Benzofuran Derivatives as Potent, Orally Active S1P1 Receptor Agonists: A preclinical Lead Molecule for MS

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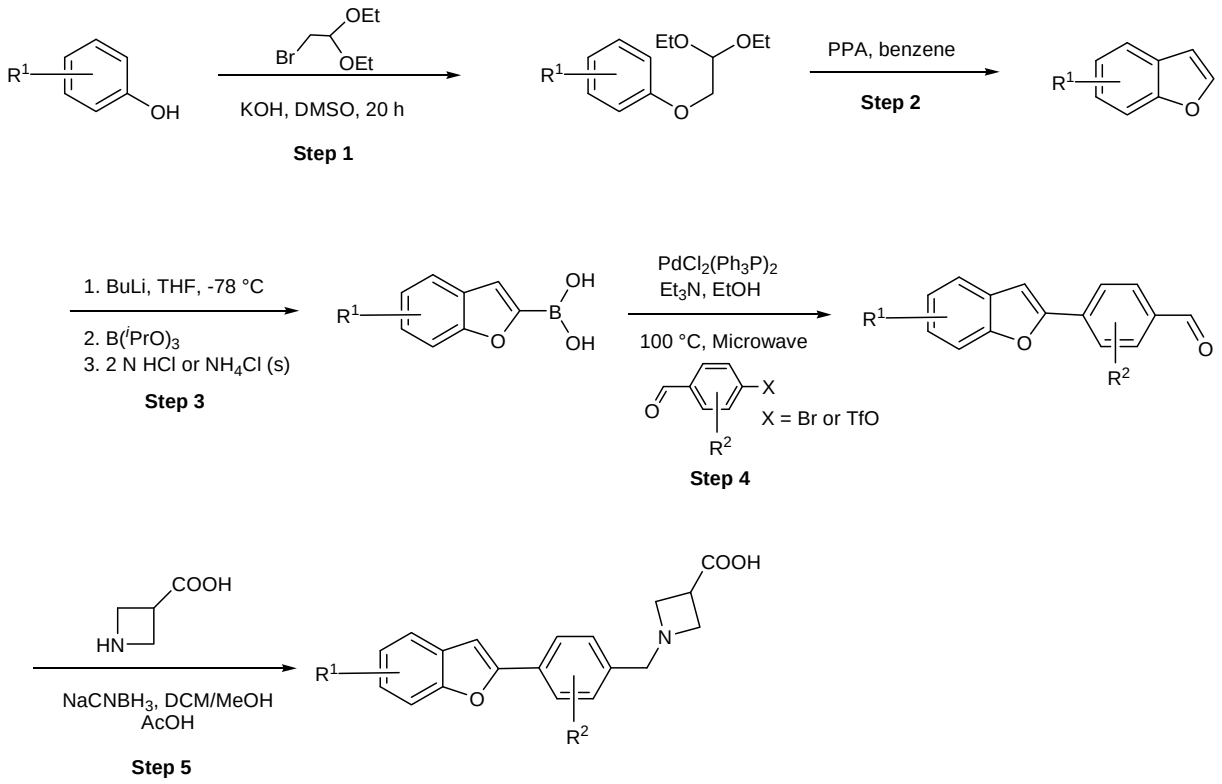
Supporting Information

Synthesis of 1-21
hS1PR cellular assays
Rat lymphocyte depletion study

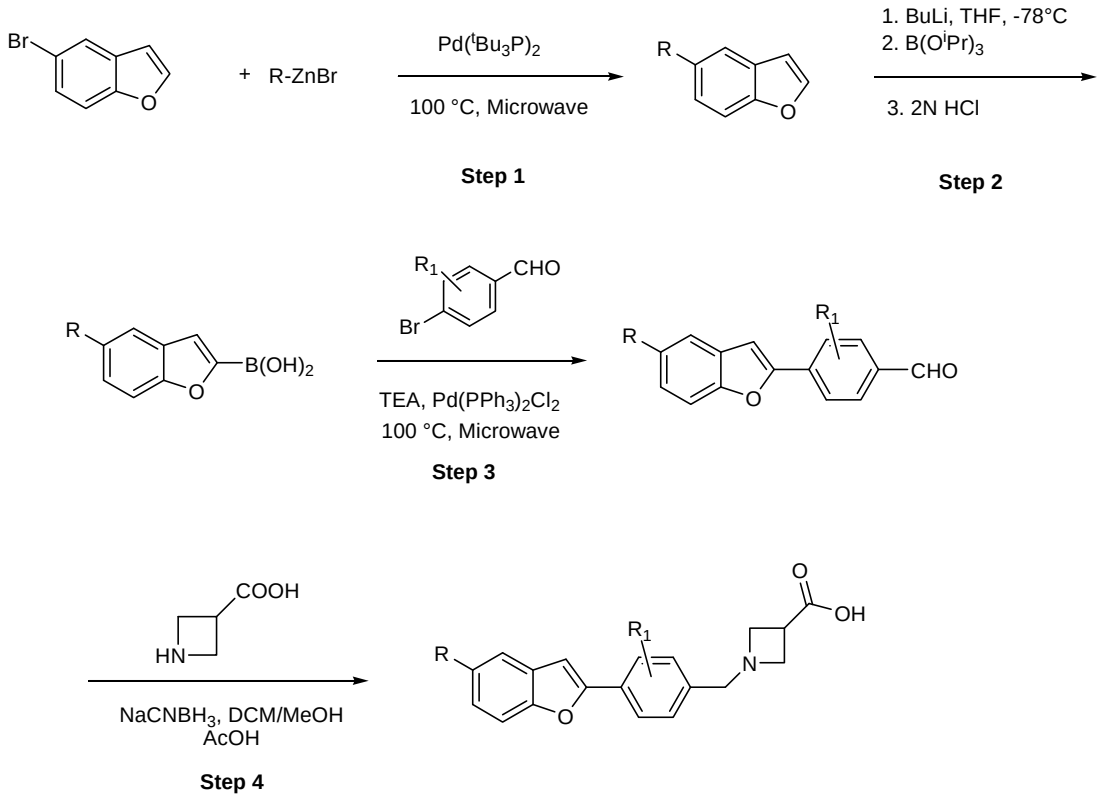
Schemes 1 to 5

General Methods: All chemicals and anhydrous solvents were obtained from commercial suppliers. All reagents and solvents were used without further purification. All synthetic compounds and intermediates gave satisfactory MS and HPLC purities. Mass spectrometry analyses were performed on Finnigan Advantage system. HPLC analyses were recorded on a HP110 system or Gilson HPLC system consisting of a 170 Diode Array detector, a 215 liquid handler, and a 322-pump. ^{1}H and ^{13}C NMR spectra were recorded at 400 MHz on a Varian AS400 NMR spectrometer with $CDCl_3$, DMSO-d_6 and MeOH-d_4 as solvents.

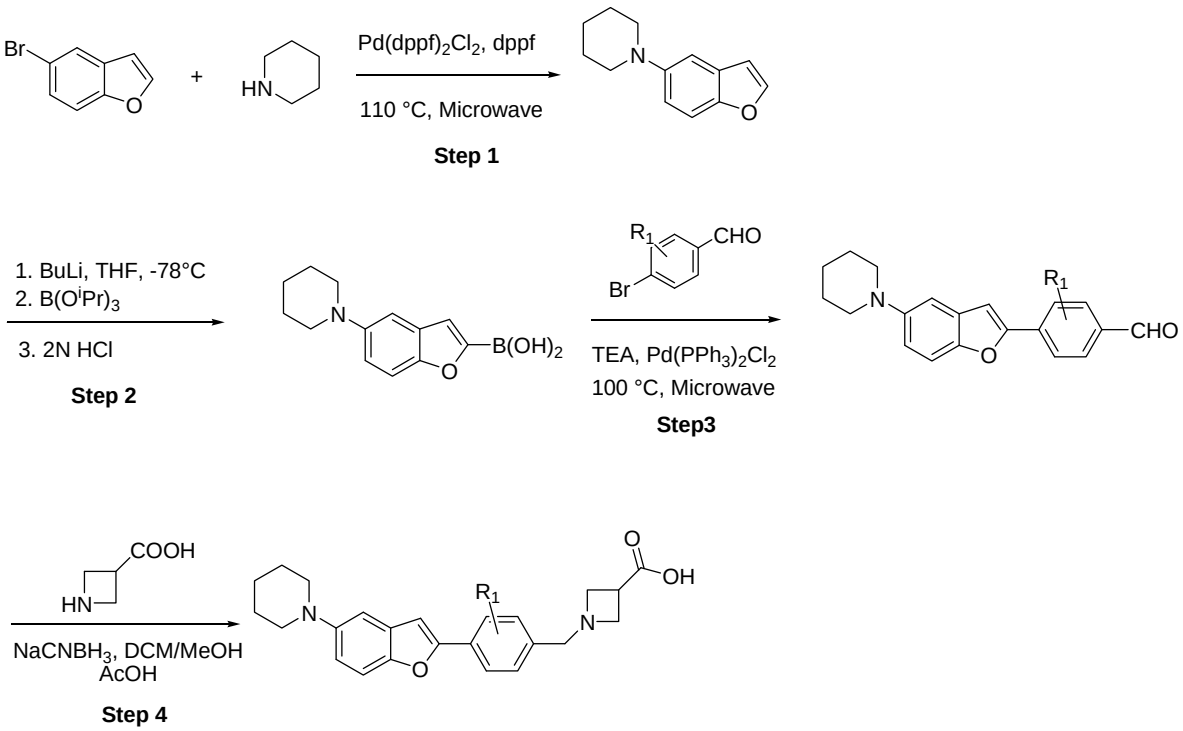
Scheme 1



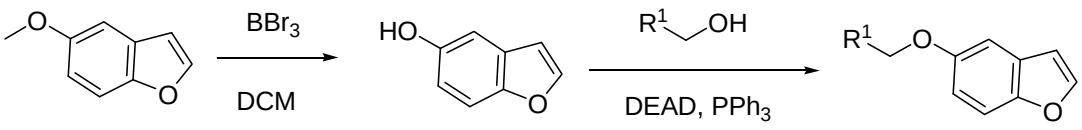
Scheme 2


Br
+ R-ZnBr
Pd(tBu3P)2
100 °C, Microwave
Step 1
R
1. BuLi, THF, -78°C
2. B(OiPr)3
3. 2N HCl
Step 2
R
B(OH)2
R1
CHO
Br
TEA, Pd(PPh3)2Cl2
100 °C, Microwave
Step 3
COOH
HN
NaCNBH3, DCM/MeOH
AcOH
Step 4
OH
N

