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Discovery of structurally novel, potent and orally efficacious GPR119 agonists

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ABSTRACT

Screening hit **5** was identified in a biochemical screen for GPR119 agonists. Compound **5** was structurally novel, displayed modest biochemical activity and no oral exposure, but was structurally distinct from typical GPR119 agonist scaffolds. Systematic optimization led to compound **36** with significantly improved in vitro activity and oral exposure, to elevate GLP1 acutely in an in vivo mouse model at a dose of 10 mg/kg.

The need to address the worldwide pandemic of Type 2 diabetes (T2D) can hardly be overstated.[1] The current treatments for this chronic disorder either fail to be effective over time or present limiting safety risks, emphasizing a strong medical need for novel approaches.[2,3] The $G_{\alpha s}$-protein-coupled receptor GPR119, predominantly expressed in pancreatic β-cells and enteroendocrine L-cells in the gut, has recently emerged as a promising drug target for T2D due to its role in regulating glucose homeostasis via modulation of incretin and insulin secretion.[4–7]

Since the ground-breaking publications disclosing GPR119 agonists such as **1** and **2** by Arena and Prosidion research groups respectively,[8,9] over 100 patent applications have been filed that generally revolve around a common pharmacophore shown in Figure 1.[10,11] To date, several organizations have progressed GPR119 agonists into the clinic. Previously disclosed compounds **3** and **4**[12] are depicted in Figure 1, and structurally also fall under the common GPR119 pharmacophore. Our own efforts towards identifying novel agonists of GPR119 that display this 'classical' pharmacophore will be disclosed elsewhere. In addition to these efforts, we undertook a high-throughput screening (HTS) campaign on a ~2 million compound collection, searching for new chemical matter that did not share structural similarity with known agonists.

The screen resulted in the identification of multiple (>20) novel hit scaffolds, which were followed up by routine hit-to-lead medicinal chemistry. During this hit-to-lead process we encountered several recurring challenges across most scaffolds, namely (a) generally narrow SAR, (b) insurmountable ADMET issues due to the narrow SAR and the overall hydrophobic nature of the scaffolds, (c) varying activity across species and (d) poor correlation between the primary, cellular cAMP assays and the functional hormone secretion assays in the relevant cell lines.

A hit scaffold identified from a purchased library that merited further investigation due to its departure from the common pharmacophore is represented by **5** in Figure 2. The hit displayed moderate activity on both the human and mouse GPR119 receptor and moderate ability to induce GLP-1 and insulin secretion in the GLUTag and HIT-T15 cell lines respectively. As expected from the fairly hydrophobic substituents around the pyrazolopyrimidine core, the high $c\log P$ (~6.5) of **5** led to poor solubility and fairly high extraction ratios (>0.8) in microsomal stability assays. In addition, the plasma stability of early analogues was low, postulated to be a result of either compound aggregation, ester hydrolysis or both.

A flexible, generalized synthetic approach that allowed us to efficiently explore both pyrazolopyrimidine substituents is shown in Scheme 1. A methyl ketone **6** was converted to the enamine **7** by

