ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

Chemical Probe Identification Platform for Orphan GPCRs Using Focused Compound Screening: GPR39 as a Case Example

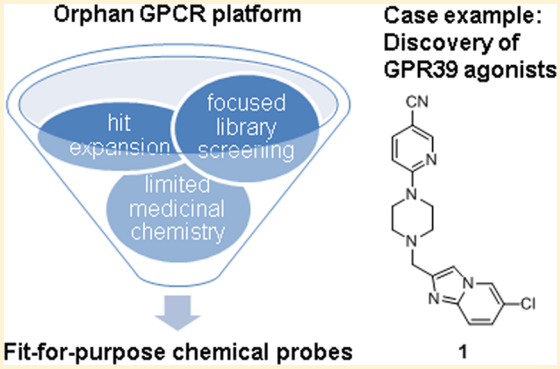
Markus Boehm,[‡] David Hepworth,[‡] Paula M. Loria,[§] Lisa D. Norquay,[†] Kevin J. Filipski,[‡] Janice E. Chin,[†] Kimberly O. Cameron,[‡] Martin Brenner,[†] Peter Bonnette,[†] Shawn Cabral,[‡] Edward Conn,[‡] David C. Ebner,[‡] Denise Gautreau,[†] John Hadcock,[†] Esther C. Y. Lee,[‡] Alan M. Mathiowetz,[‡] Michelle Morin,[†] Lucy Rogers,[§] Aaron Smith,[‡] Maria VanVolkenburg,[†] and Philip A. Carpino*[,‡]

[†]Departments of Cardiovascular, Metabolic and Endocrine Diseases and [‡]Cardiovascular, Metabolic and Endocrine Diseases Medicinal Chemistry, Pfizer Worldwide Research and Development, 620 Memorial Drive, Cambridge, Massachusetts 02139, United States

[§]Department of Pharmacokinetics, Dynamics and Metabolism—New Chemical Entities, Primary Pharmacology Group, Pfizer Worldwide Research and Development, Eastern Point Road, Groton, Connecticut 06340, United States

Supporting Information

ABSTRACT: Orphan G protein-coupled receptors (oGPCRs) are a class of integral membrane proteins for which endogenous ligands or transmitters have not yet been discovered. Transgenic animal technologies have uncovered potential roles for many of these oGPCRs, providing new targets for the treatment of various diseases. Understanding signaling pathways of oGPCRs and validating these receptors as potential drug targets requires the identification of chemical probe compounds to be used in place of endogenous ligands to interrogate these receptors. A novel chemical probe identification platform was created in which GPCR-focused libraries were screened against sets of oGPCR targets, with a goal of discovering fit-for-purpose chemical probes for the more druggable members of the set. Application of the platform to a set of oGPCRs resulted in the discovery of the first reported small molecule agonists for GPR39, a receptor implicated in the regulation of insulin secretion and preservation of beta cells in the pancreas. Compound **1** stimulated intracellular calcium mobilization in recombinant and native cells in a GPR39-specific manner but did not potentiate glucose-stimulated insulin secretion in human islet preparations.



KEYWORDS: *Orphan GPCRs, chemical probe identification platform, GPCR-focused chemical library, GPR39*

G-protein coupled receptors (GPCRs) are a diverse class of integral seven-transmembrane proteins that facilitate intracellular signal transduction by extracellular stimuli via recruitment of $G\alpha_s/G\alpha_i/G\alpha_q$ and GPCR receptor kinases.[1] GPCRs play important roles in many biological processes and have become attractive targets for drug discovery in multiple therapeutic areas. The majority of GPCR targets for which drugs are currently in development or on the market have been deorphanized, that is endogenous ligands or transmitters have been identified and functional roles for the receptors in pathological states have been established. GPCRs for which the endogenous ligands are unknown (i.e., orphan GPCRs) represent an untapped source of new targets.[2] Genetic technologies that have enabled the development of transgenic animal models in which a gene product is down-regulated or overexpressed have uncovered potential pharmacological roles for some oGPCRs.[3] Prosecution of drug discovery programs for these orphan receptors remains difficult due to the lack of endogenous ligands for assay development and the limited information about the receptors' signaling pathways and regulation in cells.

