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Discovery, optimisation and in vivo evaluation of novel GPR119 agonists

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ABSTRACT

GPR119 is increasingly seen as an attractive target for the treatment of type II diabetes and other elements of the metabolic syndrome. During a programme aimed at developing agonists of the GPR119 receptor, we identified compounds that were potent with reduced hERG liabilities, that had good pharmacokinetic properties and that displayed excellent glucose-lowering effects in vivo. However, further profiling in a GPR119 knock-out (KO) mouse model revealed that the biological effects were not exclusively due to GPR119 agonism, highlighting the value of transgenic animals in drug discovery programs.

GPR119 is a class A G-protein coupled receptor that is predominantly expressed in pancreatic islets and sections of the GI tract.[1] Recent work has led to the de-orphanisation of this receptor with the identification of oleoylethanolamide (OEA) and related compounds being proposed as natural agonists.[2] Studies have shown that agonism of GPR119 results in incretin release in the gut (e.g., glucagon-like peptide-1 release from L-cells) in addition to the stimulation of insulin release from β-cells in the pancreas.[3] This dual mechanism of action has generated considerable interest in the potential of GPR119 agonists as a therapeutic intervention in the treatment of diabetes.[4]

Following an initial demonstration that a synthetic GPR119 agonist was capable of controlling glucose excursions in preclinical animal models,[5] a number of groups have described their efforts in this area.[6–10] Herein we report the synthesis and structure–activity relationships of a series of novel GPR119 agonists together with their in vivo effects in both wild-type and GPR119 knock-out mouse studies.

Our initial hit **1** was identified from screening of the AstraZeneca compound collection (Fig. 1). This compound had moderate potency against the GPR119 receptor (EC_{50} = 538 nM in an in vitro cAMP assay)[11] and displayed modest efficacy (42% top effect).[12] The log*D* was measured[13] at 2.9 resulting in a reasonable ligand lipophilicity

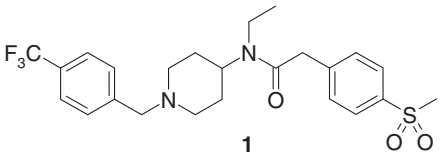

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Figure 1. Structure of the initial high-throughput screening hit.

efficiency,[14,15] LLE (pEC_{50} – log*D* = 3.4), and the compound had moderate metabolic stability in human microsomes (Cl_{int} = 8 μL/min/mg). A key issue with this compound was the measured affinity against the hERG ion channel[16] (IC_{50} = 2.5 μM) leading to a selectivity based on the ratio of primary potency/hERG of less than fivefold. Improvement of the selectivity against hERG was therefore the initial focus of the optimisation campaign.

The compounds described in this paper were prepared using a modular approach that allowed diversification of R^1 or R^2 at the final step (Scheme 1). Route A involved amide coupling of *tert*-butyl 4-(methylamino)piperidine-1-carboxylate with phenyl acetic acids (step a) using 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) followed by Boc deprotection to give the piperidine (step b). Subsequent alkylation (step c) provided compounds **2** & **8**. Route B involved alkylation of isomeric *N*-*tert*-butyl *N*-methyl-*N*-(4-piperidyl)carbamate followed by Boc deprotection to give the piperidine intermediate that was acylated to provide compounds **7** & **9**–**13**. Compound **14** was

