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LETTER

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Discovery and SAR of a Series of Agonists at Orphan G Protein-Coupled Receptor 139

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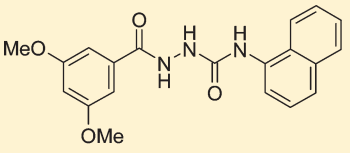
Supporting Information

ABSTRACT: GPR139 is an orphan G-protein coupled receptor (GPCR) which is primarily expressed in the central nervous system (CNS). In order to explore the biological function of this receptor, selective tool compounds are required. A screening campaign identified compound **1a** as a high potency GPR139 agonist with an EC_{50} = 39 nM in a calcium mobilization assay in CHO-K1 cells stably expressing the GPR139 receptor. In the absence of a known endogenous ligand, the maximum effect was set as 100% for **1a**. Screening against 90 diverse targets revealed no cross-reactivity issues. Assessment of the pharmacokinetic properties showed limited utility as in vivo tool compound in rat with a poor whole brain exposure of 61 ng/g and a brain/plasma (b/p) ratio of 0.03. Attempts to identify a more suitable analogue identified the des-nitrogen analogue **1s** with a reduced polar surface area of 76.7 Å^2 and an improved b/p ratio of 2.8. The whole brain exposure remained low at 95 ng/g due to a low plasma exposure.



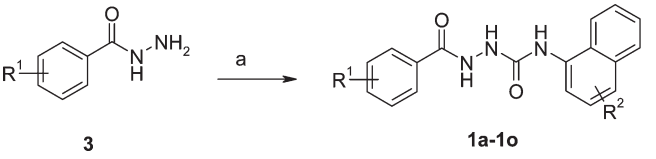
GPR139 EC_{50}= 39nM **1a**

KEYWORDS: Orphan GPR-139, agonists, G-protein coupled receptor, CNS, hydrazinecarboxamide

GPR139 is an orphan G-protein coupled receptor, which has a low sequence similarity (20−25%) to other members of the GPCR rhodopsin[1,2] family. Human and mouse GPR139 share 94% amino acid homology. Tissue distribution of mouse and human GPR139 is primarily located in the CNS. Human GPR139 is expressed in putamen, caudate nucleus, entopendundular nucleus, olfactory bulbs, hapenular nucleus, parts of the hypothalamus, substantia nigra, cerebellar nuclei, and vestibular nuclei.[1]

In order to elucidate the function of GPR139, selective ligands are required. Recently, the first surrogate agonists and antagonists have been reported.[3] In the present communication, we report the discovery of a novel GPR139 agonist series. A screening campaign was executed at our laboratory to identify GPR139 agonists. Compound **1a** was identified as a GPR139 receptor agonist with an EC_{50} of 39 nM in a calcium mobilization assay using a CHO-K1 cell line stably expressing the human GPR139 receptor. Initial assessment of compound **1a** indicated no issues with selectivity, as no cross-reactivity was observed against 90 diverse targets (data in Supporting Information). A hit exploration program was undertaken to examine whether the potency could be improved while reducing the polar surface area and hydrogen bond donor count of **1a**, which could potentially lead to a brain penetrant tool compound.[4] In this paper we outline the synthesis of analogues we undertook within the confines of the generic structure **2** (Figure 1), where we probed both the importance of peripheral substitution R^1 and R^2 and the significance of the central linker for GPR139 efficacy.

Scheme 1. Synthesis of Compounds 1a−1o[a]



3 **1a-1o**

[a] Reagents and conditions: (a) R^2-$C_{10}H_8$-NCO, DCM, rt or R^2-$C_{10}H_8$-NC(O)OPh, DMSO, 80 °C.

Variation of peripheral substituents R^1 and R^2 was achieved by treating substituted benzhydrazides (**3**) with either substituted napthyl isocyanates or substituted napthyl carbamic-acid-phenyl esters to prepare compounds **1a−1o**, as shown in Scheme 1.

