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From partial to full agonism: Identification of a novel 2,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrazole as a full agonist of the human GPR119 receptor

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ABSTRACT

A novel GPR119 agonist based on the 2,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrazole scaffold was designed through lead optimization starting from pyrazole-based GPR119 agonist **1**. The design is centered on the conformational restriction of the core scaffold, while minimizing the change in spatial relationships of two key pharmacophoric elements (piperidine-carbamate and aryl sulfone).

GPR119 is a target of significant interest to the pharmaceutical industry for the treatment of diabetes based on a recent patent analysis of the therapeutic area.[1,2] Indeed, there has been a large increase in the number of composition of matter applications for GPR119 agonists over the past 5 years. Interest in targeting this class A G-protein coupled receptor (GPCR) stems from the potential for agonists to provide improved glucose control with a low risk of hypoglycemia.[3,4] In addition, recent data suggest that the preservation of beta cell function may also be possible through the activation of this receptor.[5,6] Oleoylethanolamide (OEA) has been reported to be the endogenous agonist for this receptor,[7] although a recent report shows that other endogenous lipids may act as agonists, including monoacylglycerols (MAG).[8] While OEA has helped define some of the potential benefits of GPR119 activation, studies in transgenic mice suggest that not all of these effects are GPR119 mediated.[9] Nevertheless, there are a number of small molecule agonists described that show antihyperglycemic effects in rodents.[10–21] Most of these compounds, like most small molecule GPR119 agonists reported show two key structural elements: (i) a piperidine-carbamate group or its isostere, and (ii) an aryl sulfone group or its isostere (Fig. 1).

Recently we disclosed the importance of the conformational disposition of the piperidine-carbamate for GPR119 activation.[22] As part of our systematic approach to this target we also evaluated conformational restriction of the core region of the molecule. The proving ground for this effort was in a series of pyrazole analogs exemplified by compound **1** (Fig. 2). Herein we describe the SAR of these pyrazole-based compounds and the identification of a novel GPR119 agonist centered on a rigid 2,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrazole scaffold.

The pyrazole structure in **1** derives from our emphasis on identifying a polar core to provide flexibility in the balancing of pharmacology and ADME properties. While compound **1** shows modest potency with partial agonism in our cAMP assay,[23] by virtue of its relative polarity ($\mathrm{Log}D = 2.3$)[24] it shows reasonable human microsomal stability. In addition to addressing the deficiency in both EC_{50} and intrinsic activity, our optimization effort focused on the embedment of the aniline structural motif to mitigate potential reactive metabolite formation and idiosyncratic toxicity risks.[25] Based on these objectives, the medicinal chemistry effort commenced with the structural modification of the piperidine-carbamate group and 2-fluoro-4-methylsulfonyl aniline group.

Analogs based on **1** were synthesized through the general sequence described in Scheme 1. Starting from the known intermediate **4**,[26] reductive amination with aromatic primary amines **2** and **3** afforded the corresponding secondary amine derivatives in good yields. Oxidation of the thioether group with *m*-CPBA, cleavage of the Boc group and subsequent reactions with the various chloroformates and heteroaromatic halides furnished analogs **5–8**.

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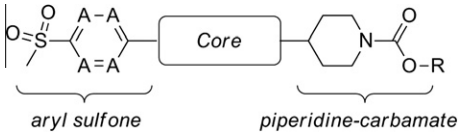
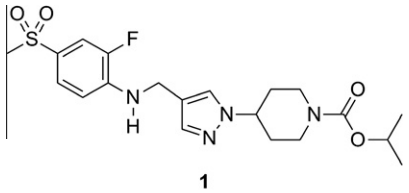


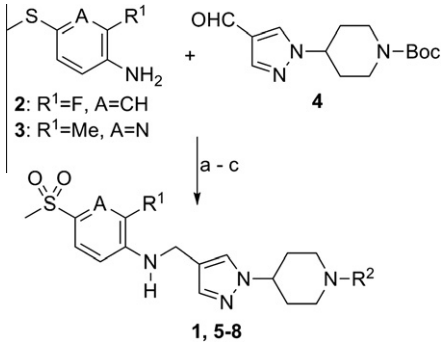
Figure 1. General structural features of GPR119 agonists.



