Structure-Activity Relationship of Novel and Selective Biaryl-Chroman GPR40 AgoPAMs

Supporting Information

1. General Methods
2. Synthesis of Key Compounds
3. Sterochemical Determination by ECD
4. Metabolites of ***AP5*** and ***14***
5. Rat IP1 EC50 for Select Compounds in Table 2
6. *In Vitro* Inositol Phosphate Turnover (IP1) Assay
7. *In Vivo* SD Rat ipGTT Assay
8. *In Vivo* GK Rat oGTT Assay
9. *In Vivo* GLP-1 Secretion Assay
10. *In Vitro* BSEP Assay
11. HPLC log D (pH 7) Determination
12. Off-Target Hits (Table 4)

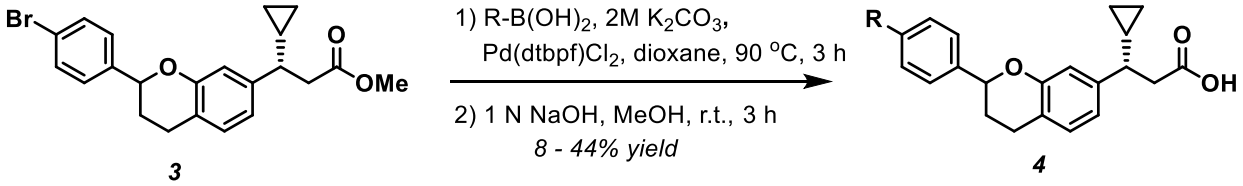
1. General Methods

Unless otherwise noted, all materials were obtained from commercial suppliers and used without purification. Dry organic solvents were purchased from Sigma-Aldrich and packaged under nitrogen in Sure Seal bottles. Reactions were monitored using thin-layer chromatography on 250 µm plates or using Agilent 1100 series LCMS with UV detection at 254 nm and a low resonance electrospray mode (ESI). Purification of title compounds was accomplished by flash column chromatography using silica gel 60 (particle size 0.04-0.063 mm, 230-400 mesh) or medium pressure liquid chromatography (MPLC) on a CombiFlash Companion (Teledyne Isco) with RediSep normal phase silica gel. ^{1}H NMR spectra were recorded on a Varian spectrometer (500 MHz) at ambient temperature. Chemical shifts are reported in ppm relative to $CDCl_3$ or CD_3OD and coupling constants (J) are reported in hertz (Hz). Supercritical fluid chromatography for the separation of isomeric mixtures was performed using a Berger multigram TM SFC (Mettler Toledo Co, Ltd) with a column temperature of 40 ^{o}C, a nozzle pressure of 100 Bar, and

monitored at a wavelength of 220 nm unless otherwise noted. Purity of final compounds was ≥95% based on analytical HPLC and NMR analysis.

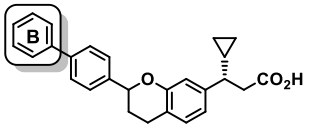
2. Synthesis of Key Compounds:

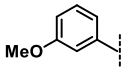
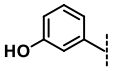
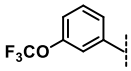
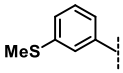
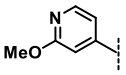
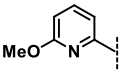
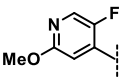
General Procedure for Suzuki coupling (Scheme 1, Table 1):



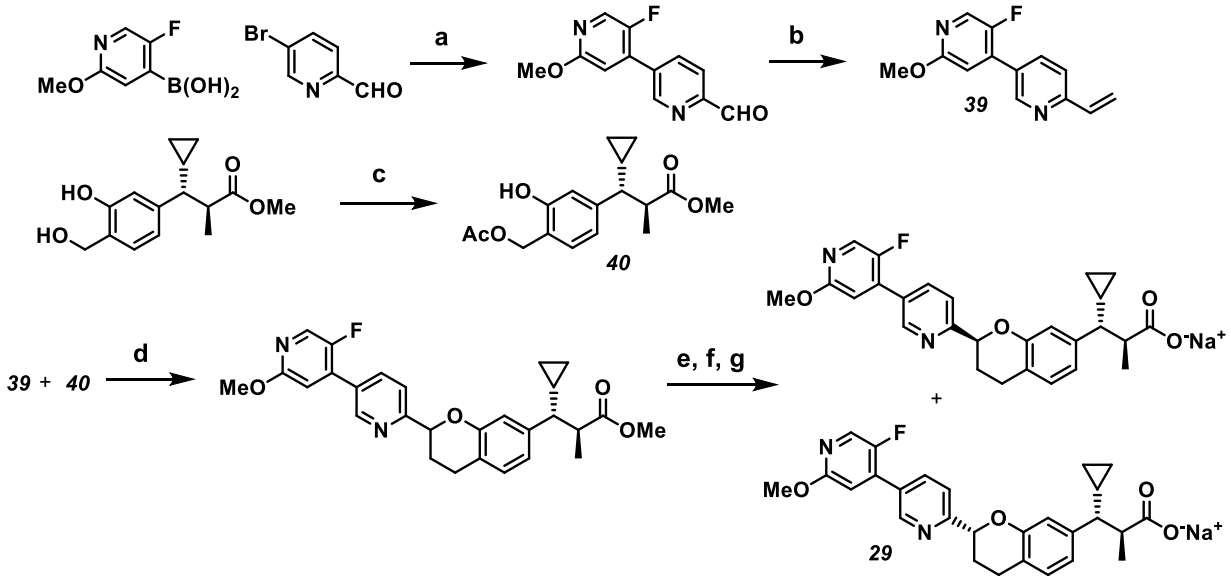
3 ***4***

To vials containing a 1 mL (0.07 mmol) solution (0.07 mmol) of (3S)-methyl 3-(2-(4-bromophenyl)chroman-7-yl)-3-cyclopropylpropanoate ***3***[17] in dioxane (made from dissolving 617 mg in 21mL dioxane) was added individual boronic acids, $Pd(dtbpf)Cl_2$ (4.6mg) , and 2M aq K_2CO_3 (0.25 mL) sequentially. The mixtures were heated at 90 °C for 3h. The aqueous layer was removed using a pipette. DMT-silica (3eq) was added and the solutions were stirred at ambient temperature for 16 h. They were then filtered. MeOH (0.5 mL) and 1N NaOH (0.5 mL) were added to the filtrate. The mixtures were stirred for 3 h. Added 2N HCl (0.5 mL) to the mixtures and stirred for 20 min. The mixtures were then concentrated on GeneVac. The residues were dissolved in 1.4 ml DMSO and filtered. The filtrates were directly purified by reverse phase chromatography (Waters Sunfire C18 5 um, 19X100 mm; flow rate = 50 ml/min; 8 minute run; gradient: 40-95% (water + 0.1% formic acid / AcCN + 0.1% formic acid)). Yields ranged from 8-44%.



R	Compound	% Isolated Yield	LC/MS (m/z) (M+H)+
	6	31%	429.2
	7	33%	399.2
	8	33%	415.2
	9	30%	483.2
	10	44%	445.2
	11	25%	430.2
	12	15%	430.2
	13	8%	448.2

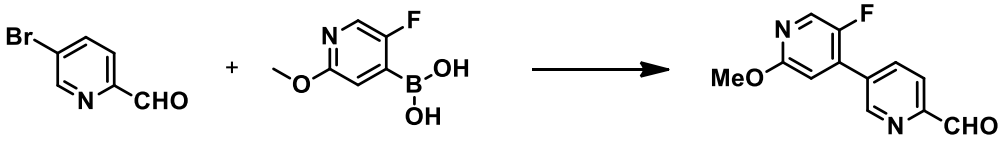
Synthesis of ***29***:



Reagents and conditions: (a) Sphos Second Generation Precatalyst, 3 M aq. K_3PO_4, THF, 80 ^oC, 59%; (b) $MePPh_3^+Br^-$, $tBuO^-K^+$, THF, 0 ^oC, 61%; (c) pyridine, AcCl, DCM, 0 ^oC, 90%; (d) xylenes, 170 ^oC , 41%; (e) 1 M aq. LiOH, MeOH/THF, 65 ^oC, 89%; (f) SFC separation: Chiral-pak AD-H (50 x 250 mm; 65% MeOH/CO_2), Diastereomer A: 33%, Diastereomer B: 34%; (g) 1 M aq. NaOH, ACN/water, 95%.

