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Design and evaluation of a 2-(2,3,6-trifluorophenyl)acetamide derivative as an agonist of the GPR119 receptor

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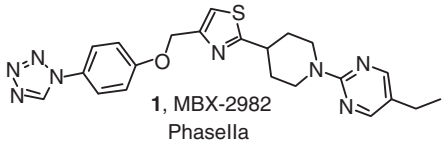
ABSTRACT

The design and synthesis of a GPR119 agonist bearing a 2-(2,3,6-trifluorophenyl)acetamide group is described. The design capitalized on the conformational restriction found in *N*-β-fluoroethylamide derivatives to help maintain good levels of potency while driving down both lipophilicity and oxidative metabolism in human liver microsomes. The chemical stability and bioactivation potential are discussed.

GPR119 is a G-protein coupled receptor found in human enteric L-cells and in pancreatic β-cells. Its role in glucose homeostasis has been the subject of numerous studies that suggest agonism of GPR119 may be a viable and highly desirable approach to the treatment of type 2 diabetes.[1,2] By way of a unique glucose-dependent mechanism, GPR119 agonism is anticipated to promote glycemic control by stimulating the secretion of insulin from the pancreas as well as gastrointestinal hormones (e.g., the incretins GLP-1 and GIP) from the gut.[3]

Ongoing research programs across the pharmaceutical industry have led to the advancement of small molecule GPR119 agonists into clinical trials (e.g., clinical candidates **1** and **2**; Fig. 1).[4] In this Letter, we report the design and synthesis of compound **3** (Fig. 2), a novel GPR119 agonist bearing an unprecedented 2-(2,3,6-trifluorophenyl)acetamide group.

Initial interest in this series originated from the identification of compound **4**, which exhibited excellent activity in our c-AMP functional assay (13 nM, 87% intrinsic activity; Table 1); however, **4** was highly lipophilic (*E* Log *D* = 4.3)[5] which resulted in high oxidative turnover in human liver microsomes (HLM). Interestingly, as exemplified by compounds **5** and **6** (Table 1), replacement of the 2,3,6-trifluorophenyl group in **4** with more classical GPR119 pharmacophores led to a decrease in potency. Systematic substitution of any of the fluorine atom on the aromatic ring by a hydrogen



1, MBX-2982
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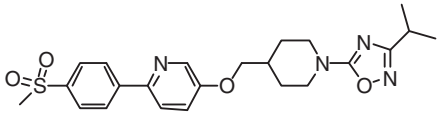

2, GSK1292263A
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Figure 1. Structure of some known GPR119 agonists in the clinic.

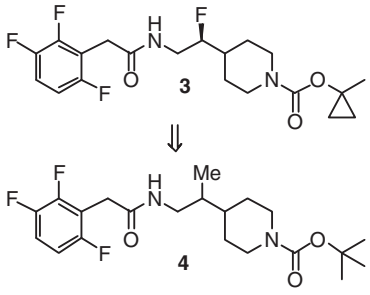

Figure 2. 2-(2,3,6-Trifluorophenyl)acetamide GPR119 agonists.

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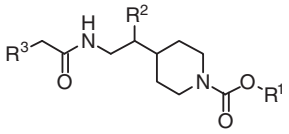
Table 1
Representative examples of analogs and their respective properties



