Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Optimisation of biphenyl acetic acid inhibitors of diacylglycerol acetyl transferase 1 – the discovery of AZD2353

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Supporting Information

DGAT 1 assay

The DGAT 1 assay was performed using human DGAT1 expressed in insect cell membranes, sf9 cells were infected with recombinant baculovirus containing human DGAT1 coding sequences and harvested after 48 hours (Proc. Nat. Acad. Sci. 1998, 95, 13018). Cells were lysed by sonication and the membranes isolated by centrifugation (1h, 28000 rpm, 4 °C, 41% sucrose gradient). The membrane fraction at the interphase was collected, washed and stored in liquid nitrogen.

The DGAT1 assay was based on a modification of that described in the literature (Methods in Enzymology, 1992, 209, 98). Compound at 0.00003-10 μM (final conc) was incubated with 4 μg/mL (final conc) membrane protein, 5 mM $MgCl_2$, and 100 μM 1,2 dioleoyl-*sn*-glycerol in acetone (10% final assay conc. of acetone) in a total assay volume of 200 μL in a 96-deep well plate. The reaction was started by adding 14C oleoyl coenzyme A (30 μM final concentration) and incubated at room temperature for 30 min. The reaction was stopped by the addition of propan-2-ol / heptane (7:1, 200 μL). The mixture was partitioned between heptane (300 μL) and carbonate buffer (pH 9.5, 100 μL). The DGAT1 activity was quantified by counting aliquots from the heptane fraction containing the radioactive trioleoylglycerol product by liquid scintillography.

ADMET assays

All quoted data, including logD values are measured rather than calculated. Protocols for generation of relevant ADMET data are described in:

D. Buttar, N. Colclough, S. Gerhardt et al. Bioorg. Med. Chem. 2010, 18, 7486.

G. Camenisch, J. Alsenz, H. van de Waterbeemd and G. Folkers, Eur. J. Pharm. Sci., 1998, 6, 313.

M. H. Bridgland-Taylor, A. C. Hargreaves, A. Easter et al. J. Pharmacological and Toxicological Methods, 2006, 54, 189.

Free-Wilson Analysis

The Free-Wilson analysis was carried out using the "FitModel" platform in JMP by the SAS institute (www.jmp.com). An illustration of this analysis for the substituents described in the paper is shown below.

Each R group was used as a descriptor and the data fitted to both the DGAT1 pIC_{50} and the logD as in the table.

Compound	R1	R2	R3	R4	R5	R6	DGAT1 pIC_{50}	LogD
1	H	H	H	H	H	H	6.2	0.3
2	Cl	H	H	H	H	H	5.2	0.6
3	H	Cl	H	H	H	H	7	0.6
4	H	H	Cl	H	H	H	8.2	0.5
5	H	H	H	Cl	H	H	5.9	0.3
6	H	Me	H	H	H	H	6.9	0.5
7	H	F	H	H	H	H	6.9	0.3
8	H	OMe	H	H	H	H	5.2	0
9	H	CN	H	H	H	H	6.5	-0.4
10	H	H	Me	H	H	H	7.7	0.4
11	H	H	F	H	H	H	7.6	0.1
12	H	H	H	H	Me	H	6.7	0.7
13	H	H	H	H	H	Me	6.1	0.7

From this fitting, predictive data for the entire matrix of combinations of the 6 substituents can be derived according to an equation of the type given below.

$$y = x_{R1}+x_{R2}+x_{R3}+x_{R4}+x_{R5}+x_{R6}+C$$

where y is the data to be predicted, xR are the derived FreeWilson coefficients for each given R group and C is a constant.

The derived coefficients for the substructures are given in the table below.

Term	DGAT1 pIC_{50} coefficient	LogD coefficient
Intercept	7.225	0.79
R1[Cl]	-0.5	0.15
R1[H]	0.5	-0.15
R2[Cl]	0.55	0.38
R2[CN]	0.05	-0.62
R2[F]	0.45	0.08
R2[H]	-0.25	0.08
R2[Me]	0.45	0.28
R2[OMe]	-1.25	-0.22
R3[Cl]	0.775	0.18
R3[F]	0.175	-0.23
R3[H]	-1.225	-0.03
R3[Me]	0.275	0.08
R4[Cl]	-0.15	0.00
R4[H]	0.15	0.00
R5[H]	-0.25	-0.20

