Discovery of a brain-penetrant $S1P_3$-sparing direct agonist of the $S1P_1$ and $S1P_5$ receptors efficacious at low oral dose

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Supporting Information

General: All solvents were purchased from Romil Ltd (Hy-Dry anhydrous solvents) and commercially available reagents were used as received. Melting points were recorded on Buchi B-545 apparatus and are uncorrected. All reactions were followed by TLC analysis (TLC plates GF254, Merck) or LCMS (liquid chromatography mass spectrometry) using a Waters ZQ instrument. NMR spectra were recorded on a Bruker AVANCE 400 spectrometer and are referenced as follows: ^{1}H (400 MHZ), internal standard TMS at $\delta = 0.00$; ^{13}C (100.6 MHz), internal standard $CDCl_3$ at $\delta = 77.23$ or DMSO-D_6 at $\delta = 39.70$. Column chromatography was performed on pre-packed silica gel columns (30-90 mesh, IST) using a biotage SP4. Mass spectra were recorded on Waters ZQ (ESI-MS) and Q-Tof 2 (HRMS) spectrometers. Mass Directed Auto Prep was performed on a Waters 2767 with a MicroMass ZQ Mass Spectrometer using Supelco LCABZ++ column.

GLOBAL gradient for chromatography are as follows (solvent B polar component, CV = column volume): 10% GLOBAL: 3% B for 2 CV, 3 to 13% B over 10 CV then 13% B for 5 CV; 20% GLOBAL: 5% B for 2 CV, 5 to 20% B over 10 CV then 20% B for 5 CV; 30% GLOBAL: 8% B for 2 CV, 8 to 38% B over 10 CV then 38% B for 5 CV; 40% GLOBAL: 10% B for 2 CV, 10 to 50% B over 10 CV then 50% B for 5 CV; 50% GLOBAL: 13% B for 2 CV, 13 to 63% B over 10 CV then 63% B for 5 CV. 100% GLOBAL: 25% B for 2 CV, 25 to 100% B over 10 CV then 100% B for 10 CV.

Abbreviations for multiplicities observed in NMR spectra: s; singulet; br s, broad singulet; d, doublet; t, triplet; q, quadruplet; p, pentuplet; spt, septuplet; m, multiplet.

The purity of all compounds was determined by LCMS and ^{1}H NMR and was always > 95%.

LCMS methodology

Method formate

LC conditions

The UPLC analysis was conducted on an Acquity UPLC BEH C18 column (50mm x 2.1mm, i.d. 1.7 μm packing diameter) at 40°C.

The solvents employed were:

A = 0.1% v/v solution of formic acid in water

B = 0.1% v/v solution of formic acid in acetonitrile

The gradient employed was:

Time (min)	Flow rate (mL/min)	%A	%B
0	1	99	1
1.5	1	3	97
1.9	1	3	97
2.0	1	0	100

The UV detection was a summed signal from wavelength of 210 nm to 350 nm.

MS conditions

MS : Waters ZQ
Ionisation mode : Alternate-scan positive and negative electrospray
Scan range : 100 to 1000 AMU
Scan time : 0.27 sec
Inter scan delay : 0.10 sec

Method high pH

LC conditions

The UPLC analysis was conducted on an Acquity UPLC BEH C18 column (50mm x 2.1mm, i.d. 1.7 µm packing diameter) at 40°C.
The solvents employed were:

A = 10 mM ammonium hydrogen carbonate in water adjusted to pH10 with ammonia solution
B = acetonitrile

The gradient employed was:

Time (min)	Flow rate (mL/min)	%A	%B
0	1	99	1
1.5	1	3	97
1.9	1	3	97
2.0	1	0	100

The UV detection was a summed signal from wavelength of 210 nm to 350 nm.

MS conditions

MS : Waters ZQ
Ionisation mode : Alternate-scan positive and negative electrospray
Scan range : 100 to 1000 AMU
Scan time : 0.27 sec
Inter scan delay : 0.10 sec

MDAP methodology

Method Formate

LC conditions

The HPLC analysis was conducted on either a Sunfire C18 column (100 mm x 19 mm, i.d 5µm packing diameter) or a Sunfire C18 column (150 mm x 30 mm, i.d. 5µm packing diameter) at ambient temperature.

The solvents employed were:

A = 0.1% v/v solution of formic acid in water

B = 0.1% v/v solution of formic acid in acetonitrile

Run as a gradient over either 15 or 25min (extended run) with a flow rate of 20 mL/min (100 mm x 19 mm, i.d 5 µm packing diameter) or 40 mL/min (150 mm x 30 mm, i.d. 5µm packing diameter).

The UV detection was a summed signal from wavelength of 210 nm to 350 nm.

MS conditions

MS	:	Waters ZQ
Ionisation mode	:	Alternate-scan positive and negative electrospray
Scan range	:	100 to 1000 AMU
Scan time	:	0.50 sec
Inter scan delay	:	0.20 sec

Method high pH

LC conditions

The HPLC analysis was conducted on either an Xbridge C18 column (100 mm x 19 mm, i.d 5µm packing diameter) or a Xbridge C18 column (100 mm x 30 mm, i.d. 5µm packing diameter) at ambient temperature.

The solvents employed were:

A = 10 mM ammonium bicarbonate in water, adjusted to pH10 with ammonia solution

B = acetonitrile

Run as a gradient over either 15 or 25 min (extended run) with a flow rate of 20 mL/min (100 mm x 19 mm, i.d 5µm packing diameter) or 40 mL/min (100 mm x 30 mm, i.d 5µm packing diameter).

The UV detection was a summed signal from wavelength of 210 nm to 350 nm.

MS conditions

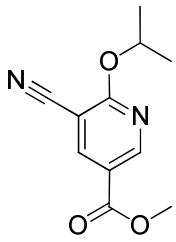
MS	:	Waters ZQ
Ionisation mode	:	Alternate-scan positive and negative electrospray
Scan range	:	100 to 1000 AMU
Scan time	:	0.50 sec
Inter scan delay	:	0.20 sec

Experimental procedures for compounds 9 – 14

Compound 9

Step1

Methyl 5-cyano-6-[(1-methylethyl)oxy]-3-pyridinecarboxylate (39).



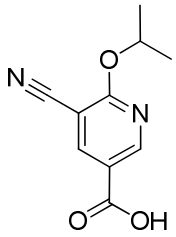
A mixture of methyl 5-cyano-6-oxo-1,6-dihydro-3-pyridinecarboxylate (2.98 g, 16.7 mmol, Enamine, EN300-27361), isopropyl iodide (6.68 mL, 66.9 mmol) and silver carbonate (9.23 g, 33.5 mmol) in $CHCl_3$ (100 mL) was stirred under nitrogen at 60°C for 4 h. The cooled mixture was filtered and the filtrate evaporated to give an orange gum. Purification of this residue on SP4, using a 40 G column (gradient 0-30% AcOEt/cyclohexane) gave the title compound (3.2 g, 87%) as a colourless solid.

LCMS (method formate): retention time 1.08 min $[M+H]^+ = 221.18$.

^1H NMR (600 MHz, $CDCl_3$) δ ppm 8.95 (d, J = 2.2 Hz, 1H), 8.45 (d, J = 2.2 Hz, 1H), 5.52 (spt, J = 6.2 Hz, 1H), 3.95 (s, 3H), 1.44 (d, J = 6.2 Hz, 6H).

Step 2

5-Cyano-6-[(1-methylethyl)oxy]-3-pyridinecarboxylic acid (40).



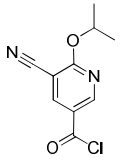
Lithium hydroxide (1.74 g, 72.7 mmol) in water (10 mL) was added to a solution of **39** (3.2 g, 14.5 mmol) in methanol (30 mL) and the resulting mixture was stirred at room temperature for 4 h. Most of the methanol was evaporated *in vacuo* and the residual aqueous phase was acidified to pH 1 with HCl (2 N in water) and then was extracted with AcOEt (3x50 mL). The combined organic extracts were washed with brine (100 mL) then dried over $MgSO_4$ and concentrated *in vacuo* to give the title compound (3.05 g, 95%) as a colourless solid.

LCMS (method formate): retention time 0.89 min $[M-H]^-$ = 205.28.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.67 (d, J = 2.3 Hz, 1H), 8.37 (d, J = 2. 3 Hz, 1H), 5.21 (spt, J = 6.1 Hz, 1H), 3.15-3.04 (br s, 1H), 1.13 (d, J = 6.1 Hz, 6H).

Step 3

5-Cyano-6-[(1-methylethyl)oxy]-3-pyridinecarbonyl chloride (41).



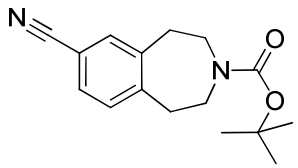
Oxalyl chloride (2.81 g, 1.94 mL, 22.1 mmol) was added to a suspension of **40** (3.04 g, 14.7 mmol) in DCM (50 mL), followed by DMF (0.011 mL, 0.15 mmol) and the resulting mixture was stirred at room temperature for 3 h then was concentrated *in vacuo*. The residue was azeotroped with toluene (3x10 mL) to give the title compound (3.5 g, 95%) as a pale yellow oil which solidified on standing under vacuum.

LCMS (method formate): retention time 1.08 min $[M+H]^+$ = 221.18 (methyl ester).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 9.04 (d, *J* = 2.5 Hz, 1H), 8.52 (d, *J* = 2.5 Hz, 1H), 5.57 (spt, *J* = 6.2 Hz, 1H), 1.46 (d, *J* = 6.2 Hz, 6H).

Step 4

1,1-Dimethylethyl 7-cyano-1,2,4,5-tetrahydro-3*H*-3-benzazepine-3-carboxylate (30).



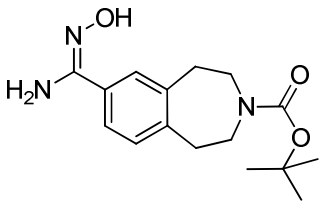
A mixture of 1,1-dimethylethyl 7-{[(trifluoromethyl)sulfonyl]oxy}-1,2,4,5-tetrahydro-*3H*-3-benzazepine-3-carboxylate (may be prepared as described in WO2002040471) (3.5 g, 8.8 mmol) and $Zn(CN)_2$ (1.25 g, 10.6 mmol) in dry DMF (100 mL) at room temperature was de-gassed under vacuum and flushed several times with nitrogen. Tetrakis(triphenylphosphine)palladium(0) (1.0 g, 0.88 mmol) was then added and the de-gassing procedure repeated. The resulting mixture was stirred under nitrogen at 100°C for 90 min then cooled to room temperature and diluted with AcOEt (100 mL). The organic phase was filtered through celite and the insoluble material rinsed with AcOEt. The combined organic phases were concentrated *in vacuo* and the residue was partitioned between AcOEt and water. The mixture was filtered again through celite, and the layers were separated. The aqueous phase was extracted with AcOEt and the combined organic phases were washed with water, dried over $MgSO_4$ and then concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel (c-hexane/AcOEt: 10 to 30% gradient) gave compound **30** (2.42 g, 95%) as a pale orange gum which solidified on standing.

LCMS (method high pH): Retention time 1.22 min, $[M+H]^+$ = 273.0.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.44 (d, *J* = 7.8 Hz, 1H), 7.41 (br s, 1H), 7.22 (d, *J* = 7.8 Hz, 1H), 3.56 (br s, 4H), 2.94 (br s, 4H), 1.52 (s, 9H).

Step 5

1,1-Dimethylethyl 7-[(hydroxyamino)(imino)methyl]-1,2,4,5-tetrahydro-3*H*-3-benzazepine-3-carboxylate (42).



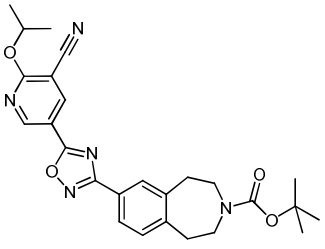
To a solution of compound **30** (29.4 g, 108 mmol) in ethanol (400 mL) was added hydroxylamine hydrochloride (33.4 g, 432 mmol) followed by the portionwise addition of $NaHCO_3$ (60.5 g, 648 mmol). The resulting mixture was stirred at 60ºC for 4 h then cooled to room temperature and concentrated *in vacuo* to 100 mL. Water (900 mL) was added and the precipitate formed was filtered off and dried under vacuum at 40ºC for 16 h to give the title compound (29.6 g, 90%) as a grey solid.

LCMS (method high pH): Retention time 0.72 min, $[M+H]^+ = 306.21$.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.53 (s, 1H), 7.46 (d, *J* = 1.5 Hz, 1H), 7.41 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.14 (d, *J* = 7.8 Hz, 1H), 5.73 (s, 2H), 3.45 (br s, 4H), 2.85 (br s, 4H), 1.41 (s, 9H).

Step 6

1,1-Dimethylethyl 7-(5-{5-cyano-6-[(1-methylethyl)oxy]-3-pyridinyl}-1,2,4-oxadiazol-3-yl)-1,2,4,5-tetrahydro-*3H*-3-benzazepine-3-carboxylate (43).

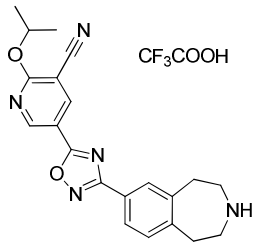


Compound **41** (657 mg, 2.92 mmol) was added portionwise to a suspension of compound **42** (850 mg, 2.78 mmol) in toluene (10 mL) and pyridine (10 mL) at room temperature under nitrogen and the resulting mixture was stirred at this temperature for 20 min then at 120 °C for 2 h, and then was cooled to room temperature and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel (Biotage SP4, loaded in DCM, column size 40 G), eluting with 0-50% AcOEt-cyclohexane gave the title compound (1.05 g, 74%) as a colourless foam.