Dysregulation of several oGPCRs have been implicated in metabolic diseases.[4] One such oGPCR is GPR39, which is a member of the ghrelin family and is expressed principally in adipose tissue, intestine, liver, pancreas, pituitary gland, and stomach.[5,6] A 23 amino acid peptide called obestatin, a cleavage product derived from the preproghrelin gene, was previously reported to be a ligand for GPR39, but later found to have no agonist activity.[7,8] The divalent metal ion Zn^{2+} has been shown to activate multiple signal transduction pathways in cells overexpressing GPR39,[8−10] resulting in production of cAMP, mobilization of intracellular Ca^{2+}, or serum response element (SRE)-mediated transcription of Rho via $G\alpha_s$, $G\alpha_q$, or $G\alpha_{12/13}$ recruitment, respectively.[6,9] Genetic knockdown of GPR39 in

Received: July 18, 2013
Accepted: September 16, 2013
Published: September 16, 2013

dx.doi.org/10.1021/ml400275z | *ACS Med. Chem. Lett.* 2013, 4, 1079−1084

mouse models leads to impaired insulin secretion due to pancreatic islet cell dysfunction.[11−13] Overexpression of GPR39 can protect mice from streptozotocin-induced β-cell failure and development of diabetes.[4] Modulation of GPR39 may represent a new approach for the treatment of diabetes, a debilitating metabolic disease that is increasing in prevalence throughout the world.

A more thorough understanding of the pharmacology and signaling pathways of GPR39 and other oGPCRs implicated in metabolic diseases cannot progress without chemical probe compounds to be used in place of endogenous ligands to interrogate these receptors. The ideal chemical probe compounds possess nanomolar binding affinities, good functional potencies, high selectivities, and good pharmacokinetic properties in animal models to allow both in vitro and in vivo characterization, but such a profile can usually only be achieved at a high cost in time and resources. In certain instances, a fit-for-purpose chemical probe compound with sufficient potency and selectivity to enable in vitro characterization in cell lines and tissues may be more appropriate and cost-effective, especially when confidence in rationale of the target is low.

GPR39 and the other oGPCRs of interest were not considered suitable candidates for chemical probe identification via high-throughput screening of large compound collections (i.e., >100 000 compounds) because these receptors lacked compelling human genetic linkage data from genome-wide association studies. We created a novel chemical probe identification platform in which GPCR-focused compound libraries were screened against sets of oGPCR targets in rapid succession, with a goal of finding lead structures and developing fit-for-purpose chemical probes for the more druggable members of the set and deprioritizing the targets with no screening hits. This platform was suitable only for the identification of compounds that either activated oGPCRs (i.e., agonists) or decreased basal activity of such receptors (i.e., inverse agonists). Focused compound screening did not require any knowledge about the key amino acids involved in the binding of native ligands to their cognate receptors. An upfront investment in the development of primary and secondary (i.e., orthogonal) assays for multiple receptors was required. Hit-to-lead optimization was restricted to several rounds of virtual screening of the Pfizer compound collection, followed by limited medicinal chemistry efforts to improve potency and efficacy. A hit was considered validated against a target if it demonstrated activity in endogenous cell lines, but showed no activity in the same cell lines when expression of the receptor was diminished via small interfering RNA technology. A fit-for-purpose chemical probe for in vitro studies of oGPCRs was defined by binding/functional potency, permeability (necessary for tissue studies), and selectivity, rather than by criteria such as stability in human or rodent liver microsomal preparations or by good oral bioavailability in animal species. Herein, we report the design of the GPCR-focused compound library and illustrate the application of the oGPCR chemical probe identification platform to the discovery of fit-for-purpose chemical probe compounds for GPR39. We also describe the pharmacological evaluation of GPR39 chemical probes in cells and native tissue systems.