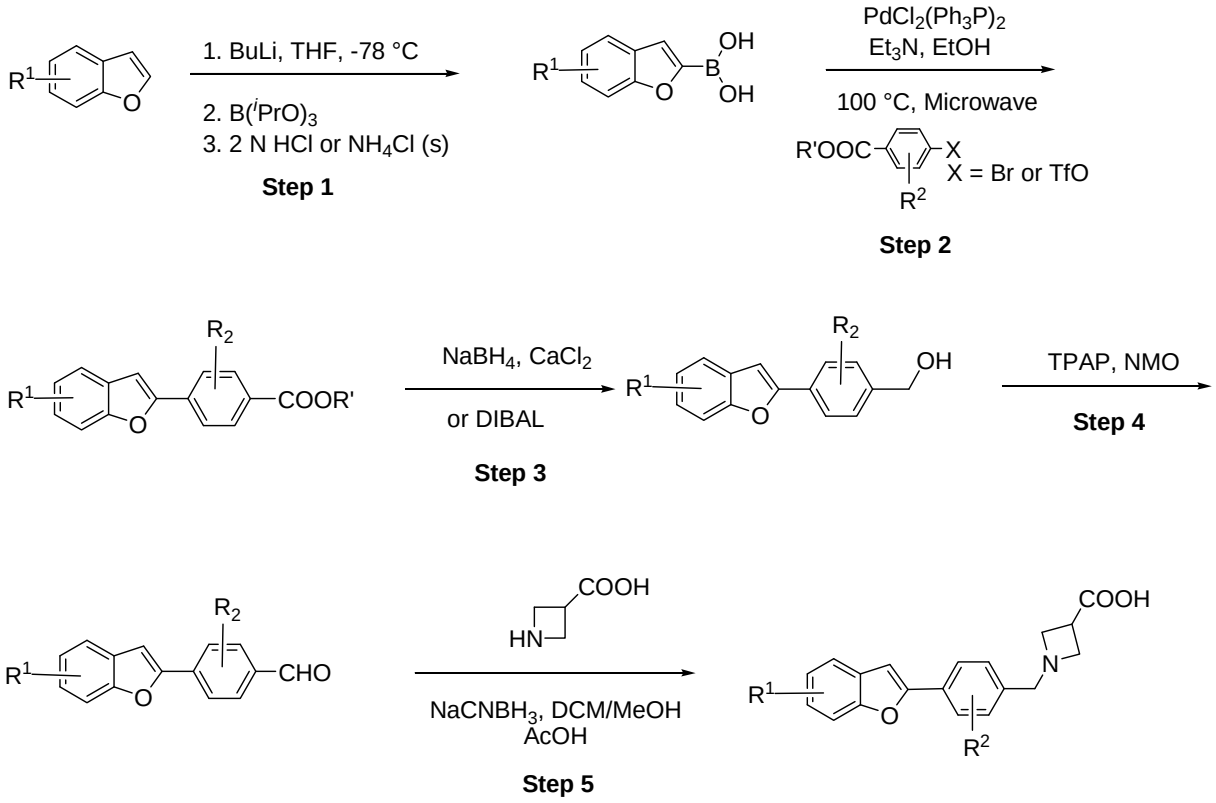
Scheme 3


Br
O
+
HN
Pd(dppf)2Cl2, dppf
110 °C, Microwave
Step 1
N
O
1. BuLi, THF, -78°C
2. B(OiPr)3
3. 2N HCl
Step 2
B(OH)2
R1
CHO
Br
TEA, Pd(PPh3)2Cl2
100 °C, Microwave
Step3
COOH
NaCNBH3, DCM/MeOH
AcOH
Step 4
OH

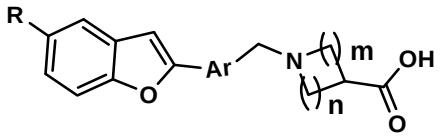
Scheme 4



Scheme 5



Compounds were prepared using the general procedures as described below:



(n, m = 0, 1, 2 respectively)

A: General procedure for C-C bond coupling with Rieke reagents

5-bromobenzofuran (1.0 mmol) was dissolved in a THF solution of Rieke reagent (0.5M, 2.9 mmol) in a microwave reaction tube. $Pd(P^tBu_3)_2$ (0.05 mmol) was added to this solution. The mixture was purged with N_2 gas for 3-5 min and heated at 100 °C for 30 min under microwave irradiation (Personal Chemistry Emrys^{TM} Optimizer microwave reactor). Upon completion of the reaction, the reaction mixture was diluted with ethyl acetate, washed with 1N HCl aqueous solution, brine, filtered through Celite. The filtrate was dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (ISCO system) to give a pure product.

B: General procedure for N-C bond coupling reaction

5-bromobenzofuran (1.0 mmol), piperidine (1.2 mmol), $Pd(dppf)Cl_2$ (0.03 mmol), dppf (0.045 mmol) and sodium *tert*-butoxide (1.5 mmol) was mixed in toluene (2 mL). The mixture was purged with N_2 gas for 3-5 min and heated at 120 °C for 30 min under microwave irradiation (Personal Chemistry Emrys^{TM} Optimizer microwave reactor). Upon completion of the reaction, the reaction mixture was directly loaded on silica gel column and purified on ISCO system (5% EtOAc in hexanes) to give a pure product.

C: General preparative procedure for formation of benzofuran boronic acids

A solution of *n*-BuLi (1.2 mmol, 2.5M solution in hexanes) was added dropwise to a solution of benzofuran compounds (1.0 mmol) in anhydrous THF (20 mL) at -78 °C. The resulting mixture was stirred at -78 °C for 20 min, and treated with $B(^iPrO)_3$ (1.5 mmol). The reaction mixture was allowed to warm up slowly to room temperature and stirred for 1 h. The reaction was cooled in ice-bath and quenched with 2N HCl or saturate NH_4Cl and extracted with Et_2O. The combined organic extracts were washed with brine, dried and concentrated under reduced pressure to yield a desired benzofuran boronic acid without further purification for next step.

D: General procedure for coupling boronic acids with aryl halides

A mixture of benzofuran boronic acid (1.1 mmol), aryl halide (1.0 mmol), triethylamine (20 mmol) and bis(triphenylphosphine)palladium(II) chloride (0.05 mmol) in ethanol (30 mL) was irradiated in a Microwave instrument at 100 °C for 20 min. The reaction mixture was cooled, and the solvent was removed. The residue was treated with water and extracted with ethyl acetate. The organic layer was dried and concentrated *in vacuo* (the aqueous work-up is optional). Purification by silica gel chromatography gave the desired product.

E: General procedure for reductive amination

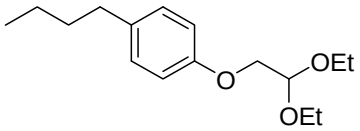
A mixture of aldehyde (1.0 mmol), acetic acid (1.5 mmol) and azetidine-3-carboxylic acid or piperidine-4-carboxylic acid (1.2-1.5 mmol) in DCM/MeOH (1:1, 10 mL) was stirred at room temperature for 1 h. Sodium cyanoborohydride (0.5 mmol) was added and the reaction mixture was stirred for 2-3 h at room temperature. After concentration of solvent under reduced pressure, the resulting residue was dissolved in DMSO, filtered and purified by reverse phase preparative HPLC (Phenomenex reverse phase Luna 5u C18(2) column, 60 x 21.2 mm ID, mobile phase: A = 0.05% TFA in water; B = 0.05% TFA in acetonitrile. The flow rate was 10-12 mL / min) to yield the desired final product with puritiy greater than 95%. All final products were obtained as the TFA salts except for Compound 59. Alternatively, the crude mixture of reductive amination can be purified by trituration with MeOH and water.

Synthesis of Compounds 1 to 21

Compound 1

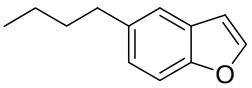
1-((4-(5-Butylbenzofuran-2-yl)phenyl)methyl)azetidine-3-carboxylic acid

1-(2,2-Diethoxyethoxy)-4-butylbenzene (step 1 in Scheme 1):



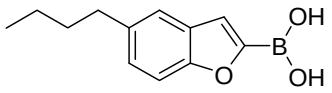
A mixture of 4-butylphenol (4.42 g, 29.4 mmol), bromoacetaldehyde diethyl acetal (4.56 mL, 29.4 mmol) and KOH (1.94 g, 29.4 mmol) in DMSO (15 mL) was stirred at reflux for 6 h. The reaction mixture was allowed to cool down to room temperature and poured over ice containing 0.60 g of KOH and diluted to 100 mL with water. The solution was extracted with Et_2O (20 mL x 3); the combined extracts were washed with 1N NaOH solution, water and brine, dried, and concentrated under reduced pressure to yield the desired product (90%) that was used without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07 (d, J = 8.8, 2H), 6.83 (d, J = 8.8, 2H), 4.83 (t, J = 5.1, 1H), 3.98 (d, J = 5.1, 2H), 3.80-3.72 (m, 2H), 3.67-3.59 (m, 2H), 2.54 (t, J = 7.7, 2H), 1.59-1.51 (m, 2H), 1.36-1.30 (m, 2H), 1.24 (t, J = 7.0, 6H), 0.91 (t, J = 7.3, 3H).

5-Butylbenzofuran (step 2 Scheme 1):



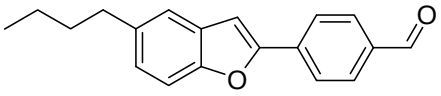
A mixture of 1-butyl-4-(2,2-diethoxyethoxy)benzene (3.28g, 12.3 mmol) and polyphosphoric acid (2.95 g, 29.4 mmol) in benzene (60 mL) was stirred at reflux for 2 h. The reaction mixture was cooled to room temperature, decanted from the PPA and filtered through a plug of silica gel, which was washed with hexanes. The filtrate and the wash were combined anc concentrated under reduced pressure to yield the crude benzofuran (91% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, J = 2.2, 1H), 7.41-7.36 (m, 2H), 7.11 (dd, J = 8.5, 1.8, 1H), 6.70 (dd, J = 2.2, 1.1, 1H), 2.70 (t, J = 7.7, 2H), 1.67-1.60 (m, 2H), 1.42-1.32 (m, 2H), 0.93 (t, J = 7.3, 3H).

5-Butylbenzofuran-2-yl-2-boronic acid (step 3 Scheme 1):



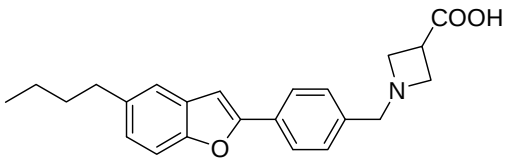
A solution of *n*-BuLi (2.0 mL, 2.5M solution in hexanes) was added dropwise to a solution of 5-butylbenzofuran (734 mg, 4.21 mmol) in anhydrous THF (20 mL) at -78 °C. The resulting mixture was stirred at -78 °C for 20 min, and treated with $B(^{i}PrO)_3$ (1.46 mL, 6.31 mmol). The reaction mixture was allowed to warm up slowly to room temperature and stirred for 1 h. The reaction was quenched with 2N HCl and extracted with Et_2O. The combined extracts were washed with brine, dried and concentrated under reduced pressure to yield the crude boronic acid (67%), that was used for next step reaction without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43-7.31 (m, 2H), 7.22-7.14 (m, 2H), 2.70 (t, *J* = 7.7, 2H), 1.67-1.59 (m, 2H), 1.41-1.32 (m, 2H), 0.93 (t, *J* = 7.3, 3H).

4-(5-Butylbenzofuran-2-yl)benzaldehyde (step 4 Scheme 1):



A solution of 5-butylbenzofuran-2-ylboronic acid (484 mg, 2.22 mmol), 4-bromobenzaldehyde (315 mg, 1.70 mmol), palladiumdichlorobis(triphenylphosphine) (60 mg, 0.085 mmol) and triethylamine (4.74 mL, 34 mmol) in EtOH was irradiated in the microwave at 100 °C for 1200 seconds. The precipitated that formed was filtered and rinsed with ethanol to yield the desired benzaldehyde (72% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.03 (s, 1H), 8.00 (d, *J* = 8.4, 2H), 7.94 (d, *J* = 8.4, 2H), 7.45-7.41 (m, 2H), 7.17-7.15 (m, 2H), 2.71 (t, *J* = 7.7, 2H), 1.68-1.61 (m, 2H), 1.41-1.33 (m, 2H), 0.94 (t, *J* = 7.3, 3H).