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0960-894X/

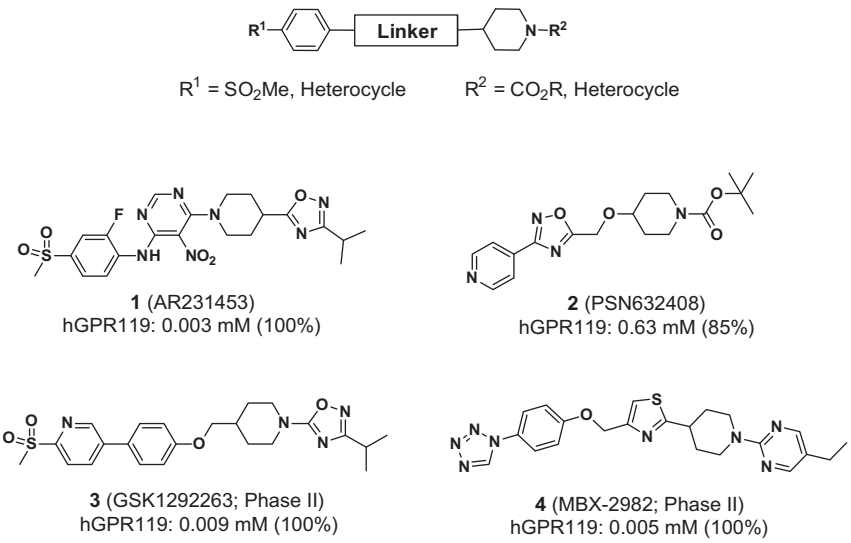


Figure 1. Common GPR119 pharmacophore and disclosed GPR119 agonists **1**–**4**, and common pharmacophore of most filed patent applications.[13]

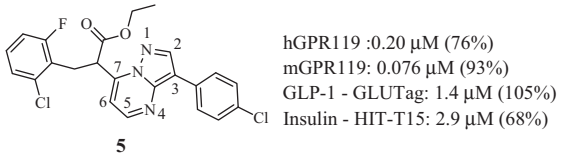
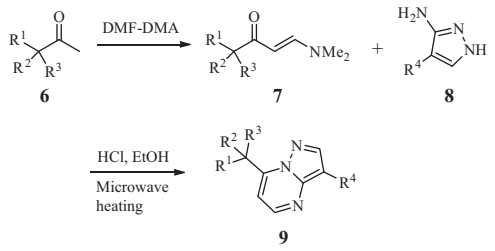


Figure 2. Structure of novel GPR119 agonist screening hit.[14]



Scheme 1. Representative scheme for the synthesis of analogs of HTS hit **5**.

treatment with dimethylformamide dimethyl acetal at elevated temperature. The synthesis of **6** could be tailored to afford mono-, di- or trisubstituted ketones where various functional groups could be incorporated either before or after the cyclization depending on the group. Next, this intermediate was condensed with a 3-aminopyrrole **8** using an acid in alcoholic solvent to afford the 3,7-disubstituted pyrazolopyrimidine **9**. The R^4 substituent was installed by either carrying through a halogen at this position followed by palladium coupling methodology or synthesizing **8** from a substituted acetonitrile by several literature-known methods.

A summary of SAR on the human GPR119 receptor (cAMP assay) for **5** and analogues **10–42** is depicted in Table 1.

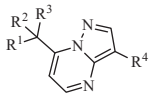
Substitution of the chlorine atom on the biphenyl unit for 'essential' elements seen in the 'common pharmacophore' such as a sulfone (**10**), a Boc-substituted piperidine (**11**) or an oxadiazole (**12**) resulted in a loss of activity on the GPR119 receptor. Similarly, adding the sulfone or a piperidine on the other side of the molecule (**13**, **14**) also resulted in inferior activity, indicating that this series does not follow the classical GPR119 pharmacophore.

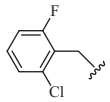
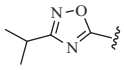
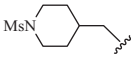
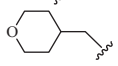
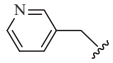
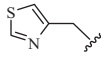
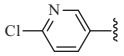
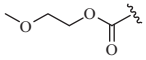
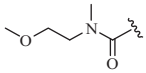
Removal of the halogens on the phenyl ring of the 7-substituent gave a roughly equipotent analogue **15**. Changing the benzylic group to either a phenyl (**16**) or a methyl group (**17**) resulted in significant loss of activity, as did removal of the chloride in the 3-phenyl substituent (**18**). Efficacies in the cAMP assay were slightly lower (50–70%) for this series when compared to typical GPR119 agonist scaffolds (represented by **1** = 100%). Based on prior experience in the characterization of several GPR119 agonist scaffolds, we observed that, generally, efficacy >80% in the cAMP assay resulted in robust effects in the downstream functional in vitro and in vivo assays. Therefore, we focused on compounds with higher efficacy for further progression.