Knowledge-based screening methods employing focused compound libraries and/or chemogenomic analyses of GPCR binding pocket residues, facilitated in part by recent disclosures of X-ray crystallographic structures, have been shown to be comparable or even superior to random screening, especially for targets from large gene families such as kinases or GPCRs.[14] With vast amounts of screening data now available, computational methods can be employed to focus selection of compounds toward coverage of the chemical and biological space defined by GPCRs.[15,16] The Pfizer in-house database contains ~230 000 unique compounds with GPCR activity data across 2000 biological screens covering over 300 GPCR targets. All compounds with activities less than 10 μM in a GPCR dose−response assay (measured as IC_{50}, EC_{50}, or K_i values) were identified. An additional 10 000 in-house compounds that showed close-in similarity to known GPCR active molecules from the literature (e.g., ChEMBL)[17] were combined with the in-house GPCR compound set in order to expand the diversity of the collection. Following the removal of compounds with no availability, a set of strict structural and physicochemical property filters was applied to ensure that hit matter derived from the screening library was preselected with a high likelihood of being suitable for medicinal chemistry follow-up. The remaining compounds were clustered using two different types of structural fingerprints (Murcko frameworks[18] and ECFP4 fingerprints[19]) in order to (1) spread chemical space coverage as evenly as possible; (2) maintain a balanced distribution of diverse chemotype scaffolds; and (3) reduce the size of larger clusters. The entire GPCR compound library was then divided into several subsets based on the GPCR activity fingerprints for each compound (i.e., activity against aminergic, peptidergic, or other GPCR target subfamilies) as well as their projected target druggability, allowing for iterative screening starting from the most attractive sets of compounds to the least attractive set. The subsets consisted of 10 000−20 000 compounds, a number small enough to enable screening to proceed in a pharmacology laboratory rather than in a fully automated HTS facility (Figure 1).

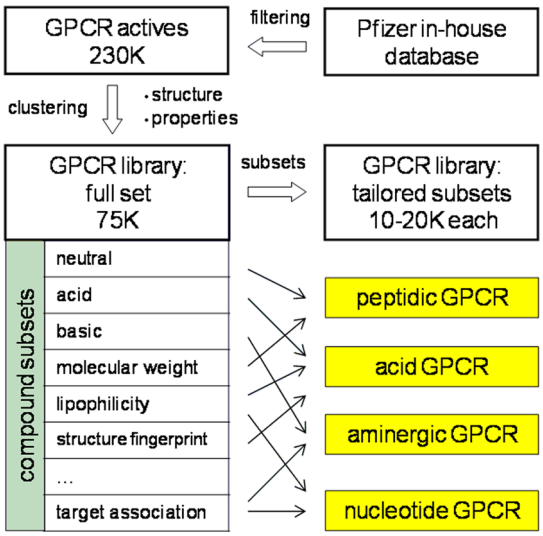


Figure 1. Design of GPCR-focused chemical library and compound subsets for screening.

The oGPCR platform required development of both primary assays for higher throughput screening and secondary orthogonal assays for eliminating compounds with target-independent activities (i.e., false positives). For the case example of GPR39, we were interested in studying the role of this oGPCR in insulin secretion and sought compounds with agonist activity at the receptor based on data from transgenic

dx.doi.org/10.1021/ml400275z | ACS Med. Chem. Lett. 2013, 4, 1079−1084

animal studies. GPR39 is coupled to signaling pathways that lead to production of cAMP or mobilization of intracellular Ca^{2+} via $G\alpha_s$ or $G\alpha_q$ pathways, respectively.[6] Activation of either cAMP/$G\alpha_s$ or Ca^{2+}/$G\alpha_q$ pathways in islets have been shown to stimulate insulin secretion from β-cells of pancreatic islets via other receptor pathways.[20] Since marketed insulin secretagogues and GLP1 receptor agonists such as exenatide have been shown to potentiate GSIS via cAMP/$G\alpha_s$ pathways, a robust, highly reproducible 384-well Cisbio assay to measure cAMP levels was established in CHO cells overexpressing human GPR39 (hGPR39). The assay was carried out in the presence of an EC_{20} concentration of the ago-allosteric GPR39 modulator Zn^{2+}, an ion that is also found in the extracellular environment of β-cells.[8] Incorporation of an allosteric agonist in the primary assay (i.e., Zn^{2+}) may result in the shift of GPR39 into more active conformations, potentially increasing the sensitivity of the assay. Heavy metals such as Zn^{2+}, which can be present as contaminants in compound samples, have been reported to increase the number of false positive screening hits in HTSs.[21] A Cisbio cAMP counter-screen in parental cells lacking hGPR39 but run in the presence of an EC_{20} concentration of Zn^{2+} was established to identify any such false positives. Compounds with activity in the cAMP screen but no activity in the parental line were evaluated in 384-well Ca^{2+} flux assays using CHO-FlpIn cells overexpressing hGPR39 and CHO cells lacking hGPR39. These assays were also carried out in the presence of EC_{20} concentrations of Zn^{2+}. A human HT29 cell line was used to assess the ability of compounds to increase cAMP and Ca^{2+} levels in cells endogenously expressing GPR39. The screening sequence as established for GPR39 in the oGPCR chemical probe identification platform was designed to discover compounds capable of activating multiple GPR39 signal transduction pathways since it was not known which of the cAMP/$G\alpha_s$ or Ca^{2+}/$G\alpha_q$ signaling pathways could potentially mediate GPR39-stimulated GSIS in β-cells (Figure 2).

