Journal of Medicinal Chemistry

BRIEF ARTICLE

pubs.acs.org/jmc

Activation of the G-Protein-Coupled Receptor 119: A Conformation-Based Hypothesis for Understanding Agonist Response

Kim F. McClure,*[,†] Etzer Darout,[†] Cristiano R. W. Guimarães,[†] Michael P. DeNinno,[†] Vincent Mascitti,[†] Michael J. Munchhof,[†] Ralph P. Robinson,[†] Jeffrey Kohrt,[†] Anthony R. Harris,[†] Dianna E. Moore,[†] Bryan Li,[†] Lacey Samp,[†] Bruce A. Lefker,[†] Kentaro Futatsugi,[†] Daniel Kung,[†] Paul D. Bonin,[‡] Peter Cornelius,[‡] Ruduan Wang,[‡] Eben Salter,[‡] Sam Hornby,[‡] Amit S. Kalgutkar,[§] and Yue Chen[§]

[†]Department of Medicinal Chemistry, [‡]Department of Discovery Biology, and [§]Department of Drug Metabolism and Pharmaceutical Sciences, Pfizer Global Research and Development, Eastern Point Road, Groton, Connecticut 06340, United States

Supporting Information

ABSTRACT: The synthesis and properties of the bridged piperidine (oxaazabicyclo) compounds **8**, **9**, and **11** are described. A conformational analysis of these structures is compared with the representative GPR119 ligand **1**. These results and the differences in agonist pharmacology are used to formulate a conformation-based hypothesis to understand activation of the GPR119 receptor. We also show for these structures that the agonist pharmacology in rat masks the important differences in human pharmacology.

INTRODUCTION

G-protein-coupled receptor 119 (GPR119) is one of several non-peptide-binding GPCR diabetes targets.[1] The recent deorphanization process has identified oleoylethanolamide (OEA) as one of the putative natural agonists of GPR119.[2−4] There are also a number of small molecule agonists that have been reported,[5,6] and many of these have recently been reviewed.[7,8] Interest in agonists of GPR119 as antidiabetics derives from their ability to stimulate an incretin response in the gut and glucose-dependent insulin release in the pancreas.[9] Currently the extent and duration of activation of the receptor needed for maximum clinical benefit are not known. Equally, it is not well understood what structural elements in the reported GPR119 ligands are responsible for triggering the agonist response. Herein we report an attempt to elucidate some of the factors involved using conformationally restricted analogs.

RESULTS AND DISCUSSION

As a baseline we undertook a detailed evaluation of **1**[10] (see Figure 1), starting with a conformational analysis. As shown in Figure 2, **1** has restricted conformations with the preferred dihedral angles (labeled 1 and 2 in Figure 2) placing the piperdinol methine approximately ±30° out of the plane of the central pyrimidine. This is true of the axial and the equatorial piperidine-ether conformations. A 0.18 kcal/mol preference for the equatorial conformation is calculated, and this axial to equatorial equilibrium is one of the more energetically accessible conformational changes.[11]

To identify pharmacologically superior agonists, we set out to further restrict the conformations of the piperidine ring. We surmised that the piperidine carbamate region makes important H-bond contacts with the receptor, similar in importance for agonist activity as has been described for acceptor contacts on the other end of the molecule.[12] This inference is based on the high prevalence of H-bond acceptor groups (cabamate, pyrimidyl, and oxadiazole) capping related piperidine structures for reported GPR119 agonists.[7,13] As one of the more common motifs found in GPR119 ligands, an understanding of the structure−function relationship for this portion of the molecule could prove important for agonist design. One of our approaches included the incorporation of a bridging ether to constrain the piperidine ring.[14]

The synthesis of two such compounds is shown in Scheme 1. Double Mannich reaction followed by carbonyl reduction with sodium borohydride gave a mixture of the *syn* and *anti* alcohol isomers **3** and **4**, with the *syn* isomer precipitating out of solution in pure form. The *anti* isomer **4** was purified away from the residual **3** by silica gel chromatography. Hydrogenolysis of the benzyl group followed by carbamate formation provided **5** and **6**. The *anti* isomer **6** proved more reactive in the reaction with the chloropyrimidine **7**, accounting for the better yield of **9** relative to **8**. Compound **8** was readily crystallized for X-ray structure analysis. The conformation of the oxaazabicyclic ring in the X-ray structure of **8** showed the same dihedral angles calculated for **1** (cf. dihedrals 1 and 2 in Figure 2), placing the central pyrimidine approximately 30° out of plane with the nearby piperidinol methine. Although an X-ray structure was not obtained for **9**, the calculated preferred dihedral angles match those calculated for **1** and **8**.[15]

Table 1 shows a comparison of the properties of the isomers **8** and **9** with **1**. Despite the addition of two methylene groups, the bridged ether compounds **8** and **9** have lower log *P* than their nonbridged counterpart **1**. All three compounds have low hepatic turnover in human microsomes and excellent aqueous solubility

Received: January 3, 2011
Published: March 01, 2011

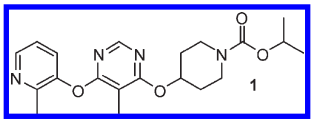


Figure 1. Representative GPR119 ligand.