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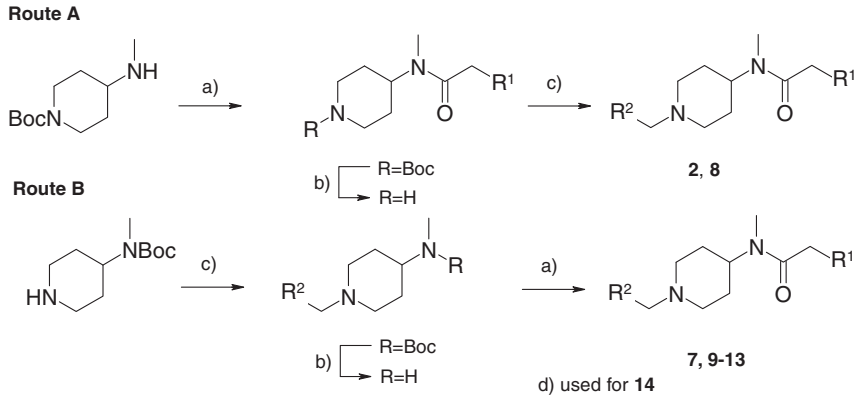
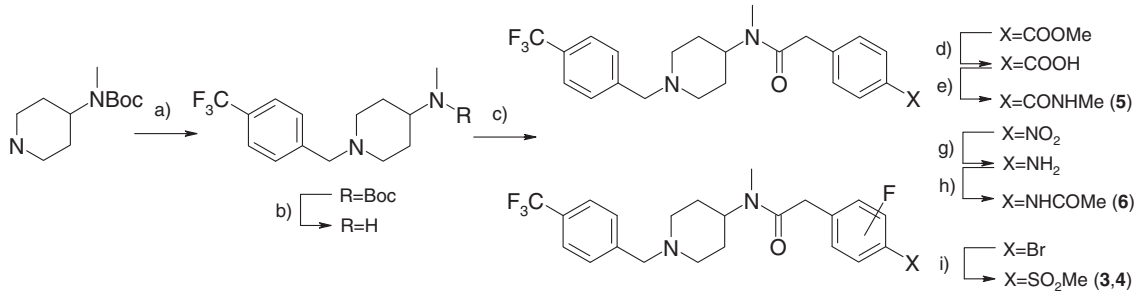


Scheme 1. General synthetic procedure. Reagents and conditions: (a) R^1CH_2COOH, DMTMM, THF, rt, 60–73%; (b) trifluoroacetic acid (TFA), CH_2Cl_2, rt, 77–100%; (c) R^2CH_2X (X = Br, Cl or OMs), $N(^iPr)_2Et$, CH_3CN, rt, 15–94%; (d) $NaBH(OAc)_3$, $N(^iPr)_2Et$, $MgSO_4$, THF, 44%.