1-((4-(5-Butylbenzofuran-2-yl)phenyl)methyl)azetidine-3-carboxylic acid (step 5 Scheme 1):

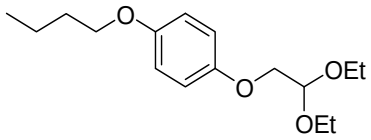


A mixture of 4-(5-butylbenzofuran-2-yl)benzaldehyde (39 mg, 0.14 mmol) and azetidine-3-carboxylic acid (30 mg, 0.28 mmol) in MeOH (1 mL) was stirred at room temperature for 1 h. Sodium cyanoborohydride (60 mg, 0.28 mmol) was added in two portions and the reaction mixture was stirred for 16 h. Concentration of the solvent under reduced pressure yielded a yellow solid that was dissolved in DMSO and purified by reverse phase prepative HPLC to yield the desired product: (42% yield): ^{1}H NMR (400 MHz, CD_3OD) δ 7.98 (d, *J* = 8.4, 2H), 7.55 (d, *J* = 8.4, 2H), 7.43-7.41 (m, 2H), 7.23 (s, 1H), 7.15 (d, *J* = 8.8, 1H), 4.40 (s, 2H), 4.25-4.23 (m, 4H), 3.52-3.46 (m, 1H), 2.71 (t, *J* = 7.7, 2H), 1.67-1.61 (m, 2H), 1.41-1.33 (m, 2H), 0.95 (t, *J* = 7.3, 3H). MS (ESI) m/z: Calculated: 363.18; Observed: 364.0 (M^++1).

Compound 2

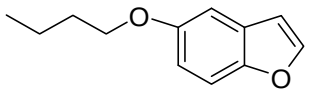
1-(4-(5-Butoxybenzofuran-2-yl)phenyl)methyl)azetidine-3-carboxylic acid

1-(2,2-Diethoxyethoxy)-4-butoxybenzene:



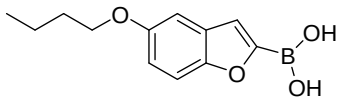
The title compound was prepared according to the procedure described for compound **1** (step 1 in Scheme 1): 84% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.86-6.80 (m, 4H), 4.81 (t, *J* = 5.1, 1H), 3.96 (d, *J* = 5.1, 2H), 3.90 (t, *J* = 6.6, 2H), 3.79-3.72 (m, 2H), 3.67-3.59 (m, 2H), 1.77-1.70 (m, 2H), 1.52-1.43 (m, 2H), 1.24 (t, *J* = 7.0, 6H), 0.96 (t, *J* = 7.4, 3H).

5-Butoxybenzofuran:



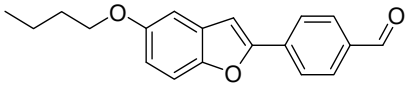
The title compound was prepared according to the procedure described for compound **1** (step 2 in Scheme 1): 81% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, *J* = 2.2, 1H), 7.38 (d, *J* = 9.2, 1H), 7.05 (d, *J* = 2.5, 1H), 6.90 (dd, *J* = 2.5, 8.8, 1H), 6.69 (br d, *J* = 2.2, 1H), 3.99 (t, *J* = 6.6, 2H), 1.82-1.75 (m, 2H), 1.56-1.47 (m, 2H), 0.99 (t, *J* = 7.3, 3H).

5-Phenylbenzofuran-2-yl-2-boronic acid (step 3 in Scheme 1):



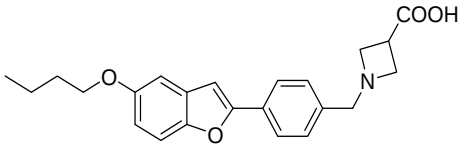
The title compound was prepared according to general procedure C: a solution of *n*-BuLi (2.5 mL, 2.5M solution in hexanes) was added dropwise to a solution of 5-butoxybenzofuran (1.0g, 5.21 mmol) in anhydrous THF (20 mL) at -78 °C. The resulting mixture was stirred at -78 °C for 20 min, and treated with B(^{i}PrO)$_3$ (1.80 mL, 7.8 mmol). The reaction mixture was allowed to warm up slowly to room temperature and stirred for 1 h. The reaction was quenched with 2N HCl and extracted with Et_2O. The combined extracts were washed with brine, dried and concentrated under reduced pressure to yield 1.2 g of crude boronic acid, that was used without further purification: (98% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, 1H), 7.30 (d, 1H), 7.06 (s, 1H), 6.98 (d, 1H), 4.44 (s, 2H), 1.81-1.71 (m, 2H), 1.58-1.50 (m, 2H), 1.00 (t, 3H).

4-(5-Butoxybenzofuran-2-yl)benzaldehyde (step 4 in Scheme 1)::



The title compound was prepared according to general procedure D: a solution of 5-phenylbenzofuran-2-yl-2-boronic acid (702 mg, 3.0 mmol), 4-bromobenzaldehyde (427 mg, 2.30 mmol), palladiumdichlorobis(triphenylphosphine) (80 mg, 0.11 mmol) and triethylamine (6.5 mL, 45 mmol) in EtOH (2mL) was irradiated in the microwave at 100 °C for 1200 s. The precipitate that formed was filtered and rinsed with ethanol to yield 620 mg of crude product, which upon column chromatography afforded 375 mg of the desired compound (43%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.03 (s, 1H), 8.05 (d, 2H), 7.98 (d, 2H), 7.82 (d, 1H), 7.18 (d, 1H), 7.16 (d, 1H), 6.94 (s, 1H), 4.44 (s, 2H), 1.81-1.71 (m, 2H), 1.58-1.50 (m, 2H), 1.00 (t, 3H). MS (ESI) m/z: Calculated: 294.34; Observed: 295.2 (M^++1).

1-(4-(5-Butoxybenzofuran-2-yl)phenyl)methyl)azetidine-3-carboxylic acid (step 5 in Scheme 1):



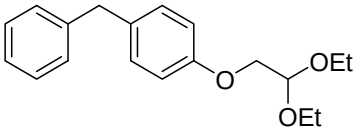
The title compound was prepared according to general procedure E: a mixture of 4-(5-butoxybenzofuran-2-yl)benzaldehyde (70 mg, 0.30 mmol), azetidine-3-carboxylic acid (46 mg, 0.45 mmol) and acetic acid (0.50 mmol) in MeOH-DCM (3:1; 2 mL) was stirred at room

temperature for 1 h. Sodium triacetoxyborohydride (211 mg, 1.00 mmol) was added and the reaction mixture was stirred for 16 h. Concentration of the solvent under reduced pressure yielded a yellow solid that was dissolved in DMSO (3 mL) and filtered to give a yellow solution that was purified by HPLC to afford 6 mg of desired product (5% yield) : ^{1}H NMR (400 MHz, CD_3OD) δ 7.97 (d, 2H), 7.55 (d, 2H), 7.40 (d, 1H), 7.21 (s, 1H), 7.10 (d, 1H), 6.92-6.89 (dd, 1H), 4.44 (s, 2H), 4.37 (q, 4H), 4.00 (t, 2H), 3.72-3.64 (m, 1H), 1.81-1.71 (m, 2H), 1.58-1.50 (m, 2H), 1.00 (t, 3H). MS (ESI) m/z: Calculated: 379.45; Observed: 380.3 (M^++1).

Compound 3

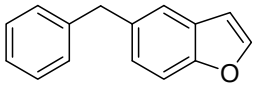
1-((4-(5-Benzylbenzofuran-2-yl)phenyl)methyl)azetidine-3-carboxylic acid

1-(4-(2,2-Diethoxyethoxy)benzyl)benzene:



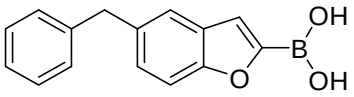
The title compound was prepared according to the procedure described for compound **1** (step 1 in Scheme 1): 84% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30-7.25 (m, 2H), 7.20-7.15 (m, 3H), 7.09 (d, *J* = 8.8, 2H), 6.84 (d, *J* = 8.8, 2H), 4.82 (t, *J* = 5.5, 1H), 3.98 (d, *J* = 5.5, 2H), 3.92 (s, 2H), 3.79-3.72 (m, 2H), 3.66-3.59 (m, 2H), 1.24 (t, 7.1, 3H).

5-Benzylbenzofuran:



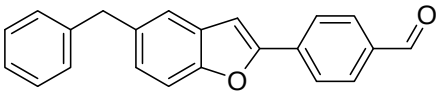
The title compound was prepared according to the procedure described for compound **1** (step 2 in Scheme 1): 89% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, *J* = 2.2, 1H), 7.42-7.40 (m, 2H), 7.31-7.7.26 (m, 3H), 7.25-7.12 (m, 3H), 6.70 (m, 1H), 4.08 (s, 2H).

5-Benzylbenzofuran-2-yl-2-boronic acid:



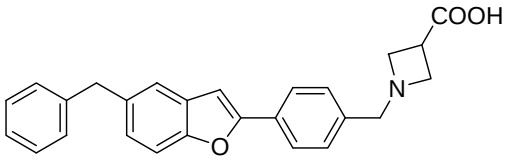
The title compound was prepared according to general procedure C (66% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (m, 1H), 7.42 (d, *J* = 8.4, 1H), 7.32-7.26 (m, 4H), 7.25-7.19 (m, 3H), 4.81 (s, 2H), 4.08 (s, 2H).

4-(5-Benzylbenzofuran-2-yl)benzaldehyde:



The title compound was prepared according to general procedure D (76% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.03 (s, 1H), 7.99 (d, *J* = 8.4, 2H), 7.94 (d, *J* = 8.4, 2H), 7.46-7.41 (m, 2H), 7.32-7.17 (m, 6H), 7.13 (br s, 1H),4.08 (s, 2H).

1-((4-(5-Benzylbenzofuran-2-yl)phenyl)methyl)azetidine-3-carboxylic acid:

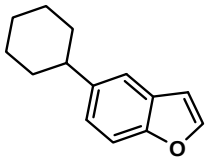


The title compound was prepared according to general procedure E(62% yield): ^{1}H NMR (400 MHz, CD_3OD) δ 7.98 (d, *J* = 8.4, 2H), 7.55 (d, *J* = 8.4, 2H), 7.45-7.42 (m, 2H), 7.28-7.15 (m, 7H), 4.44 (s, 2H), 4.37-4.22 (m, 4H), 4.06 (s, 2H), 3.72-3.64 (m, 1H). MS (ESI) m/z: Calculated: 397.17; Observed: 398.0 (M^++1).