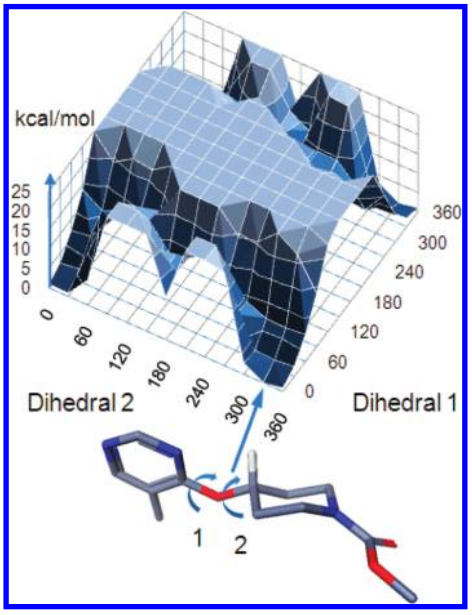


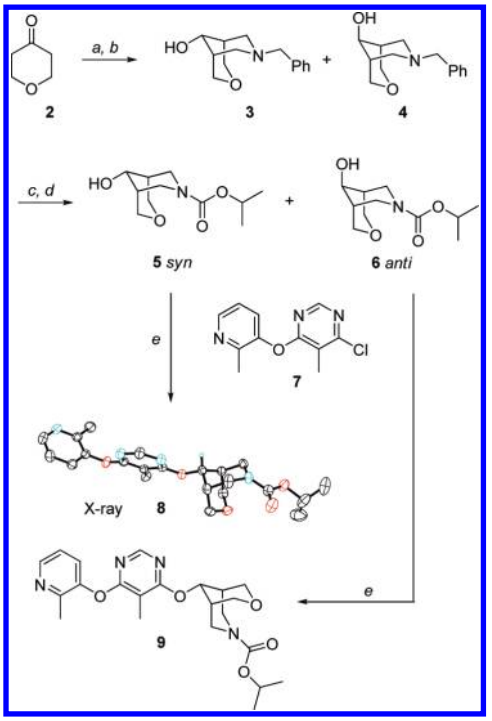
Figure 2. Energy map for a compound **1** model system.

properties in contrast to other reported agonists.[6] Although **1** is reported to be an agonist across a variety of species,[12] in our cAMP functional assay it is only a weak partial agonist of the human GPR119 receptor. The most striking comparisons are with the IA difference between **8** and **9** and the significantly greater intrinsic activity of **9** relative to **1**. The lack of an agonist response with **8** could be misinterpreted if not for its binding K_i showing that the fundamental interaction with the receptor is essentially equivalent to **1** and **9**.[16] Further functional characterization in antagonist mode (**8** in the presense of an agonist) confirms the functional antagonist profiles of **1** and **8**. Tested in this way, **8** can fully antagonize an agonist response (K_b = 25 nM), while **1** can antagonize to approximately 20% of maximum, equivalent to its intrinsic activity in agonist mode.

Taken together with the pharmacology results, the conformational analysis of **1**, **8**, and **9** led us to propose the agonist conformation hypothesis shown in Figure 3. Compound **1** can explore the agonist and antagonist forms from the accessible axial and equatorial conformations and shows partial agonist activity. Compound **8** is constrained to the "antagonist conformation" and is an antagonist. The "agonist conformation" is energetically preferred by **9**, and this accounts for the greater functional response observed.

The hypothesis reinforces the proposal that the H-bond acceptor interaction of the carbamate, presumably toward the interior of the GPCR, has a large contribution toward the agonist activity. There are several ways to interpret the differences in pharmacology based on the hypothesis. Because the agonist and antagonist conformations place the carbamate in different spacial regions, it is possible the carbamates in each have different H-bond interactions with the receptor.