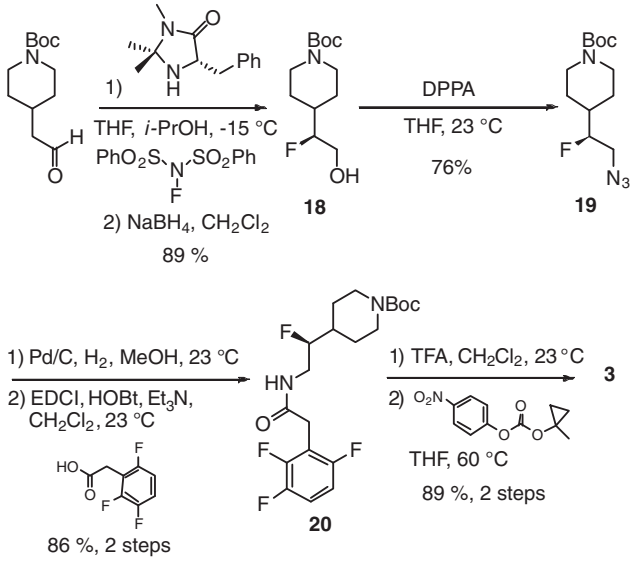
Entry	Compound	R^1	R^{2a}	R^3	EC_{50} ± SD[b] nM	IA ± SD[c] %	CL_{int} (HLM)[d] (mL/min/kg)	*E* Log *D*
1	**3**	1-MecPr	F (*S*)	2,3,6-Trifluorophenyl	80 ± 41 (39)	107 ± 10 (39)	42.4	3.4
2	**4**	*tert*-Bu	Me	2,3,6-Trifluorophenyl	13 ± 8 (4)	87 ± 12 (4)	230	4.3[e]
3	**5**	*tert*-Bu	Me	*p*-$PhSO_2Me$	884 (2)	47 (2)	136	2.8[e]
4	**6**	*tert*-Bu	Me	*1-p*-Ph-1*H*-tetrazole	267 ± 235 (4)	72 ± 14 (4)	>270	3.1[e]
5	**7**	*tert*-Bu	Me	2,3-Difluorophenyl	75 (2)	107 (2)	>300	4.2[e]
6	**8**	*tert*-Bu	Me	2,5-Difluorophenyl	78 (2)	104 (2)	>300	4.2[e]
7	**9**	*tert*-Bu	Me (*S*)[f]	2,6-Difluorophenyl	120 ± 28 (3)	101 ± 9 (3)	nd	3.9
8	**10**	*tert*-Bu	Me	2-Fluorophenyl	808 (2)	97 (2)	>300	4.1[e]
9	**11**	*tert*-Bu	Me	3-Fluorophenyl	340 (2)	102 (2)	>300	4.2[e]
10	**12**	*tert*-Bu	Me	Phenyl	5980 (1)	100 (1)	247	4.0[e]
11	**13**	*i*-Pr	Me	2,3,6-Trifluorophenyl	32 ± 14 (4)	88 ± 8 (4)	205	4.0
12	**14**	1-MecPr	Me (*S*)[f]	2,3,6-Trifluorophenyl	44 ± 15 (5)	124 ± 16 (5)	>300	3.9
13	**15**	1-MecPr	Me (*R*)[f]	2,3,6-Trifluorophenyl	236 ± 44 (5)	97 ± 7 (5)	187	3.9
14	**16**	*i*-Pr	H	2,3,6-Trifluorophenyl	409 ± 285 (4)	68 ± 9 (4)	nd	3.4
15	**17**	1-MecPr	F (*R*)	2,3,6-Trifluorophenyl	1380 (1)	100 (1)	63.8	3.4
16	**21**	1-MecPr	H	2,3,6-Trifluorophenyl	868 ± 301 (3)	111 ± 11 (3)	nd	3.5

nd: not determined.
[a] The configuration of the stereocenter is indicated in parentheses.
[b] Potency in the human recombinant cell-based c-AMP functional assay. See online Supplementary data for more details. Number of runs indicated in parentheses. SD: standard deviation of the mean (for n >2).
[c] Intrinsic Activity in the c-AMP assay. Number of runs indicated in parentheses. SD: standard deviation of the mean (for n >2).
[d] CL_{int} refers to total intrinsic clearance obtained from scaling in vitro HLM half-lives.
[e] Calculated Log *D*.
[f] The configuration was arbitrarily assigned.

atom also led to compounds of increasingly suboptimal potency (compare **4** vs **7**–**12**). Furthermore, compounds **7**–**12** had high metabolic turnover in HLM as anticipated by their lipophilicity similar to **4**. Replacement of the acid labile *tert*-butyl carbamate by an isopropyl (*i*-Pr) or a 1-methylcyclopropyl (1-MecPr) carbamate also led to compounds of similar lipophilicity and high clearance in vitro (compounds **13**–**15**).

The presence of the methyl group (compare **13** vs **16**) and the configuration of the stereocenter (compare **14** vs **15**) both had an influence on potency. Thus, although a productive interaction with the receptor cannot be ruled out, it appeared reasonable to suggest that the stereocenter helps reduce the configurational entropic penalty upon binding.[6,7] Mindful of the *gauche effect* found in *N*-β-fluoroethylamides,[8] we proposed the synthesis of conformationally restricted compounds such as **3**. The fluorine-containing stereocenter was expected to positively impact the conformational and/or vibrational component of the configurational entropy, and thereby help maintain potency.[7] In addition, this fluorine atom could help reduce lipophilicity and metabolic turnover.