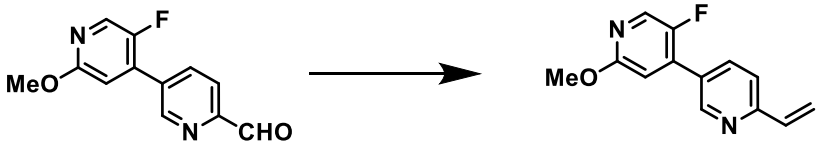
Preparation of 5'-fluoro-2'-methoxy-6-vinyl-3,4'-bipyridine

Step A:



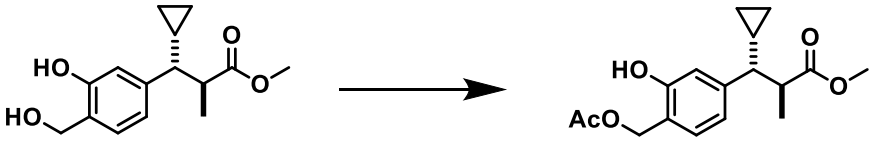
To a microwave vial containing a mixture of 5-bromopicolinaldehyde (1.5 g, 8.06 mmol), (5-fluoro-2-methoxypyridin-4-yl)boronic acid (1.68 g, 9.83 mmol), and S-Phos Second Generation Precatalyst (0.291 g, 0.403 mmol) which had been evacuated and filled with N_2 (3X), was added a N_2-sparged mixture of THF (anh.) (40.3 ml)/ 3M aqueous solution of potassium phosphate tribasic (8.06 ml, 24.19 mmol). The reaction vessel was evacuated and filled with N_2 (3X), then heated conventionally in a heating block to 80 °C for 2 h. The reaction was cooled to ambient temperature and partitioned between EtOAc and sat. NH_4Cl. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting crude residue was purified *via* MPLC (ISCO 80 g; prod. elutes at 24% EtOAc/hexane) with gradient elution 0-50% EtOAc/hexane to give a yellow solid (1.1072 g, 59%). ^{1}H NMR (500 MHz, $CDCl_3$): 3.96 (s, 3 H), 6.85 (d, 1 H), 8.10-8.06 (m, 2 H), 8.15 (d, 1 H), 8.98 (s, 1 H), 10.13 (s, 1 H).

Step B:



To a cooled mixture of methyltriphenylphosphonium bromide (2.214 g, 6.20 mmol) in THF (anh., 20 mL) at 0 °C/N_2 was added dropwise a 1 M solution of potassium tert-butoxide in THF (6.20 ml, 6.20 mmol). The resulting yellow mixture was stirred at 0 °C for 30 min. , before adding dropwise a solution of 5'-fluoro-2'-methoxy-[3,4'-bipyridine]-6-carbaldehyde (1.1072 g, 4.77 mmol) in anh. THF (10 mL). Rinsed flask with 2.0 mL of anh.THF and added to reaction. The ice bath was removed after addition, and the resulting dark green/brown reaction mixture was warmed to r.t. Stirred for 10 min., at which point the reaction was mostly complete by TLC (30% EtOAc/hexane). The reaction mixture was partitioned between EtOAc and sat. NH_4Cl. The aqueous layer was back-extracted with EtOAc and the combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting crude residue was purified *via* MPLC (ISCO 40 g; prod. elutes at 20% EtOAc/hexane) with gradient elution 0-40% EtOAc/hexane to give a yellow solid (0.6733 g, 61%). ^{1}H NMR (500 MHz, $CDCl_3$): 3.95 (s, 3 H), 5.57 (dd, 1 H, *J* = 10.79, 1.12 Hz), 6.30 (dd, 1 H, *J* = 17.46, 1.13 Hz), 6.89-6.81 (m, 2 H), 7.44 (d, 1 H), 7.88 (dt, 1 H, *J* = 8.17, 1.97 Hz), 8.09 (d, 1 H), 8.78 (s, 1 H).

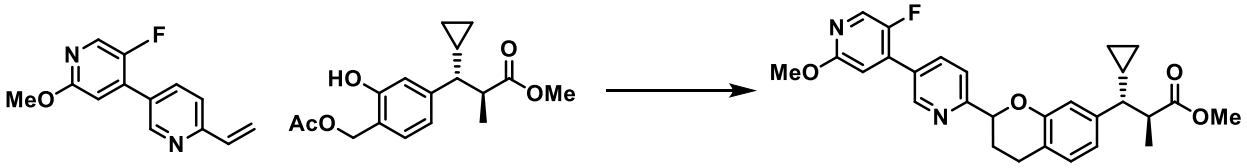
Preparation of (*2S,3R*)-methyl 3-(4-(acetoxymethyl)-3-hydroxyphenyl)-3-cyclopropyl-2-methylpropanoate (Step C)



Pyridine (4.0 ml, 49.5 mmol) was added dropwise to a stirred, cooled mixture of (2S,3R)-methyl 3-cyclopropyl-3-(3-hydroxy-4-(hydroxymethyl)phenyl)-2-methylpropanoate[15, 18] (10.94 g, 41.4 mmol) in CH_2Cl_2 (anh.) (200 ml) at 0 °C. A solution of acetyl chloride (2.94 ml, 41.4 mmol) in 7 mL of CH_2Cl_2 (anh.) was added dropwise to the resulting solution. The reaction was stirred at 0 °C for 10 min. The reaction was partitioned between DCM and sat. NH_4Cl. The layers were separated and the organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated in vacuo. The resulting residue was purified *via* MPLC (ISCO 220 g; prod. elutes at 28% EtOAc/hexane) with gradient elution 0-100% EtOAc/hexane to give a colorless viscous/sticky oil (11.3974 g, 90%). ^{1}H NMR (500 MHz, $CDCl_3$): 0.01 (m, 1 H), 0.23 (dq, 1 H), 0.35-0.29 (m, 1 H), 0.57-0.52 (m, 1 H), 0.93 (d, 3 H), 1.02 (m, 1 H), 1.87 (t, 1 H), 2.12 (s, 3 H), 2.82-2.76 (m, 1 H), 3.72 (s, 3 H), 5.09 (s, 2 H), 6.70 (d, 1 H), 6.76 (s, 1 H), 7.18 (d, 1 H), 7.91 (s, 1 H).

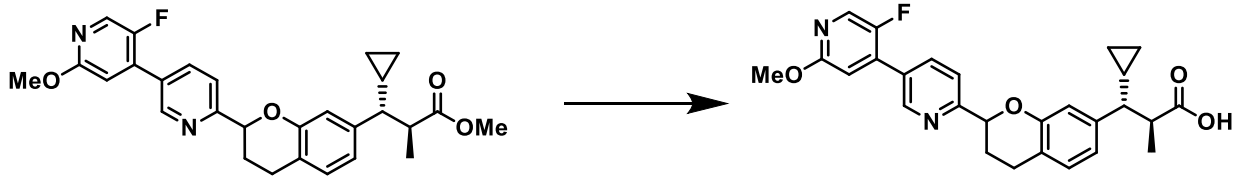
Preparation of sodium (*2S,3R*)-3-cyclopropyl-3-(2-(5-(2-fluoro-5-methoxyphenyl)-6-methylpyridin-2-yl)chroman-7-yl)-2-methylpropanoate

Step D:



A microwave vial equipped with a stirrer bar was charged with 5'-fluoro-2'-methoxy-6-vinyl-3,4'-bipyridine (0.9733 g, 4.23 mmol), (*2S,3R*)-methyl 3-(4-(acetoxymethyl)-3-hydroxyphenyl)-3-cyclopropyl-2-methylpropanoate (0.7607 g, 2.48 mmol), and xylenes (2.5 mL). The mixture was evacuated and filled with N_2 (3X). The reaction vessel was sealed and heated to 170 °C in a heating block for 50 min. The light brown reaction was concentrated in vacuo and the crude residue was purified *via* MPLC (ISCO 40 g; prod. elutes at 20% EtOAc/hexane) with gradient elution 0-40% EtOAc/hexane to give a pale yellow foam (0.4819 g, 41%). ^{1}H NMR (500 MHz, $CDCl_3$): 0.06 (d, *J* = 1.3 Hz, 1H), 0.25 (dt, 1 H), 0.34 (m, 1 H), 0.56 (m, 1 H), 0.97 (d, 3 H), 1.05 (m, 1 H), 1.88 (t, *J* = 10.1 Hz, 1H), 2.13 (m, 1 H), 2.48 (ddt, *J* = 12.3, 5.9, 3.4 Hz, 1H), 2.88 – 2.63 (m, 2H), 3.00 (ddd, *J* = 16.5, 10.8, 5.8 Hz, 1H), 3.72 (s, 3H), 3.96 (s, 3H), 5.24 (d, *J* = 9.7 Hz, 1 H), 6.70 (dd, *J* = 7.7, 1.6 Hz, 1 H), 6.78 (t, *J* = 1.7 Hz, 1 H), 6.84 (d, *J* = 5.2 Hz, 1 H), 7.04 (d, *J* = 7.7 Hz, 1 H), 7.71 (d, *J* = 8.2 Hz,1 H), 7.97 (dd, , *J* = 8.2, 1.7 Hz, 1H), 8.11 (d, *J* = 2.1 Hz, 1H), 8.80 (s, 1 H).

Step E:



To a solution of (*2S,3R*)-methyl 3-cyclopropyl-3-(2-(5'-fluoro-2'-methoxy-[3,4'-bipyridin]-6-yl)chroman-7-yl)-2-methylpropanoate (0.2187 g, 0.459 mmol) in THF (4.6 ml)/MeOH (4.6 ml) was added 1 M LiOH (4.6 mL, 4.6 mmol) at ambient temperature. The resulting mixture was heated to 65 °C for 19 h. The reaction was concentrated in vacuo. Suspended residue in EtOAc and added 4.8 mL of 1 N HCl . The mixture was partitioned between EtOAc and brine. The aqueous layer was back-extracted with EtOAc (2X). The combined organic layers were dried over $MgSO_4$, and concentrated in vacuo. Purified residue *via* MPLC (ISCO 24 g; prod. elutes at 40% EtOAc/hexane) with gradient elution 0-50% EtOAc/hexane to give a white foam (0.1896 g, 89%). LC/MS (*m/z*): 463.2 $(M+H)^+$.

