Chemistry Materials and Methods

Starting materials and reagents were purchased from commercial suppliers and used without further purification. Thin-layer chromatography (TLC) was performed on silica gel 60 F254 (Merck), and column chromatography was carried out on pre-loaded BiotageR SNAP columns with BiotageR KP-Sil. Evaporation was done *in vacuo* on a Buchi rotary evaporator. Celite 545R was used during palladium filtrations. Reaction temperatures refer to the temperature of the cooling/heating bath unless otherwise stated. Proton nuclear magnetic resonance (1H NMR) spectra were recorded on a Varian Mercury Vx-400 equipped with a 4 nucleus auto switchable probe and z-gradient or a Bruker Avance-400 equipped with a QNP (Quad Nucleus Probe) or a BBI (Broad Band Inverse) and z-gradient. Chemical shifts are given in parts per million (ppm) with the residual solvent signal used as reference. NMR abbreviations are used as follows: s = singlet, d = doublet, dd = doublet of doublet, ddd = doublet of doublet of doublets, dt = doublet of triplet, t = triplet, tt = triplet of triplets, q = quartet, m = multiplet, bs = broad singlet.

Analytical HPLC/MS was conducted on a PE Sciex API 150EX mass spectrometer with an electrospray source, using a Shimadzu Inc. LC-10A VP UV detector monitoring at 214 nm, Analyst 1.2 software, and either (a) a Gilson 215 autosampler and an Alltech Prevail™ C18 column (5 μ m, 250 mm × 4.6 mm), using a gradient of 5% v/v CH3CN (containing 1% v/v TFA) in H2O (containing 1% v/v TFA) (*t* = 0.0 min) to 95% v/v CH3CN in H2O (*t* = 6.0 min), 3.5 mL/min or (b) a PE 200 autosampler and a Supelco DiscoveryR C18 column (5 μ m, 50 mm × 2.1 mm), using a gradient of 5% v/v CH3CN (containing 1% v/v TFA) in H2O (containing 1% v/v TFA) (*t* = 0.0 min) to 95% v/v CH3CN in H2O (*t* = 5.0 min), 0.75 mL/min.

High resolution mass spectra (HRMS) samples were analyzed in the negative ion mode using a QTOF Micro (Waters) time-of-flight mass spectrometer. The mass spectrometric detection was achieved using negative electrospray ionization. The source conditions were as follows: 3000 V capillary voltage, 9 V sample cone voltage, 2 V extraction cone voltage, 300 °C desolvation temperature, 120 °C source temperature, and 6 V collision energy (26 V collision energy for the MS^2 analysis).

Preparation of *(R)*-2-(7-(4-Cyclopentyl-3-(trifluoromethyl)benzyloxy)-1,2,3,4-tetrahydrocyclopenta[*b*]indol-3-yl)acetic acid (APD334, **4**):

Step A. Preparation of Ethyl 2-(7-Hydroxy-1,2,3,4-tetrahydrocyclopenta[b]indol-3-yl)acetate. 2-Iodo-4-methoxyaniline (2.0 g, 8.03 mmol) and ethyl 2-(2-oxocyclopentyl)acetate (2.05 g, 12.1 mmol) were dissolved in N,N-dimethylformamide (30 mL) and tetraethyl orthosilicate (2.12 g, 10.4 mmol) and pyridinum *p*-toluenesulfonate (0.081 g, 0.321 mmol) were added. The reaction mixture was heated and stirred at 135 °C

for 4 h. After cooling to 120 °C, diisopropylethylamine (3.11 g, 24.09 mmol) and palladium (II) acetate (0.054 g, 0.241 mmol) were added. The reaction mixture was stirred for 3 h and then partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate. The combined organic extracts were dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The resultant solution was diluted with 50% ethyl acetate in hexanes and filtered through a pad of silica gel. The filtrate was concentrated and purified by silica gel column chromatography to give 1.9 g of ethyl 2-(7-methoxy-1,2,3,4-tetrahydrocyclopenta[b]indol-3-yl)acetate containing residual ethyl 2-(2-oxocyclopentyl)acetate. The mixture was dissolved in dichloromethane (80 mL) and cooled to 0 °C. Boron tribromide (1.0 M solution in dichloromethane, 21.0 mL, 21.0 mmol) was added and the reaction was stirred for 1.5 h. Ice water was added and the reaction mixture was allowed to reach room temperature. The aqueous mixture was extracted with dichloromethane. The combined organics were dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (650 mg). LCMS *m/z* = 260.3 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.29 (t, *J* = 7.2 Hz, 3H), 2.05-2.14 (m, 1H), 2.50 (dd, *J* = 16.8, 11.2 Hz, 1H), 2.68-2.86 (m, 4H), 3.48-3.58 (m, 1H), 4.16-4.24 (m, 2H), 6.66 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.85 (d, *J* = 2.4 Hz, 1H), 7.15 (d, *J* = 8.7 Hz, 1H), 8.4 (s, 1H).

Step B. Preparation of Methyl 4-Chloro-3-(trifluoromethyl)benzoate: To a solution of 4-chloro-3-(trifluoromethyl)benzoic acid (10.4 g, 46.2 mmol) in methanol (100 mL) was added concentrated sulfuric acid (0.51 mL, 9.24 mmol). The mixture was heated under reflux overnight. The mixture was allowed to cool to room temperature and concentrated under reduced pressure to form a solid. The solid was filtered and washed with water. The solid was then stirred with saturated aqueous sodium bicarbonate solution to remove any residual sulfuric acid, filtered and dried under vacuum to give the title compound as a white solid (10.18 g, 92% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.96 (s, 3H), 7.60 (d, *J* = 8.3 Hz, 1H), 8.14 (dd, *J* = 8.3, 2.0 Hz, 1H), 8.37 (d, *J* = 2.0 Hz, 1H).

Step C. Preparation of Methyl 4-Cyclopentyl-3-(trifluoromethyl)benzoate: To zinc(II) chloride (0.5 M solution in tetrahydrofuran, 88.0 mL, 44.0 mmol) was added cyclopentylmagnesium chloride (2 M solution in ether, 20.5 mL, 41.0 mmol). The resulting suspension was stirred at room temperature for 1 h. To the above suspension was added methyl 4-chloro-3-(trifluoromethyl)benzoate (7.00 g, 29.3 mmol) and *bis*(tri-*tert*-butylphosphine)palladium (1.35 g, 2.64 mmol) at room temperature. The mixture was heated under reflux for 2 h. The mixture was allowed to cool to room temperature, quenched with saturated aqueous sodium bicarbonate solution and filtered. The filtrate was extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate, concentrated under reduced pressure and purified by silica gel column

chromatography to give the title compound as colorless oil (7.64 g, 96% yield). LCMS *m/z* = 273.2 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ ppm 1.57-1.66 (m, 2H), 1.68-1.82 (m, 2H), 1.82-1.94 (m, 2H), 2.04-2.21 (m, 2H), 3.33-3.49 (m, 1H), 3.93 (s, 3H), 7.54 (d, *J* = 8.2 Hz, 1H), 8.13 (dd, *J* = 8.3, 1.8 Hz, 1H), 8.27 (s, 1H).

Step D. Preparation of (4-Cyclopentyl-3-(trifluoromethyl)phenyl)methanol: To a solution of methyl 4-cyclopentyl-3-(trifluoromethyl)benzoate (8.16 g, 30.0 mmol) in 1,4-dioxane (200 mL) was added lithium borohydride (2.0 M solution in tetrahydrofuran, 30.0 mL, 6.0 mmol). The mixture was heated under reflux for 2.5 h. The mixture was allowed to cool to room temperature and carefully quenched with 1 N aqueous hydrochloric acid solution to pH 5. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over anhydrous sodium sulfate, concentrated under reduced pressure and purified by silica gel column chromatography to give the title compound as a colorless oil (5.46 g, 75% yield). ^1H NMR (400 MHz, $CDCl_3$) δ ppm 1.56-1.63 (m, 2H), 1.66-1.77 (m, 2H), 1.81-1.91 (m, 2H), 2.03-2.15 (m, 2H), 3.37 (quintet, *J* = 8.0 Hz, 1H), 4.71 (d, *J* = 4.3 Hz, 2H), 7.45-7.47 (m, 1H), 7.49 (d, *J* = 1.1 Hz, 1H), 7.60 (s, 1H).

Step E. Preparation of 4-(Chloromethyl)-1-cyclopentyl-2-(trifluoromethyl)benzene: To (4-cyclopentyl-3-(trifluoromethyl)phenyl)methanol (1.21 g, 4.95 mmol) was added thionyl chloride (5.5 mL, 74.2 mmol). The mixture was heated at 50 °C for 2 h before it was allowed to cool to room temperature and stir overnight. The mixture was poured into ice and stirred for 5 minutes before it was extracted with dichloromethane. The organic extract was washed with saturated aqueous sodium bicarbonate solution, dried over anhydrous sodium sulfate and concentrated under reduced pressure to give the title compound as a light yellow oil (1.16 g, 89% yield). ^1H NMR (400 MHz, $CDCl_3$) δ ppm 1.55-1.63 (m, 2H), 1.69-1.77 (m, 2H), 1.82-1.90 (m, 2H), 2.05-2.13 (m, 2H), 3.37 (quintet, *J* = 8.6 Hz, 1H), 4.58 (s, 2H), 7.46 (d, *J* = 8.0 Hz, 1H), 7.52 (d, *J* = 8.0 Hz, 1H), 7.61 (d, *J* = 1.5 Hz, 1H).

Step F. Preparation of Ethyl 2-(7-(4-Cyclopentyl-3-(trifluoromethyl)benzyloxy)-1,2,3,4-tetrahydrocyclopenta[*b*]indol-3-yl)acetate : To a solution of ethyl 2-(7-hydroxy-1,2,3,4-tetrahydrocyclopenta[b]indol-3-yl)acetate (50.0 mg, 0.193 mmol) and 4-(chloromethyl)-1-cyclopentyl-2-(trifluoromethyl)benzene (152.0 mg, 0.578 mmol) in N,N-dimethylformamide (3 mL) was added cesium carbonate (75.0 mg, 0.231 mmol). The mixture was stirred at room temperature overnight, filtered through Celite®, and concentrated under reduced pressure. The residue was purified by HPLC to give the title compound as a light pink oil (38.7 mg, 41% yield). LCMS *m/z* = 486.5 $[M+H]^+$.