Scheme 1. Synthesis of 3-Oxa-7-azabicyclo[3.3.1]nonane Analogues[a]



[a] Reagents and conditions: (a) aq formaldehyde, benzylamine, acetic acid, reflux; (b) $NaBH_4$ (one pot, 45%); (c) $Pd(OH)_2$, H_2, 50 psi, EtOH, 23 °C (91%); (d) isopropyl chloroformate, *i*-Pr_2NEt (82%); (e) NaHMDS, **7**, dioxane, 105 °C (36% for **8**) (51% for **9**). ORTEP of **8** shown at 30% probability; one calculated hydrogen is shown for comparison with Figure 2.

Table 1. Property Comparison of 1, 8, and 9

	1	8	9
ClogP/ElogD [17]	3.4/4.2	3.3/3.3	3.3/3.6
cAMP EC_{50} [a]	14 ± 8 nM (21 ± 3% IA) n = 37	>10 μM (antagonist) n = 10	65 ± 31 nM (78 ± 4% IA) n = 10
K_i [a]	23 ± 15 nM n = 21	33 ± 38 nM n = 8	20 ± 17 nM n = 8
$Cl_{int,app\ scaled}$ [b] ((mL/min)/kg)	15	<6	<8
solubility (mg/mL)	>40[c]	8.6[d]	>66[d]

[a] Human GPR119 receptor. [b] Human microsomes. [c] Reference 7. [d] Crystalline form in simulated gastric fluid, pH 1.2.

Alternatively, the carbamates could be making the same H-bond contacts but their different conformational preferences transmit different changes to the other end of the molecule, inducing a functional response only in the case of **9**. Notably, this is one of the only examples[18,19] that we are aware of where complete switching in the functional profile can be attributed to conformational differences in the ligand. Typically, changes in pharmacology for a class A GPCR ligand is achieved by ensuring that the molecule spans beyond the agonist pocket, which is generally a subset of the larger antagonist pocket.[20]

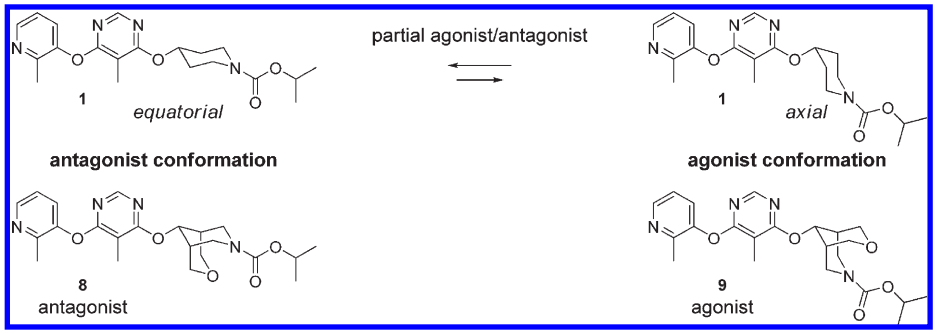
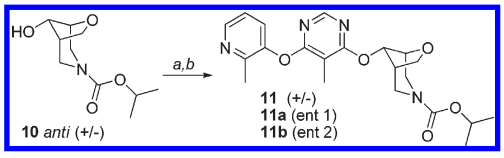


Figure 3. Agonist vs antagonist conformation hypothesis.

Scheme 2. Synthesis of the 3-Oxaazabicyclo[3.2.1]octane 11[a]



[a] Reagents and conditions: (a) LiHMDS, 7, dioxane, 85 °C (52%); (b) chiral separation.

Table 2. Human in Vitro GPR119 Pharmacology for 11

	11	11a	11b
cAMP EC_{50}	1239 ± 696 nM (84 ± 7% IA) $n = 6$	740 ± 151 nM (87 ± 10% IA) $n = 6$	2067 ± 655 nM (101 ± 1% IA) $n = 6$
K_i	1599 ± 844 nM $n = 3$	1176 ± 385 nM $n = 3$	2270 ± 384 nM $n = 3$

To exploit this concept of an "agonist conformation" while attempting to further optimize the molecules, we sought to remove one of the bridging methylene groups from **9**. No significant changes in dihedral angles were calculated for these chiral *nor* analogues of **9**. Scheme 2 shows the synthesis of the proposed oxaazabicyclic[3.2.1] system. Starting with known **10**,[21] the oxaazabicyclic[3.2.1]octane **11** was prepared in racemic form in a single step. Chiral chromatography provided the enantiomers **11a** and **11b**.