LCMS (method formate): retention time 1.54 min $[M\text{-}tBu+CH_3CN]^+ = 461.03$.

Step 7

2-[(1-Methylethyl)oxy]-5-[3-(2,3,4,5-tetrahydro-1H-3-benzazepin-7-yl)-1,2,4-oxadiazol-5-yl]-3-pyridinecarbonitrile trifluoroacetate (9).



Trifluoroacetic acid (0.51 mL, 6.6 mmol) was added dropwise to a solution of compound **43** (1.05 g, 2.2 mmol) in DCM (15 mL) at 0°C under nitrogen and the resulting mixture was allowed to warm to room temperature and stirred for 16 h. Toluene (20 mL) was added and the solvent was evaporated *in vacuo* to give a colourless solid which was triturated with Et_2O (20 mL) and filtered off to give compound **9** (1.03 g, 98%) as a colourless solid.

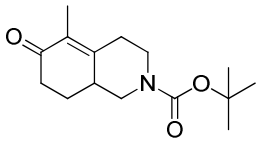
LCMS (method formate): retention time 0.97 min $[M+H]^+ = 376.24$.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.21 (d, J = 2.3 Hz, 1H), 8.98 (d, J = 2.3 Hz, 1H), 7.96 (s, 1H), 7.93 (d, J = 7.8 Hz, 1H), 7.47 (d, J = 7.8 Hz, 1H), 5.51 (spt, J = 6.1 Hz, 1H), 3.46-3.38 (br s, 2H), 3.31-3.13 (m, 8H), 1.42 (d, J = 6.1 Hz, 6H).

Compound 10

Step 1

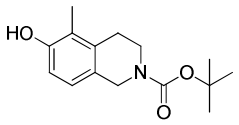
1,1-Dimethylethyl 5-methyl-6-oxo-3,4,6,7,8,8a-hexahydro-2(1*H*)-isoquinolinecarboxylate (17).



1,1-Dimethylethyl 4-oxo-1-piperidinecarboxylate **16** (70 g, 350 mmol, Aldrich) and pyrrolidine (43.6 mL, 530 mmol) were dissolved in toluene (310 mL) and the resulting mixture was refluxed under Dean-Stark conditions for 24 h then concentrated *in vacuo*. The residue was dissolved in anhydrous toluene (270 mL) and treated with hydroquinone (0.40 g) and 1-penten-3-one (29.6 g, 350 mmol). The resulting solution was refluxed for 24 h then diluted with AcOEt (300 mL). The mixture was washed with HCl (0.5 N in water, 500 mL) and the aqueous phase extracted with AcOEt (300 mL). The combined organic phases were dried ($MgSO_4$) and concentrated. Purification of the residue by flash chromatography on a silica cartridge (1.5 kg) gave the title compound (55.2 g, 59.2 %) as pale yellow oil which crystallised on standing.
LCMS (method formate): Retention time 1.04 min, $[M+H]^+$ = 266.24.
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.16-4.02 (m, 2H), 3.08-3.01 (m, 1H), 2.77-2.71 (m, 1H), 2.58-2.49 (m, 3H), 2.39-2.26 (m, 2H), 2.06-2.00 (m, 1H), 1.79 (s, 3H), 1.59-1.52 (m, 1H), 1.49 (s, 9H).

Step 2

1,1-Dimethylethyl 6-hydroxy-5-methyl-3,4-dihydro-2(*1H*)-isoquinolinecarboxylate (18).



Lithium bis(trimethylsilyl)amide (1M in THF, 246 mL, 250 mmol) was added dropwise to a solution of compound **17** (54.4 g, 210 mmol) in THF (200 mL) at -63°C, and the mixture was stirred for an additional 30 min. Chloro(trimethyl)silane (31.4 mL, 250 mmol) was added dropwise and the resulting mixture was stirred for 2 h at -70°C. The reaction was warmed to room temperature over 20 min and diluted with Et_2O (800 mL). The reaction was added to a saturated Na_2CO_3 aqueous solution and the phases were separated. The aqueous phase was extracted with Et_2O (300 mL) and the combined organic phases were dried (Na_2SO_4) and concentrated *in vacuo*. The residue was dissolved in acetonitrile (200 mL) and palladium (II) acetate (46.0 g, 210 mmol) was added. The resulting mixture was cooled (water bath) to maintain a reaction temperature below 35°C and stirred for 16 h. The reaction was filtered through Celite and the residue rinsed with AcOEt (3x300 mL). The filtrate was further filtered through a 1" pad of silica gel and concentrated *in vacuo*. The residue was dissolved in AcOEt (500 mL), treated with tetrabutylammonium fluoride (1 M in THF, 200 mL, 200 mmol). The resulting mixture was allowed to stand for 30 min, then was washed with HCl (0.5 N in water, 300 mL) and a 10% sodium thiosulphate solution, dried ($MgSO_4$) and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel (300 g column) eluting with 0-60% gradient AcOEt/cyclohexane gave the title compound (29.9 g, 55%) as a white solid.

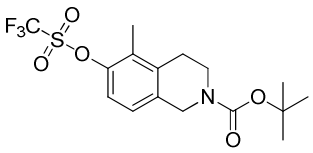
LCMS (method formate): Retention time 1.07 min, $[M+H]^+ = 264.12$.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.03 (s, 1H), 6.75 (d, J = 8.1 Hz, 1H), 6.64 (d, J = 8.1 Hz, 1H), 4.36 (s, 2H), 3.52 (t, J = 6.1 Hz, 2H), 2.62 (t, J = 6.1 Hz, 2H), 2.01 (s, 3H), 1.41 (s, 9H).

HRMS calculated for $C_{15}H_{22}NO_3$: 264.1600, found: 264.1605.

Step 3

1,1-Dimethylethyl 5-methyl-6-{[(trifluoromethyl)sulfonyl]oxy}-3,4-dihydro-2(*1H*)-isoquinolinecarboxylate (19).



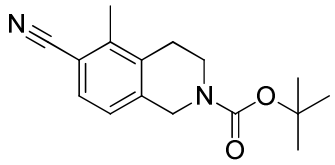
To a solution of **18** (3.16 g, 12 mmol) in dichloromethane (50 mL) at room temperature under nitrogen was added pyridine (1.94 mL, 24 mmol) and the resulting solution was cooled to -30°C before trifluoromethanesulfonic anhydride (2.2 mL, 13.2 mmol) was added dropwise. The resulting mixture was stirred for 40 min at this temperature, warmed to room temperature and concentrated *in vacuo*. The residue was diluted with AcOEt and washed sequentially with HCl (1 N in water), a saturated $NaHCO_3$ aqueous solution and brine. The organic phase was then dried over $MgSO_4$ and concentrated *in vacuo* to give the title compound (4.85 g, 102%) as a red oil which was used in the next step without further purification.

LCMS (method high pH): Retention time 1.46 min, $[M-H]^-$ = 394.22.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.10 (d, *J* = 8.1 Hz, 1H), 7.02 (d, *J* = 8.1 Hz, 1H), 4.58 (s, 2H), 3.68 (t, *J* = 5.8 Hz, 2H), 2.76 (t, *J* = 5.8 Hz, 2H), 2.25 (s, 3H), 1.50 (s, 9H).

Step 4

1,1-Dimethylethyl 6-cyano-5-methyl-3,4-dihydro-2(*1H*)-isoquinolinecarboxylate (20).



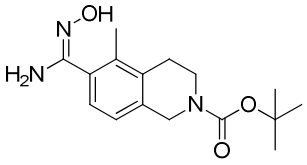
A solution of compound **19** (26.1 g, 66 mmol) in DMF (200 mL) was de-gassed for 10 min under vacuum then flushed with nitrogen. The solution was treated with tetrakis(triphenylphosphine)palladium (7.6 g, 6.6 mmol) and zinc cyanide (10.1 g, 86 mmol) and the resulting mixture was stirred at 100°C under nitrogen for 6 h then was cooled to room temperature. The mixture was filtered, the residue washed with AcOEt and most of the solvent evaporated *in vacuo*. The residue was dissolved in AcOEt and the organic phase was washed twice with a saturated $NaHCO_3$ aqueous solution. The combined aqueous phases were extracted twice with AcOEt and the combined organic phases were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel eluting with an 0-50% AcOEt/cyclohexane gradient gave the title compound (16.6 g, 92%) as a white solid.

LCMS (method high pH): Retention time 1.24 min, $[M+H]^+$ = 273.25.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.57 (d, *J* = 8.1 Hz, 1H), 7.22 (d, *J* = 8.1 Hz, 1H), 4.56 (s, 2H), 3.59 (t, *J* = 6.1 Hz, 2H), 2.72 (t, *J* = 6.1 Hz, 2H), 2.39 (s, 3H), 1.44 (s, 9H).
HRMS calculated for $C_{16}H_{21}N_2O_2$: 273.1603, found: 273.1608.

Step 5

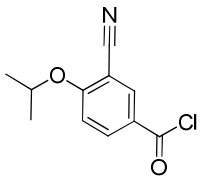
1,1-Dimethylethyl 6-[(hydroxyamino)(imino)methyl]-5-methyl-3,4-dihydro-2(*1H*)-isoquinolinecarboxylate (21).



A mixture of 1compound **20** (16.6 g, 61 mmol), $NaHCO_3$ (30.7 g, 370 mmol) and hydroxylamine hydrochloride (25.4 g, 370 mmol) in ethanol (250 mL) was refluxed for 28 h then was allowed to cool to room temperature. The reaction was filtered and the residue washed with ethanol. The combined filtrate and washings were concentrated *in vacuo*. The residue was poured into water (100 mL) and stirred at room temperature for 20 min. The precipitated solid was isolated by filtration and dried under vacuum at 40°C for 16 h to give the title compound (16 g, 86%) as a white solid.
LCMS (method high pH): Retention time 0.93 min, $[M+H]^+$ = 306.17.
^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.21 (s, 1H), 7.07 (d, *J* = 8.9 Hz, 1H), 6.99 (d, *J* = 8.9 Hz, 1H), 5.65 (s, 2H), 4.48 (s, 2H), 3.58 (t, *J* = 5.9 Hz, 2H), 2.67 (t, *J* = 5.9 Hz, 2H), 2.20 (s, 3H), 1.43 (m, 9H).
HRMS calculated for $C_{16}H_{24}N_3O_3$: 306.1818, found: 306.1817.

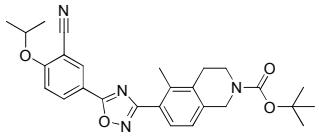
Step 6

3-Cyano-4-[(1-methylethyl)oxy]benzoyl chloride (22).



Oxalyl chloride (6.4 mL, 73 mmol) was added to a solution of 3-cyano-4-[(1-methylethyl)oxy]benzoic acid (10.7 g, 52 mmol, Biopharma Inc.) in CH_2Cl_2 (100 mL) followed by the addition of DMF (0.044 mL, 0.57 mmol) and the resulting mixture was stirred at room temperature for 4 h. The reaction mixture was filtered and concentrated *in vacuo*. The residue was co-evaporated with cyclohexane (2x50 mL) to give the title compound (11.7 g, 100%) as a pale yellow oil which solidified on standing.

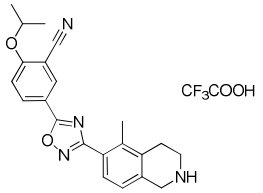
1,1-Dimethylethyl 6-(5-{3-cyano-4-[(1-methylethyl)oxy]phenyl}-1,2,4-oxadiazol-3-yl)-5-methyl-3,4-dihydro-2(*1H*)-isoquinolinecarboxylate (23).



To a suspension of compound **21** (4.6 g, 15 mmol) in toluene (30 mL) and pyridine (30 mL) at room temperature under nitrogen was slowly added compound **22** (3.5 g, 16 mmol) in toluene (15 mL). After 15 min, the resulting mixture was refluxed for 90 min (internal temperature 110°C) then was cooled to room temperature. The solution was decanted from the brown precipitate, the precipitate washed with toluene and the combined organics concentrated *in vacuo*. The residue was dissolved in AcOEt and the resulting solution washed with HCl (2 N in water). The aqueous phase was extracted with AcOEt and the combined organic phases were washed sequentially with a saturated $NaHCO_3$ aqueous solution and brine, dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue on SP4 using a 30% GLOBAL gradient (AcOEt in hexanes) gave the title compound (3.62 g, 51%) as a white foam.
LCMS (method high pH): Retention time 1.55 min, $[M+H]^+$ = 475.31.
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.42 (d, *J* = 2.3 Hz, 1H), 8.33 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.76 (d, *J* = 8.1 Hz, 1H), 7.16-7.08 (m, 2H), 4.79 (spt, *J* = 6.1 Hz, 1H), 4.64 (s, 2H), 3.72 (t, *J* = 5.8 Hz, 2H), 2.84 (t, *J* = 5.8 Hz, 2H), 2.52 (s, 3H), 1.51 (s, 9H), 1.48 (d, *J* = 6.1 Hz, 6H).

Step 7

2-Isopropoxy-5-(3-(5-methyl-1,2,3,4-tetrahydroisoquinolin-6-yl)-1,2,4-oxadiazol-5-yl)benzonitrile trifluoroacetate (24).