Step G. Preparation of 2-(7-(4-Cyclopentyl-3-(trifluoromethyl)benzyloxy)-1,2,3,4-tetrahydrocyclopenta[*b*]indol-3-yl)acetic Acid (Compound **15**): To a solution of ethyl 2-(7-(4-cyclopentyl-3-(trifluoromethyl)benzyloxy)-1,2,3,4-tetrahydrocyclopenta[b]indol-3-yl)acetate (38.7 mg, 0.080 mmol) in a mixed solvent of methanol (1.5 mL), tetrahydrofuran (0.5 mL), and water (0.5 mL) was added lithium hydroxide hydrate (11.7 mg, 0.279 mmol). The mixture was stirred at room temperature overnight and then acidified to pH 4 with 1 N aqueous hydrochloric acid and extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The foam was triturated with water to give a solid. The solid was filtered to give the title compound as a light pink solid (25.7 mg, 70% yield). LCMS *m/z* = 458.4 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.56-1.70 (m, 4H), 1.80-1.87 (m, 2H), 1.95-2.11 (m, 3H), 2.34 (dd, *J* = 16.0, 9.0 Hz, 1H), 2.59-2.74 (m, 4H), 3.21-3.25 (m, 1H), 3.41-3.49 (m, 1H), 5.11 (s, 2H), 6.70 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.92 (d, *J* = 2.3 Hz, 1H), 7.19 (d, *J* = 8.7 Hz, 1H), 7.61 (d, *J* = 8.0 Hz, 1H), 7.68 (d, *J* = 8.0 Hz, 1H), 7.70 (s, 1H), 10.45 (s, 1H), 12.18 (bs, 1H).

Step H. *(R)*-2-(7-(4-Cyclopentyl-3-(trifluoromethyl)benzyloxy)-1,2,3,4-tetrahydrocyclopenta[*b*]indol-3-yl)acetic acid (APD334, Compound 4): Racemic 2-(7-(4-Cyclopentyl-3-(trifluoromethyl)benzyloxy)-1,2,3,4-tetrahydrocyclopenta[*b*]indol-3-yl)acetic acid was resolved by normal phase preparative chiral chromatography using a ChiralCel OD column (50 X 500mm ID, 20 µm particle size) and a 8/92 solution of isopropanol (0.05% trifluoroacetic acid) and hexanes (0.05% trifluoroacetic acid) as eluent. The method was isocratic with a flow rate of 60 ml/minute and the detector was set to 220nm. APD334 is the second eluting enantiomer (most retained) with a retention time of 48.4 minutes. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.54-1.75 (m, 4H), 1.79-1.92 (m, 2H), 1.95-2.16 (m, 3H), 2.39 (dd, *J* = 16.0, 8.8 Hz, 1H), 2.61-2.83 (m, 4H), 3.23-3.34 (m, 1H), 3.45-3.56 (m, 1H), 5.14 (s, 2H), 6.74 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.92 (d, *J* = 2.3 Hz, 1H), 7.24 (d, *J* = 8.8 Hz, 1H), 7.64 (d, *J* = 8.1 Hz, 1H), 7.72 (d, *J* = 8.6 Hz, 1H), 7.74 (s, 1H), 10.50 (s, 1H), 12.24 (bs, 1H). ^{13}C APT NMR (100 MHz, *DMSO-d_6*): δ up (*C*, *C*H_2): 23.1, 25.5, 35.5, 35.6, 68.6, 117.0, 124.7 (q, *J* = 273 Hz), 124.2, 126.8 (q, *J* = 28 Hz), 128.7, 136.1, 136.2, 144.6, 147.0, 151.9, 173.4; down (*CH*, *C*H_3): 35.0, 40.5, 102.1, 110.0, 112.4, 124.1 (q, *J* = 5.7 Hz), 128.4, 131.7. ^{19}F NMR (400 MHz, *DMSO-d_6*) δ ppm -57.4. LCMS (ESI+): calcd for $C26H27F3NO3^+$ [M+H] 458.19; found, 458.4. HRMS (ESI-): calcd for $C26H25F3NO3^-$ [M-H] 456.1792; found, 456.1776.

Preparation of **7**, 2-(7-(3-Cyano-5-(trifluoromethoxy)benzyloxy)-1,2,3,4-tetrahydrocyclopenta[*b*]indol-3-yl) acetic acid: Ethyl 2-(7-hydroxy-1,2,3,4-tetrahydrocyclopenta[b]indol-3-yl)acetate (100 mg, 0.386 mmol) and 3-(hydroxymethyl)-5-(trifluoromethoxy)benzonitrile (84 mg, 0.386 mmol) were dissolved in tetrahydrofuran (3.0 mL) and cooled to 0 °C. Triphenylphosphine (202 mg, 0.771 mmol) and

diisopropylazodicarboxylate (DIAD) (0.15 mL, 0.771 mmol) were added. The mixture was warmed to room temperature and stirred for 1 h. Additional DIAD (0.15 mL, 0.771 mmol) and triphenylphosphine (202 mg, 0.771 mmol) were added and the reaction mixture was stirred for 1 h. The reaction mixture was diluted with water and extracted with ethyl acetate. The combined organics were dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give 50.8 mg of impure ethyl 2-(7-(3-cyano-5-(trifluoromethoxy)benzyloxy)-1,2,3,4-tetrahydrocyclopenta[b]indol-3-yl)acetate. The material was dissolved in dioxane (1.3 mL) and 1.0 M aqueous lithium hydroxide (0.33 mL, 0.33 mmol) was added. The reaction was monitored by LCMS until judged complete and then acidified to pH 2 with 1.0 M hydrochloric acid. The aqueous mixture was extracted with ethyl acetate. The combined organics were dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography followed by HPLC to give the title compound (1.1 mg). LCMS *m/z* = 431.2 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD) δ ppm 2.11-2.20 (m, 1H), 2.50 (dd, *J* = 15.8, 8.0 Hz, 1H), 2.66-2.84 (m, 4H), 3.51-3.60 (m, 1H), 5.18 (s, 2H), 6.78 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.95 (d, *J* = 2.4 Hz, 1H), 7.20 (d, *J* = 8.9 Hz, 1H), 7.64 (s, 1H), 7.73 (s, 1H), 7.84 (s, 1H).

Preparation of **8**, 2-(7-(3,5-Bis(trifluoromethyl)benzyloxy)-1,2,3,4-tetrahydrocyclopenta[*b*]indol-3-yl)acetic Acid:

Step A. Preparation of Ethyl 2-(7-(3,5-Bis(trifluoromethyl)benzyloxy)-1,2,3,4-tetrahydrocyclopenta[b]indol-3-yl)acetate: Ethyl 2-(7-hydroxy-1,2,3,4-tetrahydrocyclopenta[b]indol-3-yl)acetate (61 mg, 0.235 mmol) was dissolved in N,N-dimethylformamide (1.0 mL) and cesium carbonate (77 mg, 0.235 mmol) and 1-(bromomethyl)-3,5-bis(trifluoromethyl)benzene (72 mg, 0.235 mmol) were added. The reaction mixture was stirred at room temperature for 16 h and then filtered through a pad of Celite®. The filtrate was diluted with water and extracted with ethyl acetate. The combined organics were dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (28.6 mg). LCMS *m/z* = 486.4 $[M+H]^+$.

Step B. Preparation of 2-(7-(3,5-Bis(trifluoromethyl)benzyloxy)-1,2,3,4-tetrahydrocyclopenta[b]indol-3-yl)acetic Acid: Ethyl 2-(7-(3,5-bis(trifluoromethyl)benzyloxy)-1,2,3,4-tetrahydrocyclopenta[b]indol-3-yl)acetate (28.6 mg, 0.059 mmol) was dissolved in dioxane (1.0 mL) and 1.0 M aqueous lithium hydroxide solution (0.166 mL, 0.166 mmol) was added. The solution was stirred at room temperature for 3 h before it

was acidified to pH 3 with 1.0 M hydrochloric acid and extracted with ethyl acetate. The combined extracts were dried over sodium sulfate, filtered and concentrated under reduced pressure to give the title compound (23 mg). LCMS *m/z* = 458.3 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.12-2.24 (m, 1H), 2.61 (dd, *J* = 17.0, 10.7 Hz, 1H), 2.73-2.89 (m, 4H), 3.53-3.63 (m, 1H), 5.19 (s, 2H), 6.86 (dd, *J* = 8.6, 2.5 Hz, 1H), 7.0 (d, *J* = 2.5 Hz, 1H), 7.24 (d, *J* = 8.8 Hz, 1H), 7.82 (s, 1H), 7.94 (s, 2H), 8.33 (s, 1H).

Preparation of **9**, 2-(7-(3-Cyano-4-(trifluoromethoxy)benzyloxy)-1,2,3,4-tetrahydrocyclopenta[*b*]indol-3-yl)acetic Acid:

Ethyl 2-(7-hydroxy-1,2,3,4-tetrahydrocyclopenta[b]indol-3-yl)acetate (0.100 g, 0.386 mmol) and cesium carbonate (0.138 g, 0.424 mmol) were dissolved in N,N-dimethylformamide (1.0 mL), stirred at room temperature for 10 min, followed by addition of 5-(chloromethyl)-2-(trifluoromethoxy)benzonitrile (0.100 g, 0.424 mmol) in N,N-dimethylformamide (0.300 mL) at 0 °C. This mixture was stirred at room temperature for 3 h. The reaction mixture was quenched with water and extracted with ethyl acetate. The organic phase was washed with brine and concentrated. The residue was taken up in dioxane (4 mL) before aqueous 1 N lithium hydroxide (1.3 mL) was added. The mixture was stirred at room temperature for 2.5 h before it was quenched with water and acidified to pH 3 using aqueous 3 N hydrochloric acid. The mixture was purified by HPLC to give the title compound (0.040 g). LCMS *m/z* = 431.2 $[M+H]^+$, ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 2.00-2.20 (m, 1H), 2.01-2.21 (m, 1H), 2.23-2.43 (m, 1H), 2.55-2.83 (m, 4H), 3.33-3.57 (m, 1H), 5.16 (s, 2H), 6.73 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.94 (s, 1H), 7.21 (d, *J* = 8.8 Hz, 1H), 7.69 (dd, *J* = 8.7, 1.4 Hz, 1H), 7.94 (dd, *J* = 8.7, 2.1 Hz, 1H), 8.09 (s, 1H), 10.40 (s, 1H).

Preparation of **16**, 2-(7-(4-Cyclobutyl-3-(trifluoromethyl)benzyloxy)-1,2,3,4-tetrahydrocyclopenta[*b*]indol-3-yl)acetic Acid.