1

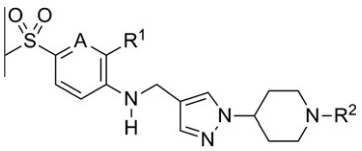
cAMP EC_{50}= 181 nM, 41% IA
CLint (HLM)= 9.7μL/min/mg
LogD= 2.3

Figure 2. In vitro profile of **1**.



Scheme 1. Synthesis of pyrazole-based GPR119 agonists. Reagents and conditions: (a) **2** or **3** (1 equiv), $NaBH(OAc)_3$ (2 equiv), AcOH (2.2 equiv), DCE, rt, 92% (from **2**), 71% (from **3**); (b) *m*-CPBA (2.5 equiv), DCM, rt, 85% (from **2**), 48% (from **3**); (c) HCl/MeOH or AcOEt, rt, followed by RCOCl (1 equiv), NMM/DCM or 2-chloro-5-ethylpyrimidine (1 equiv), DIPEA (4 equiv), DMSO, 120 °C, 31–62%.

Table 1
SAR around **1**



Compd	R^1	A	R^2	EC_{50} ± SD[a] (nM)	IA ± SD[a] (%)	Log D^{24}
1	F	CH	CO_2*i*Pr	181 ± 76	41 ± 6	2.3
5	Me	N	CO_2*i*Pr	>10000	N.D.[b]	1.6
6	F	CH	CO_2*i*Bu	240±82	24 ± 3	2.8
7	F	CH	CO_2*c*Bu	462±135	42 ± 9	2.5
8	F	CH	5-Et-2-Pyrimidyl	47±27	43 ± 3	3.0

[a] Values are arithmetic means of at least three experiments. See Ref. 22 for details.
[b] N.D. = not determined.

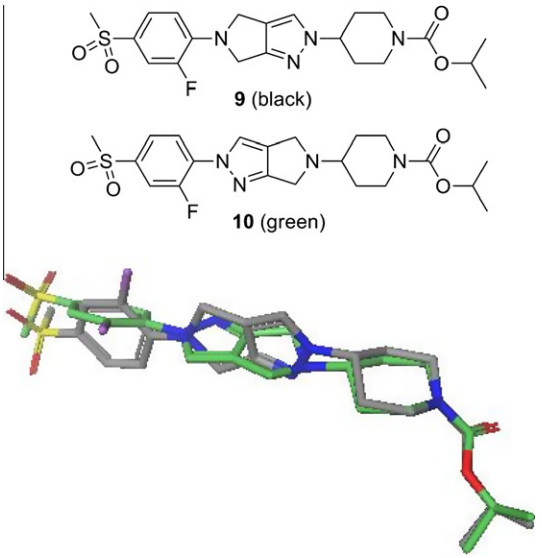


Figure 3. Superposition of **9** and **10**.

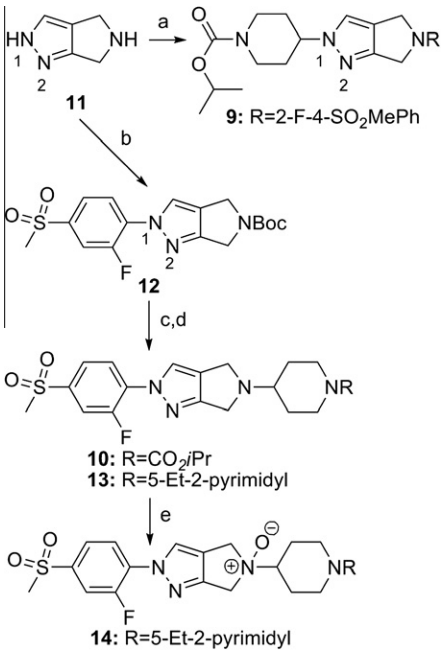
As shown in Table 1, changes made to the carbamate or aromatic sulfone had little impact on the intrinsic activity of these compounds. The attempt to replace the aniline motif in **1** with an aminopyridine derivative **5** led to a significant decrease in potency. Modifications to the carbamate group varied the potency with minimal impact on the intrinsic activity. The lowest EC_{50} in this compound set was achieved when the *i*Pr carbamate group was replaced by 5-Et-2-pyrimidyl group (**8**), however, again no increase in intrinsic activity was achieved.

