Bioorganic & Medicinal Chemistry 20 (2012) 6442–6451

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and structure–activity relationship of fused-pyrimidine derivatives as a series of novel GPR119 agonists

Kenji Negoro *, Yasuhiro Yonetoku, Ayako Moritomo, Masahiko Hayakawa, Kazuhiko Iikubo, Shigeru Yoshida, Makoto Takeuchi, Mitsuaki Ohta

Drug Discovery Research, Astellas Pharma Inc., 21 Miyukigaoka, Tsukuba, Ibaraki 305-8585, Japan

ARTICLE INFO

Article history:
Received 23 July 2012
Revised 22 August 2012
Accepted 22 August 2012
Available online 7 September 2012

Keywords:
Type 2 diabetes mellitus
Glucose-dependent insulin secretion
G-protein-coupled receptor 119
GPR119 agonist

ABSTRACT

A series of fused-pyrimidine derivatives have been discovered as potent and orally active GPR119 agonists. A combination of the fused-pyrimidine structure and 4-chloro-2,5-difluorophenyl group provided the 5,7-dihydrothieno[3,4-*d*]pyrimidine 6,6-dioxide derivative **14a** as a highly potent GPR119 agonist. Further optimization of the amino group at the 4-position in the pyrimidine ring led to the identification of 2-{1-[2-(4-chloro-2,5-difluorophenyl)-6,6-dioxido-5,7-dihydrothieno[3,4-*d*]pyrimidin-4-yl]piperidin-4-yl}acetamide (**16b**) as an advanced analog. Compound **16b** was found to have extremely potent agonistic activity and improved glucose tolerance at 0.1 mg/kg po in mice. We consider compound **16b** and its analogs to have clear utility in exploring the practicality of GPR119 agonists as potential therapeutic agents for the treatment of type 2 diabetes mellitus.

1. Introduction

The onset and progression of type 2 diabetes mellitus (T2DM) are due to the depletion of glucose-dependent insulin secretion (GDIS) from pancreatic β cells.[1] Oral agents that stimulate insulin secretion, such as sulfonylureas (SUs), reduce blood glucose and have been used as a first-line T2DM therapy for nearly 30 years.[2] However, these agents act to force β cells to secrete insulin continuously regardless of blood glucose levels, and thereby tend to promote hypoglycemia and accelerate the loss of islet function and, eventually, diminish their efficacy.[3] Despite the availability of a range of agents for T2DM, many diabetic patients fail to achieve or to maintain glycemic targets.[4] In addition, stricter glycemic guidelines have been proposed to define a direction for diabetes prevention through the identification and treatment of the pre-diabetes state.[5] Agents that can promote GDIS have great potential to replace SUs as first-line therapy for treating T2DM. The recent emergence of glucagon-like peptide 1 (GLP-1)-based GDIS agents,[6] including inhibitors of dipeptidyl peptidase-4[7] and peptidase-stable analogs such as exendin-4,[8] is undoubtedly a major advance in this direction. Nevertheless, it remains to be confirmed whether the effects of GLP-1-related agents on β cells mass and function are truly durable and beneficial.

The molecular pharmacology of lipid and lipid-like mediators that signal through G protein-coupled receptors (GPCRs) has expanded significantly over the past few years. To date, several orphan GPCRs have been paired with lysophospholipids, bile acids, arachidonic acid metabolites, dioleoyl phosphatidic acid, and short-, medium-, and long-chain free fatty acids.[9] From these discoveries, GPR40, GPR119, and GPR120 have been reported to play a role in regulating GDIS and to therefore have potential as novel targets for the treatment of type 2 diabetes.[10] GPR119, which is abundantly expressed in pancreatic β cells and the gastrointestinal tract, was identified as a receptor for lysophospholipids and certain ethanolamide derivatives of long-chain fatty acids, such as lysophosphatidylcholine and oleoylethanolamide.[11] Activation of GPR119 directly promotes GDIS and indirectly increases GLP-1 level through the upregulation of intracellular cAMP followed by a reduction in blood glucose level,[12] suggesting that GPR119 may present an attractive drug target for treating T2DM, and that its agonists may represent potential new insulin secretagogues without the risk of hypoglycemia. Thus, considerable effort has been invested in exploring GPR119 agonists as novel antidiabetic drugs,[13] such as AR231453 (**1**; Fig. 1) or PSN632408 (**2**), which were reported to show both insulinotropic and antiobesity effects. Clinical trials of several other GPR119 agonists are currently ongoing.[13]

Our continued interest in GPR119 as a drug target for the treatment of T2DM prompted an investigation into novel GPR119 agonists. In the course of structure–activity relationship (SAR) studies around our lead compound **5**,[14,15] we found that introduction of a 2,4,5-trihalogenatedphenyl group at the 2-position in the pyrimidine ring markedly improved GPR119 agonistic activity and discovered the pyrimidine derivative **6** as a potent GPR119 agonist. In addition, compound **6** was found to improve not only glucose tolerance in ICR mice but also blood glucose, plasma insulin,

* Corresponding author. Tel.: +81 (0)29 847 6631; fax: +81 (0)29 847 1519.
E-mail address: kenji.negoro@astellas.com (K. Negoro).