Compound **3** was synthesized in high yield and high enantioselectivity as shown in Scheme 1. α-Fluorination of *N*-Boc-4-piperidineacetaldehyde using MacMillan's organocatalytic methodology



Scheme 1. Synthesis of compound **3**.

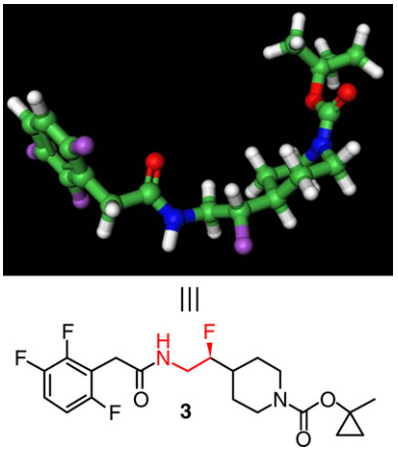


Figure 3. Averaged conformation of **3** in solution on the NMR timescale.

produced compound **18** (98% ee),[9] which was then converted to the α-fluoro azide **19** in 76% yield via a Mitsunobu-type reaction. Reduction of the azide to the amine followed by amide bond coupling under classical conditions (EDCI/HOBt/Et_3N) gave intermediate **20**. This intermediate was converted to the desired product **3** by BOC removal and subsequent installation of the 1-MecPr carbamate.[10]

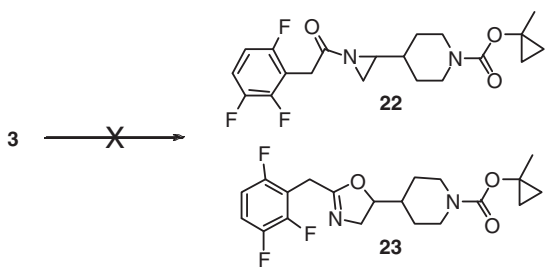
Compound **3** proved to be a full agonist in our functional c-AMP assay and its EC_{50} remained within two fold of the methyl analog **14**. Importantly, **3** was markedly more stable in HLM incubations than the corresponding methyl-substituted compounds **14** and **15** as well as less lipophilic.

We next sought to confirm the *gauche effect* hypothesis by studying the conformation of compound **3** in solution. Indeed, as depicted in Figure 3, NMR spectroscopic analysis (600 MHz/$CDCl_3$/300 K) showed that the *N*-β-fluoroethylamide favors adopting a *gauche* conformation, which constitutes about 75% of the conformer population on the NMR timescale.[11]

As observed previously in the methyl case (compounds **14** and **15**), the configuration of the stereocenter in **3** had an impact on potency in the functional c-AMP assay (compare **3** vs **17**). Again, since the topology of the receptor is not known, one can only speculate around the causes of this selectivity. Although it could be the result of the conformational bias induced by the stereocenter,[11] a productive interaction between the fluorine atom of **3** and the receptor (and/or an unfavorable one in the case of **17**) cannot be ruled out. The decrease in potency from **3** to **21** is also worth mentioning. In this case, conformational analysis of simplified versions of compounds **3** and **21** revealed energetically similar conformer distributions.[11] The low energy conformers adopted by the fluoro-substituted analog **3**, including the most stable conformation shown in Figure 3, are easily accessible to the unsubstituted analog **21**. This suggests that the energy required to achieve the bioactive conformation, whatever this might be, is not the main source of the potency increase from **21** to **3**. While the conformer distribution does not change significantly, torsional scans for the dihedral angles flanking the F atom revealed energy wells that are narrower for the fluoro-substituted analog **3** compared to **21**.[11] Therefore, it is conceivable that **3** experiences a less significant loss of vibrational entropy upon binding and that this factor contributes to its increase of potency over **21**.[7] However, in this case too, a productive interaction between the fluorine atom and the receptor cannot be ruled out.