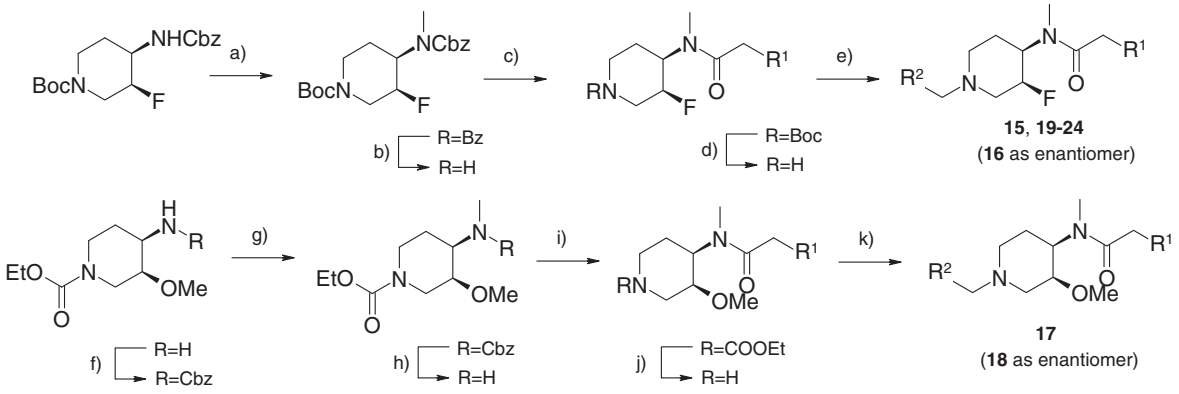


Scheme 2. Functional group conversion for diversification of R^1. Reagents and conditions: (a) *p*-CF_3PhCH_2Br, $N(^iPr)_2Et$, CH_2Cl_2, 94%; (b) TFA, CH_2Cl_2, 100%; (c) $N(^iPr)_2Et$, CH_2Cl_2, R^1CH_2COCl, 71–86%; (d) LiOH, H_2O/THF, 88%; (e) HATU, $N(^iPr)_2Et$, DMF, $MeNH_2$, 44%; (g) H_2, Pd/C, 60%; (h) MeCOCl, Pyridine, CH_2Cl_2, 65%; (i) $MeSO_2Na$, Cu(I)OTf. toluene, $Me_2NCH_2CH_2NMe_2$, DMSO, 12–23%.

synthesised using route B with a reductive amination of the corresponding aldehyde (step d) as the final step.

Other examples (**3**–**6**) were synthesised by elaboration of functional groups (Scheme 2). Amide **5** was synthesised from an ester intermediate through hydrolysis and coupling with methylamine (steps d, e) whereas the reversed amide **6** was synthesised through reduction of a nitro group followed by coupling with an acid chloride (steps g, h). Fluoro substituted aromatic examples **3** & **4** were synthesised via formation of the bromo intermediates followed by conversion to sulphone (step i) using copper catalysis.

For the fluorinated piperidine examples (Scheme 3), the known (*3S,4R*)-3-fluoro-piperazine intermediate[17] was methylated (step a) and the carboxybenzyl (Cbz) protecting group removed under hydrogenating conditions (step b). Coupling with the required phenyl acetic acid using HATU (2-(7-Aza-*1H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) (step c) and deprotection of the Boc group (step d) afforded an intermediate that could be alkylated (step e) to afford the final compounds **15** & **19**–**24**. Compound **16** was synthesised from the enantiomeric (*3R,4S*) intermediate using the same synthetic sequence. For the methoxy analogues, the known (*3S,4R*)-3-methoxy-piperazine intermediate[18] was carboxybenzyl protected (step f) and then methylated (step g). Removal of the carboxybenzyl protecting group (step h) was followed by coupling with the required phenyl



Scheme 3. General synthetic procedure for substituted piperazines. Reagents and conditions: (a) NaH, MeI, DMF, 91%; (b) H_2, Pd/C, EtOH, 90%; (c) R^1CH_2COOH, HATU, $N(^iPr)_2Et$, DMF, 44–100%; (d) TFA, CH_2Cl_2, 100%; (e) R^2CH_2X (X = Br,Cl or OMs), $N(^iPr)_2Et$, CH_3CN, r, 36–86%; (f) BnOCOCl, $N(^iPr)_2Et$, CH_2Cl_2, 99%; (g) NaH, MeI, DMF, 79%; (h) H_2, Pd/C, EtOH; (i) 2-[4-(tetrazol-1-yl)phenyl]acetic acid, DMTMM, THF; 85% (over two steps); (j) TMSI, CH_2Cl_2, 54%; (k) *p*-CF_3PhCH_2Br, $N(^iPr)_2Et$, CH_3CN, 85%.

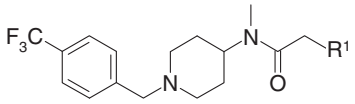
acetic acid (step i) using DMTMM. Deprotection of the ethyl carbamate was carried out using trimethylsilyl iodide (step j) and the product was then alkylated (step k) to afford the final compound **17**. Compound **18** was synthesised from the enantiomeric (*3R,4S*) intermediate using the same synthetic sequence.