R5[Me]	0.25	0.20
R6[H]	0.05	-0.20
R6[Me]	-0.05	0.20

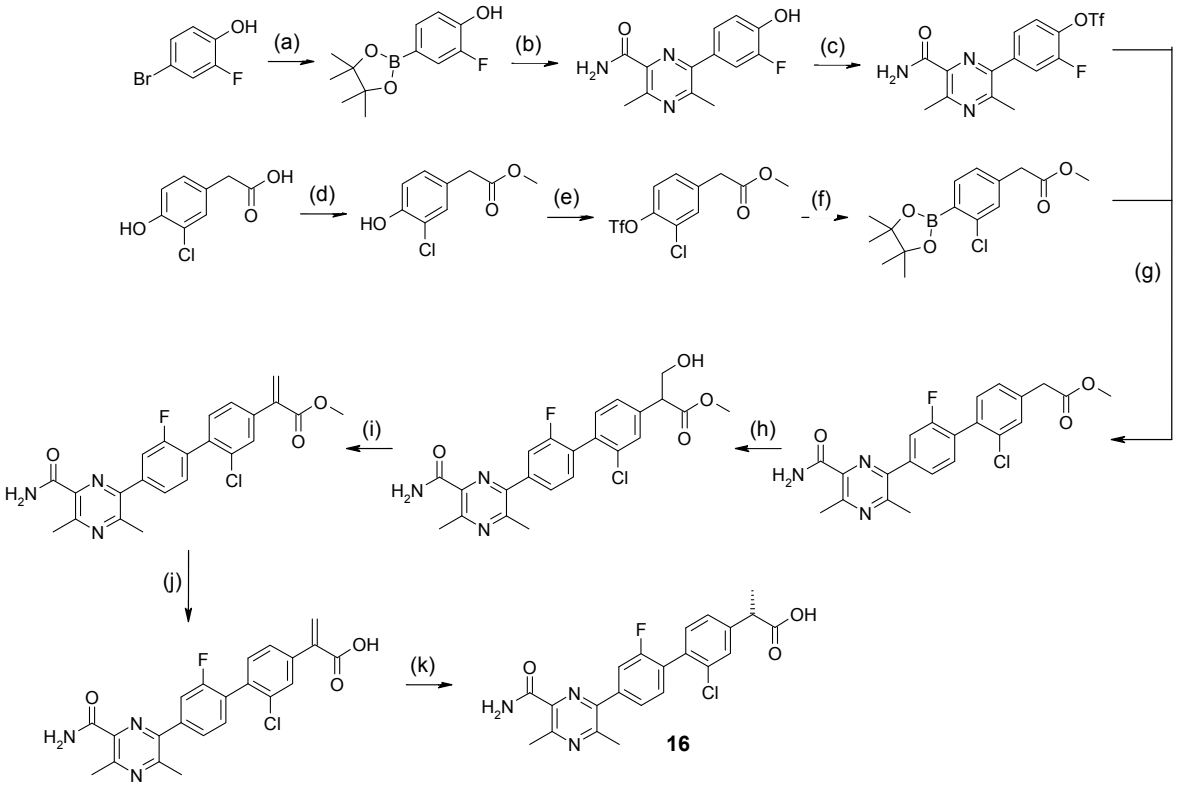
From these predicted data, targets within the matrix predicted to have high potency and desirable logD values were selected as synthesis targets. Predicted data compared to those measured for the compounds in Table 5 are given below. In reality more examples were included in the model, the data for which are not presented here and, of course, more numerous examples allow for more accurate predictions.

Example no	R1	R2	R3	R4	R5	R6	Predicted DGAT1 pIC_{50}	Measured DGAT1 pIC_{50}	Predicted LogD	Measured logD
4*	H	H	Cl	H	H	H	8.2	8.2	0.5	0.5
14	H	Cl	Cl	H	H	H	9.0	8.1	0.8	1.0
15	H	H	Cl	H	Me	H	8.7	7.9	0.9	1.1
16	H	F	Cl	H	Me	H	9.4	8.1	0.9	1.0

*compound is in the training set of the model

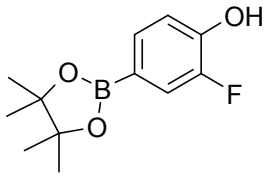
Synthesis of 16

All solvents and chemicals used were reagent grade. Anhydrous solvents tetrahydrofuran (THF), dimethoxyethane (DME) were purchased from Aldrich and used as such. Pd(dppf)Cl_2.DCM adduct (CAS No. 95464-05-4) was purchased from Aldrich. Flash column chromatography was carried out using prepacked silica cartridges (from 4g up to 330g) from 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), gas chromatography–mass spectroscopy (GC-MS) and NMR analytical techniques. ^{1}H NMR were recorded on a Bruker Avance DPX400 (400 MHz), DPX300 (300 MHz) and AV700 (700 MHz) and were determined in $CHCl_3$-*d* and, DMSO-d_6 with trimethylsilane (TMS) (0.00 ppm) or solvent peaks as the internal reference. Chemical shifts are reported in ppm relative to solvent signal and coupling constant (*J*) values are reported in Hertz (Hz). Splitting patterns are indicated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad peak. Merck precoated thin layer chromatography (TLC) plates (silica gel 60 F_{254}, 0.25 mm, art. 5715) were used for TLC analysis.