In considering further profiling of **3**, we were initially concerned about the formation of a potentially reactive aziridine such as **22** under physiologic conditions (Scheme 2). However, exposure of **3** to various conditions expected to promote cyclization did not lead to the formation of **22** or dihydrooxazole **23**.[12,13]

Although the risk of aziridine formation appeared low, subsequent studies assessing the bioactivation potential of **3** in NADPH- and glutathione (GSH)-supplemented HLM revealed a risk of reactive species formation by metabolic activation. Formation of a GSH adduct was clearly observed: m/z = 720 (MH^+); m/z = 645 (loss of glycine); m/z = 591 (loss of glutamate).[14,15] A proposed structure for the GSH conjugate of **3** that is consistent with the observed mass spectrum is compound **24** shown in Figure 4.[16] This GSH adduct is postulated to occur via an ipso substitution of one of the pendant fluorine atoms in the course of P450 catalyzed phenyl ring epoxidation.[17] The formation of **24** was NADPH-dependent, suggesting the involvement of cytochrome P450 in the bioactivation of **3**. Also, inclusion of the specific CYP3A4 inhibitor ketoconazole in the HLM incubations eliminated conjugate formation and overall metabolism, implicating that CYP3A4 was responsible for the oxidative metabolism/bioactivation of **3**.



Scheme 2. Attempts of intramolecular displacement of fluorine in **3**.

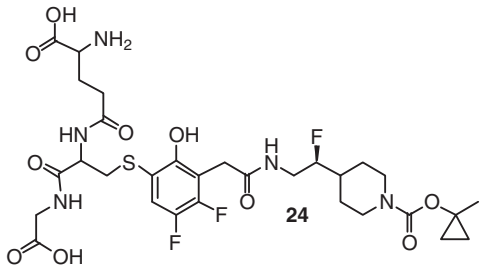


Figure 4. Representative structure of glutathione adduct **24**.

In conclusion, we have described the design and synthesis of compound **3**, a novel GPR119 agonist bearing a *N*-β-fluoroethylamide motif as key element of design. This group helped maintain good agonist potency while reducing the lipophilicity and oxidative metabolism in HLM in vitro. However, recognizing the potential for immune-mediated toxicity due to reactive metabolite formation, further pharmacologic and pharmacokinetic profiling of **3** was suspended.[18]

Supplementary data

Supplementary data (details related to the in vitro c-AMP functional assay, NMR and computational studies around compound **3**, collision-induced dissociation spectrum of compound **24**) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.01.088.

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13. Some of the conditions tried were: (a) heating in DMSO for 30 min at temperatures up to 200 °C in presence of *i*-Pr_2NEt; (b) heating in DMSO for 60 min at temperatures up to 100 °C in presence of NaHMDS.

14. Compound **3** (10 μM) was incubated in phosphate buffer (0.1 M, pH 7.4) containing $MgCl_2$ (3.3 mM), HLM (2.0 mg/mL), NADPH (1.3 mM) and GSH (5 mM). Reactions were initiated with the addition of microsomes. Control incubations were run in parallel in the absence of NADPH and/or GSH. All incubations were conducted in a shaking water bath maintained at 37 °C open to the air. After 60 min, the incubations were terminated by addition of ice-cold acetonitrile containing 0.1% formic acid, mixed vigorously, and the precipitated materials were removed by spinning in a centrifuge (3000g) for 5 min. Aliquots of the supernatants were analyzed for metabolite formation by liquid chromatography tandem mass spectrometry (LC–MS/MS). The HPLC system consisted of an Accela quaternary solvent delivery pump and autoinjector, a Surveyor PDA Plus photodiode array detector (Thermo Electron Corporation, Waltham, MA). Chromatography was performed on a Phenomenex, Synergy RP column, 150 × 4.6 mm, 5 μm (Phenomenex, Torrance, CA). LC analysis was performed at a constant flow rate of 1000 μL/min using a binary solvent system: Solvent A, 5 mM ammonium formate buffer (pH ~3.0) with 0.1% formic acid and Solvent B, acetonitrile. The initial HPLC gradient system was held at 5% B for 3 min and linearly increased to 80% B in 35 min, followed by a return to initial conditions for column re-equilibration. Post-column flow passed through the PDA detector to provide UV (λ = 200–400 nm) detection prior to being split to the mass spectrometer such that mobile phase was introduced into the electrospray source at a rate of 50 μL/min. The LC system was interfaced to a Thermo Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Xcalibur software version 2.0 was used to control the HPLC/MS system. Mass spectroscopy analyses were carried out in the positive ion mode using full-scan MS with a mass range of 100–1000 Da. Full scan data and data-dependent MS/MS acquisition on the two most intense ions were collected at 15,000 resolutions. All experimental data were acquired using external calibration prior to data acquisition.
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16. The regiochemistry of GSH attachment was not investigated. The regiochemistry as shown in Figure 4 is arbitrary and for illustrative purposes only.
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