Supplementary Material for:

Optimisation of aqueous solubility in a series of G protein coupled receptor 119 (GPR119) agonists

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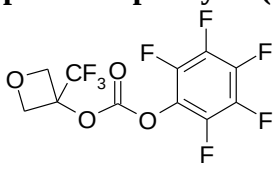
General experimental details: All solvents and chemicals used were reagent grade. Flash column chromatography was carried out using prepacked silica cartridges (from 4 g up to 330 g) from Redisep, Biotage, or Crawford and eluted using an Isco Companion system. Purity and characterization of compounds were established by a combination of liquid chromatography-mass spectroscopy (LC-MS) and NMR analytical techniques and was >95% for all compounds. ^{1}H NMR were recorded on a Bruker Avance DPX400 (400 MHz) and were determined in $CDCl_3$ or DMSO-d_6. ^{13}C NMR spectra were recorded at 101 or 175 MHz. Chemical shifts are reported in ppm relative to tetramethylsilane (TMS) (0.00 ppm) or solvent peaks as the internal reference and coupling constant (J) values are reported in Hertz (Hz). Splitting patterns are indicated as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Merck precoated thin layer chromatography (TLC) plates (silica gel 60 F_{254}, 0.25 mm, art. 5715) were used for TLC analysis. Solutions were dried over anhydrous magnesium sulfate, and solvent was removed by rotary evaporation under reduced pressure.
Synthetic details for compounds described within this paper can be found in:
Birch, Alan M.; Broo, Dan A.; Butlin, Roger J.; Clarke, David S.; Davidsson, Ojvind P.; De La Motte, Hanna; Johansson, Kjell E.; Leach, Andrew; MacFaul, Philip Al.; O'Donnell, Charles J.; Scott, James S.; Whittamore, Paul R. O. PCT Int. Appl. (**2011**), 164 pp. *WO2011030139*

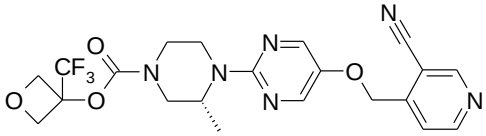
perfluorophenyl 3-(trifluoromethyl)oxetan-3-yl carbonate



Tetrabutylammonium fluoride (1M in THF, 2.86 mL, 2.86 mmol) was added to oxetan-3-one (2.06 g, 28.59 mmol) and trimethyl(trifluoromethyl)silane (2M in THF, 23.58 mL, 47.17 mmol) in THF (25 mL) at 20 ºC under nitrogen. An ice bath was used to control the resultant exotherm. The resulting dark brown solution was stirred at 20 °C for 2 hours. 6M HCl (60 mL) was added at 0 °C then the temperature was allowed to rise to and stirred at 20 °C for 2 hours. The reaction mixture was diluted with Et_2O (100 mL), and washed with saturated brine (50 mL). The aqueous layer was extracted with CH_2Cl_2 (2 x 100 mL). The combined organic layers were dried over Na_2SO_4 and filtered. The mixture was gently evaporated (stopped at ~ 400 mbar) to remove solvent then to the mixture was was added bis(perfluorophenyl) carbonate (13.52 g, 34.31 mmol) and acetonitrile (15 mL) To this solution was added triethylamine (12.75 mL, 91.49 mmol) dropwise at 0 °C over a period of 5 minutes under nitrogen. The resulting solution was allowed to warm to room temperature then stirred at 20 °C for 18 hours. All volatiles were removed under reduced pressure to leave a purple oil. The crude product was purified by flash silica chromatography, elution gradient 0 to 100% CH_2Cl_2 in isohexane. Pure fractions were evaporated to dryness to afford perfluorophenyl 3-(trifluoromethyl)oxetan-3-yl carbonate (8.38 g, 83%) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.07 – 5.02 (2H, m), 4.93 – 4.88 (2H, m); LRMS (EI+) m/z $(M)^+$ = 352.

(*R*)-3-(trifluoromethyl)oxetan-3-yl 4-(5-((3-cyanopyridin-4-yl)methoxy)pyrimidin-2-yl)-3-methylpiperazine-1-carboxylate. (11)



Triethylamine (0.579 mL, 4.15 mmol) was added to (*R*)-4-((2-(2-methylpiperazin-1-yl)pyrimidin-5-yloxy)methyl)nicotinonitrile hydrochloride (240 mg, 0.69 mmol) and perfluorophenyl 3-(trifluoromethyl)oxetan-3-yl carbonate (268 mg, 0.76 mmol) in chloroform (5 mL) at 20 °C. The reaction was stirred at 110 °C for 30 minutes. The reaction mixture was diluted with CH_2Cl_2 (50 mL), and washed with 2M K_2CO_3 aq. (20 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated to afford crude material that was purified by flash alumina chromatography, elution gradient 10 to 50% EtOAc in isohexane and by flash silica chromatography, eluting with 10-50% EtOAc in CH_2Cl_2. Pure fractions were evaporated to dryness and triturated with ether / isohexane to afford (*R*)-3-(trifluoromethyl)oxetan-3-yl 4-(5-((3-cyanopyridin-4-yl)methoxy)pyrimidin-2-yl)-3-methylpiperazine-1-carboxylate (129 mg, 39%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91 (s, 1H), 8.85 (d, *J* = 5.2 Hz, 1H), 8.19 (s, 2H), 7.71 – 7.62 (m, 1H), 5.22 (s, 2H), 5.11 – 5.02 (m, 1H), 5.01 – 4.94 (m, 1H), 4.92 – 4.78 (m, 3H), 4.48 – 4.31 (m, 1H), 4.20 – 3.86 (m, 2H), 3.34 – 2.91 (m, 3H), 1.18 (d, *J* = 6.7 Hz, 3H). HRMS (ESI) calc. for $C_{21}H_{22}O_4N_6F_3$ $(M+H)^+$ 479.1649 found 479.1646.