Unfortunately, **11** has significantly reduced binding affinity for the receptor (Table 2). The sideward placement of the bridging oxygen in **11**, relative to *meso* **8** and **9**, potentially has a negative interaction with the receptor. While this is also reflected in a weaker functional EC_{50}, the maximum response is equal to or greater than that of **9**, consistent with the hypothesis.

To understand the translation of in vitro pharmacology to preclinical in vivo efficacy, we also investigated the in vitro rat pharmacology of these compounds. Interestingly the intrinsic activities are significantly greater in rat for **1** and **8** (see Table 3). Differences in receptor density between the human and rat in vitro systems are unlikely to account for this difference, as **9** does not show the same increase in intrinsic activity. It is likely that sequence differences between the rat and human receptors are a major contributor.[22] The rat in vitro agonist activity for **8** is supported by in vivo rat glucose tolerance tests, showing a decrease in glucose AUC and increases in GLP-1 approaching the maximal effects we have observed for this mechanism. The lack of activity when testing **8** against a panel of common targets reduced concerns that these effects were off-target mediated. This example underscores the challenge in translational pharmacology for an agonist program.

Table 3. Rat in Vitro GPR119 Pharmacology for 1, 8, 9

	1	8	9
cAMP EC_{50}	84 ± 33 nM (70 ± 6% IA) $n = 6$	228 ± 135 nM (69 ± 7% IA) $n = 6$	227 ± 148 nM (49 ± 8% IA) $n = 3$
K_i	140 ± 66 nM $n = 11$	125 ± 28 nM $n = 7$	32 ± 15 nM $n = 5$

In summary, we prepared and characterized the conformationally restricted oxaazabicylic **8**, **9**, and **11**. Comparison of their human GPR119 pharmacology with that of **1** showed that the disposition of the piperidine-carbamate has little impact on the binding affinity of the compounds for the receptor but is a crucial determinant of the functional response. The "agonist conformation" proposed for **9** and **11** is supported by their significantly greater intrinsic activity relative to the parent piperidine **1**. The differences in pharmacology are restricted to the human GPR119 receptor, and the results in rat may not be predictive of human efficacy.

EXPERIMENTAL SECTION

Compounds of pharmacologic interest are greater than 95% pure as judged by five separate UPLC methods, using the method reporting the largest percent impurity.

(1*R*,5*S*,9r)-7-Benzyl-3-oxa-7-azabicyclo[3.3.1]nonan-9-ol (3) and (1*R*,5*S*,9s)-7-Benzyl-3-oxa-7-azabicyclo[3.3.1]nonan-9-ol (4). To a 20 L reactor equipped with a reflux condenser were added MeOH (8.00 L), benzylamine (4.00 mol, 428 g), tetrahydro-4*H*-pyran-4-one **2** (400 g, 4.00 mols), and acetic acid (4.00 mol, 240 g). The jacket temperature was maintained at 15−25 °C during the addition. The mixture was heated at reflux (66 °C). Aqueous formaldehyde (7.99 mol, 600 mL, 649 g) was combined with MeOH (2 L). The resulting solution was added over 1 h to the mixture while keeping the reaction at reflux. The mixture was heated for 10 min at reflux after the completion of the formaldehyde addition and cooled to 10−20 °C. $NaHCO_3$ (4.00 mol, 336 g) was added. The mixture was cooled to 10 °C, and $NaBH_4$ (4.20 mol, 159 g) was added portionwise ($NaBH_4$ tablets were used, ∼1 g each tablet). After the $NaBH_4$ addition was complete, the mixture was stirred at 15−25 °C for 40 min. Diatomaceous earth (400 g) was added to the mixture, followed by water (2 L) and 1 N sodium hydroxide solution (4.00 L). The mixture was stirred at 15−25 °C for 1 h and filtered. The filter cake was rinsed with MeOH/water (1:1 mixture, 800 mL). The filtrate was concentrated at 40−45 °C under vacuum to remove most of the

MeOH. The resulting aqueous mixture was extracted with 2-methyltetrahydrofuran (2-Me-THF) (1 × 6.00 L). The 2-Me-THF layer was washed with brine (2.00 L, 2.38 kg), concentrated under partial vacuum with a pot temperature of 40−45 °C to give an oil, which was collected in a 5 L container (Naljug). The reactor was rinsed with 1 L of acetonitrile, and the rinse was combined with the crude oil product. After 12 h standing at 10−15 °C, crystallization occurred in the Naljug. Filtration of the mixture gave **3** (193 g, 98% de). The filtrate was purified by silica gel chromatography (mobile phase, toluene/heptane/diethylamine 70/30/5, isocratic), followed by another chromatography using ChiralPak AD (mobile phase, isopropanol/heptane/diethylamine 5/95/0.2) to give additional crop of **3** (86.3 g, 99% de) and **4** (96.5 g, 99% de).