To a solution of ethyl 2-(7-(4-chloro-3-(trifluoromethyl)benzyloxy)-1,2,3,4-tetrahydrocyclopenta[*b*]indol-3-yl)acetate (202.8 mg, 0.449 mmol) in tetrahydrofuran (1 mL) was added 0.5 M cyclobutylzinc(II) bromide solution in tetrahydrofuran (8.98 mL, 4.49 mmol) and *bis*(tri-*t*-butylphosphine)palladium (46.8 mg, 0.090 mmol) at room temperature. The mixture was heated at 90 °C for 63 h. The mixture was then quenched with 1 N aqueous hydrochloric acid solution and filtered through celite®. The filtrate was extracted with ethyl acetate. The organic layer was washed with brine solution to remove excess

hydrochloric acid and concentrated under reduced pressure. The residue was purified by HPLC to afford the title compound as a pink solid (32.3 mg). LCMS *m/z* = 444.6 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.78-1.87 (m, 1H), 1.93-2.02 (m, 1H), 2.03-2.12 (m, 1H), 2.16-2.28 (m, 4H), 2.34 (dd, *J* = 16.0, 9.0 Hz, 1H), 2.60-2.75 (m, 4H), 3.41-3.51 (m, 1H), 3.74-3.84 (m, 1H), 5.12 (s, 2H), 6.70 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.92 (d, *J* = 2.4 Hz, 1H), 7.19 (d, *J* = 8.8 Hz, 1H), 7.66-7.78 (m, 3H), 10.46 (s, 1H), 12.20 (bs, 1H).

The following compounds were prepared by the methods described above:

10, 2-(7-((3-cyano-4-methoxybenzyl)oxy)-1,2,3,4-tetrahydrocyclopenta[*b*]indol-3-yl)acetic acid: LCMS *m/z* = 377.4 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.04-2.13 (m, 1H), 2.35 (dd, *J* = 16.4, 9.0 Hz, 1H), 2.58-2.77 (m, 4H), 3.41-3.50 (m, 1H), 3.92 (s, 3H), 5.02 (s, 2H), 6.69 (dd, *J* = 8.9, 2.6 Hz, 1H), 6.91 (d, *J* = 2.3 Hz, 1H), 7.19 (d, *J* = 8.7 Hz, 1H), 7.26 (d, *J* = 8.8 Hz, 1H), 7.75 (dd, *J* = 8.8, 2.0 Hz, 1H), 10.5 (s, 1H), 12.2 (bs, 1H).

11, 2-(7-(4-Cyano-3-(trifluoromethyl)benzyloxy)-1,2,3,4-tetrahydrocyclopenta[*b*]indol-3-yl)acetic acid: LCMS *m/z* = 415.4 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.01-2.14 (m, 1H), 2.35 (dd, *J* = 16.0, 9.0 Hz, 1H), 2.57-2.78 (m, 4H), 3.38-3.53 (m, 1H), 5.29 (s, 2H), 6.75 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.94 (d, *J* = 2.5 Hz, 1H), 7.22 (d, *J* = 8.7 Hz, 1H), 7.97 (s, 1H), 8.07 (s, 1H), 8.19 (d, *J* = 8.0 Hz, 1H), 10.50 (s, 1H), 12.18 (bs, 1 H)

12, 2-(7-(4-Chloro-3-(trifluoromethyl)benzyloxy)-1,2,3,4-tetrahydrocyclopenta[*b*]indol-3-yl)acetic Acid: LCMS *m/z* = 424.2 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.09-2.19 (m, 1H), 2.61 (dd, *J* = 17.3, 11.0 Hz, 1H), 2.72-2.89 (m, 4H), 3.53-3.63 (m, 1H), 5.10 (s, 2H), 6.83 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.97 (d, *J* = 2.4 Hz, 1H), 7.22 (d, *J* = 8.6 Hz, 1H), 7.50 (d, *J* = 8.2 Hz, 1H), 7.57 (dd, *J* = 8.2, 1.6 Hz, 1H), 7.80 (d, *J* = 1.8 Hz, 1H), 8.33 (bs, 1H).

13, 2-(7-(3-Cyano-4-cyclohexylbenzyloxy)-1,2,3,4-tetrahydrocyclopenta[*b*]indol-3-yl)acetic Acid: LCMS *m/z* = 429.6 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.21-1.31 (m, 1H), 1.33-1.54 (m, 4H), 1.68-1.74 (m, 1H), 1.74-1.88 (m, 4H), 2.08 (dd, *J* = 4.9, 3.5 Hz, 1H), 2.34 (dd, *J* = 16.0, 9.0 Hz, 1H), 2.61-2.76 (m, 4H), 2.81-2.89 (m, 1H), 3.45 (bs, 1H), 5.07 (s, 2H), 6.70 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.91 (d, *J* = 2.4 Hz, 1H), 7.19 (d, *J* = 8.7 Hz, 1H), 7.52 (d, *J* = 8.1 Hz, 1H), 7.72 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.81 (d, *J* = 1.5 Hz, 1H), 10.46 (s, 1H), 12.18 (bs, 1H).

14, 2-(7-(4-cyclohexyl-3-(trifluoromethyl)benzyloxy)-1,2,3,4-tetrahydrocyclopenta[*b*]indol-3-yl)acetic acid: LCMS m/z = 472.2 ($M+H^+$); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.38-1.50 (m, 4H), 1.76-1.85 (m, 6H), 2.11-2.17 (m, 1H), 2.58-2.65 (m, 1H), 2.75-2.93 (m, 5H), 3.56-3.60 (m, 1H), 5.07 (s, 2H), 6.84 (dd, *J* = 8.8,

2.5 Hz, 1H), 7.00 (d, *J* = 2.4 Hz, 1H), 7.21 (d, *J* = 8.8 Hz, 1H), 7.46 (d, *J* = 8.1 Hz, 1H), 7.59 (d, *J* = 8.1 Hz, 1H), 7.70 (d, *J* = 1.2 Hz, 1H), 8.27 (s, 1H).

17, 2-(7-(4-Cyclopropyl-3-(trifluoromethyl)benzyloxy)-1,2,3,4-tetrahydrocyclopenta[*b*]indol-3-yl)acetic Acid: LCMS *m/z* = 430.5 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.74-0.88 (m, 2H), 0.98-1.07 (m, 2H), 2.02-2.12 (m, 2H), 2.34 (dd, *J* = 16.0, 9.0 Hz, 1H), 2.59-2.76 (m, 4H), 3.39-3.52 (m, 1H), 5.10 (s, 2H), 6.69 (dd, *J* = 8.7, 2.5 Hz, 1H), 6.91 (d, *J* = 2.4 Hz, 1H), 7.10-7.26 (m, 2H), 7.61 (d, *J* = 8.3 Hz, 1H), 7.72 (d, *J* = 1.1 Hz, 1H), 10.45 (s, 1H), 12.20 (bs, 1H).

18, 2-(7-(4-Propyl-3-(trifluoromethyl)benzyloxy)-1,2,3,4-tetrahydrocyclopenta[*b*]indol-3-yl)acetic Acid: LCMS *m/z* = 432.5 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3CN) δ ppm 0.98 (t, *J* = 7.3 Hz, 3H), 1.57-1.71 (m, 2H), 2.04-2.18 (m, 1H), 2.60 (d, *J* = 7.5 Hz, 2H), 2.66-2.83 (m, 5H), 3.46-3.57 (m, 1H), 5.10 (s, 2H), 6.76 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.96 (d, *J* = 2.4 Hz, 1H), 7.23 (d, *J* = 8.8 Hz, 1H), 7.44 (d, *J* = 7.80 Hz, 1H), 7.62 (d, *J* = 8.0 Hz, 1H), 7.73 (s, 1H), 8.86 (bs, 1H).

19, 2-(7-(4-Neopentyl-3-(trifluoromethyl)benzyloxy)-1,2,3,4-tetrahydrocyclopenta[*b*]indol-3-yl)acetic Acid: LCMS *m/z* = 460.6 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.96 (s, 9H), 2.08-2.19 (m, 1H), 2.61 (dd, *J* = 17.0, 10.9 Hz, 1H), 2.73-2.90 (m, 6H), 3.54-3.63 (m, 1H), 5.09 (s, 2H), 6.85 (dd, *J* = 8.8, 2.5 Hz, 1H), 7.0 (d, *J* = 2.5 Hz, 1H), 7.22 (d, *J* = 9.0 Hz, 1H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.55 (d, *J* = 8.1 Hz, 1H), 7.74 (d, *J* = 1.1 Hz, 1H), 8.29 (bs, 1H).

20, 2-(7-(4-Isobutyl-3-(trifluoromethyl)benzyloxy)-1,2,3,4-tetrahydrocyclopenta[*b*]indol-3-yl)acetic Acid: LCMS *m/z* = 446.7 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.89 (d, *J* = 6.6 Hz, 6H), 1.87-1.98 (m, 1H), 2.03-2.13 (m, 1H), 2.35 (dd, *J* = 15.9, 9.0 Hz, 1H), 2.60-2.76 (m, 6H), 3.42-3.50 (m, 1H), 5.12 (s, 2H), 6.71 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.93 (d, *J* = 2.4 Hz, 1H), 7.20 (d, *J* = 8.7 Hz, 1H), 7.47 (d, *J* = 8.0 Hz, 1H), 7.67 (d, *J* = 8.2 Hz, 1H), 7.75 (d, *J* = 1.4 Hz, 1H), 10.47 (bs, 1H).

21, 2-(7-(4-(Cyclohexylmethyl)-3-(trifluoromethyl)benzyloxy)-1,2,3,4-tetrahydrocyclopenta[*b*]indol-3-yl)acetic acid: LCMS *m/z* = 486.3 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.96-1.06 (m, 2H), 1.14-1.22 (m, 3H), 1.55-1.70 (m, 6H), 2.05-2.10 (m, 1H), 2.40 (dd, *J* = 16.0 and 9.0 Hz, 1H), 2.62-2.75 (m, 6H), 3.42-3.50 (m, 1H), 5.12 (s, 2H), 6.72 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.94 (d, *J* = 2.4 Hz, 1H), 7.21 (d, *J* = 8.8 Hz, 1H), 7.45 (d, *J* = 8.0 Hz, 1H), 7.66 (d, *J* = 7.8 Hz, 1H), 7.75 (s, 1H), 10.48 (s, 1H), 12.20 (bs, 1H).