Biological Protocols:

cAMP assay:GPR119 agonists were tested on HEK293S cells over-expressing human GPR119. Changes in cAMP concentrations were assessed using the cAMP dynamic 2 HTRF kit (Cisbio). Cells were diluted in assay buffer (20 mM HEPES pH 7.4, Hank´s Balanced Salt Solution, 0.01 % BSA, 1 mM IBMX) and used at $2x10^3$ cells/well in 384-well plates. Cells were incubated with compound for 45 min before addition of HTRF lysis and detection reagents according to the manufacturer's protocol. Fluorescence readings were captured using an Envision plate reader and cAMP concentrations calculated using a standard curve. A typical standard deviation in logEC_{50} when a compound is repeated is 0.20 and 0.27 for the human and mouse assays respectively. This translates to 95% of EC_{50} values within 2.5-fold (human) and 3.5-fold (mouse) of a compound's "true" EC_{50}. The intrinsic activity was expressed as the percent effect compared to that of the control, 50 µM oleoylethanolamide, defined as 100% as per; Ariens, E. J. Affinity and intrinsic activity in the theory of competitive inhibition. I. Problems and theory. *Arch. Int. Pharmacodyn. Ther.* **1954**, *99*, 32-49.

Crystallography:

General:

The data was collected with graphite monochromated MoK(α) radiation on a KappaCCD Single-Crystal X-Ray diffractometer equipped with an κ-axis goniometer and a CCD area detector (Nonius, 1998). The diffraction raw data were processed within the Denzo-SMN program package (Otwinowski & Minor, 1998) converting the information from the digital image frame to a file containing h, k, l indices, background and Lp corrected intensities of the diffraction spots, along with estimate of errors. All crystallographic information has been deposited with the Cambridge Crystallographic Database.

Crystal data for 13: $C_{20}H_{22}N_6O_4$, M_r=410.4, triclinic, space group P-1 (No. 2), a=5.2885(7) Å, b=11.2227(15) Å, c=16.881(2) Å, α =85.165(6)°, β=83.895(6)°, γ=76.853(5)°, V=968.3(2) Å^3, Z=2, D_{calc}=1.408 gcm^{-3}, μ(Mo Kα)=1.01 cm^{-1}, R=0.055, wR^2=0.148, GOF=1.07. The data were collected at 200 K on a Bruker APEX-II CCD diffractometer with graphite monochromatized MoKα radiation (λ = 0.71073 Å) and employing a 0.23 mm×0.06 mm×0.05 mm crystal (R_{int}=0.03).

Procedures for determination of physicochemical properties:
log$D_{7.4}$, plasma-protein binding and solubility measurements were made as described in; Buttar, D.; Colclough, N.; Gerhardt, S.; MacFaul, P. A.; Phillips, S. D.; Plowright, A.; Whittamore, P.; Tam, K.; Maskos, K.; Steinbacher, S.; Steuber, H. A. Combined spectroscopic and crystallographic approach to probing drug–human serum albumin interactions. *Bioorg. Med. Chem.* **2010**, *18*, 7486-7496.

log$D_{7.4}$:
Log$D_{7.4}$ measurements were made using a shake-flask method where the extent of partitioning between pH 7.4 buffer and octanol was measured. Compounds were dissolved in a known volume buffer, and following the addition of a known amount of octanol, the solutions were shaken for 30 min. Following centrifugation, analysis of the aqueous layer was performed by LC–UV to quantify the amount of compound in solution and then compared to analysis of the compound in solution before the addition of octanol to calculate the partitioning coefficient, $D_{7.4}$.

Solubility:
Assessments of aqueous solubility were made after an incubation of 24 h in pH 7.4 phosphate buffer. After centrifugation, analysis of the supernatant liquid was performed by LC–UV to quantify the amount of compound in solution.

Protein binding strength *via* equilibrium dialysis:
Dialysis membranes (Spectra/Por 2, 12–14 kDa molecular weight cut-off, 47 mm diameter, Spectrum Laboratories) were prepared for use by washing with distiled water and subsequent soaking in phosphate buffer (pH 7.4). Membranes were then blotted dry and placed between two 1 mL Teflon dialysis half-cells (Braun ScienceTec, Les Ulis, France). Each half-cell was filled individually with 1 mL of protein solution containing the compound of interest, while the corresponding half-cell was filled with 1 mL of isotonic phosphate buffer. Dialysis units were immersed in a 37 °C temperature-controlled water bath and rotated at 30 rpm for 18–19 h using a Dianorm apparatus (Braun ScienceTec). After this period, samples from both the half-cell containing buffer (protein free) and the half-cell containing protein were submitted for HPLC analysis using an Agilent 1100 series HPLC with a 110 binary pump and a UV diode ray detector. Acquisition and integration were carried out using Chemstation software (Agilent Technologies) version A.06.03 with relevant customised macro software. Integration of the subsequent chromatograms, are used to calculate the concentration of drug in the protein containing solution (Dp) and in the protein-free solutions (Df), which are then used to derive the binding constant for the test compound (K_1) assuming a 1:1 binding model as shown in Eq. 1 where the compound can only bind to a single site on the protein molecule. This is expressed mathematically in Eq. 2 where D and Df are the total and free drug concentrations, respectively, and Pr is the total protein concentration.

$$D + P \overset{K_1}{\rightleftharpoons} DP \qquad \text{Eq. 1}$$

$$D = (D_f + D_p) = \frac{K_1 \cdot D_f \cdot Pr}{1 + K_1 \cdot D_f} + D_f \qquad \text{Eq. 2}$$