Isopropyl 9-*syn*-Hydroxy-3-oxa-7-azabicyclo[3.3.1]nonane-7-carboxylate (5) and Isopropyl 9-*anti*-Hydroxy-3-oxa-7-azabicyclo[3.3.1]nonane-7-carboxylate (6). The individual isomers **3** and **4** can be taken through the following two steps separately with the conditions described. However, the specific experiments below pertain to the mixture of the *syn* and *anti* isomers which are separated as **5** and **6**. The starting mixture of *syn* and *anti* isomers **3** and **4** (3.71 g, 15.9 mmol) was dissolved in ethanol (120 mL), and $Pd(OH)_2$ (450 mg) was added. The mixture was shaken for 2.5 h under 50 psi of hydrogen in a Parr shaker at 23 °C. The mixture was filtered through diatomaceous earth, and the collected solid was washed three times with MeOH. The filtrate was concentrated in vacuo to give an oily solid. This oily solid was dissolved in EtOAc, and heptane was added. The solution was concentrated in vacuo to give a mixture of *syn* and *anti* isomers of 3-oxa-7-azabicyclo[3.3.1]nonan-9-ol as a white solid (2.08 g, 91%). This material was used in the next step without further purification. LCMS (ES+): 144.1 (M + 1). To a CH_2Cl_2 (15 mL) solution of the mixture of *syn* and *anti*-isomers of 3-oxa-7-azabicyclo[3.3.1]nonan-9-ol (2.08 g, 14.5 mmol) and *N*,*N*-diisopropylethylamine (2.80 mL, 16.0 mmol) at 0 °C was added isopropyl chloroformate (14.2 mL, 14.2 mmol, 1.0 M in toluene) dropwise. The mixture was allowed to warm to room temperature over 14 h. The mixture was then diluted with aqueous 1 M HCl (50 mL) and the aqueous layer separated. The organic layer was washed sequentially with water (50 mL) and brine (50 mL) and then dried over Na_2SO_4. The mixture was filtered, and the filtrate was concentrated in vacuo to give a colorless oil. This oil was dissolved in EtOAc. Heptane was added, and the mixture was concentrated. The resulting oil was dried under vacuum to give the mixture of **5** and **6** as a clear oil (2.74 g, 82%). LCMS (ES+): 230.1 (M + 1). A mixture of **5** and **6** (5.04 g, 35.1 mmol) was separated via preparatory high pressure liquid chromatography utilizing a Chiralpak AD-H column (21 mm × 250 mm) with mobile phase of 85:15 carbon dioxide/MeOH at a flow rate of 65 mL/min. The wavelength for monitoring the separation was 210 nm. The analytical purity of each isomer was determined using analytical high pressure chromatography using a Chiralpak AD-H (4.6 mm × 25 cm) column with a mobile phase of 85:15 carbon dioxide/MeOH at a flow rate of 2.5 mL/min. The wavelength for monitoring the peaks was 210 nm. The following two isomers were obtained. **5** (1.34 g): clear oil which solidified on standing. Retention time (t_R) = 2.3 min. ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.12 (d, 1H, J = 2.8 Hz), 4.76−4.71 (m, 1H), 4.20 (d, 1H, J = 13 Hz), 4.16 (d, 1H, J = 13 Hz), 3.96−3.92 (m, 2 H), 3.79 (d, 1H, J = 3 Hz), 3.55 (s, 1H), 3.52 (s, 1H), 3.08 (d, 1H, J = 13 Hz), 2.98 (d, 1H, J = 13 Hz), 1.47 (m, 2 H) 1.16 (d, 3 H, J = 3 Hz), 1.15 (d, 3 H, J = 3 Hz). LCMS (ES+): 230.2 (M + 1). **6** (1.70 g): amber oil, t_R = 3.08 min. ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.11 (d, 1H, J = 2.8 Hz), 4.74−4.67 (m, 1H), 3.89 (d, 1H, J = 13 Hz), 3.84−3.78 (m, 2 H, J = 11 Hz), 3.80 (d, 1H, J = 6 Hz), 3.78 (d, 1H, J = 3 Hz), 3.52−3.47 (m, 2 H), 3.35−3.30 (m, 1H), 3.24−3.20 (m, 1H), 1.53 (s, 1H), 1.51 (s, 1H), 1.13 (d, 3 H, J = 1 Hz), 1.16 (d, 3 H, J = 1 Hz). LCMS (ES+): 230.2 (M + 1).