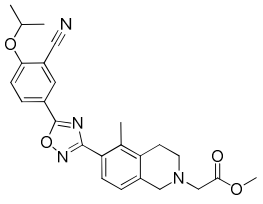
Trifluoroacetic acid (20 mL) was added dropwise at room temperature over 5 min to a stirred solution of compound **23** (6.9 g, 14.5 mmol) in dichloromethane (50 mL). The resulting mixture was then stirred at room temperature for 2 h then was concentrated *in vacuo*. The residue obtained was co-evaporated twice with toluene. Trituration of the residue formed with Et_2O gave the title compound (6.84 g, 14 mmol, 96%) as a colourless solid.

LCMS (method formate): Retention time 0.83 min, $[M+H]^+$ = 375.02.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.17 (br s, 2H), 8.51 (d, *J* = 2.3 Hz, 1H), 8.40 (dd, *J* = 9.1, 2.3 Hz, 1H), 7.78 (d, *J* = 8.1 Hz, 1H), 7.56 (d, *J* = 9.1 Hz, 1H), 7.28 (d, *J* = 8.1 Hz, 1H), 4.98 (spt, *J* = 6.1 Hz, 1H), 4.38 (br s, 2H), 3.49 (t, *J* = 6.1 Hz, 2H), 2.99 (t, *J* = 6.1 Hz, 2H), 2.47 (s, 3H), 1.39 (d, *J* = 6.1 Hz, 6H).

Step 8

Methyl 2-(6-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-5-methyl-3,4-dihydroisoquinolin-2(*1H*)-yl)acetate (44).



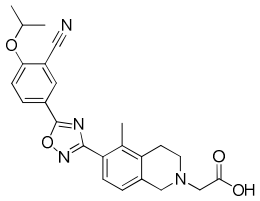
Methyl bromoacetate (626 mg, 377 µl, 4.1 mmol) was added to a stirred mixture of compound **24** (2.0 g, 4.1 mmol) and K_2CO_3 (1.4 g, 10.2 mmol) in dry acetonitrile (30 mL). The resulting mixture was stirred at 50ºC for 1 h then was cooled to room temperature and concentrated *in vacuo*. The residue was dissolved in AcOEt (50 mL) and the organic phase was washed twice with water then brine, dried over $MgSO_4$ and concentrated *in vacuo* to give the title compound (1.6 g, 3.6 mmol, 88%) as a colourless solid which was used in the next step without further purification.

LCMS (method high pH): Retention time 0.96 min, $[M+H]^+$ = 447.29.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.57 (d, *J* = 2.1 Hz, 1H), 7.48 (dd, *J* = 9.0, 2.1 Hz, 1H), 6.87 (d, *J* = 8.1 Hz, 1H), 6.26 (d, *J* = 9.0 Hz, 1H), 6.16 (d, *J* = 8.1 Hz, 1H), 3.94 (spt, *J* = 6.1 Hz, 1H), 3.00 (s, 2H), 2.92 (s, 3H), 2.60 (s, 2H), 2.14 - 2.01 (m, 4H), 1.65 (s, 3H), 0.63 (d, *J* = 6.1 Hz, 6H).

Step 9

2-(6-(5-(3-Cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-5-methyl-3,4-dihydroisoquinolin-2(*1H*)-yl)acetic acid (45).



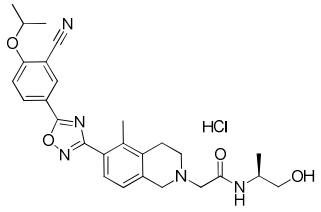
A solution of compound **44** (1.6 g, 3.6 mmol) in ethanol (20 mL) was treated with NaOH (2 N in water, 20 mL, 40 mmol) and the resulting mixture was stirred at room temperature for 16 h. Most of the ethanol was removed *in vacuo*. The residue was diluted with water (20 mL) and the mixture was acidified with glacial acetic acid then was stirred for 20 min. The precipitate formed was filtered off and washed with water then dried under vacuum to give the title compound (1.4 g, 90%) as a colourless solid.

LCMS (method formate): Retention time 0.91 min, $[M+H]^+$ = 433.27.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.50 (d, *J* = 2.1 Hz, 1H), 8.40 (dd, *J* = 9.0, 2.1 Hz, 1H), 7.63 (d, *J* = 8.0 Hz, 1H), 7.55 (d, *J* = 9.0 Hz, 1H), 7.05 (d, *J* = 8.0 Hz, 1H), 4.98 (spt, *J* = 6.1 Hz, 1H), 3.70 (s, 2H), 2.85 (s, 2H), 2.77 (m, 4H), 2.42 (s, 3H), 1.38 (d, *J* = 6.1 Hz, 6H).

Step 10

(S)-2-(6-(5-(3-Cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-5-methyl-3,4-dihydroisoquinolin-2(*1H*)-yl)-N-(1-hydroxypropan-2-yl)acetamide hydrochloride (10).



Compound **45** (86 mg, 0.2 mmol) was finely crushed before any attempt of dissolving the substance in a mixture of N,N-dimethylformamide (DMF) (2 mL) and N-methyl-2-pyrrolidone (NMP) (2 mL). The solution was warmed with a heat gun to maximise the chance of solubilising the reactant then was cooled to room temperature. DIPEA (0.11 mL, 0.6 mmol) was added followed by HATU (76 mg, 0.2 mmol) and after 5 min, by (2S)-2-amino-1-propanol (22.5 mg, 0.3 mmol). The resulting deep yellow mixture was stirred at room temperature for 1 h then DIPEA (0.11 mL, 0.6 mmol) and HATU (76 mg, 0.2 mmol) were further added followed after 5 min by (2S)-2-amino-1-propanol (22.5 mg, 0.3 mmol). The resulting solution was stirred for 20 min at room temperature then was partitioned between AcOEt and a saturated $NaHCO_3$ aqueous solution. The layers were separated and the aqueous phase was extracted twice with AcOEt. The combined organic phases were washed with a saturated $NaHCO_3$ aqueous solution, dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by MDAP (method high pH) gave a residue which was dissolved in 1,4-dioxane (1 mL). The solution was treated with HCl (4 N in 1,4-dioxane, 0.5 mL, 2 mmol) and the resulting mixture was stirred at room temperature for 5 min then concentrated *in vacuo*. Trituration of the residue with Et_2O gave the title compound (46 mg, 0.13 mmol, 44%) as a white solid.

LCMS (method high pH): Retention time 1.19 min, $[M+H]^+$ = 490.38.

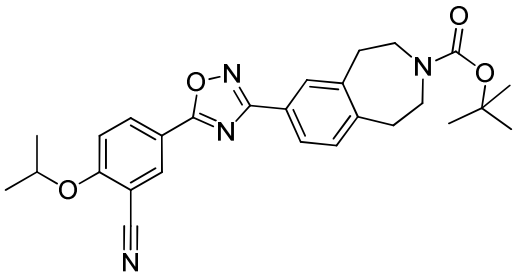
^{1}H NMR (CD_3OD) δ ppm 8.43 (d, *J* = 1.9 Hz, 1H), 8.40 (dd, *J* = 8.8, 2.2 Hz, 1H), 7.83 (d, *J* = 8.0 Hz, 1H), 7.43 (d, *J* = 8.8 Hz, 1H), 7.21 (d, *J* = 8.2 Hz, 1H), 4.99-4.90 (m, 1H), 4.46 (s, 2H), 4.10-4.01 (m, 1H), 3.98-3.88 (m, 2H), 3.61-3.57 (m, 2H), 3.57 (dd, *J* = 11.0, 4.9 Hz, 1H), 3.52 -3.46 (m, 1H), 3.17 (t, *J* = 6.2 Hz, 2H), 2.54 (s, 3H), 1.45 (d, *J* = 6.0 Hz, 6H), 1.18 (d, *J* = 6.9 Hz, 3H).

HRMS calculated for $C_{27}H_{32}N_5O_4$: 490.2454, found: 490.2454.

Compound 11

Step 1

1,1-Dimethylethyl 7-(5-{3-cyano-4-[(1-methylethyl)oxy]phenyl}-1,2,4-oxadiazol-3-yl)-1,2,4,5-tetrahydro-3*H*-3-benzazepine-3-carboxylate (46).



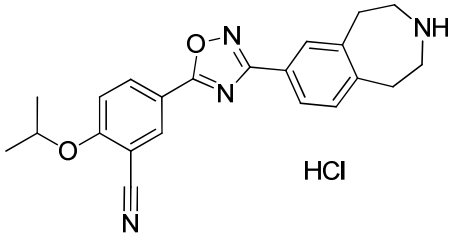
To a suspension of compound **9** (4.58 g, 15 mmol) in toluene (30 mL) and pyridine (30 mL) was slowly added under nitrogen at room temperature compound the title compound **22** (3.52 g, 15.75 mmol) in toluene (15 mL). After 15 min, the resulting mixture was refluxed for 90 min then cooled to room temperature. The solvent was decanted from the brown precipitate formed and concentrated *in vacuo*. The residue was dissolved in AcOEt and the organic phase washed with HCl (2 N in water). The aqueous phase was extracted with AcOEt and the combined organic phases were washed with a saturated $NaHCO_3$ aqueous solution and brine, dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel (c-hexane/AcOEt: 5 to 25% gradient) gave the title compound (5.5 g, 78%) as a white foam.

LCMS (method high pH): Retention time 1.56 min, $[M+H]^+ = 475.23$.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.43 (d, *J* = 2.3 Hz, 1H), 8.33 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.93-7.91 (m, 2H), 7.30-7.24 (m, 1H), 7.12 (d, *J* = 8.8 Hz, 1H), 4.80 (spt, *J* = 6.1 Hz, 1H), 3.60 (br s, 4H), 2.99 (br s, 4H), 1.50 (s, 9H), 1.48 (d, *J* = 6.1 Hz, 6H).

Step 2

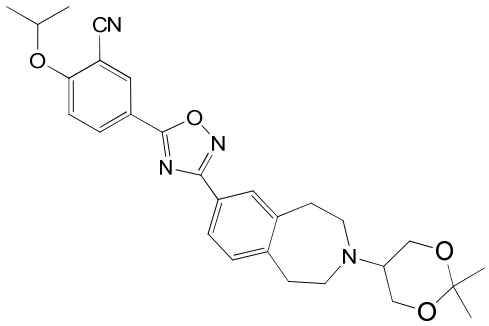
2-[(1-Methylethyl)oxy]-5-[3-(2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)-1,2,4-oxadiazol-5-yl]benzonitrile hydrochloride (47).



A solution of compound **46** (81 g, 171 mmol) in DCM (350 mL) was added dropwise to HCl (4 N in 1,4-dioxane, 427 ml, 1.71 mol) over 10 min. The resulting mixture was stirred for 3 h, then Et_2O (1.5 L) was added and the resulting slurry stirred for 20 min, then filtered off and the solid collected by filtration. The solid collected was washed with Et_2O (2x300 mL) and dried under vacuum (ca 15 mbar) at 40ºC to the title compound (73.9 g, 95 %) as a colourless solid.
LCMS (method high pH): Retention time 0.92 min, $[M+H]^+$ = 375.26.
^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.36 (br s, 2H), 8.50 (d, *J* = 2.3 Hz, 1H), 8.40 (dd, *J* = 9.2, 2.3 Hz, 1H), 7.95 (d, *J* = 1.5 Hz, 1H), 7.91 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.56 (d, *J* = 9.2 Hz, 1H), 7.45 (d, *J* = 7.8 Hz, 1H), 4.98 (spt, *J* = 6.1 Hz, 1H), 3.27-3.19 (m, 8H), 1.39 (d, *J* = 6.1 Hz, 6H).

Step 3

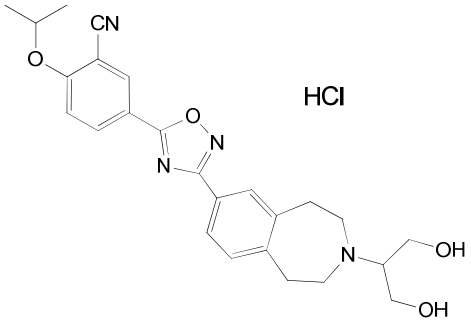
5-{3-[3-(2,2-Dimethyl-1,3-dioxan-5-yl)-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl]-1,2,4-oxadiazol-5-yl}-2-[(1-methylethyl)oxy]benzonitrile (48).



Sodium triacetoxyborohydride (1.06 g, 5 mmol) was added to a stirred mixture of compound **47** (411 mg, 1 mmol) and 2,2-dimethyldioxan-5-one (260 mg, 239 µl, 2 mmol) in DCM (10 mL). The reaction mixture was stirred at room temperature for 48 h then was treated with a saturated $NaHCO_3$ aqueous solution (15 mL), and the resulting mixture was vigorously stirred for 30 min. The phases were separated, and the organic phase was washed with water then brine. dried over $MgSO_4$ and concentrated *in vacuo* to give the title compound (100% yield assumed) which was used in the next step without further purification.
LCMS (method formate): retention time 1.00 min, $[M+H]^+$ = 489.27.

Step 4

5-(3-{3-[2-Hydroxy-1-(hydroxymethyl)ethyl]-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl}-1,2,4-oxadiazol-5-yl)-2-[(1-methylethyl)oxy]benzonitrile hydrochloride (11).



A solution of compound **48** (488 mg, 1 mmol), in THF (5 mL) was treated with HCl (2 N in THF, 5 mL, 10 mmol), and the resulting mixture was stirred at room temperature for 16 h. Most of the THF was removed *in vacuo*. The residue was partitioned between a saturated $NaHCO_3$ aqueous solution and AcOEt and the layers were separated. The aqueous phase was extracted with AcOEt (3x15 mL) and the combined organic phases were dried over $MgSO_4$ then concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel (gradient: 0-6% methanol in DCM) gave a product which was dissolved in AcOEt (2 mL) and the resulting solution was treated with HCl (1 N in Et_2O, 1.2 mL, 1.2 mmol). After 5 min, most of the solvent was removed *in* vacuo. Trituration of the residue with Et_2O (15 mL) gave the title compound (190 mg, 39%) as an off-white solid.