Another concern was the potential liability of the secondary ester functionality. In fact, the ester group could be chemically removed by spontaneous decarboxylation after saponification of hit **5** to afford **19**. The complete lack of activity of this compound indicated that the ester was an important pharmacophore feature. One way to significantly stabilize the ester was to quaternize the benzylic carbon and make it sterically less accessible. Installing a methyl group led to **20**, which maintained potency but increased efficacy to almost 80%. After racemate **20** was chromatographically separated to afford **21** and **22**, we observed that GPR119 activity mainly resided with the *R* enantiomer (determined crystallographically) **21**, while the *S* enantiomer **22** had diminished (>10 μM) activity. Increasing the size of the quaternizing substituent, exemplified by a propyl group (**23**), resulted in a loss of activity. We were encouraged by the observation that the ester group of the quaternized analogue **21** was highly inert to chemical or metabolic activation, as well as to plasma hydrolases (>99% parent remaining after 3 h incubation in human and rodent plasma).

With these important findings in hand, we turned our attention back to further optimization of the aryl substituents around the pyrazolopyrimidine core. Probing the substitution pattern around the benzylic group off position 7 of the core heterocycle, we noticed that *meta* substitution was usually better tolerated than *ortho* or *para* substitution (cf. analogues **25** vs **24** and **26**). Substitution of the benzyl group with a variety of aliphatic and heterocyclic substituents resulted in significant activity loss (isopropyl- (**27**), tetrahydopyranyl- (**28**), 3-pyridine- (**29**) and thiazolo- (**30**) analogues). We next turned our attention to the chlorophenyl substituent and determined that moving the chlorine of the phenyl in position 4 of the core heterocycle to the 3- (**31**) or 2- (**32**) position significantly reduced activity, as did the elongation to a 4-chlorobenzyl substituent (**33**). Changing the 4-chloro-phenyl to a 4-chloro-pyridyl substitution (**34**) was fairly well tolerated. Changing the chlorine

Table 1
SAR of substituents on **3**



Entry	R^1	R^2	R^3	R^4	Chirality	hGPR119	
						EC_{50} (nM)	Efficacy (%)
5	CO_2Et	H		4-Cl-Ph	Racemic	200	76
10	CO_2Et	H	(2-Cl-6-F-Bn)	4-SO_2Me-Ph	Racemic	1300	68
11	CO_2Et	Me	Bn		Racemic	560	62
12	CO_2Et	Me	Bn		Racemic	880	43
13	CO_2Et	Me	4-SO_2Me-Bn	4-Cl-Ph	Racemic	>10,000	5
14	CO_2Et	Me		4-Cl-Ph	Racemic	340	50
15	CO_2Et	H	Bn	4-Cl-Ph	Racemic	16	66
16	CO_2Et	H	Ph	4-Cl-Ph	Racemic	290	63
17	CO_2Et	H	Me	4-Cl-Ph	Racemic	1200	32
18	CO_2Et	H	Bn	Ph	Racemic	190	60
19	H	H	(2-Cl-6-F-Bn)	4-Cl-Ph	Racemic	>10,000	15
20	CO_2Et	Me	Bn	4-Cl-Ph	Racemic	20	79
21	CO_2Et	Me	Bn	4-Cl-Ph	R	17	83
22	CO_2Et	Me	Bn	4-Cl-Ph	S	>10,000	15
23	CO_2Et	Pr	Bn	4-Cl-Ph	Racemic	190	68
24	CO_2Et	Me	4-Me-Bn	4-Cl-Ph	Racemic	140	66
25	CO_2Et	Me	3-Me-Bn	4-Cl-Ph	Racemic	40	76
26	CO_2Et	Me	2-Me-Bn	4-Cl-Ph	Racemic	110	73
27	CO_2Et	Me		4-Cl-Ph	Racemic	170	56
28	CO_2Et	Me		4-Cl-Ph	Racemic	63	66
29	CO_2Et	Me		4-Cl-Ph	Racemic	73	68
30	CO_2Et	Me		4-Cl-Ph	Racemic	40	63
31	CO_2Et	Me	Bn	3-Cl-Ph	Racemic	130	66
32	CO_2Et	Me	Bn	2-Cl-Ph	Racemic	180	54
33	CO_2Et	Me	Bn	4-Cl-Bn	Racemic	>10,000	10
34	CO_2Et	Me	Bn		Racemic	58	69
35	CO_2Et	Me	Bn	4-CF_3-Ph	Racemic	11	88
36	CO_2Et	Me	Bn	4-CF_3-Ph	R	10	80
37	CO_2Et	Me	Bn	4-CF_3-Ph	S	>300	22
38		Me	Bn	4-Cl-Ph	Racemic	4	81
39		Me	Bn	4-CF_3-Ph	R	1	85
40	NHAc	Me	Bn	4-CF_3-Ph	R	86	65
41		Me	Bn	4-CF_3-Ph	Racemic	164	50
42	SO_2Me	Me	Bn	4-CF_3-Ph	Racemic	243	57