Crystallographic Tables (APD334, 4)

Table 1. Sample and crystal data

Empirical formula	$C_{26}H_{26}F_3NO_3$	
Formula weight	457.48	
Temperature	100(2) K	
Wavelength	1.54178 Å	
Crystal size	0.500 x 0.300 x 0.200 mm	
Crystal habit	Colourless Prism	
Crystal system	Triclinic	
Space group	*P*1	
Unit cell dimensions	*a* = 10.3114(3) Å	α= 71.923(3)°
	b = 10.7766(3) Å	β= 75.453(3)°
	c = 10.9873(3) Å	γ = 77.431(3)°
Volume	1110.44(6) $Å^3$	
Z	2	
Density (calculated)	1.368 Mg/m^3	
Absorption coefficient	0.889 mm^{-1}	
F(000)	480	

Table 2. Data collection and structure refinement

Diffractometer	SuperNova, Dual, Cu at zero, Atlas
Radiation source	SuperNova (Cu) X-ray Source, CuKα
Data collection method	omega scans
Theta range for data collection	8.935 to 74.490°
Index ranges	$-12 \leq h \leq 12$, $-13 \leq k \leq 13$, $-13 \leq l \leq 13$
Reflections collected	20671
Independent reflections	8493 [R(int) = 0.0238]
Coverage of independent reflections	99.5 %
Variation in check reflections	n/a
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	1.00000 and 0.71217
Structure solution technique	Direct methods
Structure solution program	SHELXTL (Sheldrick, 2013)
Refinement technique	Full-matrix least-squares on F^2
Refinement program	SHELXTL (Sheldrick, 2013)
Function minimized	$\Sigma\, w(F_o^2 - F_c^2)^2$
Data / restraints / parameters	8493 / 3 / 609
Goodness-of-fit on F^2	1.051
Δ/σ_{max}	0.000
Final R indices	

8223 data; I>2σ(I)	R1 = 0.0466, wR2 = 0.1205
all data	R1 = 0.0483, wR2 = 0.1231
Weighting scheme	$w = 1 / [\sigma^2 (F_o^2) + (0.0613P)^2 + 0.4884P]$
	where $P=(F_o^2+2F_c^2)/3$
Absolute structure parameter	-0.06(6)
Extinction coefficient	n/a
Largest diff. peak and hole	0.332 and -0.303 eÅ^{-3}

Refinement summary:

Ordered Non-H atoms, XYZ	Freely refining
Ordered Non-H atoms, U	Anisotropic
H atoms (on carbon), XYZ	Idealized positions riding on attached atoms
H atoms (on carbon), U	Appropriate multiple of U(eq) for bonded atom
H atoms (on heteroatoms), XYZ	Freely refining
H atoms (on heteroatoms), U	Isotropic refining for H bonded to N
	Appropriate multiple of (-1.5000) U(eq) for H bonded to O
Disordered atoms, OCC	No disorder
Disordered atoms, XYZ	No disorder
Disordered atoms, U	No disorder

Table 3. Atomic coordinates and equivalent isotropic atomic displacement parameters, (Å^2)

U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x/a	y/b	z/c	U(eq)
F1B	0.8475(2)	-0.2472(2)	-0.2484(3)	0.0544(6)
F2B	0.7431(3)	-0.4122(2)	-0.1999(2)	0.0513(6)
F3B	0.7131(3)	-0.2500(2)	-0.3686(2)	0.0560(7)
O1B	0.6340(2)	-0.0185(2)	0.1203(2)	0.0337(5)
O2B	0.9359(3)	0.5253(3)	0.5376(3)	0.0514(7)
O3B	0.7658(3)	0.4067(3)	0.6120(3)	0.0507(7)
N1B	0.7360(3)	0.2669(3)	0.4155(3)	0.0386(7)
C1B	0.8239(4)	0.4922(4)	0.5272(4)	0.0425(9)
C2B	0.7789(4)	0.5672(4)	0.4036(4)	0.0454(9)
C3B	0.6591(4)	0.5265(4)	0.3759(5)	0.0503(10)
C4B	0.6815(3)	0.3916(4)	0.3522(4)	0.0396(8)
C5B	0.7181(3)	0.1811(3)	0.3507(3)	0.0337(7)
C6B	0.7579(4)	0.0454(4)	0.3725(4)	0.0397(8)
C7B	0.7295(4)	-0.0152(3)	0.2908(4)	0.0364(7)
C8B	0.6603(3)	0.0572(3)	0.1895(3)	0.0308(7)
C9B	0.6211(3)	0.1925(3)	0.1656(3)	0.0307(7)
C10B	0.6513(3)	0.2559(3)	0.2473(4)	0.0332(7)
C11B	0.6311(4)	0.3905(3)	0.2507(4)	0.0387(8)
C12B	0.5678(4)	0.5258(4)	0.1840(5)	0.0521(11)
C13B	0.6193(5)	0.6140(4)	0.2426(7)	0.0678(15)
C14B	0.5599(4)	0.0476(3)	0.0192(3)	0.0343(7)

C15B	0.5326(3)	-0.0564(3)	-0.0322(3)	0.0319(7)
C16B	0.6355(4)	-0.1160(3)	-0.1147(3)	0.0328(7)
C17B	0.6115(4)	-0.2138(3)	-0.1609(3)	0.0332(7)
C18B	0.4829(4)	-0.2512(4)	-0.1308(3)	0.0379(8)
C19B	0.3815(4)	-0.1906(4)	-0.0464(4)	0.0408(8)
C20B	0.4069(4)	-0.0961(4)	0.0022(3)	0.0372(8)
C21B	0.7276(4)	-0.2793(4)	-0.2441(4)	0.0409(8)
C22B	0.4471(5)	-0.3497(4)	-0.1850(4)	0.0480(10)
C23B	0.4160(7)	-0.4782(5)	-0.0865(5)	0.0697(16)
C25B	0.2856(10)	-0.4219(10)	-0.2618(9)	0.115(3)
C26B	0.3199(7)	-0.3027(7)	-0.2454(7)	0.0825(18)
C24B	0.3463(9)	-0.5366(8)	-0.1621(8)	0.101(3)
F1A	1.0223(2)	1.0076(2)	1.75695(19)	0.0436(5)
F2A	0.9871(2)	1.1754(2)	1.5952(2)	0.0417(5)
F3A	0.8919(2)	1.0074(2)	1.6317(2)	0.0465(5)
O1A	0.8523(3)	0.2716(3)	0.8365(3)	0.0606(9)
O2A	1.0239(4)	0.3857(4)	0.7486(3)	0.0693(11)
O3A	1.1235(2)	0.7991(2)	1.2483(2)	0.0350(5)
N1A	0.9609(5)	0.5047(4)	1.0008(5)	0.0662(13)
C1A	0.9738(5)	0.2960(4)	0.8326(4)	0.0515(11)
C2A	1.0455(5)	0.2081(4)	0.9369(4)	0.0566(11)
C3A	0.9958(4)	0.2422(4)	1.0679(4)	0.0429(8)
C4A	1.0106(4)	0.3789(4)	1.0654(5)	0.0467(9)
C5A	0.9977(5)	0.5917(4)	1.0514(5)	0.0535(11)
C6A	0.9671(6)	0.7292(5)	1.0222(6)	0.0754(18)
C7A	1.0105(5)	0.7912(4)	1.0939(5)	0.0575(12)
C8A	1.0862(3)	0.7200(3)	1.1892(3)	0.0330(7)
C9A	1.1196(3)	0.5847(3)	1.2171(3)	0.0293(7)

C10A	1.0726(3)	0.5177(3)	1.1491(3)	0.0321(7)
C11A	1.0784(3)	0.3821(3)	1.1550(3)	0.0331(7)
C12A	1.1272(6)	0.2469(4)	1.2304(4)	0.0522(11)
C13A	1.0842(5)	0.1575(4)	1.1681(5)	0.0533(11)
C14A	1.1966(4)	0.7340(3)	1.3511(3)	0.0337(7)
C15A	1.2163(4)	0.8386(3)	1.4064(3)	0.0329(7)
C16A	1.1107(3)	0.8905(3)	1.4914(3)	0.0304(7)
C17A	1.1284(3)	0.9865(3)	1.5442(3)	0.0302(7)
C18A	1.2536(4)	1.0308(3)	1.5167(3)	0.0339(7)
C19A	1.3568(4)	0.9808(4)	1.4267(4)	0.0384(8)
C20A	1.3385(4)	0.8879(4)	1.3715(3)	0.0365(7)
C21A	1.0089(4)	1.0428(3)	1.6317(3)	0.0334(7)
C22A	1.2814(4)	1.1286(4)	1.5773(4)	0.0422(9)
C23A	1.4123(5)	1.0913(6)	1.6289(5)	0.0610(13)
C24A	1.4259(6)	1.2171(7)	1.6579(5)	0.0769(19)
C25A	1.3551(6)	1.3315(7)	1.5601(6)	0.079(2)
C26A	1.2936(5)	1.2668(5)	1.4851(4)	0.0537(11)

Table 4. Selected bond lengths, (Å)

F1B-C21B	1.340(5)	F2B-C21B	1.349(4)
F3B-C21B	1.343(4)	O1B-C8B	1.380(4)
O1B-C14B	1.422(4)	O2B-C1B	1.321(5)
O2B-H2BB	1.01(6)	O3B-C1B	1.226(5)
N1B-C4B	1.376(5)	N1B-C5B	1.394(4)
N1B-H1BB	0.91(4)	C1B-C2B	1.477(6)
C2B-C3B	1.523(5)	C3B-C4B	1.514(4)
C3B-C13B	1.575(8)	C4B-C11B	1.348(6)
C5B-C6B	1.391(5)	C5B-C10B	1.414(5)
C6B-C7B	1.377(5)	C7B-C8B	1.405(5)
C8B-C9B	1.384(4)	C9B-C10B	1.408(5)
C10B-C11B	1.430(5)	C11B-C12B	1.501(5)
C12B-C13B	1.540(6)	C14B-C15B	1.504(5)
C15B-C20B	1.379(5)	C15B-C16B	1.393(5)
C16B-C17B	1.393(5)	C17B-C18B	1.400(5)
C17B-C21B	1.500(5)	C18B-C19B	1.404(5)
C18B-C22B	1.513(5)	C19B-C20B	1.384(5)
C22B-C23B	1.510(6)	C22B-C26B	1.535(8)
C23B-C24B	1.557(7)	C25B-C26B	1.478(9)
C25B-C24B	1.512(12)	F1A-C21A	1.344(4)
F2A-C21A	1.343(4)	F3A-C21A	1.342(4)
O1A-C1A	1.323(6)	O1A-H1AB	0.94(7)
O2A-C1A	1.215(5)	O3A-C8A	1.381(4)
O3A-C14A	1.428(4)	N1A-C4A	1.374(6)
N1A-C5A	1.384(5)	N1A-H1AC	0.86(6)
C1A-C2A	1.485(7)	C2A-C3A	1.531(5)
C3A-C4A	1.505(5)	C3A-C13A	1.547(6)
C4A-C11A	1.354(5)	C5A-C6A	1.397(6)
C5A-C10A	1.415(6)	C6A-C7A	1.379(6)
C7A-C8A	1.394(6)	C8A-C9A	1.377(5)
C9A-C10A	1.407(5)	C10A-C11A	1.432(5)
C11A-C12A	1.484(5)	C12A-C13A	1.531(5)
C14A-C15A	1.506(4)	C15A-C16A	1.386(5)
C15A-C20A	1.392(5)	C16A-C17A	1.395(4)
C17A-C18A	1.403(5)	C17A-C21A	1.501(4)
C18A-C19A	1.397(5)	C18A-C22A	1.517(5)
C19A-C20A	1.386(5)	C22A-C23A	1.521(7)
C22A-C26A	1.530(6)	C23A-C24A	1.527(7)
C24A-C25A	1.546(10)	C25A-C26A	1.542(6)