LCMS (method formate): retention time 0.90 min, $[M+H]^+$ = 449.34.

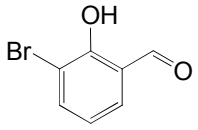
^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.20 (br s, 1H), 8.50 (d, *J* = 2.3 Hz, 1H), 8.40 (dd, *J* = 9.2, 2.3 Hz, 1H), 7.96 (s, 1H), 7.92 (d, *J* = 7.8 Hz, 1H), 7.56 (d, *J* = 9.2 Hz, 1H), 7.45 (d, *J* = 7.8 Hz, 1H), 5.39 (br s, 2H), 4.98 (spt, *J* = 6.0 Hz, 1H), 3.82 (m, 4H), 3.63 (dd, *J* = 6.7, 5.9 Hz, 2H), 3.43 (m, 1H), 3.15 (m, 3H), 1.38 (d, *J* = 6.0 Hz, 6H).

HRMS calculated for $C_{25}H_{29}N_4O_4$: 449.2189, found: 449.2189.

Compound 12

Step 1

3-Bromo-2-hydroxybenzaldehyde (31).



Reference: Hansen, T. V.; Skattelboel, L. Ortho-formylation of phenols: preparation of 3-bromosalicylaldehyde *Org. Synth*. **2005**, *82*, 64-68.

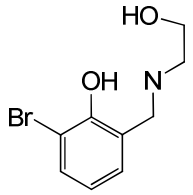
Triethylamine (72 mL, 520 mmol) was added over 10 min to a suspension of paraformaldehyde (dried 3 days at 40°C under vacuum, 23.4 g, 780 mmol) and dry magnesium chloride (49.5 g, 520 mmol, CAS # 7786-30-3, Aldrich 449172) in dry THF (1 L) under nitrogen at room temperature. The resulting mixture was stirred for 20 min, then 2-bromophenol (45 g, 260 mmol) was added over 5 min via syringe. The resulting mixture was stirred under reflux for 6 h, then cooled to room temperature and diluted with Et_2O (500 mL). The organic phase was washed with HCl (2 N in water, 500 mL). The insoluble material was removed by filtration and the phases were separated. The organic phase was washed twice with HCl (2 N in water, 500 mL), then with water (5x100 mL), dried over $MgSO_4$ and concentrated *in vacuo* to give the title compound (46.5 g, 85%) as a colourless solid which was used in the next step without further purification.

LCMS (method formate): Retention time 0.99 min, $[M+H]^+$ = 201.1 (1 Br).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 11.63 (s, 1H), 9.87 (s, 1H), 7.79 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.56 (dd, *J* = 7.7, 1.5 Hz, 1H), 6.96 (dd, *J* = 7.8, 7.7 Hz, 1H).

Step 2

2-Bromo-6-{[(2-hydroxyethyl)amino]methyl}phenol (49).



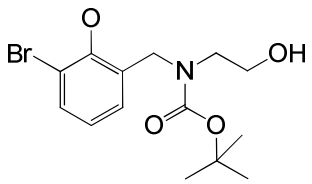
A solution of compound **31** (9.6 g, 47.8 mmol) in THF (250 mL) at 0°C under nitrogen was treated with 2-aminoethanol (3.02 g, 50.1 mmol) then with finely powdered sodium triacetoxyborohydride (10.6 g, 50.1 mmol). The resulting mixture was stirred at 0°C for 1 h, then allowed to warm to room temperature and stirred at this temperature for 16 h. Most of the solvent was removed *in vacuo* and the residue was triturated with DCM (200 mL). The solid present was removed by filtration and the filtrate was concentrated *in vacuo*. The residue obtained was dissolved in methanol (50 mL) and loaded onto an SCX ion exchange column. The column was eluted with methanol (2x100 mL) then with ammonia (2 N in methanol, 200 mL). The ammonia fractions were combined and concentrated *in vacuo* to give the title compound (10.1 g, 90%).as a colourless solid

LCMS (Method formate): Retention time 0.49 min, $[M+H]^+$ = 248.1 (1 Br).

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.35 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.01 (dd, *J* = 7.5, 1.5 Hz, 1H), 6.62 (dd, *J* = 8.1, 7.5 Hz, 1H), 5.7-5.4 (br s, 3H), 3.93 (s, 2H), 3.50 (t, *J* = 5.6 Hz, 2H), 2.60 (t, *J* = 5.6 Hz, 2H).

Step 3

1,1-Dimethylethyl [(3-bromo-2-hydroxyphenyl)methyl](2-hydroxyethyl)carbamate (32).



Compound **49** (2.3 g, 9.3 mmol) was dissolved in methanol (10 mL) and THF (40 mL) and the resulting mixture was cooled to 0°C in an ice bath. Triethylamine (1.95 mL, 14.0 mmol) was added, followed by bis(1,1-dimethylethyl) dicarbonate (2.4 mL, 10.3 mmol) and the resulting mixture was stirred at this temperature for 2.5 h, then allowed to warm to room temperature and stirred for a further 72 h. Most of the solvent was removed *in vacuo* and the residue was dissolved in EtOAc (100 mL), washed with HCl (2 N in water, 100 mL) and brine (100 mL), then dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel, eluting with 15-45% AcOEt/cyclohexane gave the title compound (3.2 g, 9.2 mmol, 98%) as pale yellow oil.

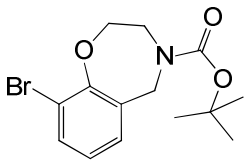
LCMS (method formate): Retention time 1.11 min, $[M+H]^+$ = 348.12 (1 Br).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 9.85 (br s, 1H), 7.47 (m, 1H), 7.08 (m, 1H), 6.70 (m, 1H), 4.45 (s, 2H), 3.77 (m, 2H), 3.50 (br s, 1H), 3.39 (m, 2H), 1.47 (9H, s).

HRMS calculated for $C_{14}H_{20}BrNNaO_4$: 368.0473, found: 368.0465 ($[M+Na]^+$).

Step 3

1,1-Dimethylethyl 9-bromo-2,3-dihydro-1,4-benzoxazepine-4(5*H*)-carboxylate (50).



A mixture of compound **49** (2.35 g, 6.79 mmol) and triphenylphospine (1.96 g, 7.47 mmol) in THF (40 mL) under nitrogen was cooled at 0°C using an ice bath, then DIAD (1.47 mL, 7.47

mmol) was added dropwise and the resulting mixture was stirred at this temperature for 1 h, then was concentrated *in vacuo*. The residue was dissolved in AcOEt (100 mL), and the organic phase was washed with brine (2x100 mL), dried over Na_2SO_4 then concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel eluting with 5-25% AcOEt/cyclohexane gave the title compound (1.35 g, 4.1 mmol, 60%) as a colourless solid.

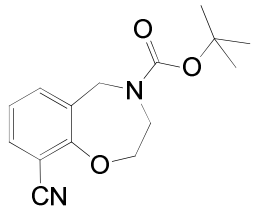
LCMS (method formate): Retention time 1.29 min, $[M+H]^+$ = 328.3 (1 Br).

^1H NMR (400 MHz, $CDCl_3$) δ ppm 7.47 (m, 1H), 7.12 (m, 1H), 6.90 (m, 1H), 4.42 (s, 2H), 4.09 (m, 2H), 3.83 (m, 2H), 1.42 (s, 9H).

HRMS calculated for $C_{14}H_{19}BrNO_3$: 328.0548, found: 328.0540.

Step 4

1,1-Dimethylethyl 9-cyano-2,3-dihydro-1,4-benzoxazepine-4(*5H*)-carboxylate (33).



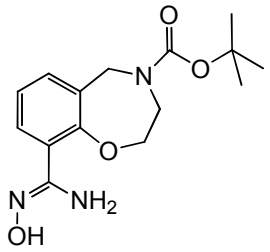
A solution of 1compound **50** (1.34 g, 4.9 mmol) in DMF (11 mL) was degassed under vacuum (3 mm Hg) for 15 min, then stirred under nitrogen. Zinc cyanide (575 mg, 4.9 mmol) and tetrakis(triphenylphospine)palladium(0) (472 mg, 0.4 mmol) were added and the resulting mixture was stirred at 100°C under nitrogen for 6 h, then cooled to room temperature and diluted with AcOEt (50 mL). The insoluble were filtered off and the filtrate was washed with brine (2x30 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel, eluting with 15-45% AcOEt/cyclohexane gave the title compound (980 mg, 83%) as a colourless solid.

LCMS (method formate): Retention time 1.10 min, $[M+H]^+$ = 275.3.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.50 (m, 1H), 7.40 (m, 1H), 7.09 (m, 1H), 4.43 (s, 2H), 4.22 (m, 2H), 3.84 (m, 2H), 1.42 (s, 9H).

Step 5

1,1-Dimethylethyl 9-[(Z)-(hydroxyamino)(imino)methyl]-2,3-dihydro-1,4-benzoxazepine-4(*5H*)-carboxylate (51).



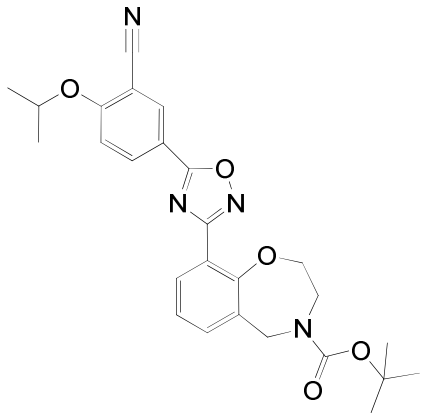
A mixture of compound **33** (2.57 g, 9.37 mmol), hydroxylamine hydrochloride (2.60 g, 37.5 mmol) and sodium bicarbonate (4.72 g, 56.2 mmol) in ethanol (40 mL) was heated at 60°C for 4 days. The mixture was then cooled to room temperature then was concentrated *in vacuo*. The residue was partitioned between water (50 mL) and AcOEt (50 mL) and the layers were separated. The aqueous phase was extracted twice with AcOEt (50 mL) and the combined organic phases were washed with brine (30 mL), dried over $MgSO_4$ and concentrated *in vacuo* to give the title compound (2.77 g, 96%) as a colourless solid.

LCMS (method formate): Retention time 0.66 min, $[M+H]^+$ = 308.03.

^{1}H NMR (400 MHz, DMSO d_6) δ ppm 9.39 (s, 1H), 7.29 (m, 1H), 7.25 (m, 1H), 7.03 (m, 1H), 5.62 (s, 2H), 4.40 (s, 2H), 4.02 (m, 2H), 3.70 (m, 2H), 1.35 (s, 9H).

Step 6

1,1-Dimethylethyl 9-(5-{3-cyano-4-[(1-methylethyl)oxy]phenyl}-1,2,4-oxadiazol-3-yl)-2,3-dihydro-1,4-benzoxazepine-4(*5H*)-carboxylate (52).



To a solution of compound **51** (8.05 g, 26.2 mmol) in toluene (50 mL) and pyridine (50 mL) at room temperature under nitrogen was slowly added 3-cyano-4-[(1-methylethyl)oxy]benzoyl chloride (compound **10**, step 5, 6.15 g, 27.5 mmol) in toluene (25 mL). The mixture was stirred for 30 min at room temperature then was refluxed for 3 h and then was cooled to room temperature and concentrated *in vacuo*. The residue was partitioned between AcOEt (300 mL) and HCl (2 N in water, 300 mL). The layers were separated and the organic phase was washed with brine (300 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue on Companion XL, using a 330 G column, 40% GLOBAL gradient (AcOEt in hexanes) gave the title compound (10.4 g, 21.8 mmol, 85%) a**s** a colourless solid.

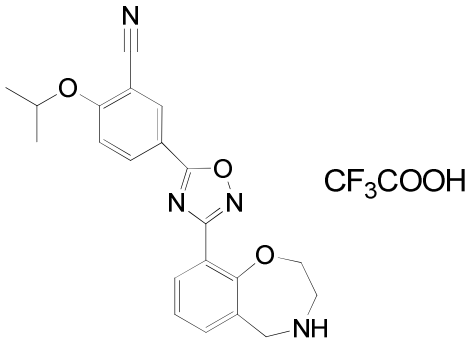
LCMS (method high pH): Retention time 1.46 min, $[M+H]^+$ = 477.23.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.43 (d, *J* = 2.3 Hz, 1H), 8.34 (dd, *J* = 9.0, 2.3 Hz, 1H), 7.93 (m, 1H), 7.45 (m, 1H), 7.20 (t, *J* = 7.6 Hz, 1H), 7.12 (d, *J* = 9.0 Hz, 1H), 4.79 (spt, *J* = 6.1 Hz, 1H), 4.57 (m, 2H), 4.18 (m, 2H), 3.88 (m, 2H), 1.48 (d, *J* = 6.1 Hz, 6H), 1.43 (s, 9H).

HRMS calculated for $C_{26}H_{29}N_4O_5$: 477.2138, found: 477.2133.

Step 7

2-[(1-Methylethyl)oxy]-5-[3-(2,3,4,5-tetrahydro-1,4-benzoxazepin-9-yl)-1,2,4-oxadiazol-5-yl]benzonitrile trifluoroacetate (53).