Two subsets of the GPCR-focused compound library were screened against GPR39 using the Cisbio cAMP assay: (1) the peptidic GPCR subset with highly favorable physicochemical properties since GPR39 is a member of the ghrelin family and (2) the aminergic GPCR subset to increase the diversity of chemotypes. A total of ~30 000 compounds were evaluated in the cAMP single point primary assay (384-well format, 172 plates total, duplicate plate screening, mean Z' 0.67 ± 0.09 SD). An activity cutoff of 40% activation was selected based on mean ± SD distribution across all plates, resulting in 550 compounds for subsequent follow-up (1.8% hit rate). These compounds were progressed through the screening cascade (Figure 2), providing several novel lead compounds that were used as query structures for iterative rounds of file mining employing virtual screening methods based on two-dimensional fingerprint and three-dimensional shape-based similarity (i.e., ECFP4[19] and ROCS[22]). No inverse agonists were identified from the screen.

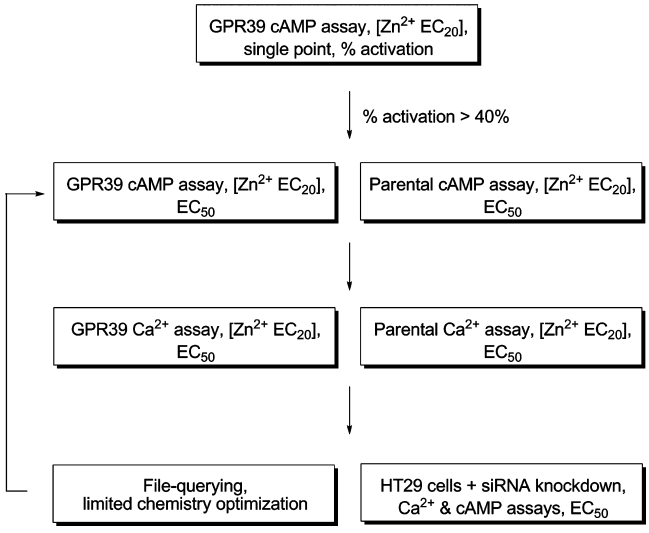
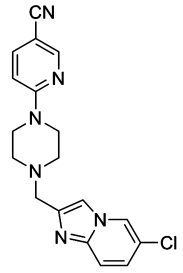


Figure 2. GPR39 screening cascade in the orphan drug chemical probe identification platform.

The piperazine derivative **1** (Figure 3) emerged from a screening hit and limited file mining as a promising lead for the discovery of GPR39 agonists, with good physicochemical properties such as low lipophilicity (SFlogD = 2.2) and moderate-to-high cellular permeability (P_{app} = 7.6 × 10^{-6} cm/sec).[23] Functional profiling of **1**, prepared initially as part of a dopamine receptor project by nucleophilic displacement of 6-chloro-2-(chloromethyl)*H*-imidazo[1,2-a]pyridine (**2**) by 6-(piperazin-1-yl)nicotinonitrile (**3**) as shown in Scheme S1 (Supporting Information) revealed good agonist activity in the hGPR39 Ca^{2+} assay (see Figure S6 in Supporting Information for representative dose−response curves), but only weak agonist activity in the hGPR39 cAMP assay. Compound **1** was a partial agonist relative to Zn^{2+} alone in the Ca^{2+} assay. No Ca^{2+} activity of **1** was observed in parental cells or in the absence of Zn^{2+}. In an endogenous HT29 cell line, **1** was shown to increase intracellular Ca^{2+} mobilization (EC_{50} = 0.9 μM), a response that was diminished with siRNA-mediated depletion of GPR39, confirming specificity-of-action and validating the lead (Figure 4). Consistent with its weaker cAMP activity in recombinant cell lines, **1** did not stimulate cAMP accumulation in HT29 cells (data not shown). In selectivity assays using a panel of 100 receptors (including dopamine receptors), ion channels, transporters, and enzymes, **1** did not display any significant inhibitory activity (Table S4 in Supporting Information). Compound **1** also exhibited very weak activity against several phosphodiesterases (PDEs), enzymes that degrade cAMP and play a role in the regulation of insulin secretion (PDE3a IC_{50} = 15 μM; PDE3b IC_{50} = 24 μM). With good physicochemical properties and selectivity over other GPCR targets, compound **1** represented a fit-for-purpose