Table 5. Selected bond angles, (°)

C8B-O1B-C14B	117.3(2)	C1B-O2B-H2BB	114(3) C4B-
N1B-C5B	106.7(3)	C4B-N1B-H1BB	129(2) C5B-
N1B-H1BB	124(2)	O3B-C1B-O2B	122.4(4)
O3B-C1B-C2B	124.6(4)	O2B-C1B-C2B	113.1(3)
C1B-C2B-C3B	117.1(3)	C4B-C3B-C2B	116.7(3)
C4B-C3B-C13B	99.2(3)	C2B-C3B-C13B	112.6(4)
C11B-C4B-N1B	111.7(3)	C11B-C4B-C3B	113.8(4)
N1B-C4B-C3B	134.3(4)	C6B-C5B-N1B	130.2(3)
C6B-C5B-C10B	121.4(3)	N1B-C5B-C10B	108.3(3)
C7B-C6B-C5B	118.2(3)	C6B-C7B-C8B	121.1(3)
O1B-C8B-C9B	124.3(3)	O1B-C8B-C7B	114.1(3)
C9B-C8B-C7B	121.5(3)	C8B-C9B-C10B	117.9(3)
C9B-C10B-C5B	119.9(3)	C9B-C10B-C11B	133.8(4)
C5B-C10B-C11B	106.3(3)	C4B-C11B-C10B	107.0(3)
C4B-C11B-C12B	111.8(3)	C10B-C11B-C12B	141.1(4)
C11B-C12B-C13B	101.8(4)	C12B-C13B-C3B	107.9(4)
O1B-C14B-C15B	107.3(3)	C20B-C15B-C16B	118.6(3)
C20B-C15B-C14B	121.2(3)	C16B-C15B-C14B	120.2(3)
C17B-C16B-C15B	120.5(3)	C16B-C17B-C18B	121.4(3)
C16B-C17B-C21B	118.3(3)	C18B-C17B-C21B	120.3(3)
C17B-C18B-C19B	116.9(3)	C17B-C18B-C22B	124.3(3)
C19B-C18B-C22B	118.9(3)	C20B-C19B-C18B	121.5(3)
C15B-C20B-C19B	121.1(3)	F1B-C21B-F3B	106.5(3)
F1B-C21B-F2B	105.1(3)	F3B-C21B-F2B	106.0(3)
F1B-C21B-C17B	113.2(3)	F3B-C21B-C17B	113.0(3)
F2B-C21B-C17B	112.3(3)	C23B-C22B-C18B	115.3(3)
C23B-C22B-C26B	102.1(5)	C18B-C22B-C26B	115.1(4)
C22B-C23B-C24B	101.8(5)	C26B-C25B-C24B	105.5(6)
C25B-C26B-C22B	105.7(6)	C25B-C24B-C23B	107.2(5)
C1A-O1A-H1AB	107(4)	C8A-O3A-C14A	117.0(3)
C4A-N1A-C5A	107.7(3)	C4A-N1A-H1AC	128(4) C5A-
N1A-H1AC	124(4)	O2A-C1A-O1A	121.9(5)
O2A-C1A-C2A	121.8(5)	O1A-C1A-C2A	116.3(4)
C1A-C2A-C3A	113.8(3)	C4A-C3A-C2A	115.3(3)
C4A-C3A-C13A	100.6(3)	C2A-C3A-C13A	111.4(3)
C11A-C4A-N1A	110.5(3)	C11A-C4A-C3A	114.3(4)
N1A-C4A-C3A	134.9(4)	N1A-C5A-C6A	129.8(4)
N1A-C5A-C10A	108.4(3)	C6A-C5A-C10A	121.7(4)
C7A-C6A-C5A	117.6(4)	C6A-C7A-C8A	121.4(4)
C9A-C8A-O3A	125.2(3)	C9A-C8A-C7A	121.6(3)
O3A-C8A-C7A	113.2(3)	C8A-C9A-C10A	118.5(3)
C9A-C10A-C5A	119.1(3)	C9A-C10A-C11A	135.1(3)
C5A-C10A-C11A	105.8(3)	C4A-C11A-C10A	107.6(3)

C4A-C11A-C12A	111.4(3)	C10A-C11A-C12A	140.9(3)
C11A-C12A-C13A	103.5(3)	C12A-C13A-C3A	109.6(3)
O3A-C14A-C15A	107.3(3)	C16A-C15A-C20A	118.4(3)
C16A-C15A-C14A	120.1(3)	C20A-C15A-C14A	121.5(3)
C15A-C16A-C17A	120.5(3)	C16A-C17A-C18A	121.7(3)
C16A-C17A-C21A	118.2(3)	C18A-C17A-C21A	120.2(3)
C19A-C18A-C17A	116.6(3)	C19A-C18A-C22A	119.3(3)
C17A-C18A-C22A	124.1(3)	C20A-C19A-C18A	121.8(3)
C19A-C20A-C15A	120.8(3)	F3A-C21A-F2A	104.9(3)
F3A-C21A-F1A	106.5(3)	F2A-C21A-F1A	106.0(3)
F3A-C21A-C17A	113.2(3)	F2A-C21A-C17A	112.4(3)
F1A-C21A-C17A	113.2(3)	C18A-C22A-C23A	115.6(3)
C18A-C22A-C26A	114.9(3)	C23A-C22A-C26A	102.8(4)
C22A-C23A-C24A	103.4(5)	C23A-C24A-C25A	105.4(4)
C26A-C25A-C24A	106.3(4)	C22A-C26A-C25A	103.1(4)

Table 6. Selected torsion angles, (°)

O3B-C1B-C2B-C3B	-5.4(5)	O2B-C1B-C2B-C3B	173.8(3)
C1B-C2B-C3B-C4B	-66.9(5)	C1B-C2B-C3B-C13B	179.4(3)
C5B-N1B-C4B-C11B	-0.5(4)	C5B-N1B-C4B-C3B	175.4(4)
C2B-C3B-C4B-C11B	-136.0(4)	C13B-C3B-C4B-C11B	-14.8(4)
C2B-C3B-C4B-N1B	48.2(6)	C13B-C3B-C4B-N1B	169.3(4)
C4B-N1B-C5B-C6B	178.0(4)	C4B-N1B-C5B-C10B	-0.2(4)
N1B-C5B-C6B-C7B	-178.5(4)	C10B-C5B-C6B-C7B	-0.5(5)
C5B-C6B-C7B-C8B	-1.0(6)	C14B-O1B-C8B-C9B	-0.9(5)
C14B-O1B-C8B-C7B	177.8(3)	C6B-C7B-C8B-O1B	-177.1(3)
C6B-C7B-C8B-C9B	1.6(5)	O1B-C8B-C9B-C10B	178.0(3)
C7B-C8B-C9B-C10B	-0.6(5)	C8B-C9B-C10B-C5B	-0.9(5)
C8B-C9B-C10B-C11B	177.9(4)	C6B-C5B-C10B-C9B	1.5(5)
N1B-C5B-C10B-C9B	179.9(3)	C6B-C5B-C10B-C11B	-177.6(3)
N1B-C5B-C10B-C11B	0.8(4)	N1B-C4B-C11B-C10B	1.0(4)
C3B-C4B-C11B-C10B	-175.8(3)	N1B-C4B-C11B-C12B	178.2(3)
C3B-C4B-C11B-C12B	1.4(5)	C9B-C10B-C11B-C4B	-180.0(4)
C5B-C10B-C11B-C4B	-1.1(4)	C9B-C10B-C11B-C12B	4.1(8)
C5B-C10B-C11B-C12B	-177.0(5)	C4B-C11B-C12B-C13B	13.1(5)
C10B-C11B-C12B-C13B	-171.1(5)	C11B-C12B-C13B-C3B	-22.1(5)
C4B-C3B-C13B-C12B	22.3(4)	C2B-C3B-C13B-C12B	146.4(4)
C8B-O1B-C14B-C15B	-174.6(3)	O1B-C14B-C15B-C20B	102.8(4)
O1B-C14B-C15B-C16B	-76.3(4)	C20B-C15B-C16B-C17B	-0.2(5)
C14B-C15B-C16B-C17B	178.9(3)	C15B-C16B-C17B-C18B	2.8(5)
C15B-C16B-C17B-C21B	-177.1(3)	C16B-C17B-C18B-C19B	-3.3(5)
C21B-C17B-C18B-C19B	176.5(3)	C16B-C17B-C18B-C22B	175.6(3)
C21B-C17B-C18B-C22B	-4.6(5)	C17B-C18B-C19B-C20B	1.5(6)
C22B-C18B-C19B-C20B	-177.5(4)	C16B-C15B-C20B-C19B	-1.7(5)
C14B-C15B-C20B-C19B	179.2(3)	C18B-C19B-C20B-C15B	1.0(6)
C16B-C17B-C21B-F1B	9.1(5)	C18B-C17B-C21B-F1B	-170.8(3)
C16B-C17B-C21B-F3B	-112.1(4)	C18B-C17B-C21B-F3B	68.0(4)
C16B-C17B-C21B-F2B	128.0(4)	C18B-C17B-C21B-F2B	-51.8(5)
C17B-C18B-C22B-C23B	112.1(5)	C19B-C18B-C22B-C23B	-69.0(6)
C17B-C18B-C22B-C26B	-129.3(5)	C19B-C18B-C22B-C26B	49.6(6)
C18B-C22B-C23B-C24B	164.9(5)	C26B-C22B-C23B-C24B	39.3(5)
C24B-C25B-C26B-C22B	24.6(8)	C23B-C22B-C26B-C25B	-41.0(6)
C18B-C22B-C26B-C25B	-166.7(5)	C26B-C25B-C24B-C23B	0.5(8)
C22B-C23B-C24B-C25B	-25.4(7)	O2A-C1A-C2A-C3A	101.1(5)
O1A-C1A-C2A-C3A	-78.6(4)	C1A-C2A-C3A-C4A	-60.1(5)
C1A-C2A-C3A-C13A	-173.8(4)	C5A-N1A-C4A-C11A	-0.3(6)
C5A-N1A-C4A-C3A	172.8(5)	C2A-C3A-C4A-C11A	-126.3(4)
C13A-C3A-C4A-C11A	-6.4(4)	C2A-C3A-C4A-N1A	60.8(7)
C13A-C3A-C4A-N1A	-179.3(5)	C4A-N1A-C5A-C6A	-177.1(6)
C4A-N1A-C5A-C10A	0.3(6)	N1A-C5A-C6A-C7A	176.1(6)