Reagents and conditions: (a) Pin_2B, KOAc, $Pd(dppf)Cl_2$, dppf, dioxane, 85 ^oC; 90%; (b) 6-chloro-3,5-dimethylpyrazine-2-carboxamide, K_3PO_4, DME/EtOH/H_2O, 80 ^oC; 91%; (c) $PhN(CF_3SO_2)_2$, K_2CO_3, THF, 120 ^oC; 75%; (d) MeOH, H_2SO_4 (conc), 75 ^oC; 90%; (e) $PhN(CF_3SO_2)_2$, K_2CO_3, THF, 120 ^oC; 69%; (f) Pin_2B, KOAc, $Pd(dppf)Cl_2$, dppf, dioxane, 85 ^oC; 79%; (g) $Pd(dppf)Cl_2$.DCM, LiCl, K_3PO_4, DME/MeOH/H_2O, 65 ^oC; 55%; (h) $(CH_2O)_n$, K_2CO_3, DMF, 0 ^oC to rt; 100%; (i) CH_3SO_2Cl, Et_3N, THF, rt; 55%; (j) KOH, *t*-BuOH, rt; 67%; (k) (*S*)-$Ru(OAc)_2$(BINAP), H_2, MeOH, 5 bar, 35 ^oC; 35%.

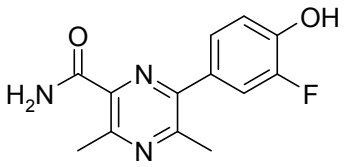
2-Fluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol -17



To a degassed solution of 4-bromo-2-fluorophenol (1.0 mL, 9.42 mmol) in dioxane (60 mL) was added KOAc (3.70 g, 37.70 mmol), Bis(pinacolato)diboron (3.59 g, 14.14 mmol), $Pd(dppf)Cl_2$ (0.465 g, 0.57 mmol) and dppf (0.317 g, 0.57 mmol). The resulting mixture was stirred at 85 °C under nitrogen for 17 h, allowed to cool, concentrated and diluted with EtOAc (100 mL). The mixture was acidified with 1N citric acid (75 mL) and filtered through celite. The aqueous phase was separated and extracted with EtOAc (150 mL) and organic extracts were combined, washed with saturated brine (150 mL), dried over $MgSO_4$, filtered and evaporated to afford crude product which was filtered through a pad of silica, washing through with EtOAc. The filtrate was evaporated to afford crude

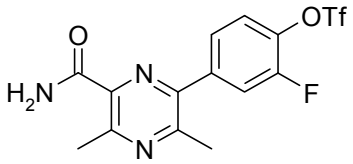
product which was purified by flash silica chromatography (0 - 20% EtOAc in isohexane) to afford 2-fluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (**17**) (2.0 g, 90 %) as a pale brown oil which solidified on standing. ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 1.33 (12 H, s), 5.38 (1 H, s), 6.98 (1 H, t, *J* 8.3), 7.46 – 7.53 (2 H, m). MS m/z (ES-) $(M-H)^-$ 237.

6-(3-Fluoro-4-hydroxyphenyl)-3,5-dimethylpyrazine-2-carboxamide



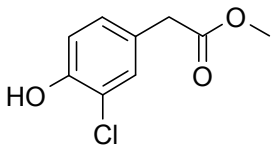
To a degassed solution of 6-chloro-3,5-dimethylpyrazine-2-carboxamide (See PCT Int. Appl. (2010), WO 2010146395, CAS no. 1166828-19-8, 0.819 g, 4.41 mmol), 2-fluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (1.05 g, 4.41 mmol) and K_3PO_4 (1.12 g, 5.29 mmol) in DME (15 mL), EtOH (7.5 mL) and water (3.75 mL) was added Pd(dppf)Cl_2.DCM adduct (0.180 g, 0.22 mmol).). The resulting mixture was stirred at 80 °C under nitrogen for 17 h, allowed to cool, concentrated and the residue was partitioned between EtOAc (75 mL), and 1N citric acid (25 mL). The precipitate was collected by filtration, washed with water (10 mL) and air dried to afford 6-(3-fluoro-4-hydroxyphenyl)-3,5-dimethylpyrazine-2-carboxamide (1.1 g, 91 %) as a beige solid. ^{1}H NMR δ_H (400 MHz, DMSO) 2.67 (3 H, s), 2.79 (3 H, s), 7.07 – 7.15 (1 H, m), 7.47 (1 H, dd, *J* 8.4, 1.4), 7.64 (1 H, s), 7.69 (1 H, dd, *J* 12.5, 2.1), 8.09 (1 H, s), 10.24 (1 H, s). MS m/z (ES+) $(M+H)^+$ 262.