Compound 4

1-(4-(5-cyclohexylbenzofuran-2-yl)benzyl)azetidine-3-carboxylic acid

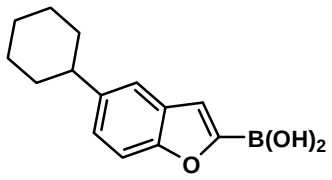
5-cyclohexylbenzofuran (step 1 in Scheme 2):



5-bromobenzofuran (500 mg, 2.55 mmol) was dissolved in a THF solution of cyclohexyl zinc(II) bromide (0.5M, 15 mL, 7.40 mmol) in a microwave reaction tube. $Pd(P^tBu_3)_2$ (65 mg, 0.128 mmol, 0.05 eqv.) was added to this solution. The mixture was purged with N_2 gas for 3-5 min and heated at 100 °C for 30 min under microwave irradiation. Upon completion of the reaction, the reaction mixture was diluted with ethyl acetate, washed with 1N HCl aqueous solution, brine, filtered through Celite. The filtrate was dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (ISCO system, 5% EtOAc in hexanes) to give 0.217 g desired product (43% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (d,

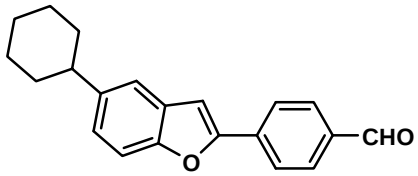
1H), 7.41 (d, 2H), 7.15 (d, 1H), 6.72 (d, 1H), 2.58 (m, 1H), 1.92-1.74 (m, 4H), 1.51-1.35 (m, 4H), 1.31-1.25 (m, 2H).

5-cyclohexylbenzofuran-2-ylboronic acid (step 2 in Scheme 2):



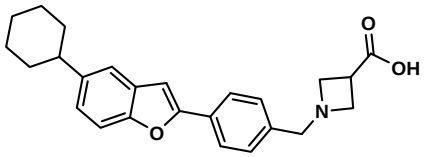
A solution of *n*-BuLi (360 uL, 0.9 mmol, 2.5M solution in hexanes) was added dropwise to a solution of 5-cyclohexylbenzofuran (150 mg, 0.75 mmol) in anhydrous THF (5 mL) at -78 °C. The resulting mixture was stirred at -78 °C for 40 min, and treated with $B(^{i}PrO)_3$ (260 uL, 1.13 mmol). The reaction mixture was allowed to warm up slowly to room temperature and stirred for 1 h. TLC indicated the completion of reaction. The reaction was cooled in ice-bath and quenched with 2N HCl (3 mL) and extracted with Et_2O. The combined organic extracts were washed with brine, dried and concentrated under reduced pressure to yield a desired boronic acid (0.156 g, 85% yield) without further purification for next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (s, 1H), 7.43 (d, 1H), 7.32 (s, 1H), 7.25 (d, 1H), 2.62 (m, 1H), 1.93-1.85 (m, 4H), 1.78-1.75 (m, 4H), 1.34-1.22 (m, 2H).

4-(5-cyclohexylbenzofuran-2-yl)benzaldehyde (step 3 in Scheme 2):



A mixture of 5-cyclohexylbenzofuran-2-ylboronic acid (75 mg, 0.37 mmol), 4-bromobenzaldehyde (62 mg, 0.34 mmol), triethylamine (1.1 mL, 7.5 mmol) and bis(triphenylphosphine)palladium(II) chloride (13 mg, 0.05 mmol) in ethanol (11 mL) was irradiated in a Microwave instrument at 100 °C for 20 min. The reaction mixture was cooled, and the solvent was removed. The residue was purification by silica gel chromatography on ISCO system gave the title compound (52 mg, 46% yield): >95% purity by LCMS, ESI-MS: 305.2 ($M+H^+$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.03 (s, 1H), 8.00 (d, 2H), 7.95 (d, 2H), 7.46 (d, 2H), 7.19 (d, 1H), 7.16 (s, 1H), 2.63-2.58 (m, 1H), 1.94-1.76 (m, 4H), 1.53-1.42 (m, 4H), 1.38-1.25 (m, 2H). MS (ESI) m/z: Calculated: 304.38; Observed: 305.2 (M^++1).

1-(4-(5-cyclohexylbenzofuran-2-yl)benzyl)azetidine-3-carboxylic acid (step 4 of Scheme 2)

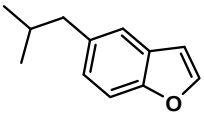


A mixture of 4-(5-cyclohexylbenzofuran-2-yl)benzaldehyde (30 mg, 0.1 mmol), acetic acid (9 uL, 0.15 mmol) and azetidine-3-carboxylic acid (15 mg, 0.15 mmol) in DCM/MeOH (1:1, 2 mL) was stirred at room temperature for 1 h. Sodium cyanoborohydride (3.1 mg, 0.05 mmol) was added and the reaction mixture was stirred for 3 h at room temperature. After concentration of solvent under reduced pressure, the resulting residue was dissolved in hot MeOH and filtered. The filtrate and the white solid, which was redisolved in hot DMSO, were both purified by reverse phase preparative HPLC (Phenomenex reverse phase Luna 5u C18(2) column, 60 x 21.2 mm ID) to yield the desired final product (16 mg, 42% yield) as a white powder: >95 % purity by LCMS, ESI-MS: 459.1 $(M+H)^+$, ^{1}H NMR (400 MHz, CD_3OD) δ 7.95 (d, 2H), 7.56 (d, 2H), 7.45 (d, 1H), 7.42 (d, 1H), 7.24 (s, 1H), 7.19 (dd, 1H), 4.45 (s, 2H), 4.34 (dd, 4H), 3.69 (m, 1H), 2.64-2.57 (d, 1H), 1.89 (t, 4H), 1.58-1.40 (m, 4H), 1.38-1.26 (m, 2H).

Compound 5

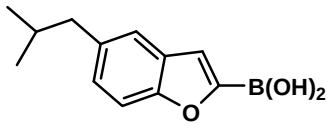
1-((4-(5-isobutylbenzofuran-2-yl)phenyl)methyl)azetidine-3-carboxylic acid (Scheme 2)

5-isobutylbenzofuran (step 1 in Scheme 2):



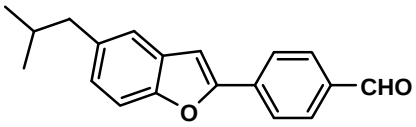
The title compound was prepared according to general procedure A: 5-bromobenzofuran (500 mg, 2.56 mmol) was dissolved in THF solution of isobutylzinc(II) bromide (0.5M, 15 mL, 7.40 mmol) in a microwave reaction tube. $Pd(P^tBu_3)_2$ (65 mg, 0.128 mmol, 0.05 eqv.) was added to this solution. The mixture was purged with N_2 gas for 3-5 min and heated at 100 °C for 30 min under microwave irradiation. Upon completion of the reaction, the reaction mixture was diluted with ethyl acetate, washed with 1N HCl aqueous solution, brine, filtered through Celite. The filtrate was dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (ISCO system, 5% EtOAc in hexanes) to give 0.331 g desired product (74% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (s, 1H), 7.35 (d, 1H), 7.07 (d, 1H), 6.70(s, 1H), 2.59 (d, 2H), 1.9 (m, 1H), 0.9 (d, 6H).

5-isobutylbenzofuran-2-ylboronic acid (step 2 in Scheme 2):



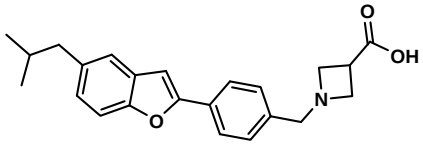
A solution of *n*-BuLi (912 uL, 2.28 mmol, 2.5M solution in hexanes) was added dropwise to a solution of 5-isobutylbenzofuran (331 mg, 1.9 mmol) in anhydrous THF (12 mL) at -78 °C. The resulting mixture was stirred at -78 °C for 40 min, and treated with $B(^{i}PrO)_3$ (658 uL, 2.85 mmol). The reaction mixture was allowed to warm up slowly to room temperature and stirred for 1 h. TLC indicated the completion of reaction. The reaction was cooled in ice-bath and quenched with 2N HCl (6 mL) and extracted with Et_2O. The combined organic extracts were washed with brine, dried and concentrated under reduced pressure to yield a crude benzofuran boronic acid (0.76 g) without further purification for next step.

4-(5-isobutylbenzofuran-2-yl)benzaldehyde (step 3 in Scheme 2):



A mixture of 5-isobutylbenzofuran-2-ylboronic acid (70 mg, 0.33 mmol), 4-bromobenzaldehyde (61 mg, 0.33 mmol), triethylamine (1.7 mL, 12.6 mmol) and bis(triphenylphosphine)palladium(II) chloride (12 mg, 0.017 mmol) in ethanol (10 mL) was irradiated in a Microwave instrument at 100 °C for 20 min. The reaction mixture was cooled, and the solvent was removed. The residue was treated with water and extracted with ethyl acetate. The organic layer was dried and concentrated *in vacuo* (the aqueous work-up is optional). Purification by silica gel chromatography on ISCO system gave the title compound (59 mg, 65% yield): >99% purity by LCMS, ESI-MS: 279.2 $(M+H)^+$.

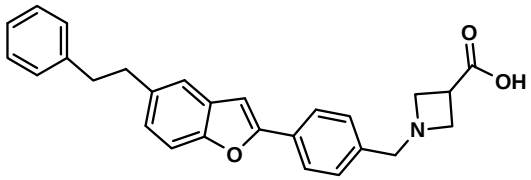
1-((4-(5-isobutylbenzofuran-2-yl)phenyl)methyl)azetidine-3-carboxylic acid (step 4 in Scheme 2):



A mixture of 4-(5-isobutylbenzofuran-2-yl)benzaldehyde (30 mg, 0.11 mmol), acetic acid (10 uL, 0.15 mmol) and azetidine-3-carboxylic acid (16 mg, 0.16 mmol) in DCM/MeOH (1:1, 2 mL) was stirred at room temperature for 1 h. Sodium cyanoborohydride (3.4 mg, 0.054 mmol) was added and the reaction mixture was stirred for 3 h at room temperature. After concentration of solvent under reduced pressure, the resulting residue was dissolved in an aliquot of DMSO and purified by reverse phase preparative HPLC (Phenomenex reverse phase Luna 5u C18(2) column, 60 x 21.2 mm ID) to yield the desired final product (25.6 mg, 65% yield) as a colorless film: >95% purity by LCMS, ESI-MS: 364.0 $(M+H)^+$, ^{1}H NMR (400 MHz, CD_3OD) δ 7.99 (d, 2H), 7.55 (d, 2H), 7.42 (d, 1H), 7.39 (s, 1H), 7.24 (s, 1H), 7.12 (dd, 1H), 4.44 (s, 2H), 4.33(d, 4H), 3.68 (m, 1H), 2.57 (d, 2H), 1.90 (m, 1H), 0.92 (d, 6H).

Compound 6

1-((4-(5-phenethylbenzofuran-2-yl)phenyl)methyl)azetidine-3-carboxylic acid

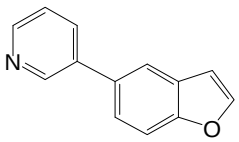


The title compound was prepared according to general procedure A, C-E: >95% purity by LCMS, ESI-MS: 411.9 $(M+H)^+$, ^{1}H NMR (400 MHz, CD_3OD) δ 7.99 (d, 2H), 7.55 (d, 2H), 7.41 (d, 1H), 7.38 (s, 1H), 7.24-7.21 (m, 3H), 7.17-7.14 (m, 4H), 4.44 (s, 2H), 4.34(d, 4H), 3.70 (m, 1H), 3.01-2.90 (m, 4H).