C10A-C5A-C6A-C7A	-1.1(9)	C5A-C6A-C7A-C8A	2.1(9)
C14A-O3A-C8A-C9A	-3.4(5)	C14A-O3A-C8A-C7A	177.5(3)
C6A-C7A-C8A-C9A	-0.7(8)	C6A-C7A-C8A-O3A	178.4(5)
O3A-C8A-C9A-C10A	179.4(3)	C7A-C8A-C9A-C10A	-1.6(5)
C8A-C9A-C10A-C5A	2.5(5)	C8A-C9A-C10A-C11A	-175.7(3)
N1A-C5A-C10A-C9A	-178.9(4)	C6A-C5A-C10A-C9A	-1.2(7)
N1A-C5A-C10A-C11A	-0.2(5)	C6A-C5A-C10A-C11A	177.5(5)
N1A-C4A-C11A-C10A	0.2(5)	C3A-C4A-C11A-C10A	-174.5(3)
N1A-C4A-C11A-C12A	177.2(4)	C3A-C4A-C11A-C12A	2.6(5)
C9A-C10A-C11A-C4A	178.4(4)	C5A-C10A-C11A-C4A	0.0(4)
C9A-C10A-C11A-C12A	2.7(8)	C5A-C10A-C11A-C12A	-175.6(5)
C4A-C11A-C12A-C13A	2.7(5)	C10A-C11A-C12A-C13A	178.2(4)
C11A-C12A-C13A-C3A	-6.7(5)	C4A-C3A-C13A-C12A	7.8(5)
C2A-C3A-C13A-C12A	130.5(4)	C8A-O3A-C14A-C15A	-175.8(3)
O3A-C14A-C15A-C16A	78.2(4)	O3A-C14A-C15A-C20A	-100.1(4)
C20A-C15A-C16A-C17A	-2.3(5)	C14A-C15A-C16A-C17A	179.4(3)
C15A-C16A-C17A-C18A	-1.9(5)	C15A-C16A-C17A-C21A	177.7(3)
C16A-C17A-C18A-C19A	4.4(5)	C21A-C17A-C18A-C19A	-175.3(3)
C16A-C17A-C18A-C22A	-176.2(3)	C21A-C17A-C18A-C22A	4.1(5)
C17A-C18A-C19A-C20A	-2.7(6)	C22A-C18A-C19A-C20A	177.8(4)
C18A-C19A-C20A-C15A	-1.4(6)	C16A-C15A-C20A-C19A	4.0(5)
C14A-C15A-C20A-C19A	-177.7(3)	C16A-C17A-C21A-F3A	-10.0(4)
C18A-C17A-C21A-F3A	169.7(3)	C16A-C17A-C21A-F2A	-128.5(3)
C18A-C17A-C21A-F2A	51.1(4)	C16A-C17A-C21A-F1A	111.4(3)
C18A-C17A-C21A-F1A	-68.9(4)	C19A-C18A-C22A-C23A	-47.3(5)
C17A-C18A-C22A-C23A	133.3(4)	C19A-C18A-C22A-C26A	72.3(5)
C17A-C18A-C22A-C26A	-107.1(4)	C18A-C22A-C23A-C24A	169.3(3)
C26A-C22A-C23A-C24A	43.3(4)	C22A-C23A-C24A-C25A	-29.1(5)
C23A-C24A-C25A-C26A	4.2(5)	C18A-C22A-C26A-C25A	-166.7(4)
C23A-C22A-C26A-C25A	-40.3(4)	C24A-C25A-C26A-C22A	22.1(5)

Table 7. Anisotropic atomic displacement parameters, (Å^2)

The anisotropic atomic displacement factor exponent takes the form: $-2\pi^2 [h^2a^{*2} U_{11} + ... + 2hka^* b^* U_{12}]$

	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
F1B	0.0527(13)	0.0456(13)	0.0619(15)	-0.0276(11)	0.0100(11)	-0.0068(10)
F2B	0.0763(16)	0.0262(10)	0.0430(11)	-0.0134(9)	0.0040(11)	-0.0029(10)
F3B	0.0898(19)	0.0456(13)	0.0277(10)	-0.0161(9)	0.0059(11)	-0.0116(12)
O1B	0.0423(12)	0.0247(11)	0.0415(12)	-0.0164(9)	-0.0162(10)	0.0001(9)
O2B	0.0567(16)	0.074(2)	0.0366(13)	-0.0243(13)	0.0045(12)	-0.0401(15)
O3B	0.0484(15)	0.0438(15)	0.0666(18)	-0.0343(14)	0.0153(13)	-0.0236(12)
N1B	0.0426(16)	0.0372(16)	0.0447(17)	-0.0244(13)	-0.0066(13)	-0.0070(13)
C1B	0.0412(19)	0.044(2)	0.052(2)	-0.0360(18)	0.0115(16)	-0.0181(16)
C2B	0.0416(19)	0.0427(19)	0.060(2)	-0.0305(18)	0.0022(16)	-0.0139(16)
C3B	0.0352(18)	0.0378(19)	0.088(3)	-0.040(2)	-0.0012(19)	-0.0053(15)
C4B	0.0323(16)	0.0325(17)	0.060(2)	-0.0286(16)	-0.0002(15)	-0.0053(13)
C5B	0.0343(16)	0.0312(17)	0.0405(18)	-0.0191(14)	-0.0037(14)	-0.0055(13)
C6B	0.048(2)	0.0292(17)	0.047(2)	-0.0137(15)	-0.0190(16)	-0.0009(15)
C7B	0.0452(19)	0.0243(15)	0.0458(19)	-0.0152(14)	-0.0171(15)	-0.0003(14)
C8B	0.0334(16)	0.0270(16)	0.0365(17)	-0.0156(13)	-0.0058(13)	-0.0047(12)
C9B	0.0305(16)	0.0265(16)	0.0377(17)	-0.0135(13)	-0.0061(13)	-0.0035(12)
C10B	0.0295(15)	0.0288(16)	0.0444(18)	-0.0169(14)	-0.0056(13)	-0.0025(12)
C11B	0.0330(16)	0.0288(17)	0.058(2)	-0.0211(15)	-0.0058(15)	-0.0022(13)
C12B	0.045(2)	0.0266(18)	0.091(3)	-0.0239(19)	-0.023(2)	0.0028(15)
C13B	0.060(3)	0.031(2)	0.128(5)	-0.037(3)	-0.041(3)	0.0089(18)
C14B	0.0406(17)	0.0272(15)	0.0370(17)	-0.0124(13)	-0.0115(14)	0.0010(13)

C15B	0.0392(17)	0.0264(15)	0.0306(16)	-0.0097(12)	-0.0105(13)	0.0011(13)
C16B	0.0400(17)	0.0294(16)	0.0286(15)	-0.0088(12)	-0.0061(13)	-0.0040(13)
C17B	0.0474(19)	0.0283(15)	0.0230(14)	-0.0074(12)	-0.0060(13)	-0.0043(14)
C18B	0.054(2)	0.0374(18)	0.0268(15)	-0.0118(13)	-0.0088(14)	-0.0112(16)
C19B	0.0426(19)	0.052(2)	0.0358(17)	-0.0201(16)	-0.0056(14)	-0.0136(16)
C20B	0.0386(17)	0.045(2)	0.0323(16)	-0.0202(15)	-0.0035(13)	-0.0056(15)
C21B	0.059(2)	0.0302(17)	0.0318(17)	-0.0124(14)	-0.0001(16)	-0.0081(16)
C22B	0.071(3)	0.049(2)	0.0340(18)	-0.0206(16)	-0.0061(18)	-0.021(2)
C23B	0.109(4)	0.065(3)	0.050(2)	-0.029(2)	0.009(3)	-0.051(3)
C25B	0.141(7)	0.149(8)	0.103(6)	-0.068(6)	-0.033(5)	-0.060(6)
C26B	0.096(4)	0.101(5)	0.086(4)	-0.058(4)	-0.040(3)	-0.014(3)
C24B	0.135(6)	0.111(5)	0.096(5)	-0.071(4)	0.014(4)	-0.081(5)
F1A	0.0614(13)	0.0406(12)	0.0256(9)	-0.0115(8)	0.0019(9)	-0.0097(10)
F2A	0.0535(12)	0.0291(10)	0.0405(10)	-0.0159(8)	0.0014(9)	-0.0047(9)
F3A	0.0411(11)	0.0496(13)	0.0561(13)	-0.0320(11)	0.0035(10)	-0.0117(10)
O1A	0.0627(19)	0.0423(16)	0.0624(19)	-0.0151(14)	0.0215(15)	-0.0163(14)
O2A	0.108(3)	0.079(2)	0.0381(15)	-0.0139(15)	-0.0035(16)	-0.066(2)
O3A	0.0475(14)	0.0270(11)	0.0371(12)	-0.0155(9)	-0.0116(10)	-0.0055(10)
N1A	0.084(3)	0.0402(19)	0.104(3)	-0.045(2)	-0.067(3)	0.0221(18)
C1A	0.074(3)	0.040(2)	0.047(2)	-0.0337(18)	0.020(2)	-0.026(2)
C2A	0.073(3)	0.0322(18)	0.060(2)	-0.0270(18)	0.014(2)	-0.0092(18)
C3A	0.0416(18)	0.0407(19)	0.057(2)	-0.0317(17)	-0.0023(16)	-0.0110(15)
C4A	0.044(2)	0.0370(19)	0.072(3)	-0.0341(19)	-0.0198(19)	0.0046(15)
C5A	0.067(3)	0.036(2)	0.078(3)	-0.035(2)	-0.042(2)	0.0102(18)
C6A	0.108(4)	0.041(2)	0.108(4)	-0.044(3)	-0.080(4)	0.027(2)
C7A	0.082(3)	0.0283(19)	0.078(3)	-0.028(2)	-0.046(3)	0.0133(19)
C8A	0.0382(17)	0.0290(16)	0.0392(17)	-0.0196(14)	-0.0071(14)	-0.0058(13)
C9A	0.0318(15)	0.0279(15)	0.0290(15)	-0.0116(12)	-0.0029(12)	-0.0039(12)