To a solution of compound **52** (12.5 g, 26.2 mmol) in DCM (100 mL) at 0°C under nitrogen was slowly added TFA (10 mL). The solution was allowed to warm to room temperature over 2 h, and then was concentrated *in vacuo*. The residue was azeotroped with toluene (2x100 mL) to give the title compound (13.6 g, 27.7 mmol, 106%) as a white solid which was used in the next step without further purification.

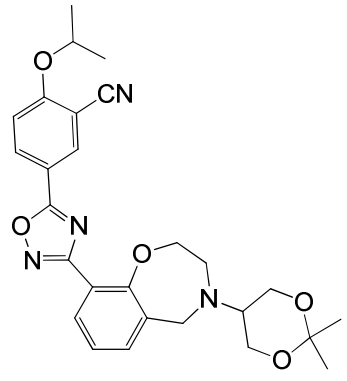
LCMS (method high pH): Retention time 1.13 min, $[M+H]^+$ = 377.2.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.33-9.21 (br s, 2H), 8.51 (d, *J* = 2.3 Hz, 1H), 8.40 (dd, *J* = 9.1, 2.3 Hz, 1H), 7.98 (dd, *J* = 7.7, 1.8 Hz, 1H), 7.71 (dd, *J* =7.7, 1.8 Hz, 1H), 7.57 (d, *J* = 9.1 Hz, 1H), 7.38 (t, *J* = 7.7 Hz, 1H), 4.99 (spt, *J* = 5.8 Hz, 1H), 4.53-4.41 (br s, 2H), 4.39 - 4.31 (br s, 2H), 4.27 - 4.31 (br s, 2H), 1.39 (d, *J* = 5.8 Hz, 6H).

HRMS calculated for $C_{21}H_{21}N_4O_3$: 377.1614, found: 377.1617.

Step 8

5-{3-[4-(2,2-Dimethyl-1,3-dioxan-5-yl)-2,3,4,5-tetrahydro-1,4-benzoxazepin-9-yl]-1,2,4-oxadiazol-5-yl}-2-[(1-methylethyl)oxy]benzonitrile (54).



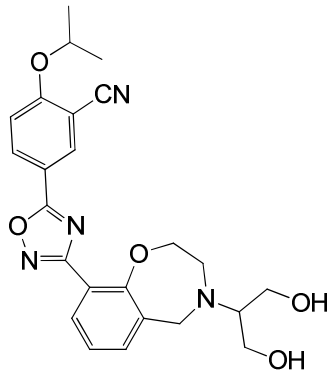
Ompound **53** (128 mg, 0.27 mmol), 2,2-dimethyl-1,3-dioxan-5-one (173 mg, 1.33 mmol) and acetic acid (0.018 mL, 0.32 mmol) were dissolved in DCM (2 mL). The resulting solution was stirred at room temperature for 15 min then was treated with sodium triacetoxyborohydride (282 mg, 1.33 mmol) and the resulting mixture was stirred at room temperature for 16 h. A saturated $NaHCO_3$ aqueous solution (10 mL) was added and the biphasic mixture was vigorously stirred for 10 min, then the layers were separated. The aqueous layer was extracted with DCM (3x10 mL). The combined organic phases were dried using a hydrophobic frit then were concentrated *in vacuo*. The crude product was dissolved in DMSO (1 mL) and methanol (1 mL) and purified by MDAP (method high pH) to give the title compound (60 mg, 0.12 mmol, 46%) as orange solid.

LCMS (method high pH): Retention time 0.99 min, $[M+H]^+$ = 491.23.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.43 (d, *J* = 2.3 Hz, 1H), 8.34 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.94 (dd, *J* = 7.7, 1.9 Hz, 1H), 7.36 (dd, *J* = 7.7, 1.9 Hz, 1H), 7.19 (t, *J* = 7.7 Hz, 1H), 7.12 (d, *J* = 8.8 Hz, 1H), 4.80 (spt, *J* = 6.1 Hz, 1H), 4.33-4.21 (m, 2H), 4.09 (s, 3H), 4.03 (dd, *J* = 12.0, 4.9 Hz, 2H), 3.92 (dd, *J* = 12.0, 7.6 Hz, 2H), 3.41-3.29 (m, 2H), 3.04-2.93 (m, 1 H) 1.48 (d, J = 6.1 Hz, 6H), 1.46 (s, 3H) 1.38 (s, 3H).

Step 9

5-(3-{4-[2-Hydroxy-1-(hydroxymethyl)ethyl]-2,3,4,5-tetrahydro-1,4-benzoxazepin-9-yl}-1,2,4-oxadiazol-5-yl)-2-[(1-methylethyl)oxy]benzonitrile (12).



Compound **54** (60 mg, 0.12 mmol) was dissolved in THF (2 mL) and the solution was treated at room temperature with HCl (2 N in THF, 0.6 mL, 1.2 mmol). The resulting mixture was stirred at this temperature for 1.5 h, and then most of the solvent was evaporated *in vacuo*. The residue was partitioned between a saturated $NaHCO_3$ aqueous solution (10 mL) and Et_2O (10 mL) and the layers were separated. The aqueous phase was extracted with Et_2O (2x10 mL) and the combined organic phases were dried over $MgSO_4$ and concentrated *in vacuo* to give the title compound (23.5 mg, 0.05 mmol, 40%) as a pale yellow solid.

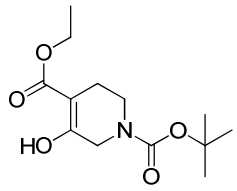
LCMS (method high pH): Retention time 0.81 min, $[M+H]^+ = 451.3$.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.43 (d, *J* = 2.3 Hz, 1H), 8.34 (dd, *J* = 8.9, 2.3 Hz, 1H), 7.92 (dd, *J* = 7.6, 1.8 Hz, 1H), 7.32 (dd, *J* = 7.6, 1.8 Hz, 1H), 7.16 (t, *J* = 7.6 Hz, 1H), 7.12 (d, *J* = 8.9 Hz, 1H), 4.79 (spt, *J* = 6.1 Hz, 1H), 4.22-4.15 (m, 2H), 4.03 (s, 2H), 3.79-3.67 (m, 4H), 3.37-3.25 (m, 2H), 3.03 (p, *J* = 6.1 Hz, 1H), 1.47 (d, *J* = 6.1 Hz, 6 H).

Compound 13

Step 1

1-*tert*-Butyl 4-ethyl 3-oxopiperidine-1,4-dicarboxylate (35).



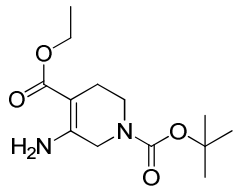
A solution of di-*tert*-butyl dicarbonate (23.57 g, 108 mmol) and triethylamine (14.6 mL, 105 mmol) in EtOH (300 mL) was added to a hydrogenation flask containing ethyl 3-oxo-1-(phenylmethyl)-4-piperidinecarboxylate hydrochloride salt **34** (29.8 g, 100 mmol, Sigma Aldrich) and palladium hydroxide (7.0 g, 20% by weight on carbon, 10 mmol). The resultant black suspension was evacuated and back filled with H_2 four times and then allowed to stir under an atmosphere of hydrogen for 20 h. The hydrogen atmosphere was removed under vacuum and backfilled with nitrogen. The suspension was filtered through a plug of celite. The celite was washed with MeOH (200 mL) and the filtrate was evaporated under reduced pressure to give pale yellow oil. The oil was diluted with CH_2Cl_2 (400 mL) and a saturated NH_4Cl aqueous solution (200 mL). The separated organic phase was washed with a saturated NH_4Cl aqueous solution (200 mL), and then the combined aqueous phases were extracted with CH_2Cl_2 (2×200 mL). The combined organic phases were passed through a hydrophobic frit and then evaporated under reduced pressure to give the title compound (27.1 g, 100%) as a pale orange oil.

LCMS (method formate): Retention time 1.23 min, $[M+H]^+ = 272.18$.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 12.10 (br s, 1H), 4.23 (q, *J* = 7.1 Hz, 2H), 4.04 (br s, 2H), 3.50 (t, *J* = 5.8 Hz, 2H), 2.35–2.32 (m, 2H), 1.48 (s, 9H), 1.32 (t, J = 7.1 Hz, 3H).

Step 2

1-*tert*-Butyl 4-ethyl 3-amino-5,6-dihydropyridine-1,4(*2H*)-dicarboxylate (36).



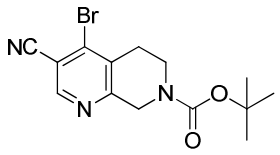
Ammonium acetate (38.5 g, 499 mmol) was added portionwise over 5 min to a stirred solution of compound **35** (27.1 g, 100 mmol) in EtOH (300 mL) at room temperature under nitrogen. The resultant suspension was stirred at room temperature for 20 min and then heated to 50°C for 1 h. The reaction was allowed to cool to room temperature and a further charge of ammonium acetate (19.25 g, 250 mmol) was added. The resultant yellow suspension was heated to 50°C for a further 1 h and then was cooled to room temperature and the solvent removed under reduced pressure. The resultant yellow solid was partitioned between CH_2Cl_2 (500 mL) and a saturated K_2CO_3 aqueous solution (500 mL). The separated organic phase was washed with a saturated K_2CO_3 aqueous solution (2×200 mL). The combined aqueous phases were then extracted with CH_2Cl_2 (2×200 mL) and the combined organic phases were passed through a hydrophobic frit and evaporated under reduced pressure to give the title compound (27.0 g, 100%) as a pale yellow solid.

LCMS (method high pH): Retention time 1.12 min, $[M+H]^+ = 271.3$.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.17 (q, *J* = 7.1 Hz. 2H), 4.00 (br s, 2H), 3.47 (t, *J* = 5.6 Hz, 2H), 2.36–2.33 (m, 2H), 1.47 (s, 9H), 1.28 (t, *J* = 7.1 Hz, 3H).

Step 3

***tert*-Butyl 4-bromo-3-cyano-5,6-dihydro-1,7-naphthyridine-7(*8H*)-carboxylate (37).**



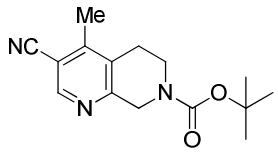
A solution of compound **36** (18.7 g, 69 mmol) and 3,3-dimethoxypropionitrile (13.54 g, 118 mmol) in THF (150 mL) was added dropwise over 10 min to a stirred suspension of potassium *tert*-butoxide (13.2 g, 118 mmol) in THF (100 mL) at 0°C under nitrogen. Following complete addition, the resultant suspension was stirred at 0°C for 5 min and then allowed to warm to room temperature over 16 h. The suspension was then heated to 70°C for 1 h and then cooled to 0°C. The reaction was quenched with HCl (4 N in 1,4-dioxane, 29.5 mL, 118.0 mmol) over 10 min. The solvent was evaporated under reduced pressure and then DMF (100 mL) and CH_2Cl_2 (100 mL) were added. The resultant dark suspension was cooled to 0°C and then PBr_3 (13.05 mL, 138.0 mmol) was added slowly over 5 min. The resulting suspension was stirred at 0°C for 30 min. Solid $NaHCO_3$ (~20 g) was added, followed by water (20 mL). When gas evolution had ceased, the suspension was diluted with CH_2Cl_2 (500 mL) and then passed through a pad of silica (10 cm × 10 cm × 10 cm) that had been pre-wetted with cyclohexane. The pad was washed with CH_2Cl_2 (1 L), the filtrate was passed through a hydrophobic frit and then evaporated under reduced pressure to give a black oil. The material was loaded in CH_2Cl_2 and purified by CombiFlash XL (750 g, silica) using a gradient of 0-20% AcOEt/cyclohexane. The appropriate fractions were combined and evaporated under reduced pressure to give the title compound (9.87 g, 42%) as a yellow solid.

LCMS (method high pH): Retention time 1.15 min, $[M-H]^-$ = 338.2 (1 Br).

^1H NMR (400 MHz, $CDCl_3$) carbamate rotamers observed δ ppm 8.67 (s, 0.4H), 8.61 (s, 0.6H), 4.74 (br s, 2H), 3.76–3.73 (m, 2H), 2.95–2.90 (m, 2H), 1.50 (s, 9H).

Step 4

***tert*-Butyl 3-cyano-4-methyl-5,6-dihydro-1,7-naphthyridine-7(*8H*)-carboxylate (38).**



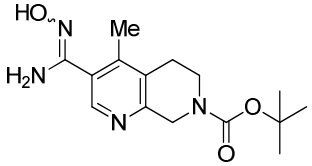
$PdCl_2$(dppf) (2.86 g, 3.90 mmol) was added to a stirred suspension of compound **37** (6.6 g, 19.5 mmol), potassium methyltrifluoroborate (9.5 g, 78 mmol) and Cs_2CO_3 (31.8 g, 98 mmol) in THF (200 mL) and H_2O (20 mL) at room temperature under nitrogen. The suspension was purged with nitrogen for 30 min and then heated to 65°C for 16 h. The suspension was allowed to cool to room temperature and then H_2O (200 mL) and AcOEt (200 mL) were added. The separated aqueous phase was extracted with AcOEt (2×200 mL) and the combined organic phases were passed through a hydrophobic frit and evaporated under reduced pressure to give a dark orange oil. The sample was loaded in DCM and purified by Biotage SP4 100 g silica using a gradient of 0-25% AcOEt/cyclohexane. The appropriate fractions were combined and evaporated under vacuum to give the title compound as a pale yellow solid (2.91 g, 55%).

LCMS (method high pH): Retention time 1.05 min, compound does not ionise.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.63 (s, 1H), 4.71 (s, 2H), 3.74 (t, *J* = 5.7 Hz, 2H), 2.80 (t, *J* = 5.3 Hz, 2H), 2.48 (s, 3H), 1.50 (s, 9H).