to more hydrophilic substituents was not tolerated, but the more lipophilic CF_3-substituent resulted in the racemic analogue **35**, displaying robust potency and efficacy. As observed before, all of the activity resided with the *R* isomer **36** and not in the *S* isomer **37**.

Only certain, fairly conservative changes to the ester were tolerated, such as the methoxyethyl ester **38**, or the corresponding methylated amide **39**, both leading to a considerable boost of activity. Interestingly, 'reverse' amides such as **40** were also somewhat tolerated, albeit with lower efficacy. Other ester isosteres such as oxazole **41** or substituting the ester group with other functionalities such as sulfone **42** also gave significantly lower efficacies. Many other functionalities were tried, but all without success.

To further probe the scope of this scaffold, we explored several alternative bicycles to the pyrazolopyrimidine core. Some interesting findings are summarized in Table 2. The pyrazolotriazine **43** nicely retained potency and efficacy, with the difference that the extra nitrogen in the triazine ring made the core much more basic

Table 2
SAR of scaffold core

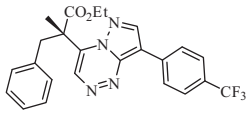
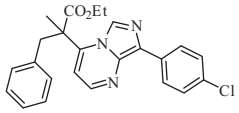
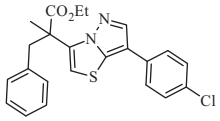
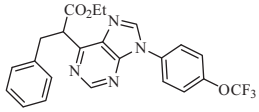
Entry	Structure	Chirality	hGPR119	
			EC_{50} (nM)	Efficacy (%)
43		R	20	88
44		Racemic	62	73
45		Racemic	53	70
46		Racemic	1100	59

Table 3
In vitro GLP1 and Insulin secretion, oral exposure and in vivo efficacy of selected compounds

Entry	hGPR119 (cAMP)		mGPR119 (cAMP)		GLP1 secretion (GLUTag)		Insulin secretion (HIT-T15)		AUCdn (balb/c) h μM	C_{max} (μM)	Active GLP-1 elevation (C57BL/6) (% elevation/dose)
	EC_{50} (μM)	Eff (%)	EC_{50} (μM)	Eff (%)	EC_{50} (μM)	Eff (%)	EC_{50} (μM)	Eff (%)			
21	0.017	83	0.076	75	0.51	110	2.2	72	0.28	1.1	No elevation up to 100 mg/kg
36	0.010	80	0.019	82	0.33	130	0.37	76	2.07	1.7	>2 fold at 10 mg/kg
39	0.001	85	0.049	71	0.03	49	0.15	74	0.50	2.3	>2 fold at 30 mg/kg
43	0.020	88	0.039	81	0.23	55	0.72	103	0.49	2.5	No elevation up to 100 mg/kg

and amenable to stable salt formation. Especially in light of the poor overall solubility profile of the series, we thought this might be of use for formulation optimization in case dissolution properties play a role in in vivo exposure. The imidazolopyrimidine **44** and 5,5-bicyclic pyrazolothiazole **45** resulted in modestly lower potency and efficacy, whereas the purine derivative **46** was not well tolerated.