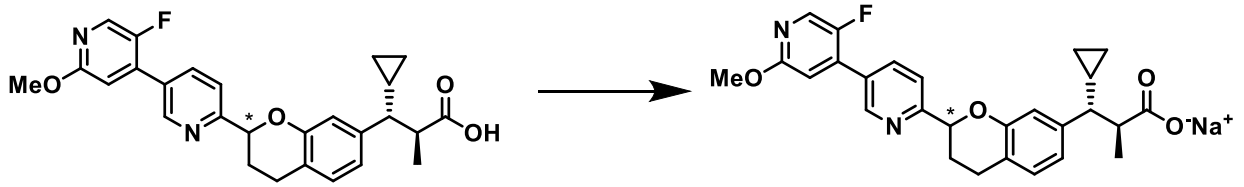
Step F:

The mixture of diastereomers were resolved on a Chiral-pak AD-H (50X250 mm; 65% MeOH/CO_2 to give 2 fractions after concentration in vacuo as pale yellow solids.

Peak 1: (0.0629 g, 33%); faster eluting peak; ^{1}H NMR (500 MHz, $CDCl_3$): 0.04 (m, 1H), 0.40-0.35 (m, 2 H), 0.60 (m, 1H), 0.94 (d, *J* = 6.6 Hz, 3H), 1.01 (m, 1H), 1.94 (t, *J* = 9.3 Hz, 1H), 2.23 – 2.06 (m, 1H), 2.45 (d, *J* = 13.7 Hz, 1H), 2.91 – 2.67 (m, 2H), 2.98 (td, *J* = 10.7, 5.5 Hz, 1H), 3.93 (s, 3H), 5.25 – 5.16 (m, 1H), 6.70 (d, *J* = 7.7 Hz, 1H), 6.77 (s, 1H), 6.81 (d, *J* = 5.2 Hz, 1H), 7.01 (d, *J* = 7.6 Hz, 1H), 7.68 (d, *J* = 8.2 Hz, 1H), 7.94 (d, *J* = 8.1 Hz, 1H), 8.09 (d, *J* = 2.0 Hz, 1H), 8.77 (s, 1H).

Peak 2: (0.0638 g, 34%); slower eluting peak; ^{1}H NMR (500 MHz, $CDCl_3$): 0.04 (m, 1H), 0.40-0.36 (m, 2 H), 0.60 (m, 1 H), 0.94 (d, *J* = 6.6 Hz, 3H), 1.03 (d, 3 H), 1.13-1.10 (m, 1 H), 1.93 (t, 1 H), 2.19 – 2.06 (m, 1H), 2.45 (d, *J* = 13.6 Hz, 1H), 2.80 – 2.68 (m, 1H), 2.98 (ddd, *J* = 16.4, 10.6, 5.8 Hz, 1H), 5.22 (d, *J* = 7.6 Hz, 1H), 6.70 (d, *J* = 8.0 Hz, 1H), 6.78 (s, 1H), 6.82 (d, *J* = 5.2 Hz, 1H), 7.01 (d, *J* = 7.5 Hz, 1H), 7.68 (d, *J* = 8.2 Hz, 1H), 7.94 (d, *J* = 8.2 Hz, 1H), 8.09 (d, *J* = 2.0 Hz, 1H), 8.78 (s, 1H).

Step G:



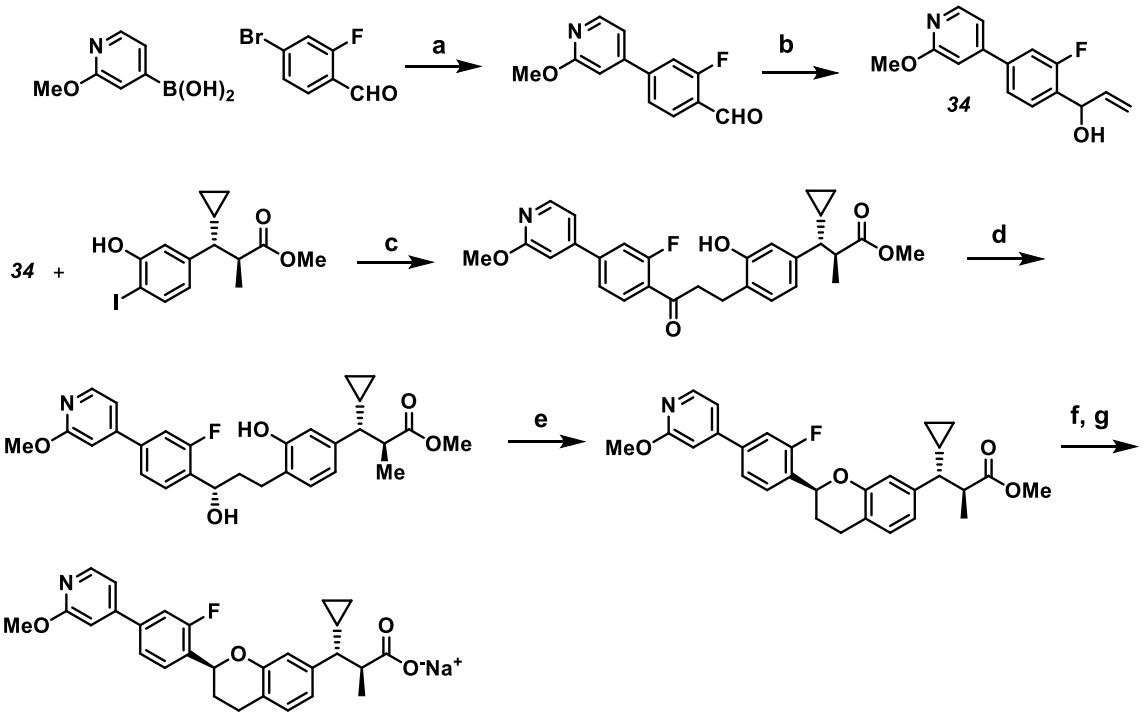
To a solution of (*2S,3R*)-3-cyclopropyl-3-(2-(5'-fluoro-2'-methoxy-[3,4'-bipyridin]-6-yl)chroman-7-yl)-2-methylpropanoic acid (Peak 2, 0.0638 g, 0.138 mmol) in Acetonitrile (2 ml)/Water (1 ml) was added 1 M NaOH (0.138 ml, 0.138 mmol) at ambient temperature. Using sonication, ensured that all was in solution. The resulting pale yellow solution was lyophilized to give a cream solid (56.2 mg, 84%).

^{1}H NMR (500 MHz, CD_3OD) δ 8.84 – 8.72 (m, 1H), 8.21 – 8.05 (m, 2H), 7.77 (dt, *J* = 8.3, 0.7 Hz, 1H), 7.00 (dd, *J* = 9.0, 6.5 Hz, 2H), 6.85 – 6.59 (m, 2H), 5.20 (dd, *J* = 9.7, 2.6 Hz, 1H), 3.94 (s, 3H), 2.98 (ddd, *J* = 16.4, 10.6, 5.8 Hz, 1H), 2.74 (dt, *J* = 16.4, 4.8 Hz, 1H), 2.62 (dq, *J* = 10.4, 6.9 Hz, 1H), 2.49 – 2.29 (m, 1H), 2.25 – 2.01 (m, 1H), 1.95 (t, *J* = 10.0 Hz, 1H), 1.07 (dtt, *J* = 9.7, 8.1, 5.0 Hz, 1H), 0.85 (d, *J* = 6.8 Hz, 3H), 0.55 (tdd, *J* = 8.7, 5.9, 4.6 Hz, 1H), 0.48 – 0.40 (m, 1H), 0.27 – 0.17 (m, 1H), -0.02 – -0.11 (m, 1H).

^{13}C NMR (126 MHz, CD_3OD) δ 183.10, 160.95, 160.33, 153.13, 147.16, 143.49, 136.71, 135.58, 133.03, 128.00, 127.66, 119.94, 119.64, 118.40, 115.25, 109.50.

HRMS calculated for $C_{27}H_{28}FN_2O_4^+$ $(M+H)^+$: 463.2028, Found: 463.2030.

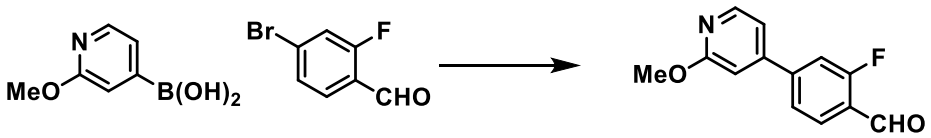
Synthesis of ***AP5***:



Reagents and conditions: (a) Sphos Second Generation Precatalyst, 3 M aq. K_3PO_4, THF, 80 °C, 89%; (b) 1 M vinyl magnesium bromide, THF, 0 °C, 65%; (c) *t*-BuXphos Palladacycle, Cy_2NMe, toluene, 90 °C, 49%; (d) formic acid, NEt_3, RuCl[(*R,R*)-Tsdpen](Mesitylene),EtOAc, quant.; (e) *t*BuP, DIAD, THF, 94%; (f) 1 M aq. LiOH, MeOH/THF, 55-58 °C, 96%; (g) 1 M aq. NaOH, AcCN/water, 95%.

