Discovery of the First Potent and Orally Efficacious Agonist of the Orphan G-Protein Coupled Receptor 119

Graeme Semple,*[,†] Beatriz Fioravanti,[†] Guillherme Pereira,[†] Imelda Calderon,[†] Jane Uy,[†] Karoline Choi,[†] Yifeng Xiong,[†] Albert Ren,[†] Michael Morgan,[‡] Vibha Dave,[§] William Thomsen,[§] David J. Unett,[§] Charles Xing,[§] Stuart Bossie,[§] Chris Carroll,[§] Zhi-Liang Chu,[§] Andrew J. Grottick,[§] Erin K. Hauser,[§] James Leonard,[§] and Robert M. Jones[†]

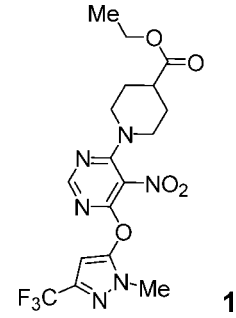
Departments of Medicinal Chemistry, DMPK, and Discovery Biology, Arena Pharmaceuticals, 6166 Nancy Ridge Drive, San Diego, California 92121

Received June 5, 2008

Abstract: GPR119 is a rhodopsin-like GPCR expressed in pancreatic β-cells and incretin releasing cells in the GI tract. As with incretins, GPR119 increases cAMP levels in these cell types, thus making it a highly attractive potential target for the treatment of diabetes. The discovery of the first reported potent agonist of GPR119, 2-fluoro-4-methanesulfonyl-phenyl)-{6-[4-(3-isopropyl-[1,2,4]oxadiazol-5-yl)-piperidin-1-yl]-5-nitro-pyrimidin-4-yl}-amine (**8g**, AR231453), is described starting from an initial inverse agonist screening hit. Compound **8g** showed in vivo activity in rodents and was active in an oral glucose tolerance test in mice following oral administration.

Injectable peptide agonists of the glucagon-like peptide-1[a] (GLP-1) receptor, such as exendin-4 (exenatide), amplify glucose-dependent insulin release and preserve pancreatic β-cell mass and have shown promise as antidiabetic agents; exenatide has been approved for this indication.[1] These compounds elevate intracellular cAMP levels in β-cells via stimulation of the GLP-1 receptor (GLP-1R), which is positively coupled to adenylate cyclase ($G\alpha_s$). GLP-1R is a class B (secretin-like) G-protein coupled receptor (GPCR), and it has proven difficult over the years to identify nonpeptide agonist ligands of this subfamily that may be suitable for oral administration. As a result, we were interested in identifying novel, class A (rhodopsin-like) $G\alpha_s$-coupled GPCRs that would have similar β-cell expression and G-protein coupling to GLP-1R. From this effort, we identified GPR119 (recently termed glucose-dependent insulinotropic receptor, or GDIR) as a constitutively active, β-cell-expressed receptor capable of elevating intracellular cAMP levels in both transfected CHO cells or pancreatic β-cell lines.[2] Additionally, we showed that GPR119 could stimulate incretin hormone release from cells localized in the GI tract, demonstrating that the receptor can regulate glucose homeostasis by multiple mechanisms.[3] Thus, our early target localization and validation studies suggested that GPR119 would be an attractive target for the treatment of type 2 diabetes, and we assumed that it may also be more amenable to the discovery of orally acting small molecule agonists as a result of its phylogenicity.

It has proven difficult to investigate functional aspects of GPR119 with native ligands, in part because the reported endogenously expressed modulators are neither potent nor selective. GPR119 is activated by phospholipids and lipid amides, such as oleoyl ethanolamide, in vitro,[4,5] but it remains unclear as to whether these are physiologically relevant ligands. A possible role for lipid amides in the activation of GPR119 is supported by its phylogenetic proximity to cannabinoid receptors,[6] however the potency of GPR119-interacting lipid amides is somewhat weak and they are also known to modulate other biological targets, including PPARα nuclear receptors and TRPV1 channels.[7] Lipid amides might also be expected to possess poor stability in vivo. Hence, to confirm early data with molecular biological tools and to enable proof of principle in appropriate pharmacological models, we required a potent and selective agonist of the receptor. A preliminary report of the discovery of weak GPR119 agonists has recently appeared,[8] but we herein describe the identification of the first highly potent ligands for GPR119 for use in target validation studies.