Isopropyl 9-*syn*-({5-Methyl-6-[(2-methylpyridin-3-yl)oxy]-pyrimidin-4-yl}oxy)-3-oxa-7-azabicyclo[3.3.1]nonane-7-carboxylate (8). Compound **5** (176 mg, 0.76 mmol) and 4-chloro-5-methyl-6-[(2-methylpyridin-3-yl)oxy]pyrimidine (**7**)[10] (164 mg, 0.69 mmol) were dissolved in anhydrous dioxane (4 mL) under an atmosphere of nitrogen. The solution was heated at 105 °C, and 1 M NaHMDS (0.85 mL, 0.84 mmol) was added. The red mixture was heated at 102−110 °C for 1 h, and then the mixture was allowed to cool to room temperature. Water (1.0 mL) was added, and the solution was transferred to a bigger flask with 2-Me-THF. The solvents were concentrated under reduced pressure to give an oil which was partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$. The layers were separated, and the aqueous layer was extracted again with CH_2Cl_2. The organic extracts were combined and washed with brine and then dried over Na_2SO_4. The mixture was filtered and the filtrate concentrated under reduced pressure to give an oil that was purified via column chromatography twice (0−5% MeOH/EtOAc and then 0−10% MeOH/CH_2Cl_2) to give the product as a clear oil. The oil was dissolved in EtOAc and heptane, and the solution was concentrated in vacuo to give an oily foam which was dried under vacuum. Triturating the foam with a solution of EtOAc and heptane produced **8** as a white solid (106 mg, 36%) after filtration. Compound **8** was crystallized from a cold (4 °C) mixture of toluene and heptane for X-ray analysis, mp 135 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (dd, 1H, J = 1.2, 4.8 Hz), 8.2 (s, 1H), 7.40 (1.2, 8.0 Hz), 7.22 (dd, 1H, J = 4.8, 8.0 Hz), 5.38 (dd, 1H, J = 3.4, 3.4 Hz), 5.021−4.97 (m, 1H), 4.65 (d, 1H, J = 13 Hz), 4.48 (d, 1H, J = 13 Hz), 4.16 (dd, 2H, J = 11, 13 Hz), 3.97 (d, 1H, J = 13 Hz), 3.89 (d, 1H, J = 13 Hz), 3.32 (d, 1H, J = 14 Hz), 3.24 (d, 1H, J = 14 Hz), 2.43 (s, 3H), 2.29 (s, 3H), 2.01 (app s, 1H), 1.96 (app s, 1H), 1.28 (d, 3H, J = 3.5 Hz), 1.27 (d, 3H, J = 3.5 Hz). LCMS: 429.0 (M + 1).

Isopropyl 9-*anti*-({5-Methyl-6-[(2-methylpyridin-3-yl)oxy]-pyrimidin-4-yl}oxy)-3-oxa-7-azabicyclo[3.3.1]nonane-7-carboxylate (9). Compounds **6** (180 mg, 0.79 mmol) and **7** (204 mg, 0.86 mmol) were dissolved in anhydrous dioxane (4 mL) under an atmosphere of nitrogen. The solution was heated at 105 °C, and 1 M NaHMDS (0.85 mL, 0.84 mmol) was added. The red mixture was heated at 105 °C for 1.3 h, and then the mixture was allowed to cool to room temperature. Water (1.0 mL) was added, and the solution was transferred to a bigger flask with 2-Me-THF. The solvents were concentrated under reduced pressure to give an oil which was partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$. The layers were separated, and the aqueous layer was extracted again with CH_2Cl_2. The organic extracts were combined and washed with brine and then dried over Na_2SO_4. The mixture was filtered and the filtrate concentrated under reduced pressure to give an oil that was purified via column chromatography (0−10% MeOH/EtOAc) to give the product as a clear oil. The oil was dissolved in EtOAc and heptane, and the solution was concentrated in vacuo to give the product as an oily foam (174 mg, 51%). A sample of the product was dissolved in acetonitrile and water and frozen in a dry ice/acetone bath. Lyophilization under vacuum produced **9** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (dd, 1H, J = 1.2, 4.8 Hz), 8.2 (s, 1H), 7.41 (dd, 1H, J = 1.2, 8.0 Hz), 7.22 (dd, 1H, J = 4.8, 8.0 Hz), 5.44 (dd, 1H, J = 3.6, 3.6 Hz), 5.02−4.97 (m, 1H), 4.36 (d, 1H, J = 13.5 Hz), 4.21 (m, 2H), 4.14 (d, 1H, J = 11 Hz), 3.86 (dd, 2H, J = 11.6 Hz), 3.52 (d, 1H, J = 13 Hz), 3.44 (d, 1H, J = 13 Hz), 2.43 (s, 3H), 2.28 (s, 3H), 2.08 (app s, 1H), 2.03 (app s, 1H), 1.28 (d, 3H, J = 3.5 Hz), 1.27 (d, 3H, J = 3.5 Hz). LCMS: 429.2 (M + 1).