Compound 7

1-(4-(5-(pyridin-3-yl)benzofuran-2-yl)benzyl)azetidine-3-carboxylic acid

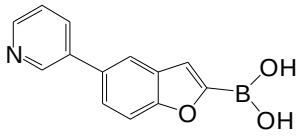
3-(benzofuran-5-yl)pyridine (step 1 in Scheme 2 except using Suzuki coulping):



A solution of 5 pyridin-3-ylboronic acid (390 mg, 3.18 mmol), 5-bromobenzofuran (500 mg, 2.54 mmol), palladiumdichlorobis(triphenylphosphine) (111 mg, 0.16 mmol) and triethylamine (8.8 mL, 63.5 mmol) in EtOH was irradiated in the microwave at 100 °C for 1200 s. Removal of the solvents followed by dissolving in CH_2Cl_2 and filtering gave the residue after concentration of the solvent under reduced pressure. The compound was purifided on ISCO to afford 316 mg of the title compound as a light yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.89

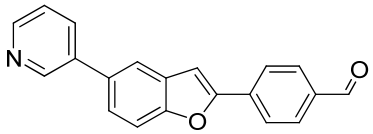
(s, 1H), 8.60 (d, 1H), 7.90 (d, 1H), 7.80 (s, 1H), 7.69 (s, 1H), 7.55 (d, 1H), 7.50 (d, 1H) 7.38 (dd, 1H), 6.85 (dd, 1H). MS (ESI) m/z: Calculated: 195.07; Observed: 196.30 (M^++1).

5-(pyridin-3-yl)benzofuran-2-ylboronic acid (step 2 in Scheme 2):



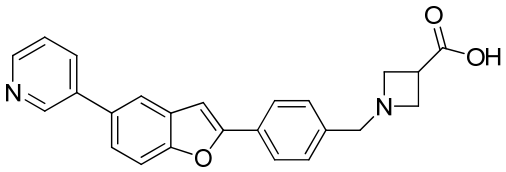
A solution of *n*-BuLi (0.76 mL, 2.5M solution in hexanes) was added dropwise to a solution of 3-(benzofuran-5-yl)pyridine (310 mg, 1.59 mmol) in anhydrous THF (10 mL) at -78 °C. The resulting mixture was stirred at -78 °C for 30 min, and treated with $B(^iPrO)_3$ (0.55 mL, 2.39 mmol). The reaction mixture was allowed to warm up slowly to room temperature and stirred for 1 h. The reaction was quenched with 2N HCl and extracted with Et_2O. The aqueous layer was neutralized with 5N NaOH (PH = 6) followed by extraction with THF: ether (1:1) three times. The combined extracts were washed with brine, dried and concentrated under reduced pressure to yield 241 mg of the crude boronic acid, which was used without further purification.

4-(5-(pyridin-3-yl)benzofuran-2-yl)benzaldehyde (step 3 in Scheme 2):



The title compound was prepared according to general procedure D (44% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.06 (s, 1H), 8.91 (br s, 1H), 8.61 (br s, 1H), 8.07 (d, 2H), 7.98 (d, 2H), 7.93 (d, 1H), 7.65 (d, 1H), 7.55 (d, 1H), 7.82 (m, 1H), 7.39 (m, 1H), 7.27 (m, 1H). MS (ESI) m/z: Calculated: 299.09; Observed: 300.30 (M^++1).

1-(4-(5-(pyridin-3-yl)benzofuran-2-yl)benzyl)azetidine-3-carboxylic acid (step 4 in Scheme 2):

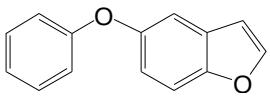


The title compound was prepared according to general procedure E (22% yield): ^{1}H NMR (400 MHz, CD_3OD) δ 9.11 (br s, 1H), 8.70 (m, 2H), 8.06 (m, 3H), 7.98 (m, 1H), 7.74 (m, 2H), 7.60 (d, 2H), 7.44 (s, 1H), 4.47 (s, 2H), 4.40-4.38 (m, 4H), 3.72 (m, 1H). MS (ESI) m/z: Calculated: 384.20; Observed: 385.00 (M^++1).

Compound 8

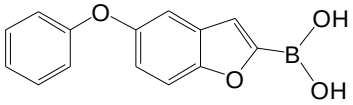
1-(4-(5-phenoxybenzofuran-2-yl)benzyl)azetidine-3-carboxylic acid

5-Phenoxy-benzofuran:



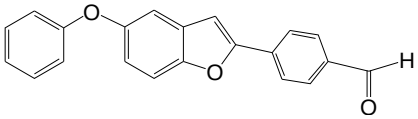
The title compound was prepared according to the procedures described for Compound **1** (step 1and 2): 65% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, 1H), 7.45 (d, 1H), 7.29 (m, 2H), 7.22 (d, 1H), 7.00-7.08 (m, 4H), 6.71 (m, 1H).

5-phenoxybenzofuran-2-yl boronic acid:



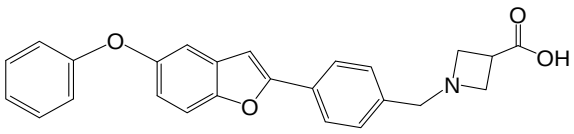
The title compound was prepared according to general procedure C (74% yield).

4-(5-phenoxybenzofuran-2-yl)benzaldehyde:



The title compound was prepared according to general procedure D (65% yield): ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.05 (s, 1H), 8.13 (d, 2H), 8.03 (d, 2H), 7.70 (d, 1H), 7.66 (br s, 1H), 7.39 (m, 4H), 7.10 (m, 2H), 7.00 (dd, 1H). MS (ESI) m/z: Calculated: 314.10; Observed: 315.10 (M^++1).

1-(4-(5-phenoxybenzofuran-2-yl)benzyl)azetidine-3-carboxylic acid:

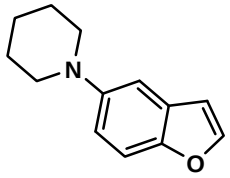


The title compound was prepared according to general procedure E (7% yield): ^{1}H NMR (400 MHz, CD_3OD) δ 7.90 (d, 2H), 7.55 (m, 3H), 7.32 (m, 2H), 7.27 (s, 1H), 7.22 (d, 1H), 7.03 (m, 4H), 4.47 (s, 2H), 4.34 (m, 4H), 3.62 (m, 1H). MS (ESI) m/z: Calculated: 399.20; Observed: 399.90 (M^++1).

Compound 9

1-(3-fluoro-4-(5-(piperidin-1-yl)benzofuran-2-yl)benzyl)azetidine-3-carboxylic acid

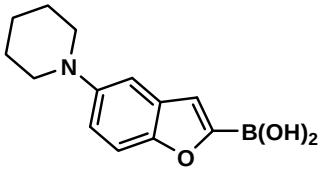
1-(benzofuran-5-yl)piperidine (step 1 of Scheme 3):



5-bromobenzofuran (2 g, 10 mmol), piperidine (1.2 mL, 12 mmol), Pd(dppf)Cl_2 (245 mg, 0.3 mmol), dppf (250 mg, 0.45 mmol) and sodium *tert*-butoxide (1.44g, 15 mmol) was mixed in toluene (10 mL). The mixture was purged with N_2 gas for 3-5 min and heated at 120 °C for 30 min under microwave irradiation (Personal Chemistry Emrys^{TM} Optimizer microwave reactor). Upon completion of the reaction, the reaction mixture was directly loaded on silica gel column and purified on ISCO system (<2% EtOAc in hexanes) to give 0.539g desired product (27% yield): ESI-MS: 202.3 $(M+H)^+$, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (s, 1H), 7.40 (d, 1H), 7.15 (s, 1H), 7.00 (d, 1H), 6.65 (s, 1H), 3.10 (m, 4H), 1.70 (m, 4H), 1.48 (m, 2H).

Note: the title compound appeared to be very volatile. The evaporation of solvent should be carried out very carefully.

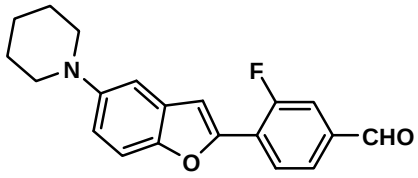
5-(piperidin-1-yl)benzofuran-2-ylboronic acid (step 2 of Scheme 3):



A solution of *n*-BuLi (334 uL, 0.83 mmol, 2.5 M solution in hexanes) was added dropwise to a solution of 1-(benzofuran-5-yl)piperidine (140 mg, 0.70 mmol) in anhydrous THF (5 mL) at -78 °C. The resulting mixture was stirred at -78 °C for 40 min, and treated with B(^{i}PrO)$_3$ (241 uL, 1.04 mmol). The reaction mixture was allowed to warm up slowly to room temperature and stirred for 1 h. TLC indicated the completion of reaction. The reaction was cooled in ice-bath and quenched with saturated NH_4Cl (1.5 mL) and extracted with Et_2O. The separated aqueous layer was neutralized to pH~5. The solution turned cloudy, which was

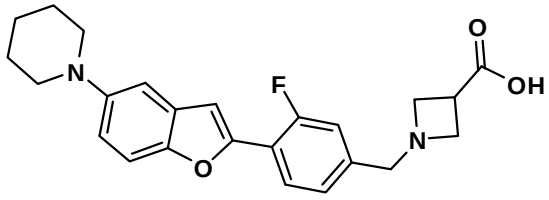
extracted with ethyl acetate (x3). The combined organic extracts were concentrated *in vacuo* yielding the desired boronic acid as brown solids (0.16g, 94% yield) without further purification for next step. ESI-MS: 246.3 $(M+H)^+$.

3-fluoro-4-(5-(piperidin-1-yl)benzofuran-2-yl)benzaldehyde (step 3 of Scheme 3):



A mixture of 5-(piperidin-1-yl)benzofuran-2-ylboronic acid (50 mg, 0.204 mmol), 4-bromo-3-fluorobenzaldehyde (37 mg, 0.184 mmol), triethylamine (0.56 mL, 4.1 mmol) and bis(triphenylphosphine)palladium(II) chloride (14 mg, 0.02 mmol) in ethanol (5 mL) was irradiated in a Microwave instrument at 100 °C for 20 min. The reaction mixture was cooled, and the solvent was removed. The residue was purification by silica gel chromatography on ISCO system yielding the title compound (15 mg, 15% yield). ESI-MS: 324.2 $(M+H)^+$, ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.00 (s, 1H), 8.19 (t, 1H), 7.75 (d, 1H), 7.67 (d, 1H), 7.43 (d, 1H), 7.35 (d, 1H), 7.14-7.11 (m, 2H), 3.13 (m, 4H), 1.77 (m, 4H), 1.59 (m, 2H).

1-(3-fluoro-4-(5-(piperidin-1-yl)benzofuran-2-yl)benzyl)azetidine-3-carboxylic acid trifluoroacetic acid salt(step 4 of Scheme 3):



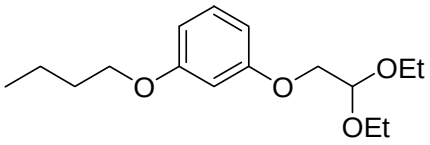
A mixture of 3-fluoro-4-(5-(piperidin-1-yl)benzofuran-2-yl)benzaldehyde (9 mg, 0.028 mmol), acetic acid (2.5 uL, 0.042 mmol) and azetidine-3-carboxylic acid (4.2 mg, 0.042mmol) in DCM/MeOH (2:1, 0.9 mL) was stirred at room temperature for 1 h. Sodium cyanoborohydride (1.0 mg, 0.014 mmol) was added and the reaction mixture was stirred for 3 h at room temperature. After concentration of solvent under reduced pressure, the resulting residue was dissolved in DMSO, and purified by reverse phase preparative HPLC (Phenomenex reverse phase Luna 5u C18 (2) column, 60 x 21.2 mm ID, mobil phase: A = 0.05% TFA in water; B = 0.05% TFA in acetonitrile. The flow rate was 12 mL / min. The gradient time was 2% B to 52 % B over 25 min.) to yield the desired final product (10.3 mg, 70% yield) as a white powder (ditrifluroacetic acid salt): >95 % purity by LCMS, ESI-MS: 409.1 $(M+H)^+$, ^{1}H NMR (400

MHz, CD_3OD) δ 8.17 (t, 1H), 8.02(d, 1H), 7.81 (d, 1H), 7.66 (dd, 1H), 7.49-7.47 (m,3H), 4.50 (s, 2H), 4.39 (dd, 4H), 3.72-3.70(m, 5H), 2.08 (m, 4H), 1.84(m, 2H).