Truncation of the *N*-ethyl to an *N*-methyl amide **2** improved the in vitro metabolic profile (Cl_{int} = 3 μL/min/mg) whilst maintaining potency (EC_{50} = 456 nM), LLE (3.8) and hERG (IC_{50} = 3 μM; now eightfold relative to primary target). We were keen to improve potency but not at the cost of additional lipophilicity and therefore had LLE as a key optimization parameter. Exploration of the R^1 substituent is shown in Table 1. Fluorination of the aryl ring (**3** & **4**) resulted in an increase in LLE score (LLE = 4.2 & 3.9, respectively) and efficacies (88% & 73%, respectively) relative to the unsubstituted analogue **2**. Although the hERG affinities had remained unaltered, the increase in primary potency resulted in an improved selectivity ratio (>20-fold in both cases). C and N linked amides were tolerated (**5** & **6**) although neither offered significant advantages over the sulphone **2**. The *N*-linked tetrazole **7** was the most potent compound identified (EC_{50} = 74 nM) and had the highest efficacy (132%) with an LLE of 4.1. Although this compound showed increased hERG affinity (1.2 μM), the increased potency resulted in an increased hERG selectivity (16×). Substitution of the aryl ring was crucial to activity as exemplified by compound **8** which was inactive in the in vitro cAMP assay, despite being considerably more lipophilic than the other compounds investigated.

Exploration of the R^2 substituent is shown in Table 2 with the R^2 substituent fixed as the *N*-tetrazole as in compound **7** (EC_{50} = 74 nM; LLE = 4.1). Incorporation of a pyridyl N (**9** & **10**) reduced potency in line with the reduction in lipophilicity (LLE scores of 4.1, 4.0 & 3.9 for compounds **7**, **9** & **10**, respectively) but with efficacy remaining high. Consequently, although reductions in hERG were observed this did not result in increased margins. Fluorination of the aryl ring (**11**) resulted in a modest potency increase and decrease in hERG affinity leading to increased selectivity (53-fold) however, increased susceptibility to metabolism was observed (Cl_{int} = 14 μL/min/mg). The CF_3 unit could be replaced by polar heterocycles such as the oxadiazole **12** with potency loss broadly in line with lipophilicity reduction (LLE = 4.3) but with efficacy maintained and selectivity against hERG remaining at similar levels (15-fold) to compound **7**. Benzothiazole **13** was identified as an interesting group that had a similar profile to the trifluoroaryl **7** with high LLE (4.3) and similar selectivity against hERG (17-fold). Attempts to saturate the aryl ring (**14**) had the desired effect of reducing the absolute affinity for hERG (IC_{50} = 7.6 μM), but led to a reduction in LLE and an increase in turnover in human microsomes (Cl_{int} = 169 μL/min/mg).

Our last area of exploration was the piperidine core as shown in Table 3. We rationalised that the basicity of the nitrogen (measured pK_a = 7.0) may be responsible for the affinity for the hERG receptor[19] and we therefore sought to moderate this by incorporation of electronegative groups at the 3-position. To this end, the

Table 1
Human GPR119 potencies, physical and DMPK properties for R^1 variation



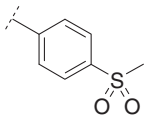
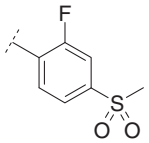
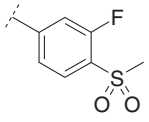
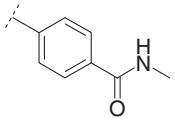
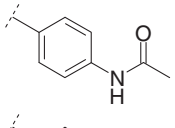
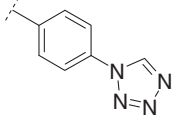
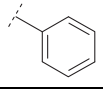
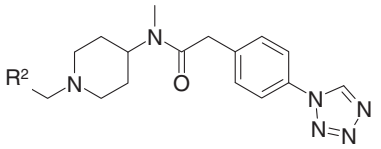
Compd	R^1	h GPR119 EC_{50} (μM)	h GPR119 activity (%)	log*D*	LLE (pEC_{50}−log*D*)	hERG IC_{50} (μM)	h Mics Cl_{int} (μL/min/mg)
2		0.456	54	2.5	3.8	3	3
3		0.122	88	2.7	4.2	2.6	18
4		0.131	73	3.0	3.9	2.9	15
5		0.489	75	2.8	3.5	2.1	<6
6		0.535	65	2.9	3.4	4.5	29
7		0.074	132	3	4.1	1.2	7
8		>30	24	>4.0	—	1.6	20

