayScan VTI automated microscope (Thermo Scientific Cellomics, Pittsburg, PA), and the degree of receptor internalization was quantitated by counting the number of GFP-containing endosomes per cell. $hS1P_1$-eGFP expressing U2OS cells were starved in serum free media for two hours prior to compound treatment. Compounds were incubated with the starved cells at 37 °C for one hour. Compound-treated cells were subsequently fixed using 4% formaldehyde, and nuclei were stained using Hoechst dye (Invitrogen/Molecular Probes, Cat. #H3570). The cells were then imaged by ArrayScan VTI, and the potency and efficacy of the compounds were determined by plotting the number of GFP-containing endosomes per cell against corresponding compound concentration.

The Ca^{2+}-mobilization assay was performed using CHO cell lines stably co-expressing $hS1P_3$ receptor and a chimeric $G_{q/i5}$ G-protein. S1P (a known agonist) or compound treatment of these cells activated the PLC-β / IP3 pathway, triggering release of Ca^{2+} from intracellular stores (e.g., the ER). Cells were loaded with Ca^{2+} sensitive fluorescent dye (Calcium Indicator Dye, Cat. #51-9000177BK, BD Biosciences) and a fluorescence quencher (PBX Signal Enhancer, Cat. #51-9006254, BD Biosciences) prior to compound treatment. Intracellular Ca^{2+} release resulted in Ca^{2+} binding to the dye and dye fluorescence (520 nm peak emission wavelength) upon excitation at 480 nm. The level of receptor activation was quantitated by measuring fluorescence intensity following compound treatment. In this assay, cells were starved in medium containing charcoal/dextran stripped serum for 16–20 hours. Compounds were added to cells loaded with Ca^{2+} sensitive dye and fluorescence quencher inside a $FLIPR^{TETRA®}$ plate reader (Molecular Devices, Sunnyvale, CA) and the fluorescence signal was measured. CHO cells expressing only the chimeric $G_{q/i5}$ G-protein were employed as a negative control. The potency and efficacy of the compounds were determined by plotting fluorescence intensity against corresponding compound concentration.

Rat lymphocyte depletion study

Female Lewis rats (250 grams, 6–8 wks) were received from Charles River Laboratories (Wilmington, MA) and allowed to acclimatize for at least one week before being placed on study. Rats (N = 5/group) were administered vehicle (20% captisol in water) or **14** (0.3, 1.0, or 3.0 mg/kg in 20% captisol/water) by oral gavage (10 mL/kg). 24 h post-dose, animals were sacrificed by CO_2 inhalation, and blood was collected by cardiac puncture. Approximately 1 mL of blood was transferred to a Microtainer® hematology tube containing EDTA (Becton Dickinson, #365973) for CBC analysis and 500 μL of plasma was placed in a Microtainer® tube containing heparin (Becton Dickinson, #365958) for subsequent pharmacokinetic analysis (plasma exposure). Differential cell counts were obtained using an Advia® 120 hematology system (Bayer Diagnostics).

Delayed-type hypersensitivity (DTH) antigen challenge study

11–13 week old female Lewis rats were obtained from Charles River Laboratory (Wilmington, MA) and were housed and maintained per IACUC standards. Chicken ovalbumin was purchased from Sigma (A5503; batch #076K7045). Complete Freund's adjuvant was purchased from DIFCO Laboratories (263810, lot 5341879). Microtainer® hematology tubes containing EDTA (Becton Dickinson, #365973; for CBC analysis) and heparin (Becton Dickinson, #365958; for pharmacokinetic analysis) were purchased from Vacutainer Systems.

On Day 0, female Lewis rats were given a subcutaneous injection of an emulsion consisting of chicken ovalbumin (OVA) in Complete Freund's Adjuvant (CFA). Rats were treated with vehicle (20% captisol/water) or **14** (0.1, 0.3, 1 or 3 mg/kg in 20% captisol/water) once daily by oral gavage (10 mL/kg). Drug treatment was started one day prior to the OVA injection, and continued once daily for 10 days (ending on day 8). On Day 7, rats were given an intra-cutaneous injection of 20 μg of OVA in saline in one ear, and an injection of saline in the contra-lateral ear (negative control). Ear thickness was measured by using a Mitutoyo micrometer on day 7, 8 and 9 (measurements were performed in a blinded manner for days 8 & 9). Blood was collected for complete blood cell count (CBC) analysis 5 days prior to the start of the study (baseline measurement), on day 2 (24 hours post third dose), and on day 9 (24 hours post tenth dose). The blood samples from day 2 and day 9 were also analyzed for test compound plasma exposure.

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