Preparation of 1-(2-fluoro-4-(2-methoxypyridin-4-yl)phenyl)prop-2-en-1-ol

Step A:



A microwave vial equipped with a stirrer was charged with 4-bromo-2-fluorobenzaldehyde (173 mg, 0.852 mmol), 2-methoxypyridine-4-boronic acid (156 mg, 1.023 mmol), and S-Phos Second Generation Precatalyst (30.7 mg, 0.043 mmol). To this mixture was added anhydrous THF (3.5 mL). The reaction vessel was degassed and filled with N_2 (3X) before adding a N_2-sparged 3M aqueous solution of potassium phosphate tribasic (0.852 mL, 2.56 mmol). The reaction vessel was sealed and heated to 80 °C in a heating block for 18 h. The reaction was allowed cool to ambient temperature, and partitioned between EtOAc and sat. NH_4Cl. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The crude residue was purified *via* MPLC (ISCO 24 g; prod. elutes at 25% EtOAc/hexane) with gradient elution 0-50% EtOAc/hexane to give a white solid (0.1758 g, 89% yield). ^{1}H

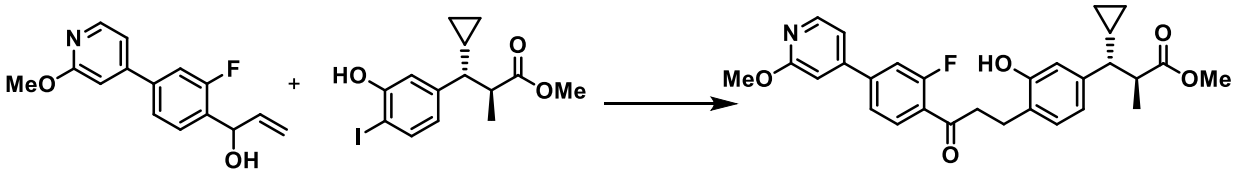
NMR (500 MHz, $CDCl_3$) δ 10.38 (s, 1H), 8.24 (d, *J* = 5.3 Hz, 1H), 8.04 – 7.89 (m, 1H), 7.57 – 7.42 (m, 1H), 7.39 (dd, *J* = 11.2, 1.6 Hz, 1H), 7.06 (dd, *J* = 5.4, 1.6 Hz, 1H), 6.99 – 6.86 (m, 1H), 3.97 (s, 3H).

Step B:

To a cold solution of 2-fluoro-4-(2-methoxypyridin-4-yl)benzaldehyde (0.1758 g, 0.760 mmol) in anhydrous THF (10 mL) at 0 °C was added dropwise a 1 M solution of vinylmagnesium bromide in THF (1 mL, 1.0 mmol). The reaction was stirred for 10 min. at 0 °C and subsequently quenched with addition of sat. NH_4Cl. The mixture was extracted with EtOAc (2X). The organic layers were collected, combined, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified *via* MPLC (ISCO 24 g; prod. elutes at 47% EtOAc/hexane) with gradient elution 0-100% EtOAc/hexane to give a white solid (0.1275 g, 65%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.18 (d, *J* = 5.4 Hz, 1H), 7.54 (t, *J* = 7.8 Hz, 1H), 7.39 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.26 (dd, *J* = 11.2, 1.7 Hz, 1H), 7.04 (dd, *J* = 5.4, 1.5 Hz, 1H), 6.95 – 6.83 (m, 1H), 6.06 (ddd, *J* = 16.6, 10.3, 5.7 Hz, 1H), 5.54 (d, *J* = 4.7 Hz, 1H), 5.38 (d, *J* = 17.1 Hz, 1H), 5.22 (dt, *J* = 10.4, 1.2 Hz, 1H), 3.96 (s, 3H), 2.27 (bs, 1H).

Preparation of Sodium (*2S,3R*)-3-cyclopropyl-3-((S)-2-(2-fluoro-4-(2-methoxypyridin-4-yl)phenyl)chroman-7-yl)-2-methylpropanoate

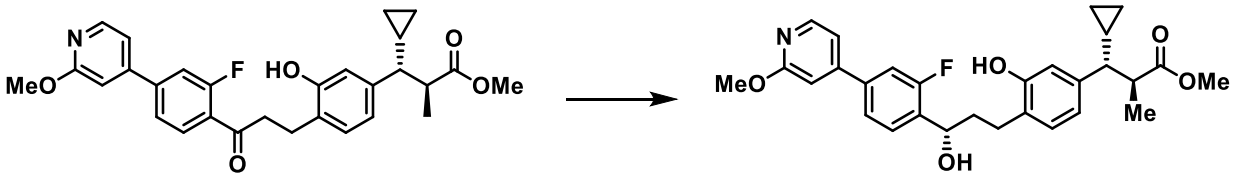
Step C:



A 1-L flask was charged with 1-(2-fluoro-4-(2-methoxypyridin-4-yl)phenyl)prop-2-en-1-ol (14.79 g, 57.0 mmol), methyl (*2S,3R*)-3-cyclopropyl-3-(3-hydroxy-4-iodophenyl)-2-methylpropanoate[15a,17] (17 g, 47.2 mmol) and anhydrous toluene (300 mL). The resulting mixture was sparged with N_2 for 10 min. before adding *t*-BuXphos palladacycle (1.2038 g, 1.753 mmol) followed by N,N-dicyclohexylmethylamine (15.16 mL, 70.8 mmol). The resulting yellow solution was sparged with N_2 for another 5 min., then heated under N_2 atmosphere to 90 °C in a heating block for 18 h. The mixture was partitioned between EtOAc and sat. NH_4Cl. The organic layer was collected, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified *via* MPLC (ISCO 330 g; prod. elutes at 47% EtOAc/hexane) with gradient elution 0-100% EtOAc/hexane to give the product as a pale yellow foam (11.3 g, 49%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25 (d, *J* = 5.4 Hz, 1H), 8.04 (t, *J* = 7.8 Hz, 1H), 7.46 (d, *J* = 8.3 Hz, 1H), 7.36 (d, *J* = 12.5 Hz, 2H), 7.05 (dd, *J* = 24.9, 6.5 Hz, 2H), 6.93 (s, 1H), 6.70 (s, 1H), 6.63 (d, *J* = 7.6 Hz, 1H), 4.00 (s, 3H), 3.69 (s, 3H), 3.45 (m, 2H), 2.99 (t, *J* = 5.8 Hz, 2H), 2.75 (dd, *J* = 10.1, 6.8 Hz, 1H), 1.81 (t, *J* = 10.0 Hz, 1H), 0.99 (m, 1H), 0.90 (d, *J* = 6.9 Hz, 3H), 0.55 – 0.48 (m, 1H), 0.35 – 0.23 (m, 1H), 0.19 (dd, *J* = 9.4, 4.6 Hz, 1H), -

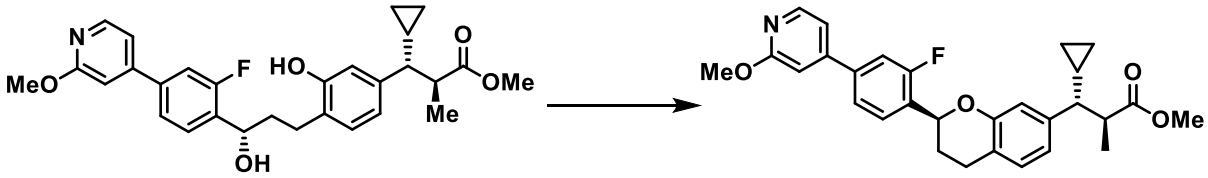
0.03 (dd, J = 9.5, 4.9 Hz, 1H).

Step D:



To a cooled solution of formic acid (10.0 mL, 230 mmol) in EtOAc (180 mL) at 0 °C was added triethylamine (12.8 mL, 92 mmol). This resulting solution was then added *via* a dropping funnel over 5 min. to a N_2-sparged solution of methyl (*2S,3R*)-3-cyclopropyl-3-(4-(3-(2-fluoro-4-(2-methoxypyridin-4-yl)phenyl)-3-oxopropyl)-3-hydroxyphenyl)-2-methylpropanoate(11.30 g, 22.99 mmol) and RuCl[(*R,R*)-TsDPEN](mesitylene) (0.716 g, 1.149 mmol) in EtOAc (110 mL). The resulting solution was sparged with N_2 for another another 3 min. before allowing to stir at ambient temperature for 22 h. The reaction was concentrated in vacuo and purified *via* MPLC (ISCO 330 g; prod. elutes at 50% EtOAc/hexane) with gradient elution 15-100% EtOAc/hexane to give the product as a foam (11.3 g, quant.). Chiral analysis (Whelk-01 4.6X250 mm; 30% IPA/CO_2) showed the product to be a 86:14 mixture of diastereomers. 1H NMR (500 MHz, $CDCl_3$) δ 8.17 (d, *J* = 5.4 Hz, 1H), 7.54 (t, *J* = 7.8 Hz, 1H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.22 (d, *J* = 11.4 Hz, 1H), 7.15 – 6.97 (m, 2H), 6.87 (s, 1H), 6.72 (bs, 1H), 6.64 (d, *J* = 5.5 Hz, 2H), 4.99 (dd, *J* = 9.0, 3.4 Hz, 1H), 3.95 (s, 3H), 3.70 (s, 3H), 3.01 (m, 1H), 2.89 (dt, *J* = 15.0, 8.2 Hz, 1H), 2.75 (dq, *J* = 13.8, 5.4, 4.2 Hz, 2H), 2.12 – 2.00 (m, 2H), 1.82 (t, *J* = 10.1 Hz, 1H), 0.99 (dq, *J* = 13.3, 6.6, 5.3 Hz, 1H), 0.91 (d, *J* = 6.8 Hz, 3H), 0.66 – 0.39 (m, 1H), 0.30 (tt, *J* = 8.8, 5.1 Hz, 1H), 0.19 (dq, *J* = 9.7, 4.8 Hz, 1H), -0.02 (dq, *J* = 9.8, 5.0 Hz, 1H).