Table 2
Human GPR119 potencies, physical and DMPK properties for R^2 variation



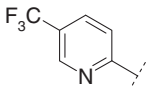
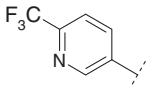
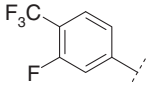
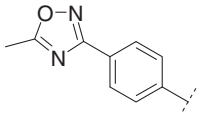
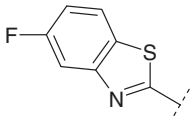
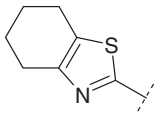
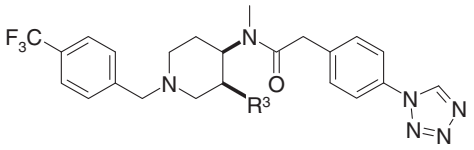
Compd	R^2	h GPR119 EC_{50} (μM)	h GPR119 activity (%)	log*D*	LLE ($pEC_{50}-\log D$)	hERG IC_{50} (μM)	h Mics Cl_{int} (μL/min/mg)
9		1.345	98	1.9	4.0	4.2	<5
10		1.89	79	1.8	3.9	13	<2
11		0.053	83	—	—	2.8	14
12		0.441	98	2.1	4.3	6.5	<2
13		0.076	85	2.9	4.3	1.3	21
14		0.396	68	2.5	3.9	7.6	169

Table 3
Human GPR119, physical and DMPK properties for R^3 variation



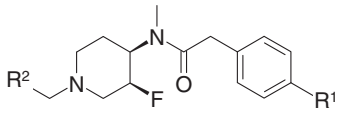
Compd	R^3 (stereo)	h GPR119 EC_{50} (μM)	h GPR119 activity (%)	log*D*	LLE ($pEC_{50}-\log D$)	hERG IC_{50} (μM)	h Mics Cl_{int} (μL/min/mg)
15	F (*3R,4S*)	0.029	104	3.3	4.2	3.6	5
16	F (*3S,4R*)	0.007	123	3.3	4.9	2.0	6
17	OMe (*3R,4S*)	0.029	84	3.1	4.4	2.0	71
18	OMe (*3S,4R*)	0.172	30	3	3.8	1.2	65

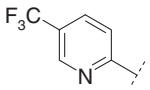
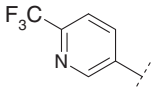
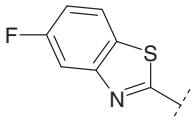
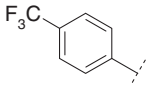
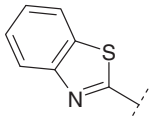
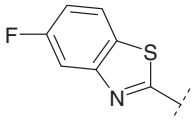
enantiomeric pairs of the *cis*-F (**15** & **16**; predicted $pK_a = 5.5$)[20] and *cis*-OMe (**17** & **18** predicted $pK_a = 6.4$) were synthesized and compared to the unsubstituted analogue **7**. For the fluoro substitution, we observed modest reduction in the absolute affinities for hERG despite an increase in lipophilicity. In the case of the (*3S,4R*) isomer **16**, we achieved a dramatic improvement in GPR119 potency ($EC_{50} = 7$ nM) with high efficacy (123%) leading to improved LLE (4.9) and hERG selectivity (>250-fold). The methoxy analogues had significant increases in metabolic instability ($Cl_{int} = 71$ & 65 μL/min/mg for compounds **17** and **18**, respectively). It was of interest to note that for the F and OMe, different enantiomers of the pairs were preferred in each case and that the potency and efficacy of the (*3S,4R*) OMe compound **18** was significantly reduced.