4-(6-Carbamoyl-3,5-dimethylpyrazin-2-yl)-2-fluorophenyl trifluoromethanesulfonate



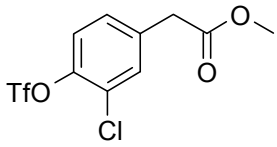
6-(3-Fluoro-4-hydroxyphenyl)-3,5-dimethylpyrazine-2-carboxamide (450 mg, 1.72 mmol), 1,1,1-trifluoro-N-phenyl-N-(trifluoromethylsulfonyl)methanesulfonamide (615 mg, 1.72 mmol) and K_2CO_3 (714 mg, 5.17 mmol) were suspended in THF (15 mL) and sealed into a microwave tube. The reaction was heated to 120 °C for 8 min in the microwave reactor and cooled to rt. The suspension was filtered, the solid was washed with EtOAc (20 mL) and the filtrate was evaporated to afford crude product which was purified by flash silica chromatography (10 - 60% EtOAc in isohexane) to dryness to afford 4-(6-carbamoyl-3,5-dimethylpyrazin-2-yl)-2-fluorophenyl trifluoromethanesulfonate (508 mg, 75 %) as a white solid. ^{1}H NMR δ_H (400 MHz, DMSO) 2.62 (3 H, s), 2.76 (3 H, s), 7.64 (1 H, s), 7.76 – 7.86 (2 H, m), 8.09 (1 H, dd, *J* 11.4, 1.9), 8.12 (1 H, s). MS m/z (ES+) $(M+H)^+$ 394.

Methyl 2-(3-chloro-4-hydroxyphenyl)acetate



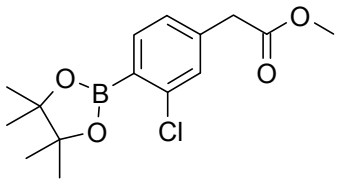
A solution of 3-chloro-4-hydroxyphenylacetic acid (6.38 g, 34.19 mmol) and conc H_2SO_4 (0.18 mL, 3.42 mmol) in MeOH (60 mL) was stirred at 75 °C for 3 h. The reaction mixture was allowed to cool, evaporated to dryness, redissolved in EtOAc (200 mL) and washed with saturated brine (2 x 150 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product which was purified by flash silica chromatography (10 - 30% EtOAc in isohexane) to afford methyl 2-(3-chloro-4-hydroxyphenyl)acetate (6.14 g, 90 %) as a colourless oil. ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 3.53 (2 H, s), 3.70 (3 H, s), 5.50 (1 H, s), 6.96 (1 H, d, *J* 8.3), 7.08 (1 H, dd, *J* 8.3, 2.1), 7.26 (1 H, s). MS m/z (ES-) $(M-H)^-$ 199.

Methyl 2-(3-chloro-4-(trifluoromethylsulfonyloxy)phenyl)acetate



Methyl 2-(3-chloro-4-hydroxyphenyl)acetate (1.14 g, 5.68 mmol), 1,1,1-trifluoro-N-phenyl-N-(trifluoromethylsulfonyl)methanesulfonamide (2.0 g, 5.68 mmol) and K_2CO_3 (2.356 g, 17.05 mmol) were suspended in THF (10 mL) and sealed into a microwave tube. The reaction was heated to 120 °C for 6 minutes in the microwave reactor and cooled to rt. The suspension was filtered, the solid was washed with EtOAc (20 mL) and the filtrate was evaporated to afford crude product. Which was purified by flash silica chromatography (0 - 10% EtOAc in isohexane) to afford methyl 2-(3-chloro-4-(trifluoromethylsulfonyloxy)phenyl)acetate (1.31 g, 69.3 %) as a colourless oil. ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 3.63 (2 H, s), 3.73 (3 H, s), 7.25 – 7.28 (1 H, m), 7.31 (1 H, d, *J* 8.5), 7.47 (1 H, d, *J* 2.2). GCMS m/z (EI+) $M^{+\cdot}$ 332.