Further characterization of selected compounds described in this Letter can be seen in Table 3. In general, analogues such as **21** that were stabilized by quaternization displayed good-to-excellent human and mouse GPR119 agonist activity. Furthermore, these compounds also showed robust GLP-1 secretion in GLUTag cells and insulin secretion in HIT-T15 cells, respectively. Curiously, switching the phenyl *para*-substituent from a chloride to a trifluoromethyl (**21** vs **36**) not only reproducibly enhanced the activity on the mouse receptor, but also raised the exposure levels from moderate to high. This may in part explain why compound **36** is efficacious in an acute study measuring active GLP-1 in C57BL/6 mice after a 10 mg/kg dose, while compound **21** is inactive at doses up to 100 mg/kg.[15] Despite being the most potent analogue on the human receptor, amide **39** is similarly active on the mouse receptor compared to the ester analogues, and is efficacious at the higher 30 mg/kg dose, perhaps due to lower exposure versus **36**. Compound **43** failed to produce a statistically significant increase of GLP-1 at doses up to 100 mg/kg, despite a similar in vitro activity and in vivo PK profile compared to **39**. The reasons for this discrepancy are currently not understood.

At this point, we concluded that the robust GLP-1 elevation of **36** observed in mice was encouraging for the series, as most other series we pursued outside the common pharmacophore never reached any level of in vivo efficacy. Compound **36** may serve as a tool compound for further in vivo investigations and as a lead into further medicinal chemistry optimization toward a drug for the treatment of T2D.

In summary, we started with the HTS-derived hit **5**, which displayed moderate in vitro activity, but no oral bioavailability. The series was subsequently proven not to fall into the common GPR119 pharmacophore widely reported to date and represents novel chemical space for further SAR studies into GPR119 agonism. Through a systematic SAR study of **5**, we were able to improve both in vitro GPR119 agonist activity and functional activity (e.g., GLP-1 and insulin secretion in the relevant cell lines) and achieve high oral exposure for selected analogues in mice. Furthermore, compound **36** induced robust elevation of active GLP-1 when dosed acutely at 10 mg/kg in C57BL/6 mice.

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13. The GPR119 agonist activity of the compounds is tested in a CHO stable cell line overexpressing human GPR119 (Lonza). The compound-induced activation of GPR119 leads to an increase of cellular cAMP production, which is measured with a cAMP HTRF kit (Cisbio). The efficacy of the receptor activation is normalized to AR231453 being 100%.
14. The compound activity on human or mouse GPR119 is measured with a cAMP HTRF assay in CHO cells overexpressing hGPR119 or mGPR119. Compound-induced GLP-1 secretion is measured in a mouse enteroendocrine cell line GLUTag. The secreted GLP-1 in the supernatant of cells treated with GPR119 agonist is detected with a HEK293 reporter cell line that co-expresses GLP-1 receptor and CRE-luciferase constructs. GPR119 agonist-induced insulin secretion in a hamster beta cell line HIT-T15 is measured with an insulin HTRF kit from Cisbio. The efficacy of the compounds in these assays is normalized to AR231453 being 100%.
15. Groups of wild-type C57BL/6J mice ($n = 8$ mice per group) were randomized into treatment groups based on their initial body weight. Mice were housed four per cage and orally dosed with vehicle, DPP-4 inhibitor alone, or DPP-4 inhibitor and GPR119 agonist, in a single dose. A glucose bolus (3 g/kg) was delivered thirty minutes post dosing. Sample was collected 2 min post glucose bolus. All animals were fasted for 16 h prior to compound administration. Blood was obtained (via retro-orbital bleeding) to measure plasma levels of active GLP-1. Approximately 200 μL samples of blood were removed for analysis at 62 min post dosing (2 min post glucose bolus). Active GLP-1 was measured using Glucagon-like peptide-1 (active) ELISA Kit, 96-well plate (Linco Research, Inc.). All procedures in this study were approved by the GNF animal care and use committee and were in compliance with the Animal Welfare Act Regulations 9 CFR Parts 1, 2 and 3, and US regulations (Guide for the Care and Use of Laboratory Animals, 1995).