Based on the above results, we next turned our attention to the modification of the central core/spacer region. It was hypothesized that restriction of free rotation around the $–CH_2NH–$ bond might lead to potency gains and/or increases in intrinsic activity.

To this end, compound **9** possessing the 2,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrazole scaffold was designed in order to highly constrain the positions available for the key pharmacophoric elements, as shown in Figure 3. Parallel to this effort, a 'flipped' version based on this bicyclic core was designed to embed the aniline structural motif from **9** (Fig. 3). This design was driven by the hypothesis that the core/spacer functions to position the two key pharmacophoric elements (piperidine-carbamate and aryl sulfone). Unsurprisingly, the superposition of **9** and **10** after global minimization[27] supported that the 2,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrazole scaffold positions the two key pharmacophoric elements of **9** and **10** in similar space.

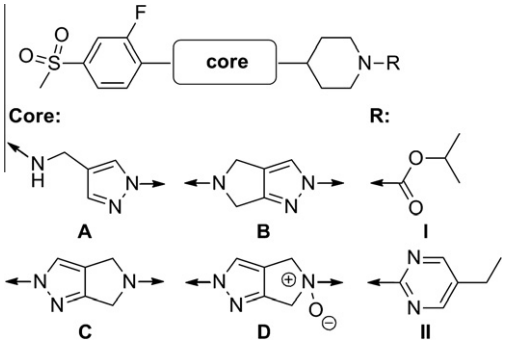
Analogs based on this hypothesis were prepared as outlined in Scheme 2. The synthesis of **10**, **13** and **14** started from the S_NAr reaction of *N*-Boc 2,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrazole, prepared from the commercially available dihydrochloride salt **11**, and 1,2-difluoro-4-(methylsulfonyl)benzene, to afford a ~1:1 mixture of *N*-1 and *N*-2 adducts. After silica gel column chromatography, the desired *N*-1 adduct **12** was obtained.[28] Boc cleavage, followed by reductive amination with various piperidone derivatives furnished analogs **10** and **13**. The corresponding *N*-oxide **14** was also synthesized by treating **13** with *m*-CPBA. Compound **9** was synthesized analogously through S_NAr reaction of **11** with 1,2-difluoro-4-(methylsulfonyl)benzene and subsequent S_N2 reaction with isopropyl 4-(methylsulfonyloxy)piperidine-1-carboxylate.

The results obtained from these core modifications are summarized in Table 2. Conformationally restricted analog **9** showed comparable potency to the lead pyrazole **1** with similar intrinsic activity, suggesting that this mode of conformational restriction is sub-optimal to elicit a full agonist response. In contrast, a significant increase in intrinsic activity was achieved when the 'flipped' core C was examined, albeit with a decrease in potency



Scheme 2. Synthesis of **9**, **10**, **13** and **14**. Reagents and conditions: (a) 1,2-difluoro-4-(methylsulfonyl)benzene (1 equiv), DIPEA (4 equiv), DMSO, 100 °C, followed by isopropyl 4-(methylsulfonyloxy)piperidine-1-carboxylate (2 equiv), Cs_2CO_3 (4 equiv), DMSO, 100 °C, 23%; (b) Boc_2O (1 equiv), DIPEA (3 equiv), DCM/MeOH, rt, followed by 1,2-difluoro-4-(methylsulfonyl)benzene (1.5 equiv), NaHMDS (2 equiv), THF, microwave, 100 °C, 35%; (c) HCl in AcOEt, rt, 77%; (d) $NaBH(OAc)_3$ (2 equiv), ketone (1 equiv), AcOH (1.2 equiv), DCE, rt, 16% (for **10**), 41% (for **13**); (e) *m*-CPBA (2 equiv), DCM, rt, 56%.