1

Figure 1. Structure of the inverse agonist screening hit.

A high-throughput screen of our compound collection, using a 96-well Flashplate membrane cyclase assay, capable of detecting both agonist and inverse agonist responses,[9] identified **1** (Figure 1) as a single tractable hit. The hit molecule was considered to be somewhat unpromising as it was an inverse agonist of the receptor ($IC_{50} = 84$ nM; $n = 17$), whereas all the preliminary data described above suggested that an agonist would be required to have a positive effect on modulation of insulin secretion and hence in diabetes models. In addition, **1** contained both an undesirable aromatic nitro group and a labile ester group. As expected, no parent compound remained 15 min after intravenous administration of **1** to rats and the major circulating metabolite was the carboxylic acid resulting from ethyl ester hydrolysis, which proved to be inactive at the receptor in our cyclase assay. However, the nitropyrimidine core structure lent itself to parallel synthesis approaches, and we therefore set out to try and generate agonist tools based on the premise that, historically, nonpeptide agonists of rhodopsin-like GPCRs reported in the literature have borne a remarkable structural similarity to antagonists or inverse agonists of the same receptor, as has been discussed previously.[10] It was clear from the lack of activity of a number of related compounds in our compound collection that the piperidine ester function was making a significant contribution to the observed activity, so despite the obvious metabolic liability, we initially elected to keep this group in place while examining the SAR at the opposite end of the molecule in the search for an agonist starting point.

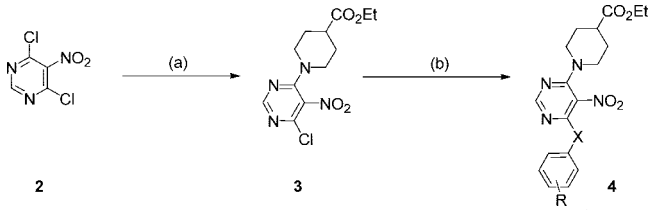
Using the simple synthetic procedure outlined in Scheme 1,[11] we replaced the trifluoromethyl pyrazole with a series of aryl ethers (Scheme 1, X = O). Reasonable inverse agonist activity was retained in the screening assay with a range of phenyl ethers substituted with simple halo-, trifluoromethyl-, cyano-, or alkyl ether groups (e.g., **4a**), but no agonists were identified among the first several dozen analogues prepared. However, when the phenyl group was substituted in the 4-position with a more complex keto-ester group, we obtained **4d**, our first example of a GPR119 agonist.

* To whom correspondence should be addressed. Phone: +1 858 453 7200. Fax: + 1 858 453 7210. E-mail: gsemple@arenapharm.com.
† Department of Medicinal Chemistry, Arena Pharmaceuticals.
‡ Department of DMPK, Arena Pharmaceuticals.
§ Department of Discovery Biology, Arena Pharmaceuticals.

[a] Abbreviations: GPR119, G-protein coupled receptor 119, also known as GDIR, Glucose-dependent insulinotropic receptor; GLP-1R, glucagon-like peptide-1 receptor; cAMP, cAMP, 3′,5′-cyclic adenosine monophosphate; $t_{1/2}$, terminal half-life; C_{max}, observed maximal plasma concentration following oral dosing; t_{max}, time to reach the C_{max}; AUC, total exposure as measured by the area under the exposure curve; %F, oral bioavailability; oGTT, oral glucose tolerance test.

10.1021/jm8006867 CCC: $40.75
Published on Web 08/13/2008

Scheme 1. Two Step Synthesis of Nitropyrimidine Core Compounds[a]



[a] Reagents and conditions: (a) Ethyl piperidine-4-carboxylate, ^{i}PrNEt, DCM, 0 °C, 91%. (b) Ph(R)XH (X = O or NH) dioxin: DMF (5/1, v/v), microwave heating, 20−65%.