Removal of the hydrazide oxygen was obtained by reaction of aldehyde **4** with hydrazine in ethanol at reflux to give 1-(3,5-dimethoxyphenyl)meth-(*E*)-ylidenehydrazine (**5**), which upon treatment with 1-napthyl isocyanate and subsequent imine reduction gave the des-oxygen analogue **1p** (Scheme 2).

Replacement of the hydrazide nitrogens was undertaken as shown in Scheme 3. *N*-BOC-glycine (**6**) was converted to 2-amino-1-(3,5-dimethoxyphenyl)ethanone hydrochloride (**7**) and then reacted with 1-napthyl isocyanate to give **1q**.

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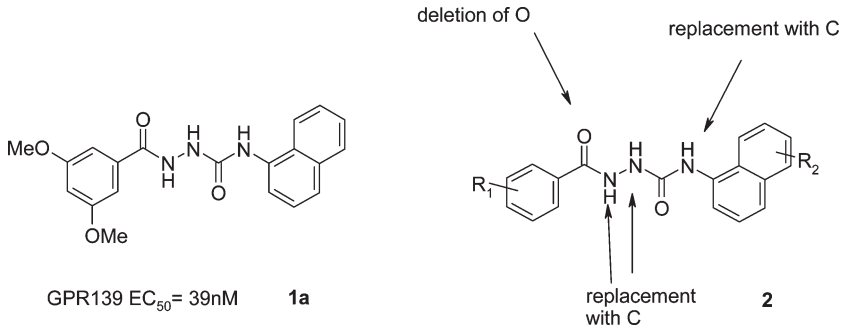
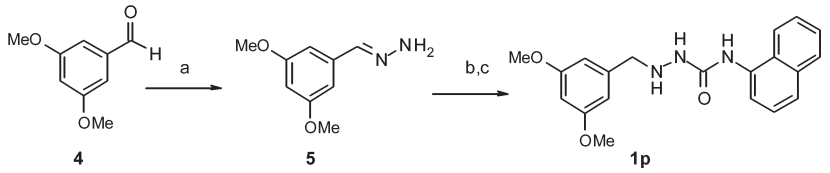


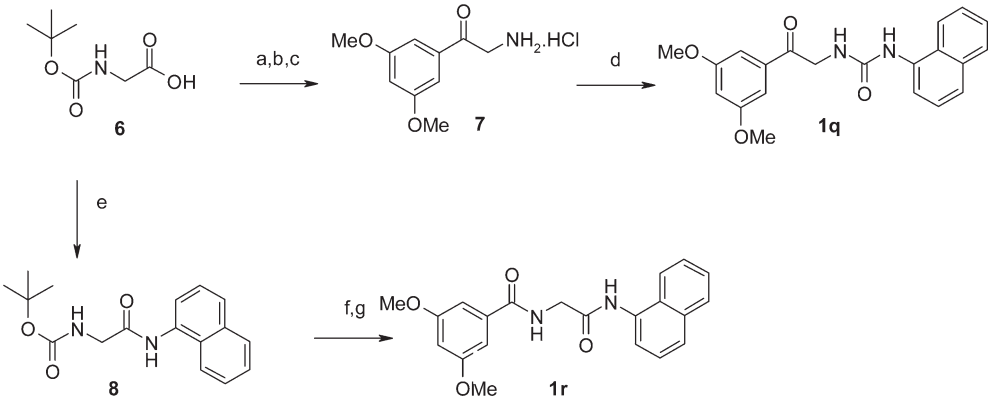
Figure 1

Scheme 2. Synthesis of Compound 1p[a]



[a] Reagents and conditions: (a) hydrazine, EtOH, reflux, 90%; (b) 1-napthylisocyanate, THF, rt, 90%; (c) $NaBH_3(CN)$, HOAc, MeOH, 91%.

Scheme 3. Synthesis of Compounds 1q and 1r[a]



[a] Reagents and conditions: (a) *N*,*O*-dimethylhydroxylamine hydroxylamine, EDCI, DMAP, Et_3N, rt, 83%; (b) 3,5-dimethoxyphenylmagnesium bromide, THF −30−50 °C, 84%; (c) HCl, 1,4-dioxane, 100%; (d) 1-napthyl isocyanate, DIPEA, THF, rt, 59%; (e) 1-napthylamine, EDCI, HOBt, DIPEA, DMF, rt, 84%; (f) HCl, 1,4-dioxane, rt, 100%; (g) 3,5-dimethoxybenzoic acid, EDCI, HOBt, DIPEA, DMF, rt, 77%.