With this knowledge of the structure–activity relationships within this series, we elected to fix the core as the (*3S,4R*)-F isomer and synthesise examples of what we felt to be optimal combinations as shown in Table 4. Incorporation of a pyridyl group into the CF_3 aryl ring delivered compounds **19** & **20** which were potent against GPR119 with hERG affinities >10 μM and that were stable in rat and human microsomes ($Cl_{int} = 4$ & 3 μL/min/mg, respectively). The benzothiazole **21** was found to have excellent potency ($EC_{50} = 12$ nM) with improved selectivity against hERG (>390-fold). Switching to a sulphone R^1 substituent, gave **22** which was the most potent compound to date ($EC_{50} = 4$ nM) and with high LLE (5.7) and robust efficacy (85%). The affinity for hERG was 4.2 μM leading to a selectivity in excess of 1000-fold, and the compound was metabolically stable ($Cl_{int} = 6$ μL/min/mg). The benzothiazole combinations **23** & **24** were potent and compound **23** was notable for having good potency ($EC_{50} = 24$ nM) in combination with low affinity for the hERG channel ($IC_{50} = 16$ μM).

In parallel with this optimisation work, further profiling of compound **7** had revealed that this compound had many attractive features. The compound displayed no inhibition towards five major isoforms of cytochrome P450 enzymes (CYP1A2, CYP2C9, CYP2C19,

Table 4
Human GPR119, physical and DMPK properties for selected combinations



Compd	R^1	R^2	h GPR119 EC_{50} (μM)	h GPR119 activity (%)	log*D*	LLE (pEC_{50}−log*D*)	hERG IC_{50} (μM)	h Mics Cl_{int} (μL/min/mg)
19			0.140	139	2.1	4.8	10	4
20			0.485	78	1.8	4.5	15	3
21			0.012	86	2.6	5.3	4.7	<7
22			0.004	85	2.7	5.7	4.2	6
23			0.024	44	2.1	5.5	16	9
24			0.018	39	2.2	5.6	7	13

CYP2D6 and CYP3A4) in a high throughput fluorescence assay, with IC_{50} values >25 μM. Plasma protein binding showed reasonable free drug levels consistently across species (mouse = 9.9% free; rat = 8.1% free; dog = 6.8% free; human = 7.6% free). The solubility as measured on crystalline material was modest at 18 μM but when coupled with good cellular permeability as measured in an in vitro CACO-2 assay (apical to basolateral $P_{app} = 57 \times 10^{-6}$ cm/s, efflux ratio = 0.4 at a compound concentration of 10 μM) or MDCK assay (apical to basolateral $P_{app} = 17 \times 10^{-6}$ cm/s, efflux ratio = 0.8 at a compound concentration of 10 μM) it was predicted that this would lead to good absorption in vivo.

The pharmacokinetic profiles of **7** were determined in vivo in three pre-clinical species; mouse, rat and dog (Table 5). The compound showed good pharmacokinetic properties across species, with low clearance (rat and dog) and good bioavailability (mouse, rat). A bile duct cannulation study in rat indicated no biliary or renal elimination. This was consistent with the excellent correlation of in vitro/in vivo scaling of clearance from hepatocytes.

Compound **7** was tested against the murine form of GPR119 and was found to retain potency (EC_{50} = 147 nM in an in vitro cAMP assay) with an efficacy of 74% relative to that of OEA. Based on the favourable mouse pharmacokinetics with this compound, it was investigated in vivo for its potential to control the glucose excursion in a mouse (C57BL6/JAX) oral glucose tolerance test (OGTT). In order to establish that this glucose control was mediated by GPR119, compound **7** was tested in an OGTT with both wild-type and GPR119 knock-out mice at a dose of 20 mg/Kg. A significant glucose lowering effect was observed in the wild-type mice however, to our surprise, an effect of similar magnitude was also observed in the knock-out mice group indicating that the effects observed were not exclusively mediated through GPR119 (Fig. 2). Reducing the dose of compound **7** led to lower glucose lowering effects (data not shown) but crucially no separation of wild-type and knock-out activities.