C10A	0.0331(16)	0.0285(17)	0.0371(16)	-0.0173(13)	-0.0036(13)	-0.0010(13)
C11A	0.0334(16)	0.0321(17)	0.0395(17)	-0.0205(14)	-0.0029(13)	-0.0057(13)
C12A	0.100(4)	0.0271(17)	0.0384(19)	-0.0083(14)	-0.021(2)	-0.020(2)
C13A	0.059(2)	0.0309(19)	0.082(3)	-0.028(2)	-0.023(2)	-0.0025(17)
C14A	0.0407(17)	0.0317(16)	0.0339(16)	-0.0178(13)	-0.0060(13)	-0.0050(14)
C15A	0.0428(18)	0.0291(16)	0.0308(16)	-0.0133(13)	-0.0071(14)	-0.0061(14)
C16A	0.0375(16)	0.0273(15)	0.0280(14)	-0.0089(12)	-0.0048(12)	-0.0077(13)
C17A	0.0391(17)	0.0264(15)	0.0253(14)	-0.0095(12)	-0.0029(12)	-0.0060(13)
C18A	0.0449(18)	0.0325(17)	0.0273(15)	-0.0125(13)	-0.0018(13)	-0.0120(14)
C19A	0.0417(19)	0.0448(19)	0.0354(17)	-0.0204(15)	0.0001(14)	-0.0152(15)
C20A	0.0393(17)	0.0411(19)	0.0329(16)	-0.0188(14)	0.0003(13)	-0.0100(15)
C21A	0.0406(18)	0.0287(16)	0.0311(16)	-0.0108(13)	-0.0010(14)	-0.0086(14)
C22A	0.050(2)	0.049(2)	0.0364(18)	-0.0258(16)	0.0063(16)	-0.0222(17)
C23A	0.057(3)	0.090(4)	0.058(3)	-0.048(3)	-0.003(2)	-0.028(2)
C24A	0.078(3)	0.127(5)	0.056(3)	-0.061(3)	0.021(3)	-0.063(4)
C25A	0.091(4)	0.091(4)	0.080(4)	-0.061(3)	0.029(3)	-0.064(3)
C26A	0.072(3)	0.051(2)	0.046(2)	-0.0292(19)	0.016(2)	-0.034(2)

Table 8. Hydrogen atom coordinates and isotropic atomic displacement parameters, (Å^2)

	x/a	y/b	z/c	U
H2BB	0.974(6)	0.469(6)	0.617(6)	0.077
H1BB	0.766(4)	0.240(4)	0.492(4)	0.029(9)
H2BC	0.8568	0.5600	0.3307	0.055
H2BD	0.7553	0.6615	0.4029	0.055
H3BA	0.5793	0.5329	0.4487	0.060
H6BA	0.8034	-0.0041	0.4417	0.048
H7BA	0.7571	-0.1076	0.3032	0.044
H9BA	0.5752	0.2410	0.0964	0.037
H12C	0.4676	0.5350	0.2050	0.063
H12D	0.5992	0.5465	0.0880	0.063
H13C	0.5475	0.6884	0.2574	0.081
H13D	0.6991	0.6506	0.1819	0.081
H14C	0.6131	0.1100	-0.0515	0.041
H14D	0.4735	0.0978	0.0540	0.041
H16B	0.7227	-0.0896	-0.1396	0.039
H19B	0.2935	-0.2150	-0.0221	0.049
H20B	0.3366	-0.0579	0.0602	0.045
H22B	0.5254	-0.3706	-0.2542	0.058
H23C	0.3541	-0.4629	-0.0059	0.084
H23D	0.4997	-0.5367	-0.0638	0.084
H25C	0.3247	-0.4306	-0.3513	0.138
H25D	0.1860	-0.4179	-0.2457	0.138

H26C	0.2444	-0.2625	-0.1869	0.099
H26D	0.3383	-0.2364	-0.3308	0.099
H24C	0.4136	-0.5978	-0.2062	0.121
H24D	0.2746	-0.5860	-0.1012	0.121
H1AB	0.822(7)	0.330(7)	0.763(7)	0.091
H1AC	0.921(6)	0.529(5)	0.935(6)	0.065(15)
H2AC	1.1436	0.2133	0.9078	0.068
H2AD	1.0336	0.1159	0.9498	0.068
H3AB	0.8992	0.2282	1.1033	0.051
H6AB	0.9181	0.7782	0.9555	0.090
H7AB	0.9885	0.8844	1.0781	0.069
H9AB	1.1731	0.5376	1.2807	0.035
H12E	1.0841	0.2312	1.3241	0.063
H12F	1.2269	0.2324	1.2217	0.063
H13E	1.0321	0.0924	1.2366	0.064
H13F	1.1655	0.1085	1.1238	0.064
H14E	1.2854	0.6871	1.3168	0.040
H14F	1.1448	0.6688	1.4198	0.040
H16C	1.0254	0.8606	1.5139	0.037
H19C	1.4418	1.0113	1.4027	0.046
H20C	1.4102	0.8576	1.3091	0.044
H22C	1.2047	1.1375	1.6521	0.051
H23E	1.4903	1.0666	1.5627	0.073
H23F	1.4059	1.0168	1.7092	0.073
H24E	1.5225	1.2252	1.6450	0.092
H24F	1.3812	1.2169	1.7489	0.092
H25E	1.2831	1.3870	1.6070	0.095
H25F	1.4213	1.3877	1.4991	0.095

H26E	1.2037	1.3159	1.4692	0.064
H26F	1.3540	1.2623	1.4004	0.064

Table 9. Selected hydrogen bond information , (Å and °).

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
N1B-H1BB...O3B	0.91(4)	2.54(4)	3.096(4)	120(3)
O1A-H1AB...O3B	0.94(7)	1.79(7)	2.703(5)	166(6)
O2B-H2BB...O2A	1.01(6)	1.59(6)	2.600(5)	175(6)

In Vitro / In Vivo Pharmacology Materials and Methods

Animals: Rats and mice were used in the in vivo pharmacology assays. All animal studies were performed according to the Guide for the Care and Use of Laboratory Animals published by the National Academy of Sciences (1996). All study protocols were reviewed and approved by Arena Pharmaceuticals Institutional Animal Care and Use Committee (IACUC). Animals were housed in standard cages and were maintained at 25$\pm$1^{o}C under 12 hour light and dark cycles. Water and standard diet was provided *ad libitum*.

Chemicals: APD334, (R)-2-(7-(4-cyclopentyl-3-(trifluoromethyl)benzyloxy)-1,2,3,4-tetrahydro cyclopenta[b]indol-3-yl)acetic acid, was synthesized at Arena Pharmaceuticals. APD334 was suspended in 0.5% methyl cellulose (MC). MTX was purchased from Sigma, St. Louis, MO. Gilenya® was purchased from Cayman, Ann Arbor, MI. and was used to synthesize Gilenya®-P. Bovine type II collagen was purchased from Elastin Products, Owensville, MO. Freund's complete adjuvant was purchased from

Difco, Detroit, MI for CIA experiments and from Chondrex, Redmond, WA, for EAE experiments. MOG_{35-55} peptide was purchased from NeoMPS, San Diego, CA.

Agonist HTRF cAMP Assay: HTRF cAMP assays were performed according to the manufacturer's instructions (Cisbio, cAMP Dynamic 2 Assay Kit 62AM4PEJ). HEK 293 cells stably expressing either recombinant human, mouse, rat, dog or monkey $S1P_1$ receptor were harvested with trypsin/EDTA, quenched with Trypsin Inhibitor-Soybean Protein (Invitrogen 17075) and suspended in PBS supplemented with 0.05% fatty acid free BSA (Sigma A8806). 5 µL of this suspension was plated into PE Proxiplate 384-Plus (Perkin Elmer 6008280) plates. Cell number per well was receptor dependent but averaged approximately 1500. Compounds were suspended and serially diluted in DMSO using 5-fold dilutions to generate a 10-point dose-response curve with a top concentration of 10 µM. Serial diluted compounds were uniformly diluted further in PBS-based assay buffer containing 10 µM forskolin (F6886), 1 mM IBMX (Sigma I5879) supplemented with 0.05% fatty acid free BSA (Sigma A8806) to achieve a 2X stock. Five µL per well of compound solution was transferred to the assay plates in triplicate along with a cAMP standard curve. After 1 hour at room temperature, 5 µl of cAMP-D2 reagent diluted in Lysis Buffer was added followed by 5 µL of Cryptate reagent (all supplied with kit). Plates were then incubated at room temperature for 1 hour prior to reading. Time-resolved fluorescence measurements were collected on PerkinElmer Envision or BMG Pherastar microplate readers. Dose response curves were generated using a nonlinear least squares curve fitting program to obtain EC_{50} values.

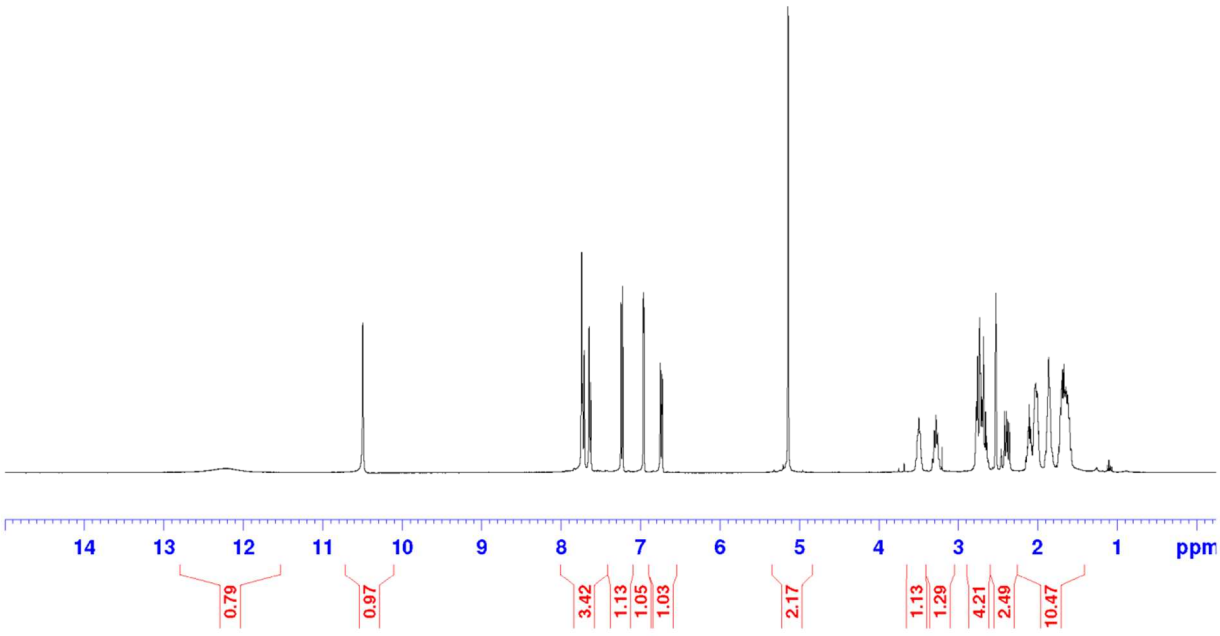
Mouse and Rat Lymphopenia: APD334 induced effects on blood lymphopenia were determined in male BALB/c mice and male Sprague-Dawley rats. Briefly, male rats and mice were given a 0 (vehicle only), 0.03 (mice only), 0.1, 0.3 or 1 mg/kg oral dose of APD334 formulated in 0.5% methylcellulose (MC) in water. Mouse blood samples were taken at 0, 1, 3, 5, 8, 16, 24 and 32 hours post-dose. Rat blood samples were collected at 0, 1, 3, 5, 8, 16, 24, 32, 48 and 72 hours post-dose. Blood lymphocyte levels were determined using an Abbott Cell-Dyn 3700 instrument (Abbott Diagnostics, Il).