Alternatively, 1-napthylamine was coupled with N-BOC-glycine to give **8**. Removal of the protecting group and coupling to 3,5-dimethoxybenzoic acid yielded the glycine derivative, **1r**.

The synthesis of the carbon analogue was obtained (Scheme 4) by coupling 1-napthyl acetic acid (**9**) with 3,5-dimethoxybenzhydrazide to give **1s**.

Introduction of methyl groups on the hydrazide nitrogens was achieved by the use of *N*-methylhydrazine in treatment with 3,5-dimethoxybenzoyl chloride or 1-napthylacetyl chloride to give **1t** and **1u**, respectively, as shown in Schemes 4 and 5.

The compounds were tested in CHO-K1 cells stably expressing the human GPR139 receptor for a calcium mobilization response measured using a Hamamatsu FDSS7000 imaging plate reader. In the absence of a known endogenous GPR139 agonist, compound **1a** was defined as a full agonist (i.e., E_{max} = 100% stimulation). This assumption was supported by the observation that a saturating dose of **1a** typically produced a calcium mobilization response close to the response to ionomycin, a potent and selective Ca^{2+} ionophore causing re-equilibration of calcium gradients between mitochondria, cytosol, and the extracellular space (data not shown). A detailed description of assay conditions is provided in the Supporting Information.

Peripheral substitution of **2**, first examining the effect of R^1 substitution while maintaining the unsubstituted napthyl, showed that the unsubstituted analogue **1b** was significantly less potent. Substitution in the 2 position with 2-ethoxy (**1d**) markedly reduced activity while the smaller 2-methoxy was tolerated (**1c**). Interestingly, the 3,5 diethyl analogue, **1e**, was inactive. The 4 position tolerated fluorine (**1g**) while the introduction of an extra methoxy in the 4 position in the tris-trimethoxy analogue (**1h**) was not detrimental to activity. The bulky phenoxy substituent (**1i**) was also tolerated in the 4 position.

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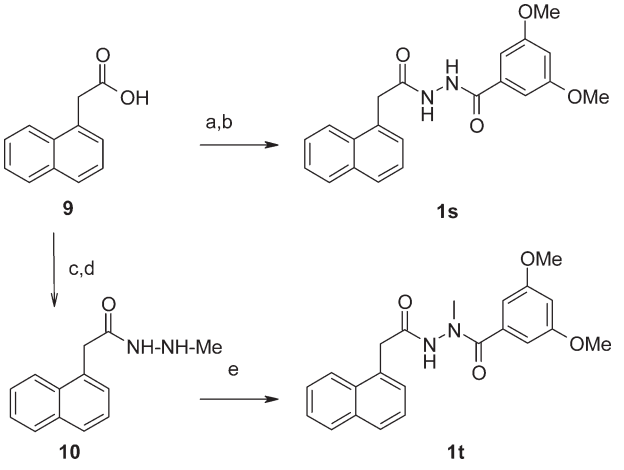
A limited examination (see Table 1) of R^2 while maintaining R^1 as the preferred 3,5-bis-methoxy showed toleration of only the 2-methyl substitutent (**1l**) whereas Br, CN, and Cl substitution in the 4 position (**1j**, **1k**, **1m**), Br in the 5 position (**1n**), or MeO in the 7 position (**1o**) all reduced activity.

Examination of the central linker (see Table 2) showed that the hydrazide oxygen plays an important role, as its removal in **1p** completely removed activity whereas replacement of the first hydrazide nitrogen with carbon to give the ketone analogue (**1q**) maintained micromolar potency. In contrast, replacement of the other nitrogens in **1r** and **1s** did not markedly affect activity, suggesting that only the amide NH was vital for activity. However, methylation of both of these nitrogens also markedly affected activity (**1t** and **1u**).