Compound 10

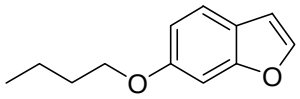
1-((4-(6-Butoxybenzofuran-2-yl)phenyl)methyl)azetidine-3-carboxylic acid

1-(2,2-Diethoxyethoxy)-3-butoxybenzene:



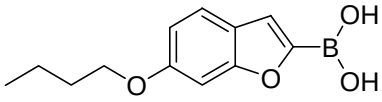
The title compound was prepared according to the procedure described for compound **1** (step 1 in Scheme 1): 86% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15 (t, *J* = 7.4), 6.52-6.49 (m, 3H), 4.83 (t, *J* = 5.1, 1H), 3.99 (d, *J* = 5.1, 2H), 3.93 (t, *J* = 6.6, 2H), 3.80-3.72 (m, 2H), 3.67-3.60 (m, 2H), 1.79-1.72 (m, 2H), 1.53-1.43 (m, 2H), 1.25 (t, *J* = 7.3, 6H), 0.97 (t, *J* = 7.3, 3H).

6-Butoxybenzofuran:



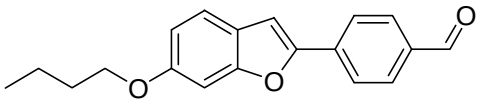
The title compound was prepared according to the procedure described for compound **1** (step 2 in Scheme 1): 83% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, *J* = 2.2, 1H), 7.44 (d, *J* = 8.5, 1H), 7.03 (d, *J* = 2.2, 1H), 6.87 (dd, *J* = 8.8, 2.5, 1H), 6.69-6.68 (m, 1H), 4.00 (t, *J* = 6.6, 2H), 1.83-1.76 (m, 2H), 1.56-1.47 (m, 2H), 0.99 (t, *J* = 7.4, 3H).

6-Butoxybenzofuran-2-yl-2-boronic acid:



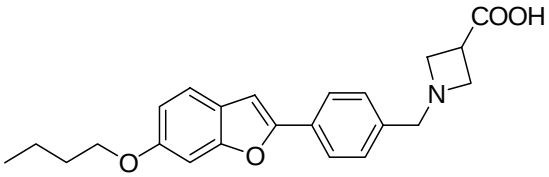
The title compound was prepared according to general procedure C (76% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52-7.42 (m, 2H), 7.00 (br s, 1H), 6.90-6.85 (m, 1H), 4.00 (t, *J* = 6.6, 2H), 1.82-1.78 (m, 2H), 1.56-1.48 (m, 2H), 0.98 (t, *J* = 7.3, 3H).

4-(6-Butoxybenzofuran-2-yl)benzaldehyde:



The title compound was prepared according to general procedure D (62% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.94 (s, 1H), 7.94-7.89 (m, 4H), 7.45 (d, *J* = 8.5, 2H), 7.10 (s, 1H), 7.05 (br d, *J* = 2.2, 1H), 6.89 (dd, *J* = 8.5, 2.2, 1H), 4.02 (t, *J* = 6.2), 1.85-1.78 (m, 2H), 1.57-1.52 (m, 2H), 1.00 (t, *J* = 7.3, 3H).

1-((4-(6-Butoxybenzofuran-2-yl)phenyl)methyl)azetidine-3-carboxylic acid:

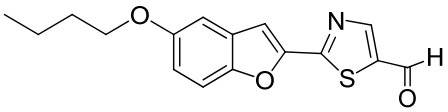


The title compound was prepared according to general procedure E (46% yield): ^{1}H NMR (400 MHz, CD_3OD) δ 7.94 (d, *J* = 8.4, 2H), 7.53 (d, *J* = 8.4, 2H), 7.47 (d, *J* = 8.5, 1H), 7.21 (s, 1H), 7.11 (br d, *J* = 2.2, 1H), 6.88 (dd, *J* = 8.5, 2.2), 4.43 (s, 2H), 4.34-4.32 (m, 4H), 4.04 (t, *J* = 6.2), 3.71-3.63 (m, 1H), 1.81-1.76 (m, 2H), 1.57-1.52 (m, 2H), 1.01 (t, *J* = 7.3, 3H). MS (ESI) m/z: Calculated: 379.18; Observed: 379.8 (M^++1).

Compound 11

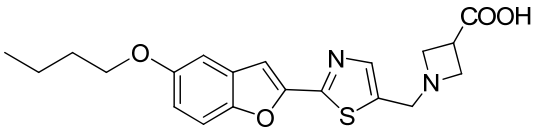
1-((2-(5-butoxybenzofuran-2-yl)thiazol-5-yl)methyl)azetidine-3-carboxylic acid

2-(5-butoxybenzofuran-2-yl)thiazole-5-carbaldehyde:



The title compound was prepared according to general procedure D except using 2-bromothiazole-5-carbaldehyde (29% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.07 (s, 1H), 8.46 (dd, 1H), 7.45 (dd, 2H), 7.03 (dd, 2H), 4.01 (dd, 2H), 1.74 (m, 2H), 1.54 (m, 2H), 1.01 (t, 3H). MS (ESI) m/z: Calculated: 301.10; Observed: 302.10 (M^++1).

1-((2-(5-butoxybenzofuran-2-yl)thiazol-5-yl)methyl)azetidine-3-carboxylic acid:

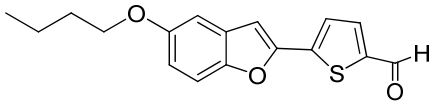


The title compound was prepared according to general procedure E (36% yield): ^{1}H NMR (400 MHz, CD_3OD) δ 8.06 (br s, 1H), 7.344 (m, 2H), 7.18 (m, 1H), 7.01 (ddd, 1H), 4.79 (s, 2H), 4.36 (m, 4H), 3.98 (m, 2H), 3.69 (m, 1H), 1.75 (m, 2H), 1.50 (m, 2H), 1.00 (t, 3H). MS (ESI) m/z: Calculated: 386.13; Observed: 386.90 (M^++1).

Compound 12

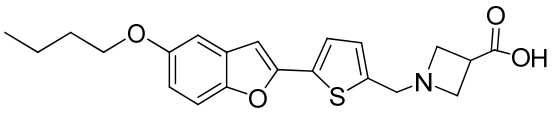
1-((5-(5-butoxybenzofuran-2-yl)thiophen-2-yl)methyl)azetidine-3-carboxylic acid

5-(5-butoxybenzofuran-2-yl)thiophene-2-carbaldehyde:



The title compound was prepared according to general procedure D except using 5-bromothiophene-2-carbaldehyde (32% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.92 (s, 1H), 7.73 (d, 1H), 7.51 (dd, 1H), 7.39 (d, 1H), 7.96 (m, 2H), 6.94 (dd, 1H), 3.98 (dd, 2H), 1.80 (m, 2H), 1.70 (m, 2H), 1.01 (t, 3H).

1-((5-(5-butoxybenzofuran-2-yl)thiophen-2-yl)methyl)azetidine-3-carboxylic acid:

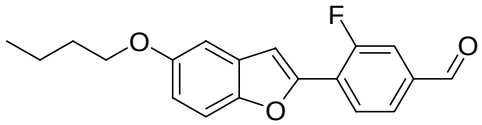


The title compound was prepared according to general procedure E (27% yield): ^{1}H NMR (400 MHz, CD_3OD) δ 7.49 (br s, 1H), 7.35 (m, 2H), 7.03 (d, 2H), 6.89 (dd, 1H), 4.67 (s, 2H), 4.35 (m, 4H), 3.98 (m, 2H), 3.67 (m, 1H), 1.73 (m, 2H), 1.51 (m, 2H), 0.99 (t, 3H). MS (ESI) m/z: Calculated: 385.13; Observed: 385.70 (M^++1).

Compound 13

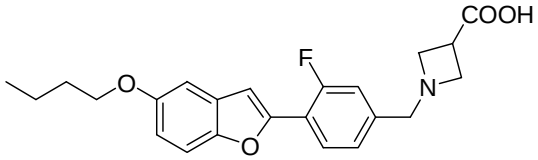
1-((4-(5-Butoxybenzofuran-2-yl)4-fluorophenyl)methyl)azetidine-3-carboxylic acid

4-(5-Butoxybenzofuran-2-yl)4-fluorobenzaldehyde:



The title compound was prepared according to general procedure C (36% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.02 (s, 1H), 8.18 (t, *J* = 7.7, 1H), 7.73 (d, *J* = 8.0, 1H), 7.66 (d, *J* = 11.2, 1H), 7.44-7.39 (m, 2H), 7.09 (d, *J* = 2.4, 1H), 6.92 (dd, *J* = 2.4, 8.8, 1H), 4.01 (t, *J* = 6.2), 1.81-1.76 (m, 2H), 1.57-1.51 (m, 2H), 1.01 (t, *J* = 7.2, 3H).

1-((4-(5-Butoxybenzofuran-2-yl)4-fluorophenyl)methyl)azetidine-3-carboxylic acid:

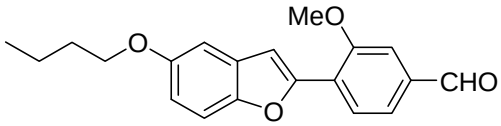


The title compound was prepared as Compound **1** (step 5 in Scheme 1) in the general method E described above (51% yield): ^{1}H NMR (400 MHz, CD_3OD) δ 8.08 (t, *J* = 7.7, 1H), 7.44-7.37 (m, 3H), 7.25 (d, *J* = 3.7, 1H), 7.14 (d, *J* = 2.2, 1H), 6.94 (dd, *J* = 8.8, 2.2), 4.35 (s, 2H), 4.18-4.15 (m, 4H), 4.01 (t, *J* = 6.2), 3.45-3.37 (m, 1H), 1.82-1.75 (m, 2H), 1.57-1.49 (m, 2H), 1.00 (t, *J* = 7.2, 3H). MS (ESI) m/z: Calculated: 397.17; Observed: 397.9 (M^++1).

Compound 14

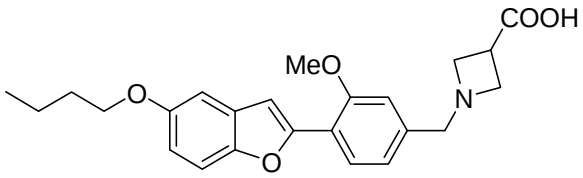
1-((4-(5-Butoxybenzofuran-2-yl)-3-methoxyphenyl)azetidine-3-carboxylic acid

4-(5-butoxybenzofuran-2-yl)-3-methoxybenzaldehyde:



The title compound was prepared as Example Compound **1** (step 4 in Scheme 1) in the general method D described above (65% yield): ^{1}H NMR (400 MHz, CD_3Cl) δ 10.03 (s, 1H), 8.22 (d, 1H), 7.59 (s, 1H), 7.50 (s, 1H), 7.45 (d, 1H), 7.41 (s, 1H), 7.08 (d, 1H), 6.93 (d, 1H), 4.16 (s, 3H), 4.05 (t, 2H), 1.84 (m, 2H), 1.61 (m, 2H), 1.04 (t, 3H). MS (ESI) m/z: Calculated: 324.14; Observed: 324.9 (M^++1).