In order to investigate whether this off-target activity was related to a particular structural feature of this compound or was characteristic of the series, compounds **5**, **13** and **17** were selected as being matched pairs with compound **7** but with structural variation in terms of the R^1, R^2 and R^3 groups, respectively. Based on the DMPK profiles of these compounds in mouse, we selected doses that would give coverage of the murine EC_{50} for the entire duration of the experiment. Upon testing these compounds in vivo, and confirming that the targeted exposures had been achieved, we again observed glucose lowering in both wild-type and knock-out animals in all three cases. This led us to conclude that the pharmacophore displayed by this chemical series was

Table 5
Pharmacokinetic parameters for selected compound **7**[a]

Species	Clp (mL/min/kg)	Vdss (L/kg)	PO half-life (h)	IV half-life (h)	PO C_{max} (μM)	Bioavailability (%)
Mouse	12	2.0	2.4	2.2	2.2	64
Rat	6.8	1.4	2.8	2.8	2.4	66
Dog	1.3	2.3	18	20	6.4	35

[a] Compounds were dosed at 2 mg/kg (IV) and 5 mg/kg (PO—mouse and rat), 13 mg/kg (PO—dog) in 5% DMSO:95% hydroxylpropyl beta cyclodextrin, and a 0.1% pluronic F127suspension, respectively at volumes of 5 mL/kg (mouse and rat) and 2 mL/kg (dog).[21]

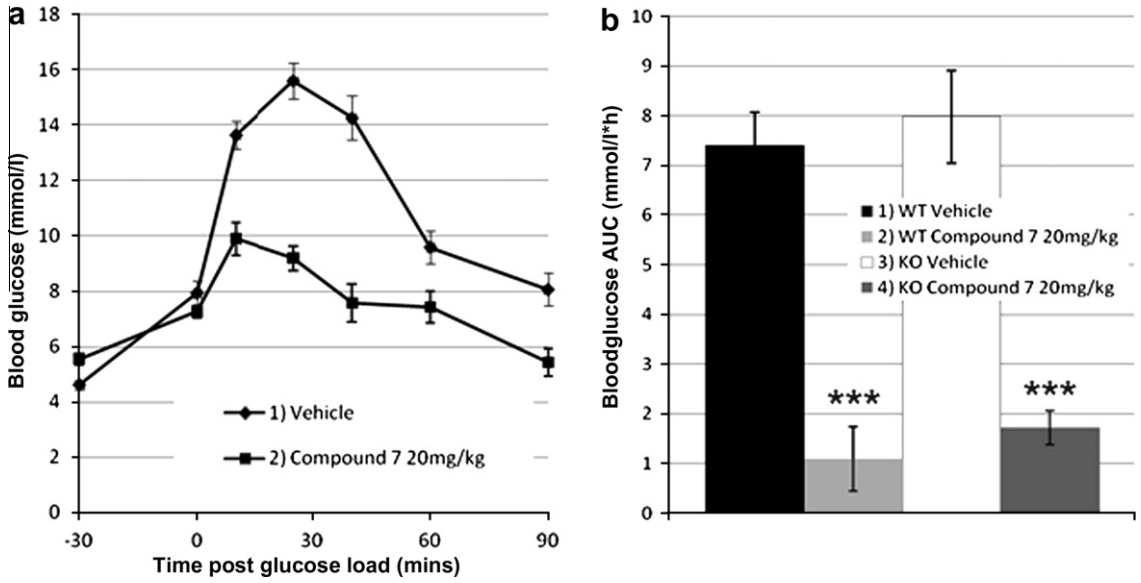


Figure 2. In vivo glucose lowering effects of compound **7** (a) OGTT blood glucose profile in C57BL6/J mice (b) OGTT blood glucose AUC GPR119 knock-out (KO) mice and wild-type (WT) controls. Compound was administered po in 0.1% pluronic F127 vehicle (n = 6–7/group) 30 min prior to a glucose load of 2 g/kg. Glucose levels were monitored for 90 min post glucose load. $^{***}p \leqslant 0.001$ versus vehicle control ANCOVA analysis.