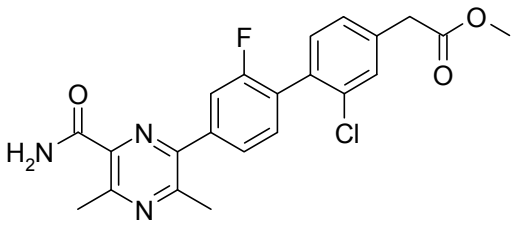
Methyl 2-(3-chloro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acetate



To a degassed solution of methyl 2-(3-chloro-4-(trifluoromethylsulfonyloxy)phenyl)acetate (6.5 g, 19.54 mmol) in dioxane (100 mL) was added KOAc (5.94 g, 60.57 mmol), bis(pinacolato)diboron (7.44 g, 29.31 mmol), dppf (0.657 g, 1.17 mmol) and $Pd(dppf)Cl_2$.DCM adduct (0.957 g, 1.17 mmol). And the suspension was degassed and then heated, under nitrogen, to 80 °C for 17 h. The reaction mixture was allowed to cool, diluted with EtOAc (300 mL) and water (100 mL). This was passed through a silica pad (3" diameter x 1" deep) washing with EtOAc. The filtrate was separated and washed with brine (200 mL) then evaporated to afford crude product which was purified by flash

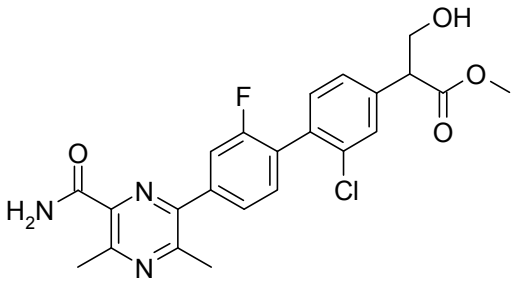
silica chromatography (0 - 30% EtOAc in isohexane) to afford methyl 2-(3-chloro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acetate (4.80 g, 79 %) as a colourless oil which solidified on standing. ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 1.29 (12 H, s), 3.52 (2 H, s), 3.61 (3 H, s), 7.08 (1 H, dd, *J* 7.6, 1.6), 7.21 (1 H, d, *J* 1.3), 7.58 (1 H, d, *J* 7.6). GCMS m/z (EI+) $M^{+\cdot}$ 310.

Methyl 2-(4'-(6-carbamoyl-3,5-dimethylpyrazin-2-yl)-2-chloro-2'-fluorobiphenyl-4-yl)acetate - 18



To a degassed solution of 6-(3-fluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-3,5-dimethylpyrazine-2-carboxamide (300 mg, 0.57 mmol) and methyl 2-(3-chloro-4-(trifluoromethylsulfonyloxy)phenyl)acetate (188 mg, 0.57 mmol), lithium chloride (42.0 mg, 0.99 mmol) and K_3PO_4 (144 mg, 0.68 mmol) in DME (5 mL), MeOH (2.5 mL) and water (1.25 mL) was added $Pd(dppf)Cl_2$.DCM adduct (23.1 mg, 0.03 mmol). The reaction mixture was degassed again and heated to reflux, under nitrogen for 8 h. The reaction mixture was allowed to cool and evaporated, the residue was partitioned between EtOAc (50 mL), and water (30 mL) and the aqueous layer was further extracted with EtOAc (50 mL). The organic layers were combined and washed with saturated brine (50 mL), dried over $MgSO_4$, filtered and silica added and then evaporated to afford crude product absorbed on silica. The residue was purified by flash silica chromatography (40 - 70% EtOAc in isohexane) to afford methyl 2-(4'-(6-carbamoyl-3,5-dimethylpyrazin-2-yl)-2-chloro-2'-fluorobiphenyl-4-yl)acetate (**18**) (133 mg, 55 %) as a cream solid. δ_H (400 MHz, $CDCl_3$) 2.74 (3 H, s), 3.01 (3 H, s), 3.68 (2 H, s), 3.75 (3 H, s), 5.52 (1 H, s), 7.29 (1 H, dd, *J* 7.9, 1.7), 7.35 (1 H, d, *J* 7.8), 7.4 – 7.48 (4 H, m), 7.77 (1 H, s). MS m/z (ES+) $(M+H)^+$ 428.