Table 1. Activity of Compounds **4a**−**4k** in a Membrane cAMP Flashplate Assay

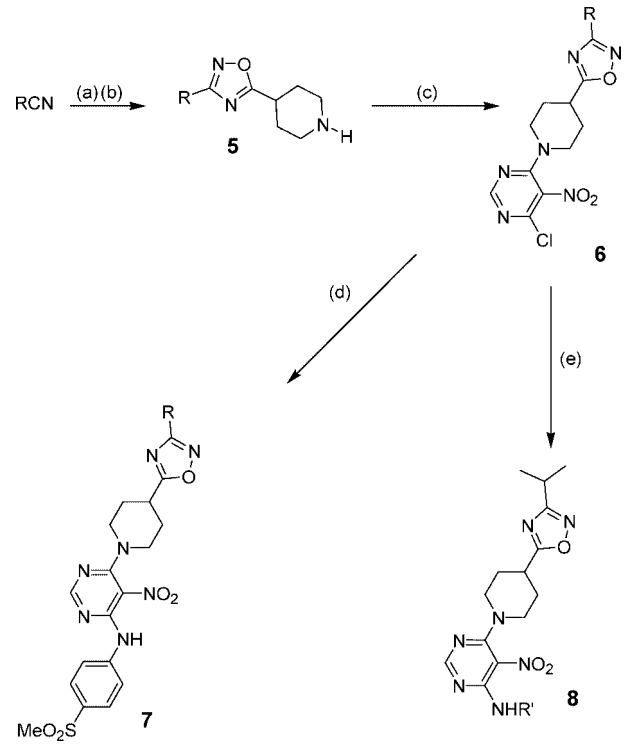
compd no.	R	X	IC_{50}/EC_{50} (μM)[a]	function
4a	4-Br	O	0.05	inverse agonist
4b	4-COEt	O	n.e.	
4c	4-$(CH_2)_2$OMe	O	n.e.	
4d	4-$COCH_2CO_2$Me	O	1.0	agonist
4e	4-$(CH_2)_2$COMe	O	1.7	agonist
4f	4-O$(CH_2)_2$OMe	O	0.5	inverse agonist
4g	4-SO_2Me	O	1.9	agonist
4h	4-SO_2NH_2	O	3.0	agonist
4i	4-(1-(1,2,4-triazole))	O	2.5	agonist
4j	4-SO_2Me	N	1.2	agonist
4k	4-(1-(1,2,4-triazole))	N	0.4	agonist

[a] Data from the flashplate assay were not run with sufficient replicates to be regarded as truly quantitative (usually $n = 1$ or $n = 2$), and so standard error data are not applicable.

Although we had succeeded in inverting the functional activity in our favor, we had done so at the expense of incorporating a second labile ester group into the molecule. Some simple examination of the SAR from the same early focused library showed that neither a simple ketone (**4b**) nor an ether (**4c**, **4f**) were sufficient to provide agonist activity, but a ketone further displaced from the ring (**4e**) also produced the desired functional response. We thus hypothesized that a hydrogen-bond accepting group was required for measurable agonist activity but that its optimal position was still undetermined. Hence we focused our further efforts on substituted phenyl analogues incorporating this type of feature with a view to more closely defining the H-bonding acceptor requirements and identifying a more stable agonist trigger group so that we could return to addressing the stability issues at the other end of the molecule. Extensive parallel SAR work led to the identification of multiple agonist triggers, selected examples of which are shown in Table 1 and include sulfones, sulfonamides, and various 5-membered heterocycles. The best activity was seen when such a group was attached to the 4-position of the phenyl ring. In addition, with the appropriate trigger in place, agonist activity was maintained with either an ether or aniline linker (**4**, X = O or NH).