Table 2
SAR of core modification



Compd	Core	R	EC_{50} ± SD[a] (nM)	IA ± SD[a] (%)	Log D^{24}	HLM CL_{int} (μL/min/mg)
1	A	I	181 ± 76	41 ± 6	2.3	9.7
8	A	II	47 ± 27	43 ± 3	3	148
9	B	I	260 ± 161	48 ± 7	3.2	36.1
10	C	I	864 ± 478	105 ± 7	2.2	10.5
13[b]	C	II	83 ± 14	118 ± 9	2.9	23
14	D	II	498 ± 255	107 ± 10	1.1	9.8

[a] Values are means of at least three experiments. See Ref. 22 for details.
[b] Tested as hydrochloride salt.

(compound **10**, Table 2). The core flipping from **9** to **10** leads to the creation of a sp^3-hybridized weakly basic nitrogen atom[29] directly connected to the piperidine-carbamate group. The profound effect on intrinsic activity of **10** could be a result of the subtle change in spatial relationships of the two key pharmacophoric elements (piperidine-carbamate and aromatic sulfone).[30]

With a full agonist **10** in hand, we next focused on potency optimization. Based on our earlier results, it was hypothesized that replacement of the *i*Pr carbamate group with a 5-Et-2-pyrimidyl group might increase potency. This transformation indeed led to the identification of **13** with increased potency relative to **10** while maintaining high intrinsic activity. Thus, the compound **13** had achieved our two initial objectives: (i) increase both potency and intrinsic activity, and (ii) embed the aniline structural motif starting from the lead compound **1**. Further in vitro profiling revealed that **13** demonstrated modest metabolic stability in human liver microsome (HLM) despite the moderate increase in log*D*, possibly as a result of its decreased flexibility. An attempt to further reduce intrinsic clearance of **13** by preparing the corresponding amine *N*-oxide **14**, with significantly reduced log*D*, led to a decrease in potency, while clearance was improved as anticipated.

In conclusion, a series of pyrazole-based GPR119 agonists were designed and characterized. Starting from a partial agonist **1**, a novel and potent GPR119 full agonist **13** was identified through a conformational restriction-core flipping strategy. The present work highlights the importance of the core optimization as a way to elicit the desired functional response. While compound **13** represented a novel lead that formed the basis of further work, **13** was roughly 10-fold less potent than exemplars from other series working in our laboratory. In order to deliver a viable clinical agent from this series, further strategies to increase potency will be required.

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23. The intrinsic activity is the percent of maximal activity of the test compound relative to the activity of a standard GPR119 agonist, 4-[[6-[(2-fluoro-4 methylsulfonylphenyl)amino]pyrimidin-4-yl]oxy]piperidine-1-carboxylic acid isopropyl ester (WO2005121121), or (*S*)-1-methylcyclopropyl 4-(1-fluoro-2-(2-(2,3,6 trifluorophenyl)acetamido)ethyl)piperidine-1-carboxylate (Bioorg. Med. Chem. Lett. **2011**, *21*, 1306) at a final concentration of 10 μM. Both standards produced a level of cAMP accumulation that was comparable to the maximum level of cAMP produced by the putative native GPR119 ligand oleoylethanolamide.
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27. A conformational analysis of **9** and **10** was performed using OPLS_2005 and the GB/SA solvation model. Few low energy conformations were selected and further refined using LMP2/6-311G** with solvent effect (water).
28. *N*-1 and *N*-2 Regio-isomers were assigned based on $^{1}H-^{19}F$ NOE and $^{1}H-^{13}C$-HMBC experiments.
29. Measured basic pK_a = 6.5.
30. MCMM conformational search using Batchmin in water indicates that there are a few axial conformers of the piperidine ring within 5 kcal/mol from the lowest energy equatrial conformer for compound **10**, while no axial conformers were found for compound **9**. The presence of these axial conformers may contribute to the increase in intrinsic activity of compound **10** and its related analogues. For the importance of the axial conformation of the piperidine ring in GPR119 agonism, see Ref 22.