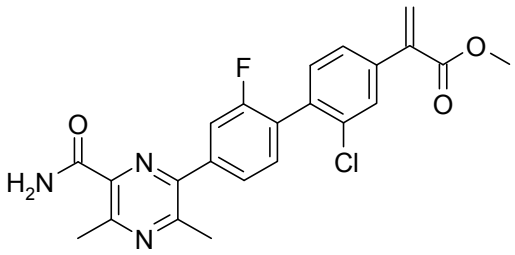
Methyl 2-(4'-(6-carbamoyl-3,5-dimethylpyrazin-2-yl)-2-chloro-2'-fluorobiphenyl-4-yl)-3-hydroxypropanoate - 19



Paraformaldehyde (0.370 g, 12.34 mmol) was added to a stirred solution of methyl 2-(4'-(6-carbamoyl-3,5-dimethylpyrazin-2-yl)-2-chloro-2'-fluorobiphenyl-4-yl)acetate (4.40 g, 10.28 mmol) and K_2CO_3 (1.42 g, 10.28 mmol) in DMF (45 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h under nitrogen, before being allowed to warm to and stir at rt for 45 minutes. The reaction

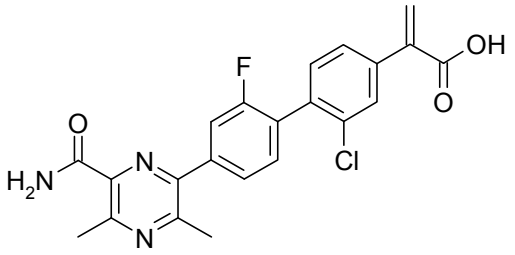
mixture was diluted with water (400 mL), treated with 2M HCl (5.2 mL) and extracted with EtOAc (2 x 100 mL). The organic extracts were combined, washed with water (50 mL) and saturated brine (2 x 50 mL) dried over $MgSO_4$, filtered and evaporated to afford crude product which was purified by flash silica chromatography (0 - 10% MeOH in DCM) to afford methyl 2-(4'-(6-carbamoyl-3,5-dimethylpyrazin-2-yl)-2-chloro-2'-fluorobiphenyl-4-yl)-3-hydroxypropanoate (**19**) (4.73 g, 100 %) as a colourless oil. ^{1}H NMR δ_H (400 MHz, DMSO) 2.79 (3 H, s), 2.95 (3 H, s), 3.73 (3 H, s), 3.77 – 3.85 (1 H, m), 3.96 – 4.06 (2 H, m), 5.16 (1 H, t, *J* 5.0), 7.46 – 7.6 (2 H, m), 7.64 (1 H, d, *J* 1.5), 7.69 (1 H, s), 7.77 (1 H, dd, *J* 7.9, 1.7), 7.87 (1 H, dd, *J* 11.0, 1.6), 8.01 (1 H, s), 8.17 (1 H, s). MS m/z (ES+) $(M+H)^+$ 458.

Methyl 2-(4'-(6-carbamoyl-3,5-dimethylpyrazin-2-yl)-2-chloro-2'-fluorobiphenyl-4-yl)acrylate - 20



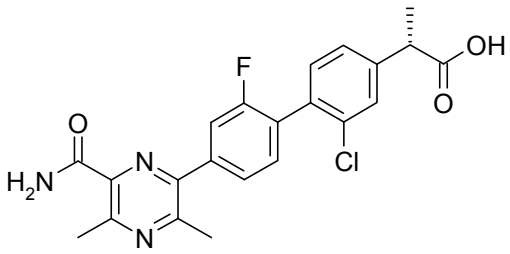
Triethylamine (1.2 mL, 8.26 mmol) was added to a stirred suspension of methyl 2-(4'-(6-carbamoyl-3,5-dimethylpyrazin-2-yl)-2-chloro-2'-fluorobiphenyl-4-yl)-3-hydroxypropanoate (1.35 g, 2.95 mmol) and methanesulfonyl chloride (0.3 mL, 3.83 mmol) in THF (20 mL) under nitrogen. The resulting suspension was stirred at rt for 17 h, the reaction was incomplete so further triethylamine (0.11 mL, 0.83 mmol) and methanesulfonyl chloride (0.030 mL, 0.38 mmol) were added and the reaction was left to stir for an additional 2 h at rt. The mixture was diluted with EtOAc (100 mL), and washed with saturated NH_4Cl (50 mL), water (50 mL), and saturated brine (2 x 50 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product which was purified by flash silica chromatography (30 - 65% EtOAc in isohexane) to afford methyl 2-(4'-(6-carbamoyl-3,5-dimethylpyrazin-2-yl)-2-chloro-2'-fluorobiphenyl-4-yl)acrylate (**20**) (0.715 g, 55 %) as a white crystalline solid. ^{1}H NMR δ_H (400 MHz, DMSO) 2.73 (3 H, s), 2.84 (3 H, s), 3.86 (3 H, s), 6.29 (1 H, s), 6.46 (1 H, s), 7.53 – 7.63 (3 H, m), 7.70 (1 H, s), 7.76 – 7.81 (2 H, m), 7.88 (1 H, dd, *J* 10.9, 1.6), 8.18 (1 H, s). MS m/z (ES+) $(M+H)^+$ 440.