<u>Step 5</u>

***tert*-Butyl 3-(N'-hydroxycarbamimidoyl)-4-methyl-5,6-dihydro-1,7-naphthyridine-7(*8H*)-carboxylate (55).**



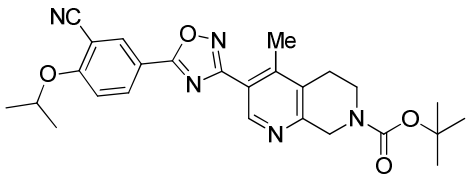
Compound **38** (2.63 g, 9.6 mmol), hydroxylamine hydrochloride (3.34 g, 48.1 mmol) and $NaHCO_3$ (4.04 g, 48.1 mmol) were mixed in EtOH (90 mL). The reaction was stirred at 90ºC under nitrogen for 16 h. The solid was removed by filtration whilst the reaction mixture was still hot, and washed on the filter with hot EtOH (60 mL). The filtrate was evaporated undervacuum to give the title compound (2.90 g, 98%) as a mixture of oxime isomers.

LCMS (method high pH): Retention time 0.76 min, $[M+H]^+ = 307.13$.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.48 (s, 0.6H), 8.31 (s, 0.4H), 8.20 (s, 0.6H), 7.89 (s, 0.4H), 7.56 (s, 0.4H), 6.90 (br s, 0.6H), 5.87 (s, 1H), 4.51 (s, 2H), 3.63–3.60 (m, 2H), 2.73–2.70 (m, 2H), 2.26 (s, 1H), 2.22 (s, 2H), 1.42 (s, 9H).

Step 6

***tert*-Butyl 3-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-4-methyl-5,6-dihydro-1,7-naphthyridine-7(*8H*)-carboxylate (56).**



To 3-cyano-4-[(1-methylethyl)oxy]benzoic acid (150 mg, 0.731 mmol, Biopharma Inc.) and HATU (417 mg, 1.1 mmol) in DMF (15 mL) was added DIPEA (0.45 mL, 2.6 mmol) and the reaction mixture stirred at room temperature for 5 min. Compound **55** (448 mg, 1.46 mmol) was added and the reaction mixture stirred at room temperature for 1.5 h. The reaction mixture was then heated at 100°C for 16 h under nitrogen then cooled to room temperature. The solvent was removed under reduced pressure, and the mixture partitioned between AcOEt (100 mL) and a saturated $NaHCO_3$ aqueous solution and the layers were separated. The organic

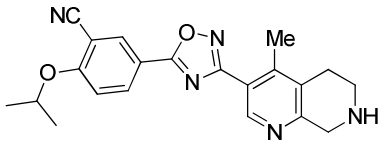
phase was washed with a saturated $NaHCO_3$ aqueous solution (2×100 mL). The aqueous layers were combined and re-extracted with AcOEt (100 mL). The combined organics phases were dried using a hydrophobic frit and evaporated under reduced pressure. The sample was dissolved in CH_2Cl_2, loaded onto a silica cartridge (100 g) and eluted using a gradient of 0-50% cyclohexane-EtOAc. The appropriate fractions were combined and evaporated under vacuum to give the title compound (305 mg, 88%) as an off-white foam.

LCMS (method formate): Retention time 1.38 min, $[M+H]^+ = 476.12$.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.83 (s, 1H), 8.52 (d, J = 2.5 Hz, 1H), 8.39 (dd, J = 9.0, 2.0 Hz, 1H), 7.56 (d, J = 9.0 Hz, 1H), 4.97 (spt, J = 6.0 Hz, 1H) 4.59 (s, 2H), 3.66 (t, J = 5.5 Hz, 2H), 2.83 (t, J = 5.5 Hz, 2H), 2.49 (s, 3H), 1.44 (s, 9H), 1.39 (d, J = 6.0 Hz, 6H).

Step 7

2-Isopropoxy-5-(3-(4-methyl-5,6,7,8-tetrahydro-1,7-naphthyridin-3-yl)-1,2,4-oxadiazol-5-yl)benzonitrile (57).



To compound **56** (570 mg, 1.2 mmol) in CH_2Cl_2 (18 mL) was added trifluoroacetic acid (2 mL) and the reaction mixture stirred at room temperature for 2.5 h. The reaction mixture was then evaporated under reduced pressure, and the residue purified using aminopropyl SPE cartridge (20 g), loaded in CH_2Cl_2 and eluted using 10% MeOH in CH_2Cl_2. The appropriate fractions were combined and evaporated under reduced pressure to give the title compound (555 mg, 123%) as a brown gum which was used in the next step without further purification.

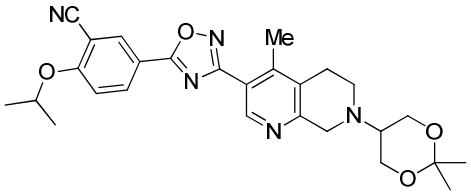
LCMS (method formate): Retention time 0.82 min, $[M+H]^+ = 376.09$.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.77 (s, 1H), 8.52 (d, J = 2.0 Hz, 1H), 8.40 (dd, J = 8.8,

2.0 Hz, 1H), 7.57 (d, *J* = 8.8 Hz, 1H), 4.98 (spt, *J* = 6.0 Hz, 1H), 3.92 (s, 2H), 3.17 (d, *J* = 6.0 Hz, 6H), 3.02 (t, *J* = 5.8 Hz, 2H), 2.71 (t, *J* = 5.8 Hz, 2H), 2.47 (s, 3H).

Step 8

5-(3-(7-(2,2-Dimethyl-1,3-dioxan-5-yl)-4-methyl-5,6,7,8-tetrahydro-1,7-naphthyridin-3-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxybenzonitrile (58).



Compound **57** (280 mg, 0.75 mmol) and 2,2-dimethyl-1,3-dioxan-5-one (582 mg, 4.47 mmol) were stirred in CH_2Cl_2 (4 mL) for 15 min at room temperature. Sodium triacetoxyborohydride (948 mg, 4.47 mmol) was added and the reaction mixture stirred at room temperature for 16 h. The reaction mixture was quenched with a saturated $NaHCO_3$ aqueous solution (50 mL) and extracted with CH_2Cl_2 (3×50 mL). The combined organic phases were dried using a hydrophobic frit and evaporated under a stream of nitrogen. The sample was dissolved in CH_2Cl_2 and loaded onto a silica cartridge (25 g) and the cartridge eluted using a gradient of 0-100% cyclohexane-EtOAc. This was followed by elution using a gradient of 0-10% CH_2Cl_2-MeOH. The appropriate fractions were combined and evaporated under vacuum to give the title compound (249 mg, 68%).

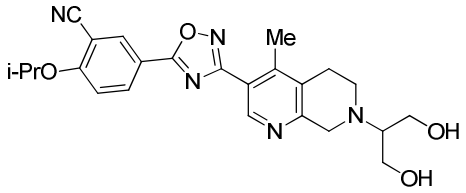
LCMS (method formate): Retention time 0.90 min, $[M+H]^+$ = 490.08.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.79 (s, 1H), 8.51 (d, *J* = 2.0 Hz, 1H), 8.39 (dd, *J* = 8.8, 2.0 Hz, 1H), 7.56 (d, *J* = 8.8 Hz, 1H), 4.97 (spt, *J* = 6.0 Hz, 1H), 3.99 (dd, J = 11.5, 5.0 Hz,

2H), 3.85–3.81 (m, 4H), 2.91 (t, *J* = 5.5 Hz, 2H), 2.80–2.71 (m, 3H), 2.47 (s, 3H), 1.39 (d, J = 6.0 Hz, 6H), 1.37 (s, 3H), 1.32 (s, 3H).

Step 9

5-(3-(7-(1,3-Dihydroxypropan-2-yl)-4-methyl-5,6,7,8-tetrahydro-1,7-naphthyridin-3-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxybenzonitrile hydrochloride (13).



To 5-(3-(7-(2,2-Dimethyl-1,3-dioxan-5-yl)-4-methyl-5,6,7,8-tetrahydro-1,7-naphthyridin-3-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxybenzonitrile (246 mg, 0.50 mmol) in THF (2 mL) was added HCl (2 N in water, 2 mL, 4 mmol) and the reaction mixture was stirred at room temperature for 1.5 h. The reaction mixture was blown down under a stream of nitrogen and then triturated with Et_2O to give 5-(3-(7-(1,3-dihydroxypropan-2-yl)-4-methyl-5,6,7,8-tetrahydro-1,7-naphthyridin-3-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxybenzonitrile hydrochloride (249 mg, 102%).

LCMS (method formate): Retention time 0.80 min, $[M+H]^+$ = 450.06.

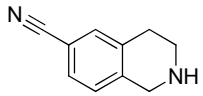
^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.79 (br s, 1H), 8.92 (s, 1H), 8.54 (d, *J* = 2.5 Hz, 1H), 8.41 (dd, *J* = 9.0, 2.5 Hz, 1H), 7.58 (d, *J* = 9.0 Hz, 1H), 4.99 (spt, J = 6.0 Hz, 1H), 4.86–4.80 (m, 1H), 4.56–4.52 (m, 1H), 4.00–3.92 (m, 5H), 3.62–3.51 (m, 2H), 3.27–3.14 (m, 2H), 2.54 (s, 3H), 1.39 (d, *J* = 6.0 Hz, 6H).

HRMS calculated for $C_{24}H_{28}N_5O_4$: 450.2141, found: 450.2144.

Compound 14

Step 1

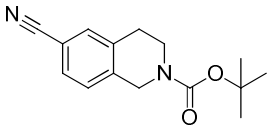
1,2,3,4-Tetrahydroisoquinoline-6-carbonitrile (27).



A mixture of 6-bromo-1,2,3,4-tetrahydroisoquinoline **26** (2.86 g, 11.5 mmol, Allichem LLC), zinc cyanide (1.76 g, 15.0 mmol) and tetrakis(triphenylphosphine)palladium(0) (1.33 g, 1.15 mmol) in N,N-dimethylformamide (DMF) (20 mL) which was split in two was placed in two 25 mL microwave vials and each mixture was heated in the microwave for 60 min at 130 °C then cooled to room temperature. The mixtures were combined and concentrated *in vacuo*. Purification of the residue by SP4 (gradient: 5 to 10% (2 M NH_3 in MeOH) in DCM) using a 40 G column gave the title compound (1.41 g, 77%) as a colourless solid.
LCMS (method formate): Retention time 0.36 min, $[M+H]^+$ = 159.16.
^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.63 (br s, 1H), 7.48 (d, *J* = 2.0 Hz, 1H), 7.44 (dd, *J* = 8.2, 2.0 Hz, 1H), 7.20 (d, *J* = 8.2 Hz, 1H), 4.20 (s, 2H), 3.33 (t, *J* = 6.2 Hz, 2H), 3.01 (t, *J* = 6.19 Hz, 2H).

Step 2

Tert-butyl 6-cyano-3,4-dihydroisoquinoline-2(*1H*)-carboxylate (28).



Di-tert-butyl dicarbonate (2.229 mL, 9.60 mmol) was added to a mixture of compound **27** (1.38 g, 8.73 mmol) and triethylamine (3.65 mL, 26.2 mmol) in dichloromethane (DCM) (25 mL) and the resulting mixture was stirred at room temperature for 60 min. The solution was then washed with water (25 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the

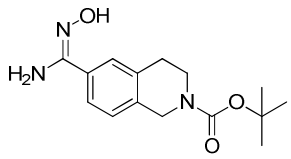
residue by SP4 (gradient: 10 to 50% AcOEt in cyclohexane) using a 40 G column gave the title compound (2.17 g, 96%) as a colourless solid.

LCMS (method high pH): Retention time 1.17 min, $[M+H]^+$ = 259.28.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.47 (d, *J* = 7.8 Hz, 1H), 7.45 (br s, 1H), 7.21 (d, *J* = 7.8 Hz, 1H), 4.62 (s, 2H), 3.66 (t, *J* = 5.7 Hz, 2H), 2.86 (t, *J* = 5.7 Hz, 2H), 1.51 (s, 9H).

Step 3

Tert-butyl 6-(N'-hydroxycarbamimidoyl)-3,4-dihydroisoquinoline-2(*1H*)-carboxylate (59).



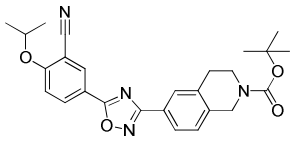
Hydroxylamine hydrochloride (3.50 g, 50.4 mmol) was added to a suspension of compound **28** (2.17 g, 8.40 mmol) and sodium bicarbonate (4.23 g, 50 mmol) in ethanol (100 mL) and the resulting mixture was stirred at 80°C for 4 h then was cooled to room temperature. The insoluble material was filtered off and the filtrate was concentrated *in vacuo* to give the title compound (2.78 g, 98%) as colourless foam which was used in the next step without further purification.

LCMS (method high pH): Retention time 0.70 min, $[M+H]^+$ = 292.30.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.54 (br s, 2H), 8.16 (br s, 1H), 7.48 (d, *J* = 7.8 Hz, 1H), 7.47 (s, 1H), 7.15 (d, *J* = 7.8 Hz, 1H), 4.49 (br s, 2H), 3.55 (t, *J* = 5.8 Hz, 2H), 2.78 (t, *J* = 5.8 Hz, 2H), 1.43 (s, 9H).

Step 4

Tert-butyl 6-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-3,4-dihydroisoquinoline-2(*1H*)-carboxylate (60).