1-((4-(5-Butoxybenzofuran-2-yl)-3-methoxyphenyl)azetidine-3-carboxylic acid:

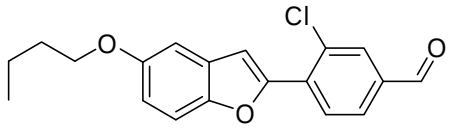


The title compound was prepared as Compound **1** (step 5 in Scheme 1) in the general method **E** described above (36% yield): ^{1}H NMR (400 MHz, CD_3OD) δ 8.04 (d, 1H), 7.39 (s, 1H), 7.38 (s, 1H), 7.21 (s, 1H), 7.15 (d, 1H), 7.08 (s, 1H), 6.83 (d, 1H), 4.44 (s, 2H), 4.38 (m, 7H), 4.02 (m, 2H), 3.62 (m, 1H), 1.82 (m, 2H), 1.63 (m, 2H), 1.01 (t, 3H). MS (ESI) m/z: Calculated: 409.19; Observed: 409.9 (M^++1).

Compound 15

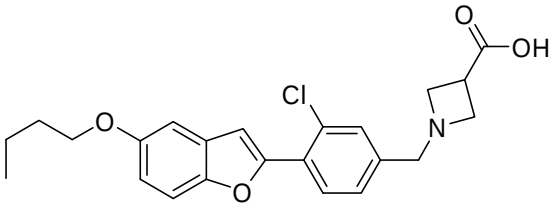
1-((4-(5-Butoxybenzofuran-2-yl)-3-chlorophenyl)methyl)azetidine-3-carboxylic acid

4-(5-Butoxybenzofuran-2-yl)-3-chlorobenzaldehyde:



The title compound was prepared according to general procedure D (72% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.00 (s, 1H), 8.25 (d, *J* = 8.0, 1H), 7.99 (d, *J* = 1.4, 1H), 7.86 (dd, *J* = 8.4, 1.5), 7.70 (s, 1H), 7.42 (d, *J* = 8.8), 7.10 (d, *J* = 2.6, 1H), 6.99 (dd, *J* = 8.8, 2.5), 4.01 (t, *J* = 6.5, 2H), 1.84-1.77 (m, 2H), 1.54-1.49 (m, 2H), 1.00 (t, *J* = 7.3, 3H).

1-((4-(5-Butoxybenzofuran-2-yl)-3-chlorophenyl)methyl)azetidine-3-carboxylic acid:

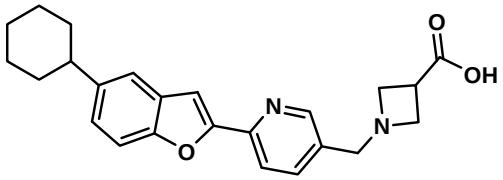


The title compound was prepared according to general procedure E (66 % yield): ^{1}H NMR (400 MHz, CD_3OD) δ 8.13 (d, *J* = 8.4, 1H), 7.70 (d, *J* = 1.8, 1H), 7.57 (s, 1H), 7.53 (dd, *J* = 8.4, 1.8, 1H), 7.42 (d, *J* = 9.1, 1H), 7.15 (d, *J* = 2.5 1H), 6.95 (dd, *J* = 9.1, 2.5), 4.45 (s, 2H),

4.40-4.32 (m, 4H), 4.00 (t, *J* = 6.5, 2H), 3.74-3.66 (m, 1H), 1.81-1.74 (m, 2H), 1.58-1.49 (m, 2H), 1.00 (t, *J* = 7.3, 3H). Calculated: 413.14; Observed: 413.9 (M^++1).

Compound 16

1-((6-(5-cyclohexylbenzofuran-2-yl)pyridin-3-yl)methyl)azetidine-3-carboxylic acid

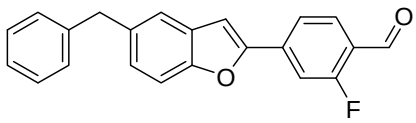


The title compound was prepared according to general procedure E (Scheme 2): >95% purity by LCMS, ESI-MS: 391.1 $(M+H)^+$, ^{1}H NMR (400 MHz, CD_3OD) δ 8.81 (d, 1H), 7.94 (d, 1H), 7.65 (d, 1H), 7.59 (s, 1H), 7.50 (m, 2H), 7.35 (m, 1H), 4.44 (s, 2H), 4.45 (s, 2H), 4.34 (dd, 4H), 3.69 (m, 1H), 2.64-2.57(d, 1H), 1.89 (t, 4H), 1.58-1.41 (m, 4H), 1.38-1.26 (m, 2H).

Compound 17

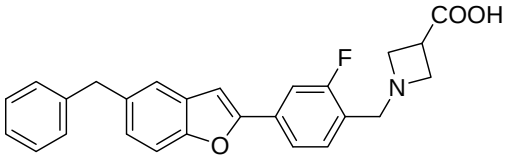
1-((4-(5-Benzylbenzofuran-2-yl)2-fluorophenyl)methyl)azetidine-3-carboxylic acid

4-(5-Benzylbenzofuran-2-yl)2-fluorobenzaldehyde:



The title compound was prepared in the same manner as Example Compound **1** except using 4-bromo-2-fluorobenzaldehyde in step-4 (Scheme 1): 67% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.35 (s, 1H), 7.92 (dd, *J* = 8.1, 7.0, 2H), 7.69 (d, *J* = 8.5, 1H), 7.63 (d, *J* = 11.4, 1H), 7.46-7.42 (m, 2H), 7.33-7.19 (m, 6H), 7.13 (s, 1H), 4.09 (s, 2H).

1-((4-(5-Benzylbenzofuran-2-yl)2-fluorophenyl)methyl)azetidine-3-carboxylic acid:

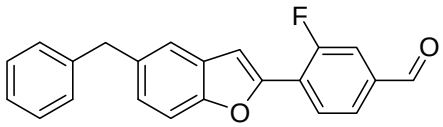


The title compound was prepared according to general procedure E (54% yield): ^{1}H NMR (400 MHz, CD_3OD) δ 7.78 (d, *J* = 8.1, 1H), 7.73 (d, *J* = 9.9, 1H), 7.58 (t, *J* = 7.7, 1H), 7.46-7.44 (m, 2H), 7.29-7.16 (m, 7H), 4.39 (s, 2H), 4.17-4.15(m, 4H), 4.06 (s, 2H), 3.72-3.64 (m, 1H). MS (ESI) m/z: Calculated: 415.16; Observed: 416.0 (M^++1).

Compound 18

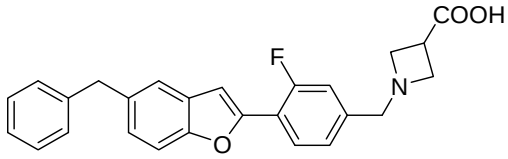
1-((4-(5-Benzylbenzofuran-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylic acid

4-(5-Benzylbenzofuran-2-yl)-3-fluorobenzaldehyde:



The title compound was prepared according to general procedure D (65% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.01 (s, 1H), 8.20 (t, *J* = 7.7, 1H), 7.77 (d, *J* = 8.0, 1H), 7.68 (d, *J* = 11.3, 1H), 7.47-7.45 (m, 2H), 7.37-7.20 (m, 7H), 4.10 (s, 2H).

1-((4-(5-Benzylbenzofuran-2-yl)-3-fluorophenyl)methyl)azetidine-3-carboxylic acid:

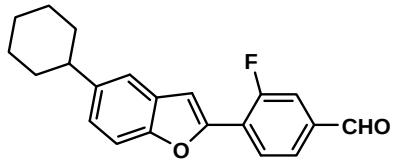


The title compound was prepared according to general procedure E (56% yield): ^{1}H NMR (400 MHz, CD_3OD) δ 8.09 (t, *J* = 7.9, 1H), 7.47-7.45 (m, 2H), 7.40-7.37 (m, 2H), 7.28-7.16 (m, 7H), 4.34 (s, 2H), 4.17-4.15 (m, 4H), 4.07 (s, 2H), 3.53-3.45 (m, 1H). MS (ESI) m/z: Calculated: 415.16; Observed: 415.9 (M^++1). HRMS (mass error) = 416.16598 (0.8 ppm). Elemental Analysis: Calcd. C: 75.17; H: 5.34; N: 3.37; F: 4.57. Found. C: 74.96; H: 5.31; N: 3.26; F: 4.46.

Compound 19

1-(4-(5-cyclohexylbenzofuran-2-yl)3-fluorophenyl)methyl)azetidine-3-carboxylic acid

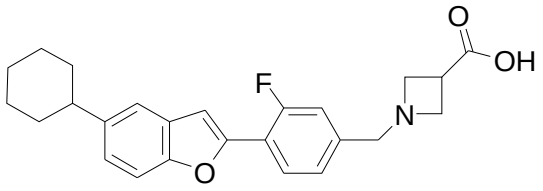
4-(5-cyclohexylbenzofuran-2-yl)2-fluorobenzaldehyde (step 3 in Scheme 2):



A mixture of 5-cyclohexylbenzofuran-2-ylboronic acid (75 mg, 0.30 mmol; see procedures described above), 4-bromo-2-fluorobenzaldehyde (48 mg, 0.24 mmol), triethylamine (1.1 mL, 7.5 mmol) and bis(triphenylphosphine)palladium(II) chloride (12 mg, 0.05 mmol) in ethanol (11 mL) was irradiated in a Microwave instrument at 100 °C for 20 min. The reaction

mixture was cooled, and the solvent was removed. The residue was treated with water and extracted with ethyl acetate. The organic layer was dried and concentrated *in vacuo* (the aqueous work-up is optional). Purification by silica gel chromatography on ISCO system gave the title compound (51 mg, 49% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.04 (s, 1H), 8.00-7.97 (m, 2H), 7.46 (s, 1H), 7.43 (d, 2H), 7.32 (s, 1H), 7.25 (d, 1H), 2.62 (m, 1H), 1.95-1.77(m, 4H), 1.58-1.56 (m, 4H), 1.46-1.44 (m, 2H). MS (ESI) m/z: Calculated: 322.27; Observed: 323.2 (M^++1).