1

hGPR39 Ca^{2+} EC_{50} = 0.056 μM (76%)
hGPR39 cAMP EC_{50} = 12 μM (100%)

Figure 3. Structure and pharmacological activities of GPR39 agonist **1** identified from oGPCR platform screening. Efficacies are relative to Zn^{2+} alone in the assays.

dx.doi.org/10.1021/ml400275z | ACS Med. Chem. Lett. 2013, 4, 1079−1084

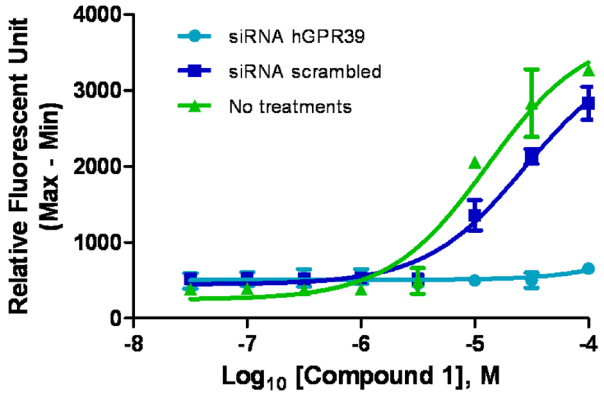


Figure 4. Treatment of **1** in HT29 cells in the presence and absence of siRNA constructs.

chemical probe for exploring Ca^{2+}-dependent GPR39 pharmacology in cellular systems.

To assess the potential role of GPR39 agonism to impact GSIS, compound **1** was evaluated in a standard static insulin secretion assay with human islet preparations from three separate donors (Table S5 in Supporting Information). Activation of $G\alpha_q/Ca^{2+}$-dependent pathways in islets have been shown to stimulate insulin secretion from β-cells in a glucose-dependent manner.[20] All three human islet preparations showed potentiation of GSIS to control stimulatory agents such as forskolin (1 μM), glybenclamide (1 μM), and GLP1 (0.1 μM). However, compound **1** showed no effect on insulin secretion at either basal or stimulatory glucose levels when used at concentrations up to 10 μM (Figure 5). The lack

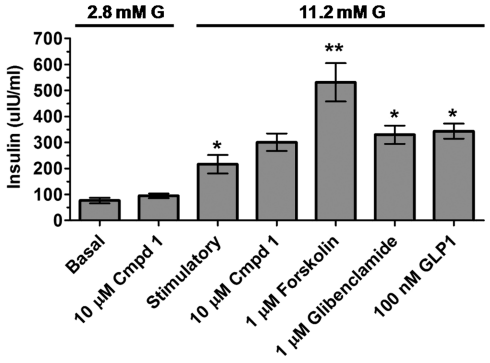


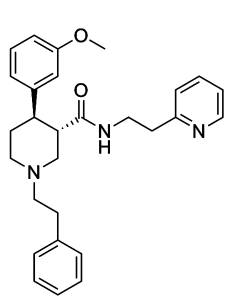
Figure 5. Effects of **1** and GLP1 control on human islet insulin secretion at low (2.8 mM) and stimulatory (11.2 mM) glucose (G) concentrations.

of a stimulatory effect of **1** on GSIS was confirmed in an INS-1 insulinoma cell line (data not shown). The addition of Zn^{2+} to the assay media was not required since Zn^{2+} is coreleased from insulin secretory granules throughout the 1 h assay period. The ability of **1** to increase intracellular Ca^{2+} mobilization in human islets (i.e., to demonstrate $G\alpha_q$ signaling) was not measured. It is unknown if an analogue of **1** with improved potency or efficacy would demonstrate potentiation of GSIS. It is also possible that GPR39 does not play a role in GSIS via a $G\alpha_q/Ca^{2+}$-dependent pathway. Evaluation of **1** in rodent models of islet health, a potential role for GPR39 identified from transgenic animal studies, was not investigated since **1** was not a suitable probe for in vivo studies.