(1*R*,5*R*,8*R*)-Isopropyl 8-(5-Methyl-6-(2-methylpyridin-3-yloxy)pyrimidin-4-yloxy)-6-oxa-3-azabicyclo[3.2.1]octane-3-carboxylate and (1*S*,5*S*,8*S*)-Isopropyl 8-(5-Methyl-6-(2-methylpyridin-3-yloxy)pyrimidin-4-yloxy)-6-oxa-3-azabicyclo[3.2.1]-octane-3-carboxylate (11, 11a, 11b). A mixture of **10**[21] (90 mg, 0.42 mmol) and **7**[10] (106 mg, 0.35 mmol) was dissolved in 1,4-dioxane (2.1 mL). A solution of 1 M LiHMDS (0.42 mL, 0.42 mmol) was added dropwise, and the mixture was stirred at room temperature for 1 h. The mixture was heated at 85 °C for 16 h. The mixture was cooled to room temperature, diluted with EtOAc, and saturated aqueous NH_4Cl. The layers were separated, and the aqueous phase was extracted twice with EtOAc. The combined organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated in vacuo. The

crude residue was purified by silica gel chromatography using 80% EtOAc/heptane to provide 90 mg (52% yield) of the desired procuct (R_f = 0.2, EtOAc/heptane 4:1). The enantiomers were separated by chiral HPLC chromatography using a Minigram-1 with a Chiralpak AD-H column (80/20) CO_2/ethanol (210 nm detection) to give peak 1 (**11a**) at t_R (3.41 min) and peak 2 (**11b**) t_R (4.40 min). ^{1}H NMR ($CDCl_3$, 500 MHz) δ ppm 1.25 (d, J = 6.08 Hz, 6 H), 2.23 (s, 3 H), 2.39 (s, 3 H), 2.74−2.84 (m, 1 H), 3.20 (d, J = 13.68 Hz, 1 H), 3.37 (t, J = 12.48 Hz), 3.80−4.15 (m, 4 H), 4.31−4.41 (m, 1 H), 4.89−4.98 (m, 1 H), 5.36 (t, J = 5.48 Hz, 1 H), 7.20 (dd, J = 8.2, 4.88 Hz, 1 H), 7.38 (dd, J = 8.0, 1.36 Hz, 1 H), 8.19 (s, 1H), 8.40 (dd, J = 4.68, 1.36 Hz). LCMS (ES+): 415.2 (M + 1).

■ ASSOCIATED CONTENT

Supporting Information. Energy maps for **1**, **8**, **9**, and **11**; X-ray experimental conditions for the structure determination of **8**; human and rat GPR119 in vitro assay conditions; plot of the agonist and antagonist mode human cAMP assays for **1** and **8**; rat oral and ip glucose tolerance test data for **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +1 860 441 8223. Fax: +1 860 686 0605. E-mail: kim.f.mcclure@pfizer.com.

■ ACKNOWLEDGMENT

The authors thank Brian Samas and Ivan Samardjiev for the X-ray structure determination of **8**.

■ ABBREVIATIONS USED

GPR119, G-protein-coupled receptor 119; OEA, oleoylethanolamide; GLP-1, glucagonlike peptide 1; GPCR, G-protein-coupled receptor; cAMP, 3′,5′-cyclic adenosine monophosphate; IA, intrinsic activity; AUC, area under the curve; ip, intraperitoneal; UPLC, ultrahigh pressure liquid chromatography

■ REFERENCES

(1) Fyfe, M. C. T.; Overton, H. A.; Procter, M. J.; Reynet, C.; White, J. R. New nonpeptide-binding GPCRs as targets for diabetes and the metabolic syndrome. *Annu. Rep. Med. Chem.* **2007**, *42*, 129–145.

(2) Overton, H. A.; Babbs, A. J.; Doel, S. M.; Fyfe, M. C. T.; 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.