2-(4'-(6-Carbamoyl-3,5-dimethylpyrazin-2-yl)-2-chloro-2'-fluorobiphenyl-4-yl)acrylic acid - 21



Powdered KOH (3.90 g, 69.57 mmol) was added to methyl 2-(4'-(6-carbamoyl-3,5-dimethylpyrazin-2-yl)-2-chloro-2'-fluorobiphenyl-4-yl)acrylate (15.3 g, 34.78 mmol) in *t*-BuOH (450 mL) and the reaction mixtue was stirred at rt. After 3 h further powdered KOH (2.0 g, 35.71 mmol) was added and the mixture was allowed to stir for 17 h at rt. Further Powdered KOH (4.0 g, 71.42 mmol) and *t*-BuOH (200 mL) were added and the reaction mixture was stirred at rt an additional 4 h. The mixture was acidified with excess saturated citric acid and the organics removed under reduced pressure. The aqueous residue was diluted with water (100 mL) and EtOAc (500 mL). The precipitate was filtered to afford crude product. The organic layer was separated, dried over $MgSO_4$, filtered and evaporated to afford further crude product. The solids were combined slurried in EtOAc (50 mL), filtered, washed with isohexane (50 mL) and dried to afford 2-(4'-(6-carbamoyl-3,5-dimethylpyrazin-2-yl)-2-chloro-2'-fluorobiphenyl-4-yl)acrylic acid (**21**) (9.98 g, 67 %) as a cream solid. ^{1}H NMR δ_H (400 MHz, DMSO) 2.73 (3 H, s), 2.84 (3 H, s), 6.06 (1 H, s), 6.29 (1 H, s), 7.52 (1 H, d, *J* 8.0), 7.56 – 7.64 (2 H, m), 7.69 (1 H, s), 7.74 – 7.81 (2 H, m), 7.87 (1 H, dd, *J* 10.9, 1.6), 8.18 (1 H, s); CO_2H not observed. MS m/z (ES+) $(M+H)^+$ 426.

(2S)-2-(4'-(6-Carbamoyl-3,5-dimethylpyrazin-2-yl)-2-chloro-2'-fluorobiphenyl-4-yl)propanoic acid - 16



2-(4'-(6-carbamoyl-3,5-dimethylpyrazin-2-yl)-2-chloro-2'-fluorobiphenyl-4-yl)acrylic acid (60 g, 119.76 mmol), diaceto[(s)-(-)-2,2'-bis(diphenylphosphino)-1-1'binaphthyl]ruthenium(II) (1.008 g, 1.20 mmol) in degassed MeOH (3500 mL) were stirred under an atmosphere of hydrogen at 5 bar and 35 °C for 3 h. The solvent was evaporated to afford crude product which was purified by flash silica chromatography (5 - 50% MeOH in DCM) to afford 34.5g, brown solid which was slurried in MeCN (400 mL) for 2 h at rt. The suspension was filtered and washed with MeCN (100 mL) and air dried to afford 33g of a brown solid, which was purified by preparative chiral-HPLC on a Prochrom 200mm 20um Chiralpak AD column, eluting isocratically with 70% isohexane in EtOH (modified with 0.2 % AcOH) as eluent to afford 22.5g of the desired compound as a light brown solid. This was slurried in MeCN (200 mL) at rt for 2 h, filtered, washed with MeCN (70 mL) and dried under vacuum at 60 °C overnight to afford (2S)-2-(4'-(6-carbamoyl-3,5-dimethylpyrazin-2-yl)-2-chloro-2'-fluorobiphenyl-4-yl)propanoic acid (**16**) (21g, 35%) as a light brown solid.
δ_H (700 MHz, DMSO) 1.40 (3 H, d, *J* 7.2), 2.63 (3 H, s), 2.74 (3 H, s), 3.78 (1 H, q, *J* 7.1), 7.36 (1 H, dd, *J* 7.9, 1.5), 7.41 (1 H, d, *J* 7.9), 7.48 (1 H, t, *J* 7.8), 7.51 (1 H, d, *J* 1.5), 7.59 (1 H, s), 7.67 (1 H, dd, *J* 7.9, 1.6), 7.76 (1 H, dd, *J* 10.8, 1.5), 8.08 (1 H, s); CO_2H not observed.