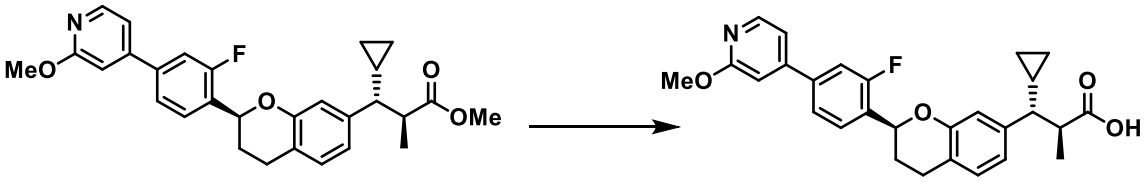
Step E:



To a cooled solution of methyl (*2S,3R*)-3-cyclopropyl-3-(4-((*S*)-3-(2-fluoro-4-(2-methoxypyridin-4-yl)phenyl)-3-hydroxypropyl)-3-hydroxyphenyl)-2-methylpropanoate (11.40 g, 23.10 mmol) in anhydrous THF (400 mL) at 0 °C was added tri-*n*-butylphosphine (6.9 mL, 27.7 mmol), followed by dropwise addition of DIAD (4.9 mL, 25.4 mmol). After 1 h, the reaction was concentrated in vacuo and the residue was *via* MPLC (ISCO 330 g; prod. elutes at 20% EtOAc/hexane) with gradient elution 0-50% EtOAc/hexane to give a white solid (10.3 g, 94%). ^{1}H NMR (500 MHz, $CDCl_3$): 0.02 (m, 1H), 0.26-0.21 (m, 1 H), 0.35 (m, 1 H), 0.57-0.53 (m, 1 H), 0.97 (d, 3 H), 1.08-1.02 (m, 1 H), 1.26 (t, 7 H), 1.86 (t, 1 H), 2.05 (m, 1 H), 2.28 (m, 1 H), 2.82-2.78 (m, 2 H), 3.06-2.99 (m, 1 H), 3.72 (s, 3 H), 3.99 (s, 3 H), 5.40 (d, 1 H), 6.73-6.69 (m, 2 H), 6.94-6.93 (m, 1 H), 7.09-7.03 (m, 2 H), 7.33 (d, 1 H), 7.45 (d, 1 H), 7.66 (t, 1 H), 8.24 (d, 1 H). LC/MS (*m/z*): 476.7$(M+H)^+$.

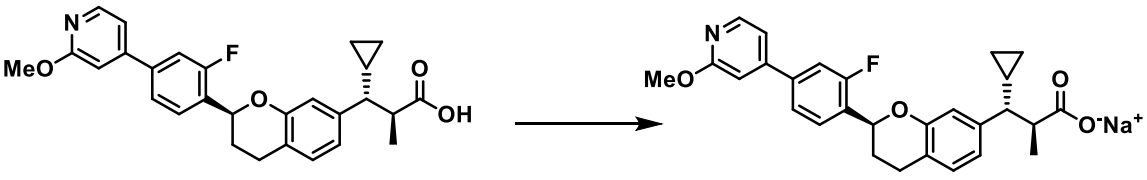
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.21 (d, *J* = 5.4 Hz, 1H), 7.64 (t, *J* = 7.8 Hz, 1H), 7.43 (d, *J* = 8.2 Hz, 1H), 7.31 (d, *J* = 11.4 Hz, 1H), 7.08 (d, *J* = 5.4 Hz, 1H), 7.02 (d, *J* = 7.8 Hz, 1H), 6.93 (s, 1H), 6.71 (s, 1H), 6.68 (d, *J* = 7.8 Hz, 1H), 5.38 (d, *J* = 9.3 Hz, 1H), 3.98 (s, 3H), 3.71 (s, 3H), 3.01 (ddd, *J* = 16.7, 11.7, 6.0 Hz, 1H), 2.90 – 2.60 (m, 2H), 2.26 (m, 1H), 2.11 – 1.96 (m, 1H), 1.85 (t, *J* = 10.1 Hz, 1H), 1.08 – 0.98 (m, 1H), 0.95 (d, *J* = 6.9 Hz, 3H), 0.60 – 0.44 (m, 1H), 0.44 – 0.27 (m, 1H), 0.23 (dt, *J* = 9.5, 4.8 Hz, 1H), 0.01 (dd, *J* = 9.4, 5.0 Hz, 1H).

Step F:



To a solution of methyl (*2S,3R*)-3-cyclopropyl-3-((*S*)-2-(2-fluoro-4-(2-methoxypyridin-4-yl)phenyl)chroman-7-yl)-2-methylpropanoate (10.3 g, 21.66 mmol) in THF (230 ml)/MeOH (130 ml) was added 1 M LiOH (170 ml, 170 mmol) at ambient temperature. The resulting mixture was heated to 55-58 °C for 17 h. The reaction was concentrated in vacuo and the residue was resuspended in EtOAc . Added 1 N HCl (170 mL) . The mixture was partitioned between EtOAc and sat. NH_4Cl/brine. The aqueous layer was back-extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, and concentrated in vacuo to give a white solid (9.5834 g, 96%). The 86:14 mixture of diastereomers was further resolved on a Chiral-pak AD-H (5X25 cm; 60% IPA/CO_2 to give the faster eluting peak as the desired diastereomer (7.0889 g, 74%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 (d, *J* = 5.3 Hz, 1H), 7.65 (t, *J* = 7.7 Hz, 1H), 7.44 (d, *J* = 8.3 Hz, 1H), 7.32 (d, *J* = 10.8 Hz, 2H), 7.10 (d, *J* = 5.3 Hz, 1H), 7.03 (d, *J* = 7.7 Hz, 1H), 6.95 (s, 1H), 6.73 (s, 1H), 6.70 (d, *J* = 7.9 Hz, 1H), 5.39 (d, *J* = 9.6 Hz, 1H), 4.01 (s, 3H), 3.13 – 2.90 (m, 1H), 2.90 – 2.71 (m, 2H), 2.27 (m, 1H), 2.16 – 2.00 (m, 1H), 1.93 (t, *J* = 10.0 Hz, 1H), 1.10 (m, 1H), 1.01 (d, *J* = 6.9 Hz, 3H), 0.61 (m, 1H), 0.50 – 0.22 (m, 2H), 0.06 (m, 1H).

Step G:



To a suspension (*2S,3R*)-3-cyclopropyl-3-((*S*)-2-(2-fluoro-4-(2-methoxypyridin-4-yl)phenyl)chroman-7-yl)-2-methylpropanoic acid (7.0889 g, 15.36 mmol) in acetonitrile (70 ml)/water (30 ml) was added 1 M NaOH (15.36 ml, 15.36 mmol) at ambient temperature. Using sonication, ensured that all was in solution. The resulting pale yellow solution was frozen and lyophilized to give a white solid (7.0643 g, 95%).

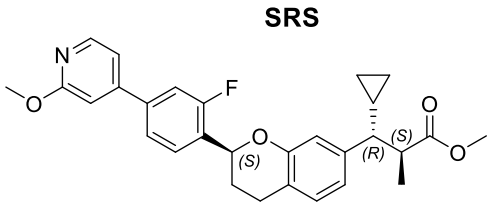
^{1}H NMR (500 MHz, CD_3OD) δ 8.18 (dd, *J* = 5.4, 0.7 Hz, 1H), 7.68 (t, *J* = 7.9 Hz, 1H), 7.57 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.50 (dd, *J* = 11.6, 1.8 Hz, 1H), 7.25 (dd, *J* = 5.4, 1.6 Hz, 1H), 7.07 (dd, *J* = 1.7, 0.7 Hz, 1H), 7.03 – 6.95

(m, 1H), 6.71 (d, *J* = 7.5 Hz, 2H), 5.37 (dd, *J* = 10.2, 2.3 Hz, 1H), 3.95 (s, 3H), 3.06 – 2.90 (m, 1H), 2.76 (ddd, *J* = 16.4, 5.0, 3.1 Hz, 1H), 2.62 (dq, *J* = 10.4, 6.8 Hz, 1H), 2.26 (ddt, *J* = 13.5, 5.8, 2.9 Hz, 1H), 2.03 (dddd, *J* = 13.5, 11.3, 10.1, 5.2 Hz, 1H), 1.94 (t, *J* = 9.9 Hz, 1H), 1.07 (dtt, *J* = 9.8, 8.1, 5.0 Hz, 1H), 0.85 (d, *J* = 6.9 Hz, 3H), 0.55 (dddd, *J* = 9.1, 8.1, 5.9, 4.6 Hz, 1H), 0.43 (ddt, *J* = 9.4, 5.7, 4.7 Hz, 1H), 0.23 (dddd, *J* = 9.1, 8.2, 5.7, 4.3 Hz, 1H), -0.07 (ddt, *J* = 9.3, 5.8, 4.5 Hz, 1H).