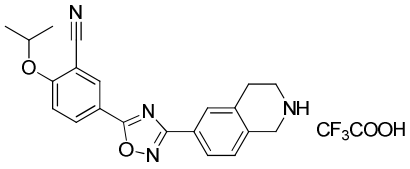
Compound **22** (1.62 g, 7.25 mmol) was added portionwise to a solution of compound **59** (2.01 g, 6.9 mmol) in toluene (20 mL) and pyridine (20 mL) at room temperature under nitrogen and the resulting mixture was stirred for 20 min at room temperature then heated at 120°C for 2 h, then cooled to room temperature. The solvent was evaporated *in vacuo* and the residue partitioned between water (15 mL) and AcOEt (15 mL) and the layers were separated. The aqueous layer was extracted twice with AcOEt (15 mL) and the combined organic phases were washed with brine (20 mL) then dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue via SP4 (gradient: 0 to 50% AcOEt in c-hexane) on a 25 G column gave the title compound (2.21 g, 69.5%) as a colourless foam.

LCMS (method high pH): Retention time 1.52 min, $[M+H]^+$ = 461.33.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.43 (d, *J* = 2.1 Hz, 1H), 8.34 (dd, *J* = 9.0, 2.1 Hz, 1H), 7.98 - 7.92 (m, 2H), 7.26 (d, *J* = 8.8 Hz, 1H), 7.12 (d, *J* = 9.0 Hz, 1H), 4.80 (spt, *J* = 6.1 Hz, 1H), 4.65 (s, 2H), 3.70 (t, *J* = 5.4 Hz, 2H), 2.94 (t, *J* = 5.4 Hz, 2H), 1.51 (s, 9H), 1.48 (d, *J* = 6.1 Hz, 6H).

Step 5

2-Isopropoxy-5-(3-(1,2,3,4-tetrahydroisoquinolin-6-yl)-1,2,4-oxadiazol-5-yl)benzonitrile trifluoroacetate (61).



A solution of compound **60** (2.21 g, 4.8 mmol) in dichloromethane (20 mL) at 0°C was treated dropwise with trifluoroacetic acid (1.85 mL, 24.0 mmol), and the resulting mixture was allowed to warm to room temperature and stirred for 16 h then was concentrated *in vacuo*. Trituration of the residue with Et_2O (2x15 mL) gave the title compound (2.2 g , 95%) as a colourless solid.

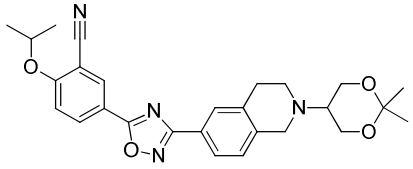
LCMS (method high pH): Retention time 0.88 min, $[M+H]^+$ = 361.25.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.17 (br s, 2H), 8.51 (d, *J* = 2.3 Hz, 1H), 8.41 (dd, *J* = 9.2, 2.3 Hz, 1H), 7.98 (s, 1H), 7.97 (d, *J* = 7.8 Hz, 1H), 7.57 (d, *J* = 9.2 Hz, 1H), 7.46 (d, *J* = 7.8 Hz, 1H), 4.99 (spt, *J* = 6.1 Hz, 1H), 4.39 (br s, 2H), 3.46 (t, *J* = 6.1 Hz, 2H), 3.14 (t, *J* = 6.1 Hz, 2H), 1.39 (d, *J* = 6.1 Hz, 6H).

HRMS calculated for $C_{21}H_{21}N_4O_2$: 361.1665, found: 361.1661.

Step 6

5-(3-(2-(2,2-Dimethyl-1,3-dioxan-5-yl)-1,2,3,4-tetrahydroisoquinolin-6-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxybenzonitrile (62).

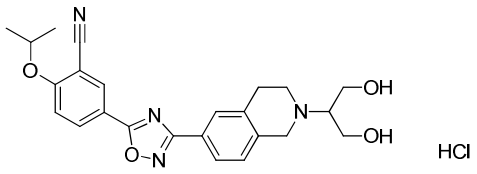


A stirred suspension of compound **61** (0.252 g, 0.531 mmol) in dichloromethane (DCM) (8 mL) under nitrogen was treated with 2,2-dimethyl-1,3-dioxan-5-one (0.19 mL, 1.59 mmol) followed by sodium triacetoxyborohydride (0.51 g, 2.39 mmol). The resulting mixture was stirred under nitrogen at room temperature for 19 h then was treated with a saturated $NaHCO_3$ aqueous solution (5 mL). The biphasic mixture was vigorously stirred for 15 min then the layers were separated. The aqueous phase was extracted with DCM (3x5 mL) and the combined organic phases were dried by filtering through a cartridge fitted with a hydrophobic frit, and the solvent was evaporated under a stream of nitrogen to give the title compound (233 mg, 0.5 mmol, 92%) as a cream solid which was used in the subsequent reaction without further purification.

LCMS (method high pH): Retention time 0.99 min, $[M+H]^+$ = 475.29.

Step 7

5-(3-(2-(1,3-Dihydroxypropan-2-yl)-1,2,3,4-tetrahydroisoquinolin-6-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxybenzonitrile hydrochloride (14).



A stirred solution of crude compound **62** (230 mg, 0.48 mmol) in tetrahydrofuran (THF) (6 mL) was treated with HCl (2 N in water, 6 mL, 18 mmol) and the resulting mixture was stirred at room temperature for 80 min. The volatiles were evaporated under a stream of nitrogen and the residue was partitioned between a saturated $NaHCO_3$ aqueous solution (5 mL) and AcOEt (5 mL). The layers were separated and the aqueous phase extracted with AcOEt (3x4 mL) and DCM (4x4 mL). The combined organic phases were dried by filtering through a cartridge fitted with a hydrophobic frit and then were evaporated to dryness under a stream of nitrogen. The residue was purified by MDAP (method high pH). The required fractions were evaporated *in vacuo* to give 5-(3-{2-[2-hydroxy-1-(hydroxymethyl)ethyl]-1,2,3,4-tetrahydro-6-isoquinolinyl}-1,2,4-oxadiazol-5-yl)-2-[(1-methylethyl)oxy]benzonitrile (63.2 mg, 0.145 mmol, 30 %) as a white solid.

This material was dissolved in methanol (2 mL) and the resulting solution was treated with HCl (1 N in Et_2O, 0.15 mL, 0.15 mmol). The mixture was allowed to stand for 5 min at room temperature before being evaporated under a stream of nitrogen and then dried *in vacuo* to give the title compound (58 mg, 0.12 mmol, 85%) as a white amorphous solid.

LCMS (method high pH): Retention time 0.86 min, $[M+H]^+$ = 435.32.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.53-10.40 (br s, 1H), 8.50 (d, *J* = 2.2 Hz, 1H), 8.40 (dd, *J* = 9.2, 2.2 Hz, 1H), 7.97 (s, 1H), 7.95 (d, *J* = 7.8 Hz, 1H), 7.56 (d, *J* = 9.2 Hz, 1H), 7.45 (d, *J* = 7.8 Hz, 1H), 5.51 (br s, 2H), 4.98 (spt, *J* = 6.1 Hz, 1H), 4.82-4.69 (m, 1H), 4.66-4.56 (m, 1H), 4.01-3.90 (m, 5H), 3.62-3.50 (m, 2H), 3.28-3.19 (m, 1H), 3.18-3.09 (m, 1H), 1.39 (d, *J* = 6.1 Hz, 6H).

HRMS calculated for $C_{24}H_{27}N_4O_4$: 435.2032, found: 435.2036.

In-vitro assays

Membrane preparation for $S1P_1$ GTPγS assay

All steps were performed at 4°C. Cells were homogenised within a glass Waring blender for 2 bursts of 15 secs in 200 mL of buffer (50 mM HEPES, 1 mM leupeptin, 25 μg/mL bacitracin, 1 mM EDTA, 1 mM PMSF, 2 μM pepstatin A). The blender was plunged into ice for 5 mins after the first burst and 10-40 mins after the final burst to allow foam to dissipate. The material was then spun at 500 g for 20 mins and the supernatant spun for 36 mins at 48,000 g. The pellet was resuspended in the same buffer as above but without PMSF and pepstatin A. The material was then forced through a 0.6mm needle, made up to the required volume, (usually x4 the volume of the original cell pellet), aliquoted and stored frozen at –80°C.

$S1P_1$ GTPγS assay

$S1P_1$ expressing RH7777 membranes (1.5 μg/well) membranes (1.5 μg/well) were homogenised by passing through a 23G needle. These were then adhered to WGA-coated SPA beads (0.125 mg/well) in assay buffer (HEPES 20 mM, $MgCl_2$ 10 mM, NaCl 100 mM and pH adjusted to 7.4 using KOH 5M). GDP 10 μM FAC and saponin 90 μg/mL FAC were also added.

After 30 minutes precoupling on ice, the bead and membrane suspension was dispensed into white Greiner polypropylene LV 384-well plates (5 μl/well), containing 0.1 μl of compound. 5 μl/well [^{35}S]-GTPγS (0.5 nM for $S1P_1$ or 0.3 nM for $S1P_3$ final radioligand concentration) made in assay buffer was then added to the plates. The final assay cocktail (10.1 μl) was then sealed, spun on a centrifuge, then read immediately on a Viewlux instrument.

Membrane preparation for $S1P_3$ GTPγS assay

All steps were performed at 4°C. Cells were homogenised within a glass Waring blender for 2 bursts of 15 secs in 200 mL of buffer (50 mM HEPES, 1 mM leupeptin, 25 μg/mL bacitracin, 1 mM EDTA, 1 mM PMSF, 2 μM pepstatin A). The blender was plunged into ice for 5 mins after the first burst and 10-40 mins after the final burst to allow foam to dissipate. The material was then spun at 500 g for 20 mins and the supernatant spun for 3 mins at 48,000 g. The

resultant pellet was resuspended in the same buffer without PMSF and pepstatin A but containing 10% w/v sucrose. The membrane suspension was then layered on top of buffer without PMSF and pepstatin A containing 40% w/v sucrose and spun at 100,000 g for 60 mins. The cloudy interface between the 2 sucrose layers was removed and resuspended in buffer without PMSF and pepstatin A. The material was spun at 48,000 g for 45 mins. The resultant cell pellet was resuspended in the required volume in buffer without PMSF and pepstatin A, (usually x4 the volume of the original cell pellet), aliquoted and stored frozen at –80°C.

$S1P_3$ GTPγS assay

$S1P_3$ expressing RBL membranes (0.44 μg/well) purified through a sucrose gradient were homogenised by passing through a 23G needle. These were then adhered to WGA-coated SPA beads (GE Healthcare 0.5 mg/well) in assay buffer (HEPES 20 mM, $MgCl_2$ 10 mM, NaCl 100 mM and pH adjusted to 7.4 using KOH 5M). 2 μg/well of Saponin was added.

After 30 minutes precoupling on ice, 5 μM GDP final assay concentration was added to the bead and membrane suspension. The bead, membrane, Saponin and GDP suspension was mixed with [^{35}S]-GTPγS (Perkin Elmer, 0.3 nM final radioligand concentration) made in assay buffer (HEPES 20 mM, $MgCl_2$ 10 mM, NaCl 100 mM and pH adjusted to 7.4 using KOH 5M). The bead, membrane and radioligand suspension was dispensed into white Greiner polypropylene 384-well plates (45 μl/well), containing 0.5 μl of a solution of test compound in 100% DMSO. The final assay cocktail (45.5 μl) was then sealed, spun on a centrifuge, then read on a Viewlux instrument following a 3 hour incubation of plates at room temperature.

$S1P_1$ β-Arrestin recruitment assay

β-Arrestin recruitment assays were carried out using the PathHunter CHO-K1 EDG1 β-Arrestin cell line (DiscoveRx Corporation) in a chemi-luminescence detection assay. This cell line stably expresses β-Arrestin 2 and $S1P_1$ fused to complementing portions of β-galactosidase ('EA' and 'pro-link', respectively) which associate upon Arrestin recruitment to form functional β-galactosidase enzyme.

Cells were grown to 80% confluency in Growth Medium (F12 nutrient HAMS supplemented with 10% heat-inactivated USA FBS, 1% L-glutamax, 800 μg/mL Geneticin and 300 μg/mL Hygromycin). Cells were harvested from the flask using Enzyme Free Cell Dissociation Buffer (Gibco) and washed from flasks with Optimem solution (Gibco). Cells were then centrifuged at

1000 rpm for 2-3 min and resuspended in Assay Buffer (Prepared from Sigma kit H1387 supplemented with 20 mL/L HEPES, 4.7 mL/L $NaHCO_3$, 0.1% pluronic acid F-68 solution, 0.1% BSA and adjusted to pH 7.4 using sodium hydroxide at $1x10^6$ cells/mL. Cells were dispensed into assay plates containing compounds (100nl/well of a solution of test compound in 100% DMSO) at $1x10^4$ cells/well and incubated at 37°C/5% CO_2 for 90 min followed by 15 min at room temperature. 5 μL detection mix (1 part Galacton Star, 5 parts Emerald II, 19 parts Assay Buffer; DiscoveRx) were added per well and the plates incubated at room temperature for 60 min. Luminescence was quantified using a Viewlux plate reader.