Although our HTS assay had served its purpose of enabling us to identify agonist molecules, the reproducibility of the assay and hence the ability to obtain truly quantitative data was relatively poor as a result of the requirement for transient transfection of the receptor prior to each preparation of membranes; cells expressing the receptor at high levels were also prone to apoptosis. This is believed to be an artifact of receptor overexpression (many times higher than under physiological conditions) and was cell type dependent. Clear agonism or inverse agonism could be readily determined, but it was difficult to rapidly and accurately determine structural features that would enhance potency as the generation of high quality data (SD < 0.4 log units) required too many replicates. Hence we changed our assay platform to a melanophore dispersion assay. The melanophore assay uses *Xenopus* cells, which when transiently transfected with GPCRs can engage their existing intracellular machinery to respond to receptor activation by changes in pigment trafficking. This results in an apparent color change

Scheme 2. Preparation of Oxadiazole Analogues[a]



[a] Reagents and conditions: (a) $NH_2OH.HCl$, Na_2CO_3, H_2O/EtOH. (b) 1-Boc-ethyl piperidine-4-carboxylate, NaH, THF, 4 M HCl/dioxan. (c) **2**, ^{i}PrNEt, DCM, 0 °C, 91%. (d) 4-$MeSO_2PhNH_2$, dioxane: DMF (5/1, v/v), microwave, 180 °C, 300s. (e) R′NH_2, K_2CO_3, dioxane, microwave, 250 °C, 300s.

that can be quantified by simple measurement of light transmission in high-throughput format to produce concentration−response curves. These cells are particularly suited to measuring Gi or Gs coupled GPCR activation (producing pigment aggregation and dispersion respectively).[12] This assay switch provided data that proved to be significantly more reproducible and consequently more suitable for more subtle SAR examination.

With a clear indication that agonist molecules could be identified within the series and a new assay in hand, we now sought to replace the ester group to provide more metabolically stable compounds. As the ester group had proven important for the initial hit identification, we elected first to replace it with a highly conservative isosteric replacement. The oxadiazole ring can provide all of the hydrogen bond donor and acceptor capabilities of the ester itself, and so we prepared a series of such derivatives as outlined in Scheme 2. Reaction of a series of alkyl and aryl nitriles with hydroxylamine provided hydroxyamidine intermediates, which were condensed directly with 1-Boc-ethyl piperidine-4-carboxylate. Removal of the Boc-protecting group under acidic conditions provided the oxadiazole building blocks **5**. Reaction of **5** with **2** under similar conditions as used previously to incorporate piperidines provided the intermediates **6**, which upon displacement of the second chloro- group from the pyrimidine with aniline-4-methyl sulfone under microwave irradiation conditions duly provided the target compounds **7**. As can be seen in Table 2, keeping constant the aniline sulfone group which we had previously shown to enable good agonist activity, allowed the identification of a number of more potent agonist molecules. The SAR of the alkyl substituent on the oxadiazole portion was particularly remarkable. Increasing the size of this substituent from methyl to ethyl provided a more than 25-fold improvement in agonist potency. The addition of more carbon atoms to the chain in a linear manner did not provide any further improvements in activity, whereas the inclusion of α-branching (but not β-branching) in this group again gave a significant improvement in potency. In addition to alkyl

Table 2. Agonist Activity of Selected Oxadiazole Analogues in the Melanophore Dispersion Assay[12]

compd no.	R	EC_{50} (nM)	log SD (n)[a]
7a	Me	260	0.26 (8)
7b	Et	10	0.19 (8)
7c	^{n}Pr	430	0.28 (4)
7d	^{c}Pr	25	0.20 (8)
7e	$CH_2{}^c$Pr	160	0.13 (3)
7f	^{i}Pr	5.8	0.12 (5)
7g	^{t}Bu	1.5	0.38 (3)
7h	3-pyridyl	660	0.28 (7)
7i	3-CF_3−Ph	24	0.27 (3)

[a] The standard deviation from the mean of the EC_{50} value. The number of determinations is shown in parentheses.