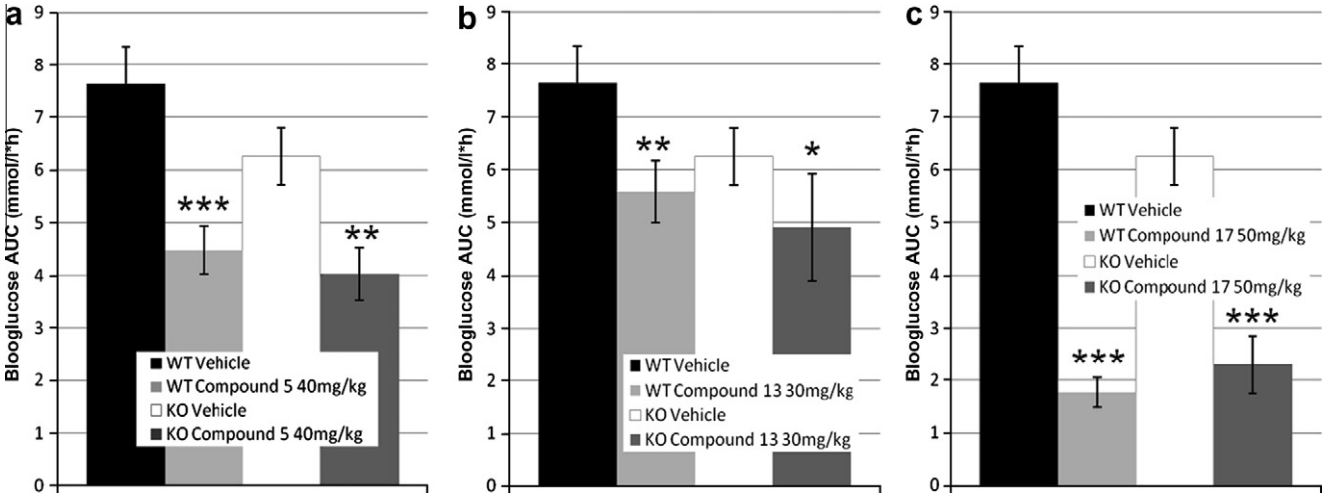


Figure 3. In vivo glucose lowering effects of (a) compound **5** (b) compound **13** (c) compound **17** in GPR119 knock-out (KO) mice and wild-type (WT) controls. Compound was administered po in 0.1% pluronic F127 vehicle (n = 11–12 vehicle, n = 8 compound treated) 30 min prior to a glucose load of 2 g/kg. Glucose levels were monitored for 90 min post glucose load and used to calculate reductions in blood glucose AUC. $^{*}p \leqslant 0.05$, $^{**}p \leqslant 0.01$, $^{***}p \leqslant 0.001$ versus vehicle control ANCOVA analysis.

giving glucose lowering effects through mechanisms other than GPR119 (Fig. 3).

In efforts to identify what target may be responsible for these effects, compound **7** was screened against a panel of 100 targets. Only three targets were identified (5HT2A, D4.2 & CCR5) that gave >75% activity at a concentration of 30 μM. In contrast, compound **13** was inactive against both D4.2 & CCR5 (<10% activity at 30 μM) yet still showed off-target activity. Compounds from other series being profiled in the project which showed glucose lowering in the wild type but not the knock-out mouse, had similar levels of activity against 5HT2A (data not shown) suggesting that this was not the target responsible. Further attempts to ascertain the cause of the 'off-target' effects have not provided a clear outcome at present.

In summary, optimisation of a novel series of GPR119 agonists allowed us to identify compounds that were potent against the receptor with reduced hERG liabilities relative to the initial lead as exemplified by compound **23**. A representative example **7** had excellent pharmacokinetic properties and displayed excellent glucose lowering in vivo. However the glucose lowering effects were also observed in a GPR119 knock-out mouse model leading us to conclude that the biological effects were not exclusively due to GPR119 agonism. Further studies on alternative chemical series that do not exhibit activity in the GPR119 knock-out mouse model will be reported in due course

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