$S1P_2$ yeast reporter assay

$S1P_2$ assays were performed using a yeast reporter system as described in Dowell, S. J.; Brown A. J. Yeast assays for G protein-coupled receptors. *Methods in Molecular Biology* (Totowa, NJ, United States), **2009**, *552* (G Protein-Coupled Receptors in Drug Discovery), 213-229. An expression construct containing $S1P_2$ under control of the *GPD* promoter was integrated at the *ura3* locus of *S. cerevisiae* strain MMY24 (Brown A. J.; Goldsworthy, S. M.; Barnes, A. A.; Eilert, M. M.; Tcheang, L.; Daniels, D.; Muir, A. I.; Wigglesworth, M. J.; Kinghorn, I.; Fraser, N. J.; Pike, N. B.; Strum, J. C.; Steplewski, K. M.; Murdock, P. R.; Holder, J. C.; Marshall, F. H.; Szekeres, P. G.; Wilson, S.; Ignar, D. M.; Foord, S. M.; Wise, A.; Dowell, S. J. The Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids *J. Bio. Chem*. **2003**, *278*, 11312-11319). Cells were seeded at 0.02 OD_{600} in minimal medium lacking histidine and supplemented with 5mM 3-amino triazole (Sigma, Poole, UK) and 100μM fluorescein-di-(β-D-glucopyranoside) (FDGlu; Invitrogen Molecular Probes). Agonist-mediated growth was measured by conversion of FDGlu to fluorescein as described in Dowell SJ and Brown AJ (2009) using an Analyst HT plate reader (LJL BioSystems, Sunnyvale, CA; ex. 485nm/em. 535nm) after 24h incubation at 30°C.

$S1P_4$ and $S1P_5$ Aequorin calcium accumulation assays

Aequorin assays were performed using CHO-K1 $S1P_4$ and $S1P_5$ aequorin cell lines purchased from Euroscreen SA (Gosselies, Belgium) and Perkin Elmer (Waltham, MA USA) respectively. In the presence of the cofactor coelenterazine, calcium released on receptor activation results in a conformational change of apo-aequorin and an oxidative decarboxylation reaction producing coelenteramide and a flash luminescence signal at 469 nm.

Luminescence was measured in a Lumilux (Perkin Elmer) reader containing internal liquid handling capacity. Frozen cells were revived into DMEM F12 medium containing 10% charcoal-stripped FBS, pre-warmed to 37°C, and all traces of freezing media were removed by centrifugation. Pelleted cells were resuspended in 50 mL of 37°C medium and allowed to recover for 30 min at 37°C. Following recovery, cells were harvested and re-suspended at $2.5x10^6$ cells/mL in loading buffer (prepared from Sigma kit H1387, supplemented with 20 mM HEPES, 4.16 mM $NaHCO_3$, 0.1% pluronic acid F-68 solution and 0.1% BSA) containing 5 µM coelenterazine. Cells were protected from light exposure and incubated at room temperature for 18-24h with gentle rotation. After loading, cells were diluted to $5x10^5$ cells/mL with assay buffer and placed, stirring, in the Lumilux. Instrument automation added 20 µL/well of diluent buffer followed by 20 µL/well of cells ($1x10^5$ cells/well) to compound plates, and agonist luminescent responses were recorded immediately.

S1P4 cat # ES-592-A
S1P5 cat # ES-593-A
DMEM F12 cat# either Sigma D6421 or Invitrogen 11320
Charcoal stripped FBS cat # Hyclone SH30068.03
Base buffer: prepared from Sigma kit H1387, supplemented with 20mM HEPES (Sigma H0887) and 4.16mM $NaHCO_3$ (Sigma S8761) and pH'd to 7.4
Loading buffer: Base buffer supplemented with 0.1% pluronic acid F-68 solution (Gibco/Invitrogen 24040-032) and 0.1% BSA (Calbiochem 126609)
Assay buffer/diluent buffer: base buffer supplemented with 0.1% pluronic acid F-68 solution (Gibco/Invitrogen 24040-032)
Coelenterazine cat # Invitrogen C6780 or Biotium BT10110-1

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Correlations

Figure 7: Correlation between Vss and ACD cLogP

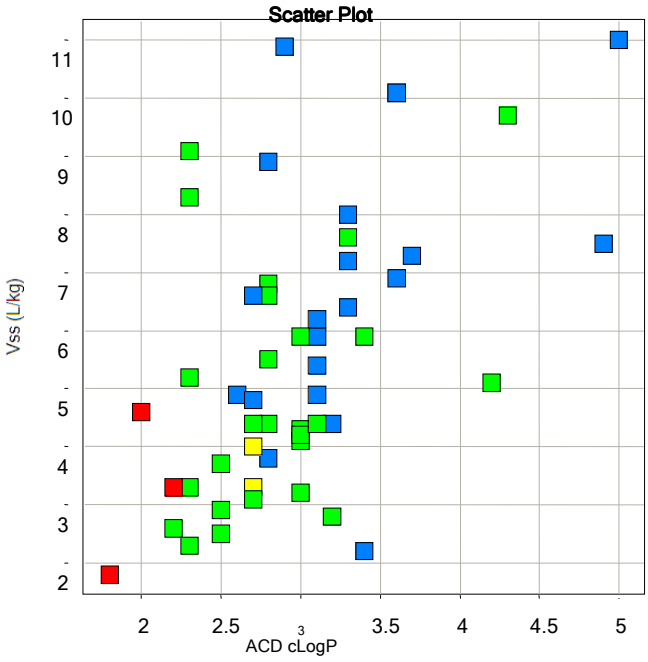


Figure 7: Correlation between Vss and PSA

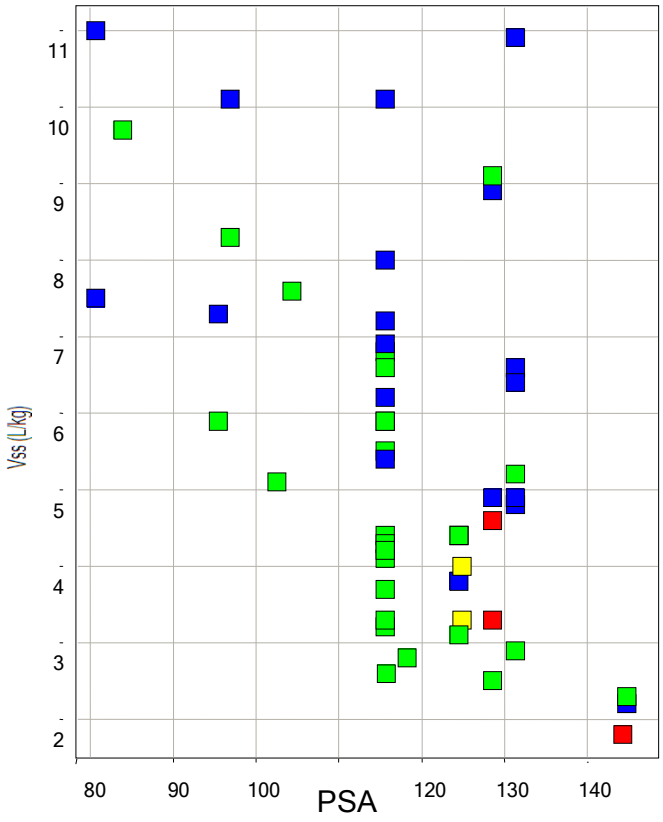
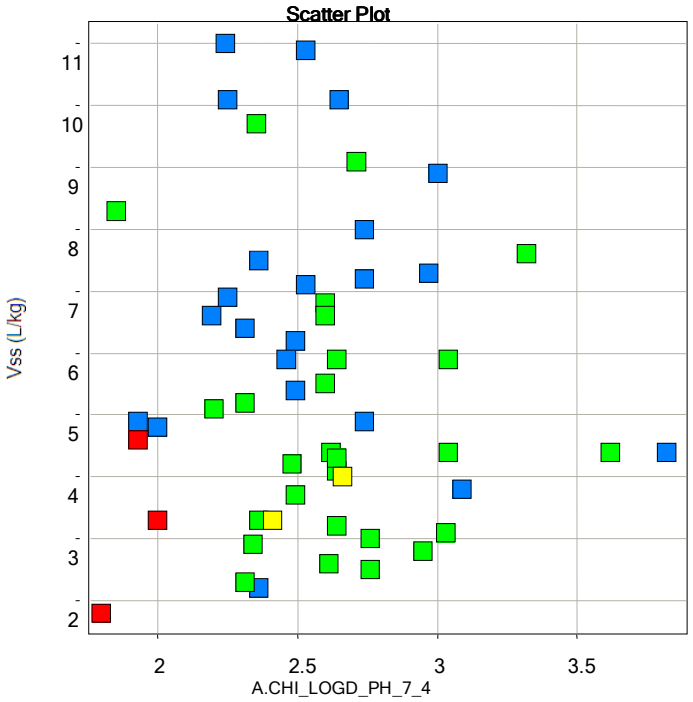


Figure 8: Correlation between Vss and CHI LogD @ pH 7.4



Rat Lymphocyte depletion study

All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals.

Rat Lymphocyte Reduction studies

Male Lewis rats (300-350g, supplied by Charles River UK Ltd.) had pre-dose blood samples (200µl) removed by direct venepuncture the day prior to oral dosing. On the study day, rats received either vehicle (1% methycellulose 4ml/kg p.o.) or compound **15** (0.1 – 3 mg/kg p.o.) and had further blood samples taken at 0.25, 0.5, 1, 2, 4, 7, 12, 24, 30, 36, 48 and 54 h post-dose. From each blood sample 50 µl was mixed with 50 µl of water for pharmacokinetic analysis. The remainder of the blood samples were analysed using the Sysmex XT2000iV automatic haematology analyser for lymphocyte counts. At the completion of the study rats were euthanized using a Schedule 1 method.

DMPK studies

All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals. For all studies, the temperature and humidity were nominally maintained at 21°C ± 2°C and 55% ± 10%, respectively.
For intravenous administration in the PK studies, test compounds were formulated in DMSO and 10% (w/v) Kleptose™ in saline (5%:95%) at a concentration of 0.2 mg/mL. The pH of the dose solution was adjusted to 8.0 using 0.1 mM NaOH, and the dose was filtered using a *ca.*0.2 μm syringe filter unit. Test compounds were administered as a 1 h iv infusion at 5 mL/kg/h to achieve a target dose of 1mg/kg. There were no known contaminants in the diet or water at concentrations that could interfere with the outcome of the studies.

Rat PK studies

Male CD rats (258-284g, supplied by Charles River UK Ltd.) were surgically prepared at GSK with implanted cannulae in the femoral vein (for drug administration) and jugular vein (for blood sampling). Each rat received Duphacillin (100 mg/kg s.c.) and Carprofen (7.5 mg/kg s.c.) as a pre-operative antibiotic and analgesic respectively. Each rat was allowed to recover for at least 2 days prior to dosing. Rats had free access to food and water throughout. Rat PK studies were conducted as a crossover design over 2 dosing occasions, with 3 days between dose administrations. On study day 1, n=3 male rats each received a 1 h intravenous infusion of the test compound. On study day 2, the same three rats each received an oral administration of test compound suspended in 1% (w/v) methylcellulose *aq*. at a concentration of 0.6 mg/mL administered by gavage at 5 mL/kg to achieve a target dose of 3 mg/kg. At the end of the study the rats were euthanised by administration of sodium pentobarbital (Euthatal™) through the jugular vein cannula.

Rat CNS Penetration studies

Male CD rats (273-286g, supplied by Charles River UK Ltd.) were surgically prepared at GSK with implanted cannulae for drug administration and sampling as described for Rat PK studies.

N=3 rats each received an intravenous bolus loading dose (0.38 mg/kg) followed by a 7 h constant rate infusion (0.8 mg/kg/h) where a steady state blood concentration of approximately 400 ng/mL was obtained. Blood samples were removed for concentration analysis at intervals between 5 and 7 h where attainment of steady state blood concentration was confirmed. At the 7 h timepoint rats were euthanised by administration of sodium pentobarbital (Euthatal^{TM}) and the brain removed for tissue concentration analysis.

Dog PK studies

One healthy, laboratory-bred, male Beagle dog (13.5 kg, supplied by Harlan Laboratories, UK) was fasted overnight prior to each dose administration and fed approximately 4 h after the start of dosing. The dog had free access to water throughout. The study was conducted as a crossover design on two dosing occasions with 6 days between dose administrations. On study day 1 the dog received a 1 h intravenous infusion of test compound via cannulation of the saphenous vein. On study day 7 the same dog received an oral administration of test compound suspended in 1% (w/v) methylcellulose *aq*. at a concentration of 0.5 mg/mL and administered by gavage at 2 mL/kg to achieve a target dose of 1 mg/kg. A temporary cannula was inserted into the cephalic vein from which serial blood samples (*ca.* 600 μL) were collected from predose up to 2 hours after the start of dosing. After 2 hr samples were taken via direct venepuncture of the jugular vein. At the end of each study the dogs were returned to the colony.

Blood Sample Analysis

Diluted blood samples (1:1 with water) were extracted using protein precipitation with acetonitrile containing an analytical internal standard. An aliquot of the supernatant was analysed by reverse phase LC-MS/MS using a heat assisted electrospray interface in positive ion mode. Samples were assayed against calibration standards prepared in control blood.

Brain Tissue Analysis

Brain tissue was homogenised with water and subsequently extracted using acetonitrile. Following centrifugation an aliquot of the supernatant was analysed by reverse phase LC-MS/MS using a heat assisted electrospray interface in positive ion mode. Samples were assayed against calibration standards prepared using control brain tissue. Brain concentrations

were corrected for residual blood concentration assuming 15 μL blood is present in 1 g of brain.

PK Data Analysis from PK studies

PK parameters were obtained from the blood concentration-time profiles using non-compartmental analysis with WinNonlin Professional 4.1a (Pharsight, Mountain View, CA).

^{1}H and ^{13}C NMRs of compound 15