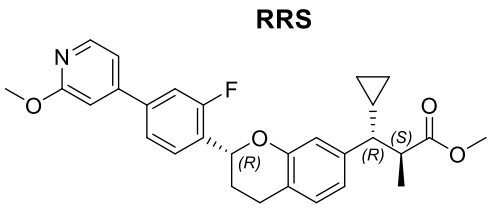
^{13}C NMR (126 MHz, CD_3OD) δ 183.05, 164.40, 159.15, 153.63, 149.25, 146.29, 143.37, 138.42, 129.29, 127.98, 127.24, 121.84, 119.77, 118.24, 115.18, 114.11, 112.54, 106.89, 70.85, 53.17, 52.10, 48.31, 28.07, 23.44, 15.55, 5.03, 1.40.

HRMS calculated for C28H29FNO4+ $(M+H)^+$: 462.2075, Found: 462.2068.

3. Sterochemical determination by ECD



Methyl ester of ***AP5***

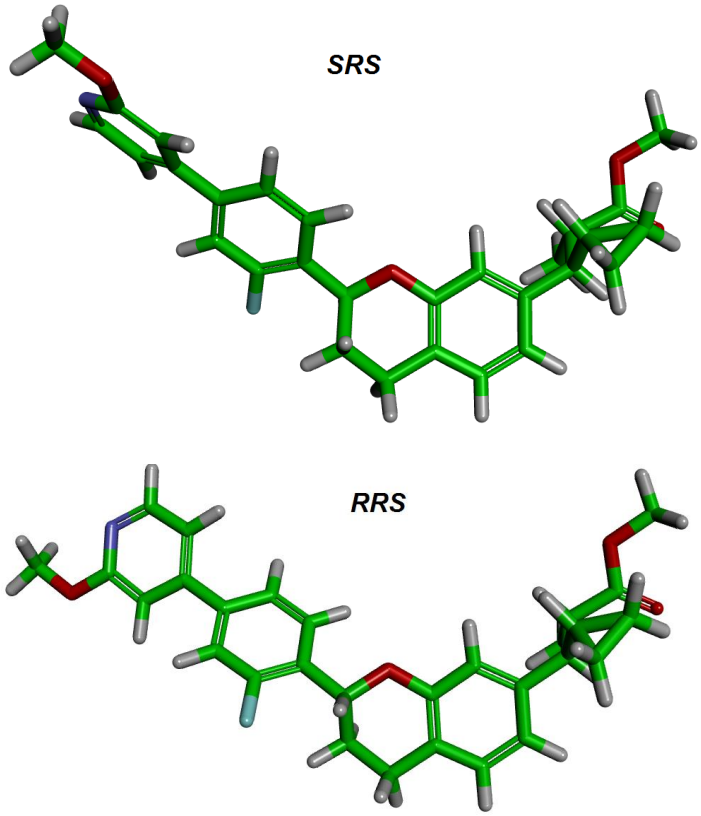


Methyl ester of ***25***

Summary: The absolute configuration of the methyl ester of ***AP5*** was assigned to be (*SRS*) using Electronic Circular Dichroism (ECD) spectroscopy with confidence. Analysis was done comparing experimental data to the calculated UV and ECD (Figure 1) spectra of the (*SRS*) configuration. The experimental ECD spectrum of the methyl ester of ***AP5*** matched well with the calculated (*SRS*) spectrum over the region from 185-350 nm, resulting in an assignment of (*SRS*).

The absolute configuration of methyl ester of ***25*** was assigned to be (*RRS*) using Electronic Circular Dichroism (ECD) spectroscopy with confidence. Analysis was done comparing experimental data to the calculated UV and ECD (Figure 2) spectra of the (*RRS*) configuration. The experimental ECD spectrum of the methyl ester of ***25*** matched well with the calculated (*RRS*) spectrum over the region from 185-350 nm, resulting in an assignment of (*RRS*).

Global Minimized Structure of the (*SRS*) and (*RRS*) diastereomers



ECD Background: Electronic circular dichroism is a method useful for determining the absolute configuration (AC) of chiral molecules. A circular dichroism event in the UV/Visible region, differences in the absorbance signal of left and right circularly polarized light passing through the sample are exploited to determine the AC. The method relies on comparing the experimentally determined UV and ECD spectra to the calculated spectra. Calculations are performed to exhaustively sample conformation space, determine structural minima, and Boltzmann-weight the calculated spectra when relevant. In this case, calculations were performed for both the free-base as well as the protonated species to account for the presence of the TFA salt. Figures output show the superposition of curves, and compare them with the enantiomreic spectra. For cases where an assignment is made with a low confidence level, those assignments should be confirmed with an orthogonal method such as NMR or small molecule crystallography.

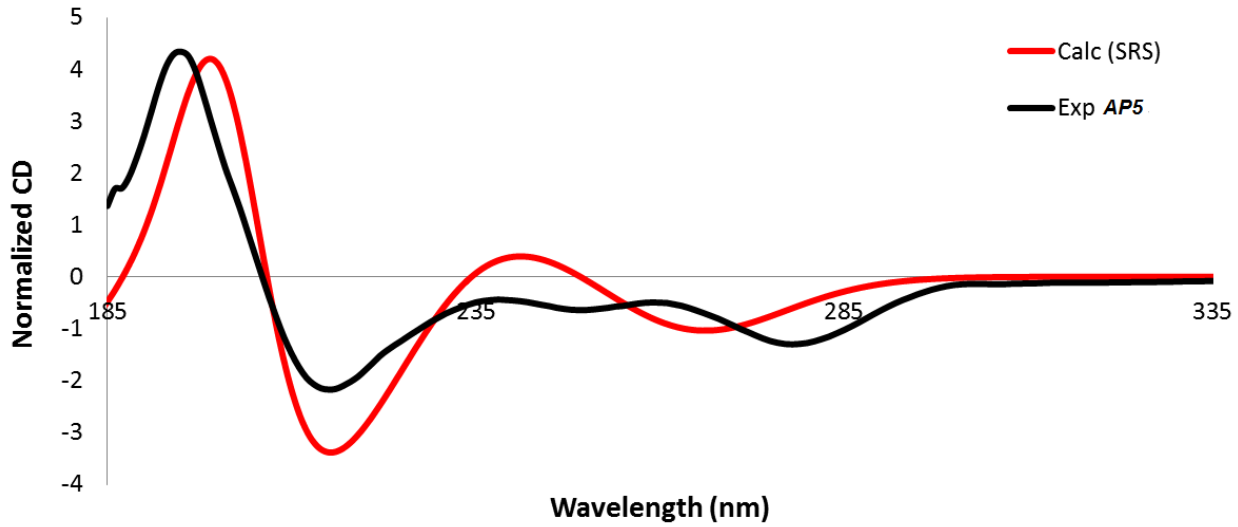


Figure 1. Comparison ECD spectra of the methyl ester of ***24*** in MeCN and calculated (*SRS*). The observed ECD spectrum of the methyl ester of ***24*** compares better with the calculated Boltzmann population-weighted spectrum of the (*SRS*) configuration for the monomer over the range 185-335 nm.