In vitro assessment of the ADME properties of the original hit **1a** for suitability as a tool compound for *in vivo* studies indicated good stability in rat and human liver microsomes with values well below the liver blood flow threshold values of 20 mL/min[5] and 1.4 L/min, respectively.[6] A high polar surface area[7] and a potential efflux issue, as indicated by the MDCK ratio for **1a**, were indeed predictive for the resulting low brain/plasma (b/p) ratio in rat of 0.03 and a whole brain exposure of 65 ng/g after a p. o. dose of 50 mg/kg dosed in PEG400. The brain concentration of **1a** corresponded to an unbound brain concentration of 6.23 nM, which is significantly lower than the EC_{50} of 39 nM (see Table 3). The most promising compound **1s** resulting from the lead optimization work reduced the PSA from 88.7 Å^2 to 76.7 Å^2 and improved the apparent permeability in MDCK cells. The brain/plasma ratio improved markedly, but low plasma levels meant that the whole brain exposure did not improve. The brain exposure of **1s** was 95 ng/g, corresponding to an unbound brain concentration of 10.4 nM (see Table 3). The aqueous solubility of both **1a** and **1s** at pH 7.4 in 25 mM phosphate buffer was low, at 1.5 μg/mL and 1.6 μg/mL, respectively.

We have identified novel agonists for the GPR139 orphan receptor. ADME assessment indicates poor brain exposure in rat brain after oral dosing, limiting the usefulness of these compounds *in vivo*.

Scheme 4. Synthesis of Compounds of 1s and 1t[a]



[a] Reagents and conditions: (a) $SOCl_2$, DCM, rt; (b) 3,5-dimethoxybenzhydrazide, DIPEA, CH_2Cl_2, 91%; (c) $SOCl_2$, MeOH, rt, 99%; (d) $CH_3(NH)NH_2$, reflux, 71%; (e) 3,5-dimethoxybenzoyl chloride, DIPEA, THF, rt, 69%.

Table 1. Effect of R^1 and R^2 on Efficacy at Human GPR139 Receptors

compd	R^1	R^2	EC_{50} (nM)*	E_{max} (%)
1a	3,5-diMeO	H	39	100
1b	H	H	2400	85
1c	2-MeO	H	620	79
1d	2-EtO	H	nd	53
1e	3,5 diEt	H	nd	6
1f	3-MeO	H	1000	100
1g	4-F	H	440	96
1h	3,4,5-triMeO	H	63	76
1i	4-PhO	H	2100	61
1j	3,5-diMeO	4-Br	nd	73
1k	3,5-diMeO	4-CN	nd	20
1l	3,5-diMeO	2-Me	86	86
1m	3,5-diMeO	4-Cl	nd	64
1n	3,5-diMeO	5-Br	nd	54
1o	3,5-diMeO	7-MeO	nd	50

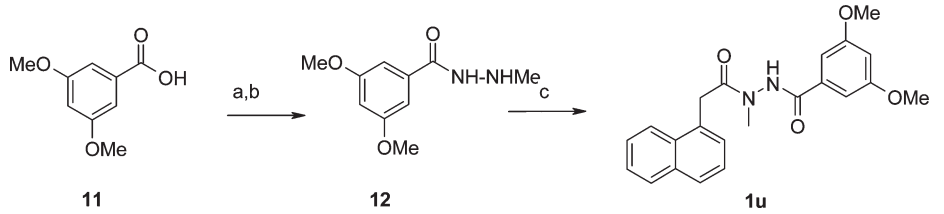
* If an EC_{50} could not be determined, the activity is reported as percent stimulation at 50 μM. nd, not determined. Data are the mean of at least three experiments. The efficacy of saturating concentrations of compound **1a** was set to 100%.

Table 2. Effect of the Central Linker on Efficacy at Human GPR139 Receptors[a]

compd	EC_{50} (nM)	E_{max} (%)
1p	>10000	
1q	3300	100
1r	180	98
1s	88	95
1t	2500	74
1u	5100	84

[a] Data are the mean of at least three experiments. The efficacy of saturating concentrations of compound **1a** was set to 100%.