(3) Chu, Z.-L.; Carroll, C.; Chen, R.; Alfonso, J.; Gutierrez, V.; He, H.; Lucman, A.; Xing, C.; Sebring, K.; Zhou, J.; Wagner, B.; Unett, D.; Jones, R. M.; Behan, D. P.; Leonard, J. *N*-Oleoyldopamine enhances glucose homeostasis through the activation of GPR119. *Mol. Endocrinol.* **2010**, *24*, 161–170.

(4) Ezzili, C.; Otrubova, K.; Boger, D. L. Fatty acid amide signaling molecules. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5959–5968.

(5) Semple, G.; Fioravanti, B.; Pereira, G.; Calderon, I.; Uy, J.; Choi, K.; Xiong, Y.; Ren, A.; Morgan, M.; Dave, V.; Thomsen, W.; Unett, D. J.; Xing, C.; Bossie, S.; Carroll, C.; Chu, Z.-L.; Grottick, A. J.; Hauser, E. K.; Leonard, J.; Jones, R. M. Discovery of the first potent and orally efficacious agonist of the orphan G-protein coupled receptor 119. *J. Med. Chem.* **2008**, *51*, 5172–5175.

(6) Wu, Y.; Kuntz, J. D.; Carpenter, A. J.; Fang, J.; Sauls, H. R.; Gomez, D. J.; Ammala, C.; Xu, Y.; Hart, S.; Tadepalli, S. 2,5-Disubstituted pyridines as potent GPR119 agonists. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2577–2581.

(7) Shah, U. GPR119 agonists: a promising new approach for the treatment of type 2 diabetes and related metabolic disorders. *Curr. Opin. Drug Discovery Dev.* **2009**, *12*, 519–532.

(8) Fyfe, M. C.; 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) 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 G protein-coupled receptor 119 in glycemic control by enhancing glucagon-like peptide-1 and glucose-dependent insulinotropic peptide release. *Endocrinology* **2008**, *149*, 2038–2047.

(10) Jones, R. M.; Lehmann, J. Modulators of Metabolism and the Treatment of Disorders Related Thereto. WO 2007035355, 2007.

(11) **LMP2/6-311G** with solvent effects (water) included was used to calculate the difference in energy between axial and equatorial conformations. The energy maps were obtained using B3LYP 6-31G* in the gas phase.

(12) Jones, R. M.; Leonard, J. N. The emergence of GPR119 agonists as anti-diabetic agents. *Annu. Rep. Med. Chem.* **2009**, *44*, 149–170.

(13) Jones, R. M.; Leonard, J. N.; Buzard, D. J.; Lehmann, J. GPR119 agonists for the treatment of type 2 diabetes. *Expert Opin. Ther. Pat.* **2009**, *19*, 1339–1359.

(14) During the course of this work the patent application (Xia, Y; Boyle, C. D.; GreenLee, W. J.; Chackalamannil, S.; Jayne, C. L.; Stamford, A. W.; Dai, X.; Harris, J. M.; Neustadt, B. R. Bicyclic Heterocycle Derivatives and Methods of use Thereof. WO2009055331, 2009) appeared, disclosing related or identical structures.

(15) See Supporting Information

(16) To our knowledge this is the first primary literature disclosure of a GPR119 binding assay.

(17) Lombardo, F.; Shalaeva, M. Y.; Tupper, K. A.; Gao, F. ElogD$_{oct}$: a tool for lipophilicity determination in drug discovery. 2. Basic and neutral compounds. *J. Med. Chem.* **2001**, *44*, 2490–2497.

(18) Bollinger, S.; Hübner, H.; Heinemann, F. W.; Meyer, K.; Gmeiner, P. Novel pyridylmethylamines as highly selective 5-HT$_{1A}$ superagonists. *J. Med. Chem.* **2010**, *53*, 7167–7179.

(19) Zimmerman, D. M.; Leander, J. D.; Cantrell, B. E.; Reel, J. K.; Snoddy, J.; Mendelsohn, L. G.; Johnson, B. G.; Mitch, C. H. Structure−activity relationships of *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine antagonists for .mu.- and .kappa.-opioid receptors. *J. Med. Chem.* **1993**, *36*, 2833–2841.

(20) Bissantz, C.; Bernard, P.; Hibert, M.; Rognan, D. Protein-based virtual screening of chemical databases. II. Are homology models of G-protein coupled receptors suitable targets?. *Proteins: Struct., Funct., Bioinf.* **2003**, *50*, 5–25.

(21) Darout, E.; Basak, A. The synthesis of azabicyclic heterocycles. *Tetrahedron Lett.* **2010**, *51*, 2998–3001.

(22) Human accession number: NM_178471. Rat accession number: AR240217