Table 3. Agonist Activity of Isopropyl Oxadiazole Analogues in the Melanophore Dispersion Assay[12]

compd no.	R′	EC_{50} (nM)	log SD (n)[a]
8a	4-(SO_2Et)-Ph	17	0.15 (3)
8b	4-($SO_2{}^n$Pr)-Ph	4.7	0.4 (5)
8c	4-($SO_2{}^i$Pr)-Ph	7.8	0.44 (7)
8d	4-(SOEt)-Ph	17.1	0.15 (3)
8e	4-(SEt)-Ph	110	0.17 (2)
8f	3-F, 4-(SO_2Me)-Ph	3.4	0.44 (3)
8g	2-F, 4-(SO_2Me)-Ph	0.68	0.37 (569)
8h	2-F, 4-(SO_2Et)-Ph	7.0	0.14 (2)
8i	2-F, (4-$SO_2{}^i$Pr)-Ph	7.9	0.24 (6)

[a] The standard deviation from the mean of the EC_{50} value. The number of determinations is shown in parentheses.

groups in this position, we also examined a range of aryl groups (two examples of which are shown in Table 2), but no further compounds were identified that matched the single-digit nanomolar potency of **7f** or **7g**. Subsequently, alternative heterocycles were also examined in place of the oxadiazole in **7** (including the isomeric oxadiazoles) and the SAR for compounds of this type will be reported elsewhere.

Having identified a stable replacement for the ester group, we turned our attention back to the other end of the molecule to re-examine whether our previous SAR observations still held true. The compounds **8a**−**8i** were all prepared from the same intermediate (**6**, R = ^{i}Pr) by nucleophilic displacement of the pyrimidine chloro- group with various anilines, with the exception of **8d**, which was prepared from **8e** by oxidation of the thioether group to the sulfoxide with *meta*-chloro-perbenzoic acid. As can be seen from Table 3, no further improvement in potency was observed by increasing the size of the sulfone alkyl group. Interestingly, the sulfoxide **8d** was comparable in potency to **7f**, whereas the thioether analogue **8e** was significantly less potent, re-emphasizing the preference for a good hydrogen bond acceptor in this region of the molecule. Addition of a further fluoro-substituent onto this aryl ring provided two additional compounds with improved potency, with **8g** (AR231453) being the first subnanomolar agonist compound that we had observed. Replacement of the sulfone with sulfonamides did not provide any further improvement, and although a similar SAR was observed with other oxadiazoles in place of the ^{i}Pr oxadiazole in this series, once more, no real potency advantage ensued.

Further profiling of **8g** showed it to be a highly selective ligand for GPR119 with no off-target activity across a 76 receptor and enzyme profiling panel (CEREP), which included the known incretin receptors and no activity against 140 known and orphan GPCRs in melanophore dispersion assays in-house. In addition, **8g** had no activity against dipeptidyl peptidase-4, a well validated target for the treatment of diabetes. Importantly for a pharmacological tool, **8g** had activity across species, albeit with somewhat lower potency in rodents (EC_{50} in melanophore for other species tested: cynomologus monkey GPR119 = 0.4 nM; mouse GPR119 = 12 nM; rat GPR119 = 14 nM; dog GPR119 = 1.6 nM). Across all species, **8g** displayed a significantly greater level of efficacy in these assays than the putative endogenous ligand oleoyl ethanolamide and has since become our standard compound for comparing the efficacy of other series of compounds at the receptor. Comparative efficacy studies of a number of naturally occurring lipid amides will be detailed in further reports from our laboratories.

Compound **8g** did not inhibit any of the major CYP enzymes and had no activity up to 10 μM at the hERG channel in a patch clamp assay. Furthermore, excellent exposure was observed after oral administration to C57/bl6J mice (10 mg/kg po dose: t_{max} = 0.5 h, C_{max} = 4975 ng/mL (9.84 μM), AUC = 19803 h·ng/mL, $t_{1/2}$ = 3.4 h; $\%F$ was not calculated as the iv PK profile was not determined in mouse), making this a promising tool for studying the function of GPR119 in this species. As has been recently reported,[2] **8g** stimulated cAMP production via endogenously expressed GPR119 in the β-cell-like hamster insulinoma line HIT-T15, with an EC_{50} of 4.7 nM, a value comparable to the EC_{50} observed in cell lines overexpressing the cloned rodent receptors.