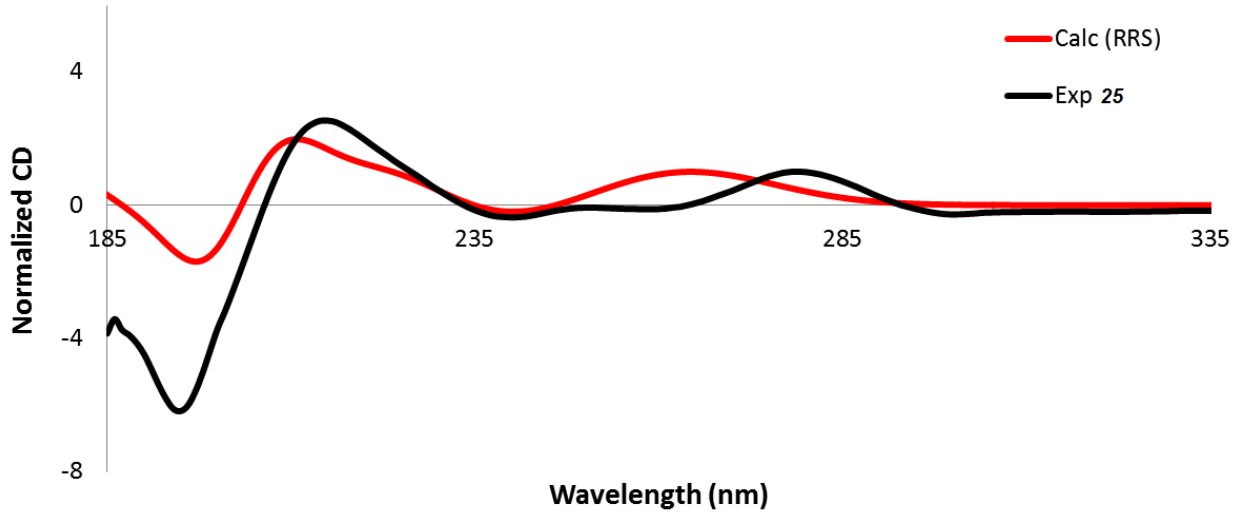
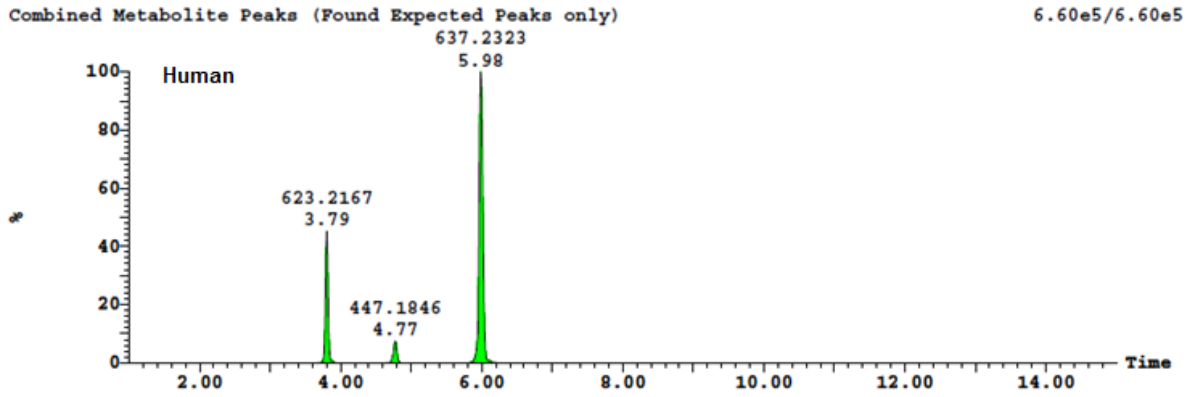
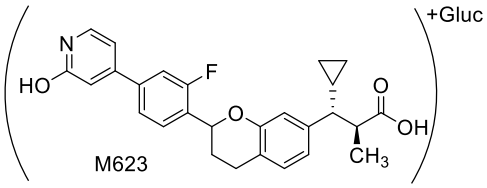
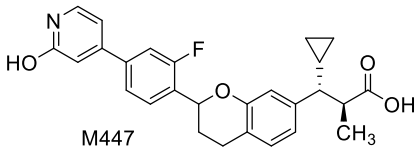
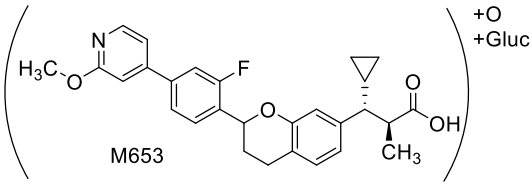
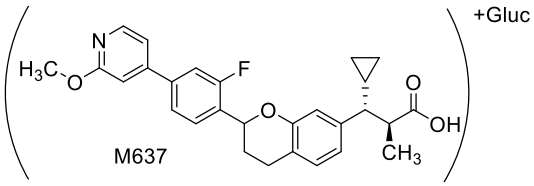


Figure 2. Comparison ECD spectra of the methyl ester of ***25*** in MeCN and calculated (*RS*). The observed ECD spectrum of the methyl ester of ***25*** compares better with the calculated Boltzmann population-weighted spectrum of the (*RRS*) configuration for the monomer over the range 185-335 nm.

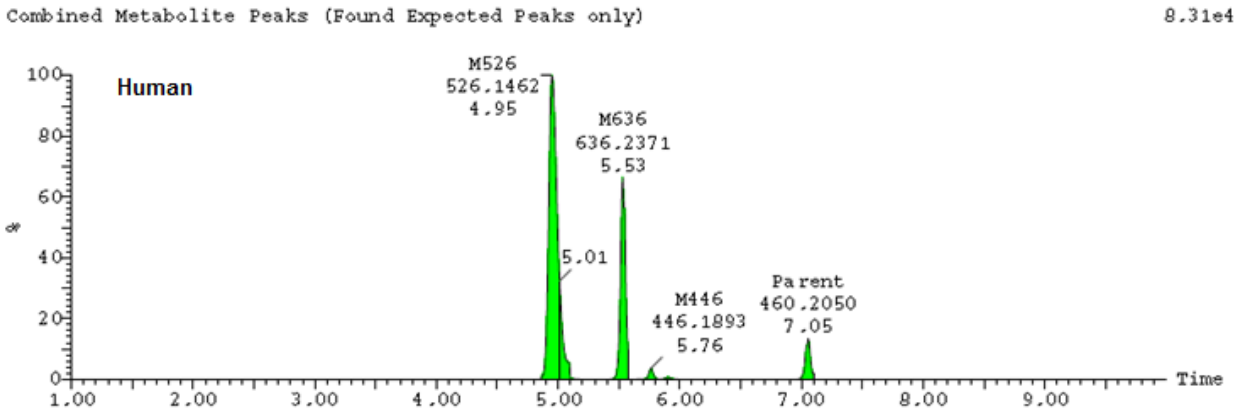
4. Metabolites of *AP5* and *14*

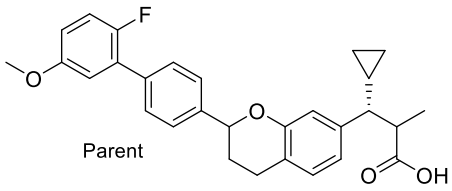
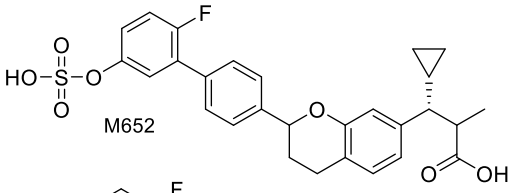
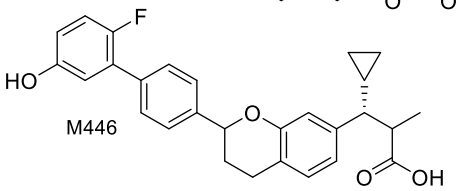
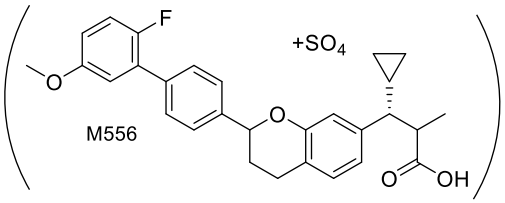
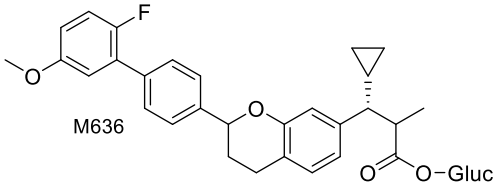
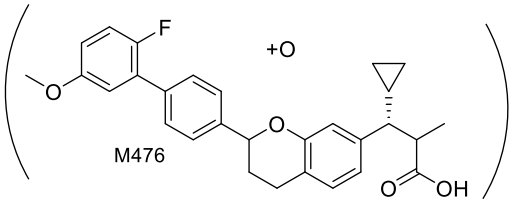
Metabolites of ***AP5***:



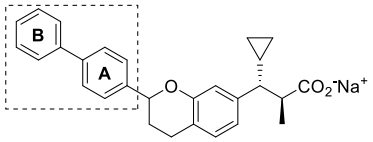
Metabolite	MW	Retention Time (min.)	% Area Rat	Dog	Monkey	Human
	623	3.79	9.0%	1.2%	10.4%	20.8%
	447	4.77	--	1.1%	1.8%	4.5%
	653	4.28	9.1%	--	--	--
	637	5.98 6.09	81% 0.9%	96.4% 1.4%	86.7% 1.1%	74.7% --

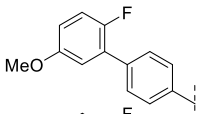
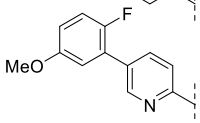
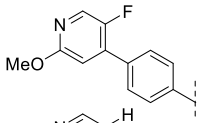
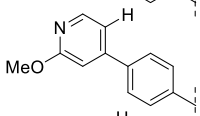
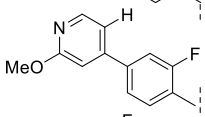
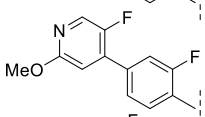
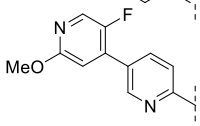
Metabolites of ***14***:



Metabolite	MW	Retention Time (min.)	% Area Rat	% Area Human
 Parent	460	7.05	14.5%	5.1%
 M652	652	4.95	--	66.2%
 M446	446	5.76	--	1.2%
 M556	556	5.02	--	1.9%
 M636	636	5.53	85.5%	25.3%
 M476	476	5.90	--	0.3%

5. Rat IP1 EC50 for Select Compounds in Table 2



	Cpd	rat IP1 EC_{50} (nM) ± SD
	***14***	1.2 ± 0.35
	15	1.7 ± 0.41
	***16***	2.3 ± 0.4
	17	3.8 ± 0.88
	***20***	1.9 ± 0.29
	21	0.52 ± 0.20
	***22***	2.6
	23	1.8
	***AP5***	0.49 ± 0.28
	25	10.8 ± 1.6
	***26***	0.17
	27	4.7 ± 1.1
	***28***	3.4 ± 0.97
	29	5.2 ± 1.6

6. *In Vitro* Inositol Phosphate Turnover (IP1) Assay

Stable cell lines expressing human GPR40 (hGPR40/HEK293), and rat GPR40 (rGPR40/CHO-K1) were cultured in DMEM media supplemented with 10% FBS, glutamine, non-essential amino acids and penicillin/streptomycin. hGPR40/HEK293 cell media was supplemented with 500 μg/mL G418 (Life Technologies), while rGPR40/CHO-K1 cells were grown in 10 μg/ml blasticidin plus 200 μg/ml hygromycin (Life Technologies). Cell stocks were maintained and grown in a sub-confluent state using standard cell culture procedures. The day before the experiment, the cells were harvested with non-enzymatic cell dissociation buffer and re-suspended in DMEM supplemented with 10% FBS, glutamine, non-essential amino acids, penicillin/streptomycin at 0.15, 0.2 and 0.3 million cells per ml for human and rat GPR40, respectively. A sterile Perkin Elmer Culturplate-384 was then seeded with 7,500, 10,000 or 15,000 human, or rat GPR40 cells in a volume of 50 μl per well. The seeded plates were incubated overnight at 37 ^{o}C.