δ_C (176 MHz, DMSO) 18.22, 22.14, 22.82, 44.05, 116.53 (d, *J* 23.6), 125.25 (d, *J* 2.5), 126.35 (d, *J* 16.0), 126.51, 128.46, 131.30 (d, *J* 2.7), 131.74, 132.21, 132.31, 139.69 (d, *J* 8.0), 141.34, 143.50, 146.72, 150.73, 151.86, 158.73 (d, *J* 245.7), 167.12, 174.72.

MS m/z (ES+) $(M+H)^+$ 428. HRMS (ESI): MH^+, found 428.1170 $C_{22}H_{20}$ ClF N_3O_3 requires 428.1177

C 61.37%; H 4.52%; N 9.75%; O 11.59% H2O 0.4%; MeCN 0.04%; DCM 0.18%; EtOAc 0.05%; MeOH 0.2%

PKPD modelling

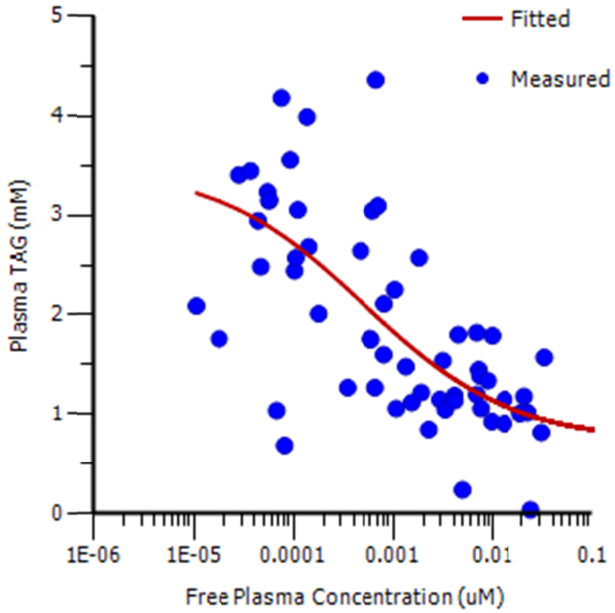
Treatment with **16** resulted in a exposure-dependent decrease in circulating plasma TAG levels. Figure 6 shows the relationship between plasma triglycerides and free compound levels in plasma for **16** in the rat OLTT assay. A direct response PK/PD model (Emax sigmoidal model) was used to succesfully fit the PK/PD data showing a clear correlation between compound levels in plasma and an effect in the OLTT. PK/PD parameters for the model fit are shown on table 1.

PK/PD parameters for model fit for **16** in the rat OLTT

Parameter	Estimate	Units	Stderr%
IC50	0.0005	uM	62
Gamma	0.6	--	56
E0	3.5	mM	11
Imax	2.7	mM	26

$$E = E0 - \frac{I_{max} \times C^{\gamma}}{IC_{50}{}^{\gamma} + C^{\gamma}}$$

PK/PD analysis of rat OLTT (plasma triglycerides Vs free compound concentrations in plasma) for **16**. A direct response (Emax sigmoidal) model was used to fit the PK/PD data.



Treatment with **16** resulted in a exposure-dependent decrease in TAG synthesis in adipose tissue in the rat. Figure 7 shows the relationship between adipose TAG synthesis (expressed

as TAG:DAG ratio in adipose tissue) and free compound levels in plasma for **16** in the rat. A direct response PK/PD model (Emax sigmoidal model) was used to succesfully fit the PK/PD data showing a clear correlation between free compound levels in plasma and an effect in the adipose TAG/DAG ratio. PK/PD parametersfor the model fit are shown on table 2.

PK/PD parameters for model fit for **16** in the rat adipose tissue TAG synthesis assay

Parameter	Estimate	Units	Stderr%
IC50	0.0022	uM	18
Gamma	1.3	--	19
E0	5.2	--	5.6
Imax	4.7	--	7.5

$$E = E0 - \frac{I_{\max} \times C^{\gamma}}{IC_{50}{}^{\gamma} + C^{\gamma}}$$

PK/PD analysis of rat rat adipose TAG synthesis assay (adipose tissue TAG:DAG ratio Vs free compound concentrations in plasma) for **16**. A direct response (Emax sigmoidal) model was used to fit the PK/PD data.