As can be seen in Figure 2, the in vivo effect of **8g** was similar in terms of % inhibition of glucose AUC in an oral glucose tolerance test (oGTT) in both C57/bl6J mice and Sprague−Dawley rats at a dose of 3 mg/kg ip despite the clear species differences in the magnitude of the glucose excursion. This similarity may be expected in light of the similar potency and efficacy of **8g** versus both mouse and rat GPR119. In addition, **8g** improved oral glucose tolerance in wild-type C57/bl6J mice after per oral administration (Figure 3). Importantly, in terms of target validation, **8g** also enhanced glucose-dependent insulin release and

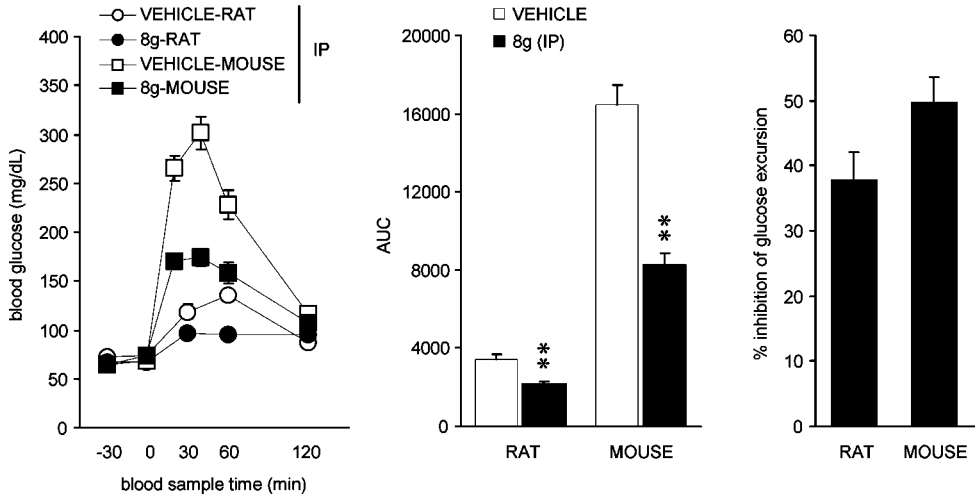


Figure 2. The effect of compound **8g** on oral glucose tolerance in mouse and rat following ip administration.

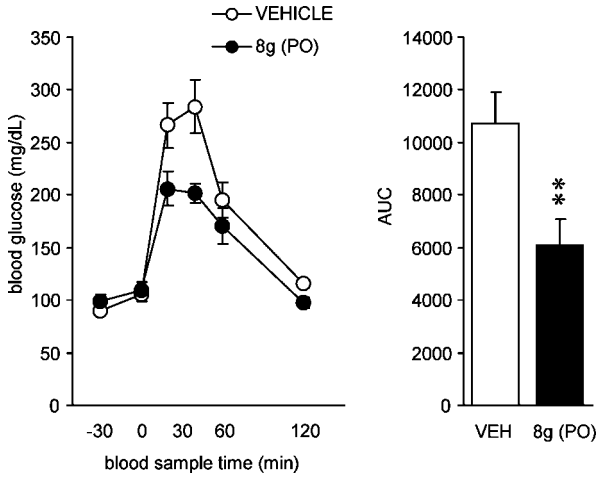


Figure 3. The effect of compound **8g** on oral glucose tolerance in mouse after oral administration.

improved oral glucose tolerance in wild-type C57/bl6J mice but not in GPR119-deficient mice at a dose of 20 mg/kg po, clearly demonstrating the receptor dependence of the observed effects.[2] Diabetic *KK/A*^{y} mice were also highly responsive to **8g**. It should be noted, however, that a statistically significant effect on glucose excursion in an oGTT following oral administration of **8g** was not observed in rats, although a trend toward efficacy was seen at a dose of 30 mg/kg (data not shown). We believe that this lack of a consistent effect results from the combination of a narrower window for measuring the decrease in glucose excursion in rats as well as the significantly poorer exposure for **8g** in rat compared to mouse following oral administration (10 mg/kg po dose: $t_{max} = 1$ h, $C_{max} = 126$ ng/mL (0.250 μM), AUC = 263 h·ng/mL, $t_{1/2} = 1.1$ h, $F = 12\%$). Taken together with the molecular biological data, these pharmacological studies with a selective receptor agonist strongly suggest that targeting GPR119 will be a useful approach to provide novel antidiabetic agents acting in a glucose-dependent fashion.