Compound **1** exhibited a biased signaling profile, activating only one of the GPR39 signal transduction pathways induced by Zn^{2+}. A racemic piperidine derivative **4** (SFlogD = 2.4; P_{app} = 3.1 × 10^{-6} cm/sec) identified from file mining around initial screening hits showed weak activity in both the cAMP and Ca^{2+} functional assays. Limited medicinal chemistry optimization of **4** in which the piperidine group was replaced by a pyrrolidine ring and the phenylethyl group by a substituted imidazolyl moiety found in other screening hits led to the discovery of enantiomers (+)-*trans*-**5** and (−)-*trans*-**5** (Figure 6). These pyrrolidine derivatives exhibited comparable physicochemical properties as **4** [(+)-*trans*-**5**, SFlogD = 2.1; (−)-*trans*-**5**, SFlogD = 2.0], although permeability was in the low-to-moderate range [(+)-*trans*-**5**, P_{app} = 0.8 × 10^{-6} cm/sec; (−)-*trans*-**5**, P_{app} = 1.2 × 10^{-6} cm/sec]. (+)-*trans*-**5** and (−)-*trans*-**5**, prepared as shown in Schemes S2 and S3 (Supporting Information), were equipotent in the Ca^{2+} assay, with similar EC_{50} and E_{max} values (partial relative to Zn^{2+} alone), but in the cAMP assay, (−)-*trans*-**5** was more potent than (+)-*trans*-**5**, although both compounds were equally efficacious, with responses similar to Zn^{2+} alone. In HT29 cells, neither (+)-*trans*-**5** nor (−)-*trans*-**5** stimulated Ca^{2+} mobilization or increased cAMP activity at concentrations up to 30 μM. Lack of Ca^{2+} activity for (+)-*trans*-**5** or (−)-*trans*-**5** in the endogenous cell line was surprising since these two compounds were only 3−6-fold less active in the Ca^{2+} assay compared to **1**. Several structural modifications to **5** failed to improve potencies in the Ca^{2+} and cAMP functional assays. Additional medicinal chemistry optimization was outside the scope of the chemical probe identification platform. A chemical probe with dual Ca^{2+} and cAMP activities was not identified.

In conclusion, a novel oGPCR screening platform was established to discover fit-for-purpose chemical probe compounds. A GPCR-focused compound library consisting of several subsets with varying physicochemical properties was created from the Pfizer in-house database and screened against multiple oGPCRs, including the potential diabetes target GPR39. The first reported small molecule agonists for GPR39 were identified from screening hits following limited file mining (e.g., **1** and **4**) or a combination of file mining and medicinal chemistry optimization [e.g., (+)-*trans*-**5** and (−)-*trans*-**5**]. These compounds were shown to activate Ca^{2+} and cAMP signal transduction pathways in recombinant cells overexpressing GPR39 but not in parental cells. Compound **1** stimulated intracellular Ca^{2+} mobilization in an endogenous HT29 cell line in a GPR39-specific manner and exhibited a good selectivity profile, satisfying our requirements as a fit-for-purpose chemical tool compound for studying GPR39 pharmacology in cellular and tissue systems and for building confidence in rationale of GPR39 as a potential target for the treatment of diabetes. Compound **1** did not potentiate GSIS in human islet preparations, possibly because the compound was not sufficiently potent or efficacious, GPR39 signaling through the Ca^{2+} pathway might not be coupled to insulin secretion, or GPR39 was not involved in insulin signaling. Compounds **1**, **4**, (+)-*trans*-**5**, and (−)-*trans*-**5** represent structurally attractive starting points for further medicinal chemistry optimization. Their discovery demonstrates the potential of a platform approach to provide chemical probe compounds in a cost-effective manner to study and better understand receptor targets lacking endogenous ligands.

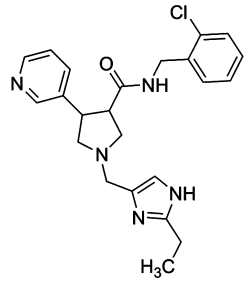
dx.doi.org/10.1021/ml400275z | ACS Med. Chem. Lett. 2013, 4, 1079−1084



racemic **4**

hGPR39 Ca^{2+} EC_{50} = 3.1 μM (82%)

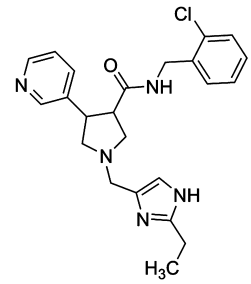
hGPR39 cAMP EC_{50} = 4.5 μM (107%)



(−)-*trans*-**5**

hGPR39 Ca^{2+} EC_{50} = 0.32 μM (66%)

hGPR39 cAMP EC_{50} = 0.31 μM (107%)



(+)-*trans*-**5**

hGPR39 Ca^{2+} EC_{50} = 0.19 μM (74%)

hGPR39 cAMP EC_{50} = 0.84 μM (99%)

Figure 6. Structures and pharmacological activities of racemic **4**, (+)-*trans*-**5**, and (−)-*trans*-**5**. Efficacies are relative to Zn^{2+} alone in the assays.