On the day of the experiment, the growth media was removed from the assay by gently patting the assay plates on an absorbent sheet and 10 μl of IP1 stimulation buffer (Cis Bio IP-one Tb HTRF kit)

supplemented with 50 mM LiCl is added to each well. Test compounds dissolved in DMSO were serially diluted in ½ log increments starting from 2 mM or 0.2 mM and 50 nl of the compound dilution was acoustically added to each well (final starting concentration 10 μM or 1 μM). Plates were then incubated for 60 minutes at room temperature and 10 μl of detection buffer (prepared as described in the Tb kit) is added to each well. The plates are then incubated one additional hour at room temperature. After the final incubation, the plates were read in a Perkin Elmer Envision with a method designed for HTRF assays (320 nm excitation, dual emission 615 and 655 nm). For each assay, a standard curve plate in which IP1 is titrated is also included. All fluorescent readings (using the 655/615 nm ratio) are back calculated to a concentration of IP1 using the IP1 standard curve and the percent activity at each concentration of test compound is determined using 0% activation (basal activity) determined in those wells that contain DMSO alone, while 100% activity is determined in wells that contained a concentration of a partial allosteric agonist know to activate GPR40. The % activity is then plotted versus the concentration of test compound and the dose response curve fitted to a standard 4-parameter non-linear regression model using a custom in-house developed software package. Maximal % activity and EC_{50} are then determined for each test compound.

For the human GPR40 IP1 assay run in the presence of 100% human serum, the assay is performed as described above except that instead of IP1 stimulation buffer, 10μl of pooled human serum containing 50 mM LiCl is added to each well. After the addition of compounds and 60 minute incubation at room temperature, the serum is removed by gently patting the assay plates on an absorbent sheet followed by the addition of 10 μl of IP1 stimulation buffer and 10 μl of detection buffer. The plates are then incubated one additional hour at room temperature, read in an Envision reader and potency/efficacy determined as described above.

7. *In Vivo* SD Rat ipGTT Assay

Male Sprague Dawley (SD) rats (7-12 weeks of age) are housed 2 per cage and given access to normal diet rodent chow and water ad libitum. Rats are randomly assigned to treatment groups and fasted 4 to 6 h. Baseline blood glucose concentrations are determined by glucometer from tail nick blood. Animals are then treated orally with vehicle (0.25% methylcellulose) or test compound. Blood glucose concentration is measured at a set time point after treatment (t = 0 min) and rats are then intraperitoneally-challenged with dextrose (3 g/kg). One group of vehicle-treated rats is challenged with saline as a negative control. Blood glucose levels are determined from tail bleeds taken at 20, 40, 60 min after dextrose challenge. The blood glucose excursion profile from t = 0 to t = 60 min is used to integrate an area under the curve (AUC) for each treatment. Percent inhibition values for each treatment are generated from the AUC data normalized to the saline-challenged controls.

8. *In Vivo* GK Rat oGTT Assay

Diabetic male Goto Kakizaki rats (9-16 weeks of age) are housed 2 per cage and given access to normal diet rodent chow and water ad libitum. Baseline blood glucose concentrations are determined by glucometer from tail nick blood (obtained at 2h post- lights on). Rats are assigned to treatment groups based on baseline glucose and bodyweight and fasted 5 h. Blood glucose is sampled at -60 min and

animals are then treated orally with vehicle (0.25% methylcellulose) or test compound. Blood glucose concentration is measured at a set time point after treatment (t = 0 min) and rats are then challenged with dextrose (1g/ kg). Blood glucose levels are determined from tail bleeds taken at 20, 40, 60, 120 min after dextrose challenge. The blood glucose excursion profile from t = 0 to t = 60 min is used to integrate an area under the curve (AUC) for each treatment. Percent inhibition values for each treatment are generated from the AUC data normalized to the healthy WKY rat controls.

9. *In Vivo* GLP-1 Secretion Assay

Twelve week old male C57BL/6 mice or GPR40 KO mice on the C57BL/6 background from Taconic Farms (Hudson, NY) were maintained on a chow diet (Purina 5053). Prior to testing, animals were fasted overnight with access to water ad libitum for ~16 h. Animals were then dosed p.o. with vehicle (0.5% methylcellulose) or test article. Animals were euthanized via CO_2 and blood was collected via cardiac puncture at 1 h post dose. This time point was determined to achieve maximal effect on GLP-1 levels over 24 h post dose (data not shown). Plasma was harvested and assayed for GLP-1 (Meso Scale Discovery, Gaithersburg, MD). Studies in lean mice show the compounds described increase total and active GLP-1 significantly compared to vehicle controls.

10. *In Vitro* BSEP Assay

The inhibitory effect of the test compound on ATP-dependent [^{3}H]TCA (1 µM, Perkin Elmer Life Sciences, Boston, MA) uptake was conducted in membrane vesicles isolated from baculovirus infected Spodoptera frugiperda (Sf9) cells containing human Bsep (*ABCB11*) (Invitrogen Life Technologies, Carlsbad, CA) essentially as described previously (Prueksaritanont et al., 2013). The experiment was performed in triplicate. Bsep-mediated [^{3}H]TCA uptake was calculated by subtracting the uptake of [^{3}H]TCA in the presence of AMP from that in the presence of ATP, and data were normalized to % control, where uptake in the absence of test compound was 100%. The IC_{50} values for inhibition of Bsep-mediated uptake were obtained by fitting the data to the formula below by nonlinear regression analysis using GraphPad Prism.

$$Control(\%) = \frac{100}{1 + I / IC_{50}}$$

Where “control (%)” represents Bsep-mediated uptake measured in the presence of various concentrations of inhibitor to that in the absence of inhibitor and “I” represents nominal inhibitor concentration.

11. HPLC log D (pH 7) Determination

The high throughput (HT) HPLC log D (pH 7) value was determined by the following method.

The chromatographic system consists of an Agilent 1200 HPLC/DAD system and ChemStation software, both from Agilent Technologies, USA.

The separations are carried out on a Supelco Ascentis Express C18, 30 mm x 3.0 mm I.D., 2.7 µm, (Sigma-Aldrich, USA). The mobile phase consists of phosphate buffered saline at pH 7 (mobile phase A) and acetonitrile (mobile phase B). The column oven temperature is set to 30 °C. The HPLC analysis begins with an isocratic step of 0.2 minutes at 5% B at 1.5 ml/min, followed by a gradient from 5% to 98% B in 1.0 minute at 1.5 ml/min. A second isocratic step of 0.2 minutes at 98% B with a changing flow rate from 1.5 to 2.0 ml/min is then followed by a gradient from 98 to 5% B in 0.1 minutes with the flow rate changing from 2.0 to 1.5 ml/min. The equilibration time between injections is 0.4 minutes at 5% B. The injection volume is 5 µL and the spectrophotometric detection is set to 215, 238 and 254 nm.

The chromatographic system is calibrated with a set of standards with published shake-flask log D values. Linear regression is used to determine the calibration line relating the retention time to log D for the calibration standards. This line is then used to determine the HT HPLC log D (pH 7) value of API from the measured retention time by the HPLC/DAD analysis of the API solution.

The sample is dissolved in DMSO to create a stock solution of 10 mM. Dilute 2.5 µl of stock solution (10 mM) into 250 µl of organic co-solvents (10% MeCN/80% MeOH/10% DMSO) to create a standard solution of 100 µM. Inject the standard solution onto the HPLC/DAD system. Based on the HPLC/DAD analysis retention time of the standard solution, the HT HPLC log D

12. Off-Target Hits (Table 4)

In vitro panel screen data was collected at Eurofins Scientific (www.eurofins.com). The specific ligand binding to the receptors is defined as the percent inhibition at 10 µM concentration in the presence of an excess of unlabeled ligand. The results (% Inh) are expressed as a percent inhibition of control specific binding.

Listed below are receptors for which binding inhibition at 10 µM was >50%:

Compound ***AP5***: 1) Adenosine A1-AA (85% inh), 2) PPAR gamma (53% inh), 3) Prostanoid EP3-Ag (64% inh), and 4) Prostanoid IP (64% inh)

Compound ***2***: Prostanoid IP (63% inh)