In summary, the manipulation of our initial inverse agonist screening hit to provide an orally bioavailable pharmacological tool compound has been outlined. The incorporation of a hydrogen-bond acceptor group to the southern portion around the nitro pyrimidine core was required but was not always sufficient for agonist activity. Replacement of the ester function in the original hit with an isosteric oxadiazole provided useful improvements in stability, and the inclusion of small branched alkyl groups on this oxadiazole gave compounds with increased activity. Finally fluorination on the southern ring provided a further enhancement in potency, resulting in the identification of **8g**, which has proven to be an excellent tool compound for target validation studies in mice. In addition, we have shown that it is possible to identify orally bioavailable agonists of the receptor, which may make GPR119 a more attractive target for future drug development then other $G\alpha_s$-coupled receptors expressed on pancreatic β-cells such as GLP-1R. Further SAR enhancements leading to the identification of a first clinical candidate for GPR119 will be reported in due course.

Supporting Information Available: Synthetic experimental procedures, overexpression of GPR119 in RIN5F cells can amplify insulin secretion in the presence of high concentrations of glucose, examples of dose−response curves for an inverse agonist (**4a**) and an agonist (**4i**) molecule in the cAMP membrane flashplate assay, data showing correlation of two assay platforms used and reproducibility of the melanophore assay. This material is available free of charge via the Internet at http://pubs.acs.org.

Note Added after ASAP Publication.

This manuscript published ASAP on August 13, 2008 with an error in Table 3. The revised version was published on August 19, 2008.

References

(1) Gallwitz, B. Exenatide in type 2 diabetes: treatment effects in clinical studies and animal study data. *Int. J. Clin. Pract.* **2006**, *60*, 1654–1661.

(2) Chu, Z.-L.; Jones, R. M.; He, H.; Carroll, C.; Gutierrez, V.; Lucman, A.; Moloney, M.; Gao, H.; Mondala, H.; Bagnol, D.; Unett, D.; Liang, Y.; Demarest, K.; Semple, G.; Behan, D. P.; Leonard, J. A role for β-Cell-Expressed GPR119/ GDIR in Glycemic Control by Enhancing Glucose-Dependent Insulin Release. *Endocrinology* **2007**, *148*, 2601–2609.

(3) Chu, Z.-L.; Carroll, C.; Alfonso, J.; Gutierrez, V.; He, H.; Lucman, A.; Pedraza, M.; Mondala, H.; Gao, H.; Bagnol, D.; Chen, R.; Jones, R. M.; Behan, D. P.; Leonard, J. A Role for Intestinal Endocrine Cell-Expressed GPR119 in Glycemic Control by Enhancing GLP-1 and GIP Release. *Endocrinology* **2008**, *149*, 20382047.

(4) Soga, T.; Ohishi, T.; Matsui, T.; Saito, T.; Matsumoto, M.; Takasaki, J.; Matsumoto, S.; Kamohara, M.; Hiyama, H.; Yoshida, S.;.; Momose, K.; Ueda, Y.; Matsushime, H.; Kobori, M.; Furuichi, K. Lysophosphatidylcholine enhances glucose-dependent insulin secretion via an orphan G-protein-coupled receptor. *Biochem. Biophys. Res. Commun.* **2005**, *326*, 744–751.

(5) Overton, H. A.; Babbs, A. J.; Doel, S. M.; Fyfe, M. C.; Gardner, L. S.; Griffin, G.; Jackson, H. C.; Procter, M. J.; Rasamison, C. M.; Tang-Christensen, M.; Widdowson, P. S.; Williams, G. M.; Reynet, C. Deorphanization of a G protein-coupled receptor for oleoylethanolamide and its use in the discovery of small-molecule hypophagic agents. *Cell Metab.* **2006**, *3*, 167–175.