■ ASSOCIATED CONTENT

Supporting Information

Synthetic schemes for **1**, racemic **4**, (+)-*trans*-**5**, and (−)-*trans*-**5**, experimental details, analytical data and physicochemical properties of compounds, broad panel selectivity data for **1**, and biological assay protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(P.A.C.) Tel: 617-551-3235. E-mail: philip.a.carpino@pfizer.com.

Notes

The authors declare the following competing financial interest(s): All authors are employees of Pfizer, Inc.

■ ABBREVIATIONS

β-cell, beta cell; cAMP, cyclic adenosine monophosphate; Ca^{2+}, calcium ion; ChEMBL, chemical database of bioactive cmpds; CHO, Chinese hamster ovary; EC_{xx}, concentration of an agonist that produces xx% of the maximum effect of that agonist; ECFP4, extended connectivity fingerprints with radius 4; GLP, glucagon-like peptide-1; GPCR, G-protein coupled receptor; GSIS, glucose-stimulated insulin secretion; hGPR39, human GPR39; HTS, high-throughput screening; mM, millimolar; oGPCR, orphan G-protein coupled receptor; IC_{50}, half maximal inhibitory concentration; P_{app}, apparent partition coefficient; PDE, phosphodiesterase; ROCS, rapid overlay of chemical structures; SFlogD, shake flask log D; siRNA, small interfering RNA; Zn^{2+}, zinc ion

■ REFERENCES

(1) Ritter, S. L.; Hall, R. A. Fine-tuning of GPCR activity by receptor-interacting proteins. *Nat. Rev. Mol. Cell Biol.* **2009**, *10*, 819−830.

(2) Civelli, O.; Reinscheid, R. K.; Zhang, Y.; Wang, Z.; Fredriksson, R.; Schioth, H. B. G protein-coupled receptor deorphanizations. *Annu. Rev. Pharmacol. Toxicol.* **2013**, *53*, 127−146.

(3) Lin, S. H. S.; Civelli, O. Orphan G protein-coupled receptors: targets for new therapeutic interventions. *Ann. Med.* **2004**, *36*, 204−214.

(4) Egerod, K. L.; Jin, C.; Petersen, P. S.; Wierup, N.; Sundler, F.; Holst, B.; Schwartz, T. W. β-Cell specific overexpression of GPR39 protects against streptozotocin-induced hyperglycemia. *Int. J. Endocrinol.* **2011**, No. 401258.

(5) McKee, K. K.; Tan, C. P.; Palyha, O. C.; Liu, J.; Feighner, S. D.; Hreniuk, D. L.; Smith, R. G.; Howard, A. D.; Van der Ploeg, L. H. T. Cloning and characterization of two human G protein-coupled receptor genes (GPR38 and GPR39) related to the growth hormone secretagogue and neurotensin receptors. *Genomics* **1997**, *46*, 426−434.

(6) Popovics, P.; Stewart, A. J. GPR39: a Zn^{2+}-activated G protein-coupled receptor that regulates pancreatic, gastrointestinal and neuronal functions. *Cell. Mol. Life Sci.* **2011**, *68*, 85−95.

(7) Gourcerol, G.; St-Pierre, D. H.; Tache, Y. Lack of obestatin effects on food intake: Should obestatin be renamed ghrelin-associated peptide (GAP)? *Regul. Pept.* **2007**, *141*, 1−7.

(8) Holst, B.; Egerod, K. L.; Schild, E.; Vickers, S. P.; Cheetham, S.; Gerlach, L.-O.; Storjohann, L.; Stidsen, C. E.; Jones, R.; Beck-Sickinger, A. G.; Schwartz, T. W. GPR39 signaling is stimulated by zinc ions but not by obestatin. *Endocrinology* **2007**, *148*, 13−20.

(9) Yasuda, S.-I.; Miyazaki, T.; Munechika, K.; Yamashita, M.; Ikeda, Y.; Kamizono, A. Isolation of Zn^{2+} as an endogenous agonist of GPR39 from fetal bovine serum. *J. Recept. Signal Transduction* **2007**, *27*, 235−246.