Scheme 5. Synthesis of Compound 1u[a]



[a] Reagents and conditions: (a) $SOCl_2$, MeOH, rt, 98%; (b) $CH_3(NH)NH_2$, reflux, 76%; (c) 1-napthylacetyl chloride, DIPEA, THF, rt, 72%.

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Table 3. In Vitro and In Vivo ADME Assessment of Compounds 1a and 1s[a]

compd	PSA (Å^2)	*c* log *P*	ClintR (mL/min)	ClintH (L/min)	MDCK P_{app} (1×10^{-6} cm/s)	MDCK ratio (BA/AB)	b/p rat 50 mg/kg p.o. @ 2 h[b]	UBP (%)	UBBr (%)	whole brain exposure
1a	88.7	3.34	8.2	1	28.8	4.1	0.03	1.5	3.5	61 ng/g
1s	76.7	3.48	34	3.1	54	2.6	2.8	0.4	4.0	95 ng/g

[a] Abbreviations: P_{app}, apparent permeability; PSA, polar surface area; ClintR, intrinsic clearance in rat liver microsomes; ClintH, intrinsic clearance in human liver microsomes; MDC, Madin−Darby canine kidney cells; b/p, brain plasma ratio; UBP, % unbound in plasma; UBBr, % unbound in brain. The basolateral-to-apical (B → A) and apical-to-basolateral (A → B) apparent permeability coefficient ratio in the same transport assay was used to determine the extent of efflux or P-gp substrate specificity of **1a** and **1s**. [b] Dosed in PEG400, $n = 2$.

■ ASSOCIATED CONTENT

Supporting Information. Description of the GPR139 assay, cross reactivity data, and experimental details for the synthesis of all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Matsuo, A.; Matsumoto, S.; Nagano, M.; Masumoto, K.; Takasaki, J.; Matsumoto, M.; Kobori, M.; Masao, K.; Shigeyoshi, Y. Molecular cloning and characterization of a novel Gq-coupled orphan receptor GPRg1 exclusively expressed in the central nervous system. *Biochem. Biophys. Res.* **2005**, *331*, 363–369.

(2) Süsens, U.; Hermans-Bormeyer, I.; Urny, J.; Schaller, H. C. Characterisation and differential expression of two very closely related G-protein-coupled receptors, GPR139 and GPR142, in mouse tissue and during mouse development. *Neuropharmacology* **2006**, *50* (4), 512–520.

(3) Hu, L. A.; Tang, P. M.; Eslahi, N. K.; Zhou, T.; Barbosa, J.; Liu, Q. Identification of Surrogate Agonists and Antagonists for Orphan G-Protein-Coupled GPR139. *J. Biomol. Screen.* **2009**, *14*, 789–799.

(4) Doan, K. M. M.; Humphreys, J. E.; Webster, L. O.; Wring, S. A.; Shampine, L. J.; Serabjit-Singh, C. J.; Adkison, K. K.; Polli, J. W. Passive permeability and P-Glycoprotein-mediated efflux differentiate central nervous system (CNS) and non-CNS marketed drugs. *J. Pharmacol. Exp. Ther.* **2002**, *303* (39), 1029–1037.

(5) Houston, B. J. Utility of *in vitro* drug metabolism data in predicting in vivo metabolic clearance. *Biochem. Pharmacol.* **1994**, *9*, 1469–1479.

(6) Kemme, M. J. B.; Burggraaf, J.; Schoemaker, R. C.; Paulson, S.; Karim, A.; Lentjes, E. G. W. M.; Childs, A.; Braeckman, R. A.; Cohen, A. F. The influence of reduced liver blood flow on the pharmacokinetics and pharmacodynamics of recombinant tissue factor pathway inhibitor **2000**, *67* (8), 504–511.

(7) Pajouhesh, H.; Lenz, G. R. Medicinal chemical properties of successful central nervous drugs. *NeuroRx* **2005**, *2*, 541–552.