1-(4-(5-cyclohexylbenzofuran-2-yl)3-fluorophenyl)methyl)azetidine-3-carboxylic acid (step 4 in Scheme 2):

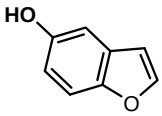


A mixture of 4-(5-cyclohexylbenzofuran-2-yl)3-fluorobenzaldehyde (40 mg, 0.12 mmol), acetic acid (10 uL, 0.15 mmol) and azetidine-3-carboxylic acid (15 mg, 0.15 mmol) in DCM/MeOH (1:1, 2 mL) was stirred at room temperature for 1 h. Sodium cyanoborohydride (3.0 mg, 0.05 mmol) was added and the reaction mixture was stirred for 3 h at room temperature. After concentration of solvent under reduced pressure, the resulting residue was dissolved in hot MeOH and filtered. The filtrate and the white solid, which was redisolved in hot DMSO, were both purified by reverse phase preparative HPLC (Phenomenex reverse phase Luna 5u C18(2) column, 60 x 21.2 mm ID) to yield the desired final product (12 mg, 42% yield) as a white powder: >95% purity by LCMS, ^{1}H NMR (400 MHz, CD_3OD) δ 8.12 (d, 1H), 7.47-7.38 (m, 4H), 7.28-7.20 (m, 2H), 4.66 (s, 2H), 4.34 (m, 4H), 3.72 (m, 1H), 2.61 (m, 1H), 1.95-1.82 (m, 4H), 1.60-1.56 (m, 4H), 1.42-1.40 (m, 2H). MS (ESI) m/z: Calculated: 407.48; Observed: 408.2 (M^++1).

Compound 20

1-(4-(5-(cyclopentylmethoxy)benzofuran-2-yl)-3-fluorobenzyl)azetidine-3-carboxylic acid

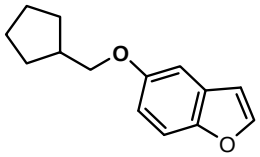
5-hydroxy benzofuan (step 1 of Scheme 4):



To an ice-cooled solution of 5-methylbenzofuran (0.5 g, 3.37 mmol) in DCM (7 mL) was added boron tribromide (3.4 mL, 3.37 mmol, 1M in DCM). The light brown solution was stirred at 0 °C for 1h, another equivalent of boron tribromide (3.4 mL) was then added. The mixture was stirred at room temperature for 2 h. TLC analysis indicated the completion of the reaction. The mixture was poured into ice and the pH was adjusted to 7 with Na_2CO_3. The aqueous was

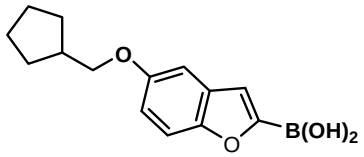
extracted with DCM (x2). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated. The resulting light brown sold gave the satisfactory purity without further purification for next step: 0.36 g (79.6% yield), ^{1}H NMR (400 MHz, CD_3OD) δ 7.59(d, J = 2.0 Hz, 1H), 7.35(d, J = 9.2 Hz, 1H), 7.01(d, J = 2.4 Hz, 1H), 6.82 (dd, J = 8.8 Hz, J = 2.8 Hz, 1H), 6.67 (m, 1H), 4.73 (s, 1H).

5-(cyclopentylmethoxy)benzofuran (step 2 of Scheme 4)



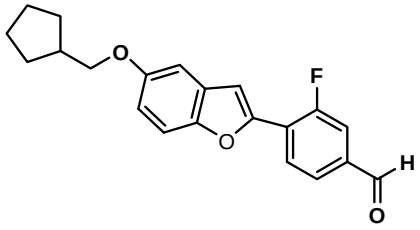
DEAD (362 mg, 2.09 mmol) was slowly added to a solution of 5-hydroxybenzofuran (200 mg, 1.49 mmol), triphenylphosphine (547 mg, 2.09 mmol) and cyclopentyl- methanol (203 mg, 2.0 2mmol) in 3 mL of THF. The mixture was stirred at room temperature for 16 hours. The solvent was removed and the residue was purified by ISCO column chromatography using 0-5% AcOEt in Hexanes. The title compound was obtained as a white solid (0.208 g, 65% yield): 84 % purity by HPLC; ^{1}H NMR (400 MHz, CD_3OD) δ 7.58 (d, 1H), 7.38 (d, J = 8.4 Hz, 1H), 7.06 (s, 1H), 6.91 (d, J = 9.2 Hz, 1H), 6.69 (m, 1H), 3.82 (d, 2H), 2.39 (m, 1H), 1.85 (m, 2H), 1.63(m, 4H), 1.39(m, 2H).

5-(cyclopentylmethoxy)benzofuran-2-ylboronic acid (step 3 of Scheme 1)



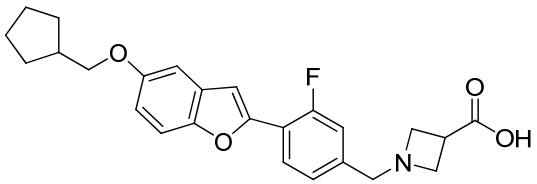
The title compound was prepared according to general procedure C (94.7% yield): ^{1}H NMR (400 MHz, CD_3OD) δ 7.39 (d, J = 9.2 Hz, 1H), 7.30 (s, 1H), 7.07 (d, 1H), 6.99 (dd, J = 9.2 Hz, J = 2.4 Hz, 1H), 3.82 (d, J = 7.0 Hz, 2H), 2.39 (m, 1H), 1.86 (m, 2H), 1.63(m, 4H), 1.39(m, 2H).

4-(5-(cyclopentylmethoxy)benzofuran-2-yl)-3-fluorobenzaldehyde (step 4 of Scheme 1)



The title compound was prepared according to general procedure D (53% yield): ESI-MS: 339.3 $(M+H)^+$, ^{1}H NMR (400 MHz, CD_3OD) δ 10.0 (s, 1H), 8.20 (t, 1H), 7.30 (s, 1H), 7.77 (d, 1H), 7.68 (d, 1H), 7.43 (d, 1H), 7.36 (d, 1H), 7.09 (s, 1H), 6.99 (dd, 1H), 3.88 (d, J = 7.0 Hz, 2H), 2.39 (m, 1H), 1.86 (m, 2H), 1.63(m, 4H), 1.39(m, 2H).

1-(4-(5-(cyclopentylmethoxy)benzofuran-2-yl)-3-fluorobenzyl)azetidine-3-carboxylic acid (step 5 of Scheme 1)

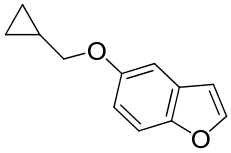


The title compound was prepared according to general procedure E (79% yield): ESI-MS: 423.9 $(M+H)^+$, ^{1}H NMR (400 MHz, CD_3OD) δ 8.11 (t, 1H), 7.45-7.40 (m, 3H), 7.28 (d, 1H), 7.15 (d, 1H), 6.95(dd, 1H), 4.46 (s, 2H), 4.36-4.34 (m, 4H), 3.88 (d, J = 7.4 Hz, 2H), 3.68 (m, 1H), 2.38 (m, 1H), 1.85(m, 2H), 1.65 (m, 4H), 1.43(m, 2H).

Compound 21

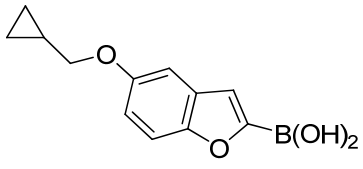
1-(4-(5-(cyclopentylmethoxy)benzofuran-2-yl)-3-fluorobenzyl)azetidine-3-carboxylic acid

5-(cyclopropylmethoxy)benzofuran (step 2 of Scheme 4):



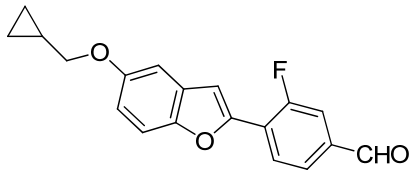
The title compound was prepared as Example Compound 20 (step 2 in Scheme 4): 49% yield; ^{1}H NMR (400 MHz, CD_3OD) δ 7.60 (d, 1H), 7.38 (d, 1H), 7.05 (s, 1H), 6.94 (d, 1H), 6.69 (m, 1H), 3.84 (d, 2H), 1.31(m, 1H), 0.66 (m, 2H), 0.37 (m, 2H).

5-(cyclopropylmethoxy)benzofuran-2-ylboronic acid (step 3 of Scheme 1)



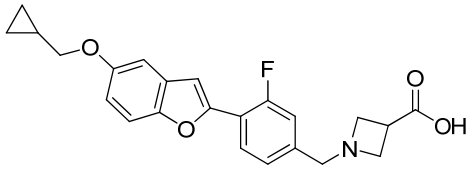
The title compound was prepared according to general procedure C (98% yield): ^{1}H NMR (400 MHz, CD_3OD) δ 7.39 (d, 1H), 7.29 (s, 1H), 7.06 (d, 1H), 7.00 (dd, 1H), 3.83 (d, J = 6.9 Hz, 2H), 1.30 (m, 1H), 0.66 (m, 2H), 0.38 (m, 2H).

4-(5-(cyclopropylmethoxy)benzofuran-2-yl)-3-fluorobenzaldehyde (step 4 of Scheme 1)



The title compound was prepared according to general procedure D (50% yield): ESI-MS: 311.2 $(M+H)^+$, ^{1}H NMR (400 MHz, CD_3OD) δ 10.01 (s, 1H), 8.20 (t, 1H), 7.78 (d, 1H), 7.69 (d, 1H), 7.44 (d, 1H), 7.36 (d, 1H), 7.08 (s, 1H), 7.01 (d, 1H), 3.85 (d, J = 7.1 Hz, 2H), 1.32 (m, 1H), 0.68 (m, 2H), 0.38 (m, 2H).

1-(4-(5-(cyclopentylmethoxy)benzofuran-2-yl)-3-fluorobenzyl)azetidine-3-carboxylic acid:



The title compound was prepared according to general procedure E (68% yield): ESI-MS: 395.9 $(M+H)^+$, ^{1}H NMR (400 MHz, CD_3OD) δ 8.01 (t, 1H), 7.35-7.30 (m, 3H), 7.17 (d, 1H), 7.04 (d, 1H), 6.87 (dd, 1H), 4.37 (s, 2H), 4.28-4.25 (m, 4H), 3.76 (d, J = 6.7 Hz, 2H), 3.60 (m, 1H), 1.18 (m, 2H), 0.54-0.51 (m, 2H), 0.28-0.26 (m, 2H).

hS1PR Cellular Assays

The hS1P1 receptor internalization assay was performed using a U2OS cell line expressing hS1P1-eGFP chimeric protein (Thermo Scientific, Søborg, Denmark). Upon compound

treatment, the hS1P1 receptor was internalized into the cytoplasm, forming GFP-containing-endosomes. This event was detected using an ArrayScan 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. HS1P1-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 ºC 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, 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 Ca2+-mobilization assay was performed using CHO cell lines stably co-expressing hS1P3 receptor and a chimeric Gq/i5 G-protein. S1P (a known agonist) or compound treatment of these cells activated the PLC-β / IP3 pathway, triggering release of Ca2+ from intracellular storage (e.g., the ER). Cells were loaded with Ca2+ 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 Ca2+ release resulted in Ca2+ binding to the dye and fluorescence (515–575 nm emission wavelength) of the dye upon excitation at 470–495 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 Ca2+ sensitive dye and fluorescence quencher inside a FLIPR plate reader (Molecular Devices, Sunnyvale, CA), and the fluorescence signal was measured. CHO cells expressing only the chimeric Gq/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 (150-175 grams, 6-8 wks) were received from Charles River Laboratories 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 compound **18** at 0.3, 1, 3 mg/kg (20% captisol in water orally (po, 10 mL/kg) at time 0. 24 h postdose, animals were sacrificed by CO_2 inhalation. Using a 18G needle and 1 cc syringe, blood was collected by cardiac puncture. Approximately 500 μL of blood was placed in a microtainer tube containing EDTA (BD #365973), and the sample was mixed thoroughly. Differential cell counts were perfomed using an Advia 120 hematology system by Bayer.