(6) Brown, A. J. Novel cannabinoid receptors. *Br. J. Pharmacol.* **2007**, *152*, 567–575.

(7) (a) Fu, J.; Gaetani, S.; Oveisi, F.; Verme, J. L.; Serrano, A.; Rodrı´guez de Fonseca, F.; Rosengarth, A.; Luecke, H.; Di Giacomo, B.; Tarzia, G.; Piomelli, D. Oleylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR-α. *Nature* **2003**, *425*, 90–93. (b) Ahern, G. P. Activation of TRPV1 by the Satiety Factor Oleoylethanolamide. *J. Biol. Chem.* **2003**, *278*, 30429–30234.

(8) Fyfe, M. C. T.; Babbs, A. J.; Bertram, L. S.; Bradley, S. E.; Doel, S. M.; Gadher, S.; Gattrell, W. T.; Jeevaratnam, R. P.; Keily, J. F.; McCormack, J. G.; Overton, H. A.; Rasamison, C. M.; Reynet, C.; Rushworth, P. J.; Sambrook-Smith, C. P.; Shah, V. K.; Stonehouse, D. F.; Swain, S. A.; White, J. R.; Widdowson, P. S.; Williams, G. M.; Procter, M. J. Synthesis, SAR, and in vivo efficacy of novel GPR119 agonists with a 4-[3-(4-methanesulfinylphenoxy)propyl]-1-Boc-piperidine core. Abstracts of Papers, 234th ACS National Meeting, Boston, MA, August 19−23, 2007, MEDI-062. The recent patent literature has also been reviewed: Fyfe, M. C. T.; McCormack, J. G.; Overton, H. A.; Procter, M. J.; Reynet, C. GPR119 agonists as potential new oral agents for the treatment of type 2 diabetes and obesity. *Expert Opin. Drug Discovery* **2008**, *3*, 403–413.

(9) HEK293 cells transiently expressing GPR119 were prepared using standard transfection protocols and crude plasma membranes were prepared 48 h post-transfection as described by Chu et al.[2] Compound stimulation of membrane adenylyl cyclase activity was measured using a 96-well FlashPlate kit (NEN Life Sciences, Boston, MA) as described by Chu et al.[3] Briefly, assay buffer contained 20 mM HEPES, pH 7.4, 10 μM GTP, 0.1 mM ATP, 500 μM IBMX, 10 mM phosphocreatine, and 10 U/50 μL creatine phosphokinase. Test compound incubations were performed for 60 min at room temperature in the presence of 15 μg of membrane protein. cAMP measurements were extrapolate from a standard curve included on each assay plate.

(10) Sugg, E. E. Nonpeptide agonists for peptide receptors: lessons from ligands. *Annu. Rep. Med. Chem.* **1997**, *32*, 277–283.

(11) Jones, R. M.; Semple, G.; Fioravanti, B.; Pereira, G.; Calderon, I.; Uy, J.; Duvvuri, K.; Choi, K.; Xiong, Y.; DaveV. Preparation of 1,2,3-trisubstituted aryl and heteroaryl derivatives, in particular pyrimidines, as modulators, in particular agonists and inverse agonists, of G-coupled protein receptor and their use in the prophylaxis and treatment of metabolic disorders such as diabetes and hyperglycemia. V. PCT Int. Appl. WO 2004065380, 2004.

(12) Cultured *Xenopus* dermal melanophores were transiently transfected with plasmid DNA encoding the GPR119 receptor and plated into clear 384-well assay plates following standard protocols. At 48 h post-transfection, the cells were treated with melatonin (10nM, 1 h) to induce pigment aggregation. Cells were then exposed to test compounds for 1 h, and the resulting GPR119-induced pigment dispersion was measured using an absorbance microplate reader. For a full description of standard protocol see: Potenza, M.; Graminski, G.; Lerner, M. A method for evaluating the effects of ligands upon Gs protein-coupled receptors using a recombinant melanophore-based bioassay. *Anal. Biochem.* **1992**, *206*, 315–322.

JM8006867