(10) Storjohann, L.; Holst, B.; Schwartz, T. W. Molecular mechanism of Zn^{2+} agonism in the extracellular domain of GPR39. *FEBS Lett.* **2008**, *582*, 2583−2588.

(11) Tremblay, F.; Richard, A. M.; Will, S.; Syed, J.; Stedman, N.; Perreault, M.; Gimeno, R. E. Disruption of G protein-coupled receptor 39 impairs insulin secretion in vivo. *Endocrinology* **2009**, *150*, 2586−2595.

(12) Verhulst, P. J.; Lintermans, A.; Janssen, S.; Loeckxt, D.; Himmelreicht, U.; Buyse, J.; Tack, J.; Depoortere, I. GPR39, a receptor of the ghrelin receptor family, plays a role in the regulation of glucose homeostasis in a mouse model of early onset diet-induced obesity. *J. Neuroendocrinol.* **2011**, *23*, 490−500.

(13) Holst, B.; Egerod, K. L.; Jin, C.; Petersen, P. S.; Oestergaard, M. V.; Hald, J.; Sprinkel, A. M. E.; Stoerling, J.; Mandrup-Poulsen, T.; Holst, J. J.; Thams, P.; Oerskov, C.; Wierup, N.; Sundler, F.; Madsen, O. D.; Schwartz, T. W. G protein-coupled receptor 39 deficiency is associated with pancreatic islet dysfunction. *Endocrinology* **2009**, *150*, 2577−2585.

(14) Martin, R. E.; Green, L. G.; Guba, W.; Kratochwil, N.; Christ, A. Discovery of the first nonpeptidic, small-molecule, highly selective somatostatin receptor subtype 5 antagonists: a chemogenomics approach. *J. Med. Chem.* **2007**, *50*, 6291−6294.

(15) Crossley, R. The design of screening libraries targeted at G-protein coupled receptors. *Curr. Top. Med. Chem.* **2004**, *4*, 581−588.

(16) Miller, J. L. Recent developments in focused library design: targeting gene-families. *Curr. Top. Med. Chem.* **2006**, *6*, 19−29.

(17) Gaulton, A.; Bellis, L. J.; Bento, A. P.; Chambers, J.; Davies, M.; Hersey, A.; Light, Y.; McGlinchey, S.; Michalovich, D.; Al-Lazikani, B.; Overington, J. P. ChEMBL: a large-scale bioactivity database for drug discovery. *Nucleic Acids Res.* **2012**, *40*, D1100−D1107.

(18) Bemis, G. W.; Murcko, M. A. The properties of known drugs. 1. Molecular frameworks. *J. Med. Chem.* **1996**, *39*, 2887−2893.

(19) Rogers, D.; Brown, R. D.; Hahn, M. Using extended-connectivity fingerprints with Laplacian-modified Bayesian analysis in high-throughput screening follow-up. *J. Biomol. Screening* **2005**, *10*, 682−686.
(20) Ahren, B. Islet G protein-coupled receptors as potential targets for treatment of type 2 diabetes. *Nat. Rev. Drug Discovery* **2009**, *8*, 369−385.
(21) Hermann, J. C.; Chen, Y.; Wartchow, C.; Menke, J.; Gao, L.; Gleason, S. K.; Haynes, N.-E.; Scott, N.; Petersen, A.; Gabriel, S.; Vu, B.; George, K. M.; Narayanan, A.; Li, S. H.; Qian, H.; Beatini, N.; Niu, L.; Gan, Q.-F. Metal impurities cause false positives in high-throughput screening campaigns. *ACS Med. Chem. Lett.* **2013**, *4*, 197−200.
(22) Hawkins, P. C. D.; Skillman, A. G.; Nicholls, A. Comparison of shape-matching and docking as virtual screening tools. *J. Med. Chem.* **2007**, *50*, 74−82.
(23) Di, L.; Whitney-Pickett, C.; Umland, J. P.; Zhang, H.; Zhang, X.; Gebhard, D. F.; Lai, Y.; Federico, J. J.; Davidson, R. E.; Smith, R.; Reyner, E. L.; Lee, C.; Feng, B.; Rotter, C.; Varma, M. V.; Kempshall, S.; Fenner, K.; El-kattan, A. F.; Liston, T. E.; Troutman, M. D. Development of a new permeability assay using low-efflux MDCKII cells. *J. Pharm. Sci.* **2011**, *100*, 4974−4985.