Experimental Autoimmune Encephalomyelitis: Female C57BL/6 mice (8-10 week old) were procured from Charles River Laboratories. Mice were immunized subcutaneously with a total of 100 mg MOG_{35-55} peptide (NeoMPS, San Diego, CA) emulsified 1:1 with Freund's complete adjuvant containing 4 mg/ml

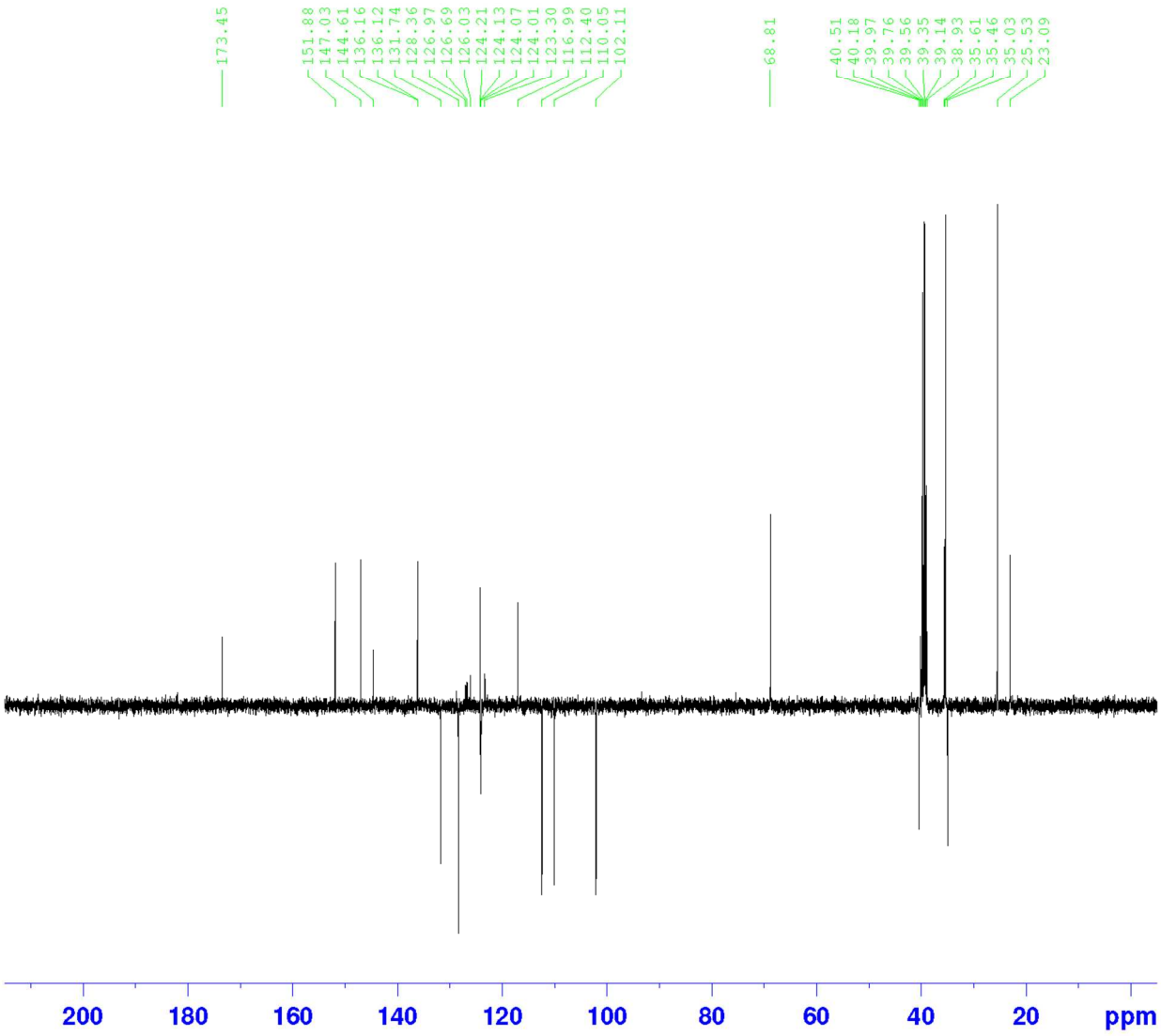
heat-killed Mycobacterium tuberculosis. Mice received 200 ng pertussis toxin intraperitoneally on day of immunization and again 48 hours later. Mice were monitored daily from day 7 for disease symptoms. The following disease scoring scale was used: 0 = Healthy; 1 = limp tail or hind limb weakness; 2 = limp tail and limb weakness or weakness of 2 or more limbs; 3 = severe limb weakness or single limb paralysis; 4 = paralysis of 2 or more limbs; 5 = death. In the prophylactic dosing study, mice were dosed orally, once daily from days 3-25; and in the therapeutic dosing study from days 18-37. Compounds were suspended in 0.5% MC vehicle using a sonicator to ensure uniform suspension. Dosing volume was 5 ml/kg. APD334 was dosed at 0.3, 1 and 3 mg/kg and Gilenya® at 1 mg/kg dose. 12 mice were used per group. On day 37, spinal cord and brain integrity was assessed by immunohistochemistry staining of 6 mM thick acetone-fixed frozen spinal cord sections. Infiltrating T cells were identified by direct immunohistochemistry using PE-conjugated anti-CD3 mAb and FITC-conjugated anti-CD4 mAb (BD Pharmingen, San Jose, CA). Nuclei were stained with DAPI and slides were mounted with Vectashield hard set mounting medium (Vector Laboratories, Burlingame, CA).

Data analysis. Statistical analysis was performed on the data from the EAE disease model using one-way analysis of variance (ANOVA) test followed by Dunnett's multiple comparison post-test. Histopathology parameters were evaluated using a Student's t-test with significance set at 5%.

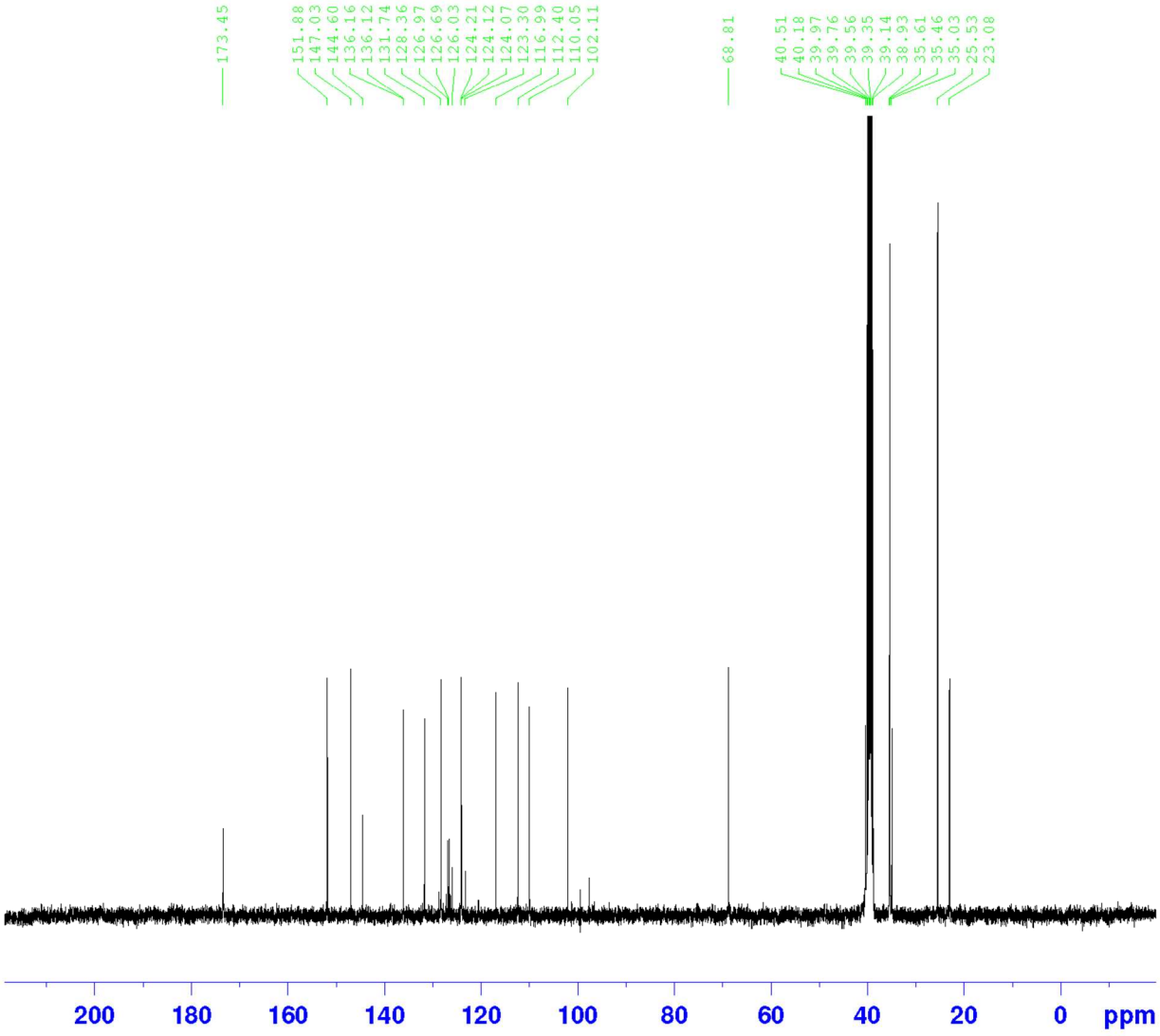
APD334 ^{1}H NMR (DMSO-d_6):



APD334 ^{13}C NMR APT (DMSO-d_6):



APD334 ^{13}C (DMSO-d_6):



APD334 ^{19}F NMR (DMSO-d_6):