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http://dx.doi.org/10.1016/j.bmc.2012.08.054

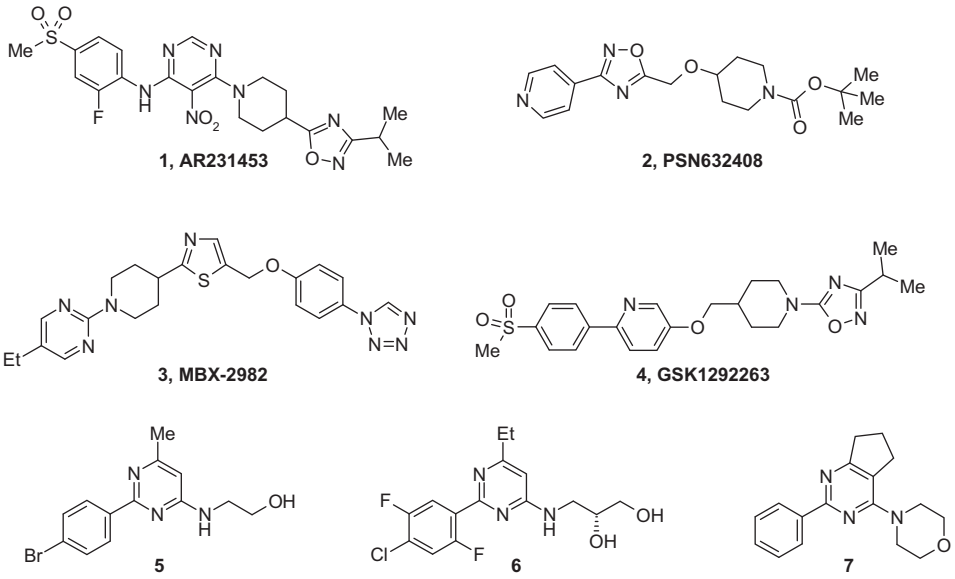


Figure 1. Structures of representative GPR119 agonists AR231453 (**1**), PSN632408 (**2**), MBX-2982 (**3**), GSK1292263 (**4**), **5**, **6** and **7**.

triglyceride levels and pancreatic insulin content in diabetic kk/Ay mice.[15] Separately to these findings, we also identified another lead compound **7**, which had a cyclopentane fused-pyrimidine structure and showed moderate in vitro GPR119 agonistic activity with an EC value of 9.1 μM. From these discoveries, we then designed novel fused-pyrimidine derivatives using a combination of the structure of compounds **6** and **7**. In this paper, we describe the synthesis and SAR studies of these fused-pyrimidine derivatives as novel GPR119 agonists.

2. Chemistry

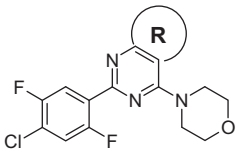
Synthesis of the 4-morpholino-fused-pyrimidine derivatives **12a**–**f** listed in Table 1 is shown in Scheme 1. Condensation of 4-chloro-2,5-difluorobenzamidine **8**[15] with the corresponding cyclic β-keto ester **9a**–**f**[16] followed by chlorination using phosphorous oxychloride afforded the 4-chloro-fused-pyrimidines **11a**–**f**. These compounds **11a**–**f** were then subjected to substitution with morpholine to obtain the 4-morpholino-fused-pyrimidine derivatives **12a**–**f**.

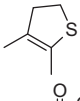
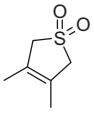
Synthesis of the 5,7-dihyd.rothieno[3,4-*d*]pyrimidine 6,6-dioxide derivatives **14a**–**f**, **15a** and **16a**–**d** listed in Table 2 is shown in Scheme 2. Oxidation of the 5,7-dihydrothieno[3,4-*d*] pyrimidine derivative **11d** with *m*-chloroperbenzoic acid (*m*-CPBA), followed by substitution with the corresponding amines gave the 4-substituted 6,6-dioxide derivatives **14a**–**j**. Hydrolysis of the ethyl esters **14g**–**j** afforded the carboxylic acid derivatives **15a**–**d**, and amidation of compound **15a**–**d** with ammonium chloride or methylamine provided the amide derivatives **16a**–**d**.

3. Results and discussion

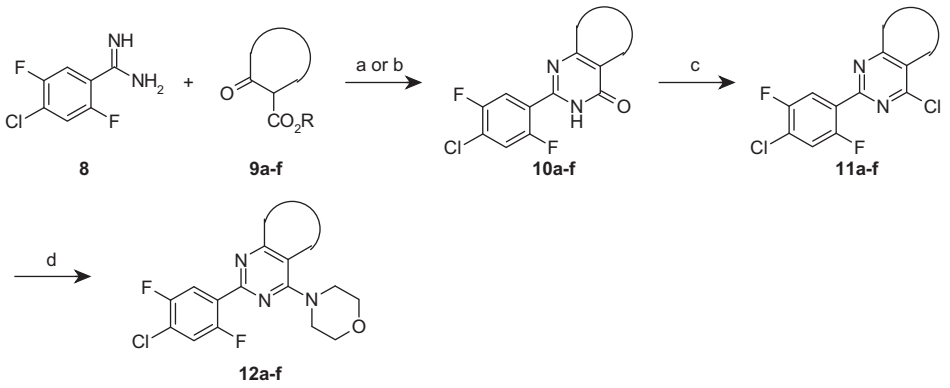
The synthesized compounds were evaluated for their agonistic activities toward GPR119 using a cAMP reporter assay system in which HEK293 cells were transfected with human GPR119 and cAMP-responsive element (pCRE)-luciferase expression plasmids.[17] In this assay system, we relatively evaluated our compounds by comparing their EC and IA values with those of lead compound **5**. EC value refers to the concentration of a tested compound in this assay system at which they showed as potent efficacy as compound **5** at 10 μM. IA value refers to the relative activity (%) of the tested compounds compared to the efficacy of compound **5** at 10 μM in the same assay system.[15a]

Table 1
In vitro SARs for analogs with replacement of the cyclopentane ring in compound **6**



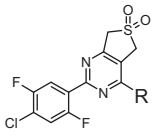
Compound	R	GPR119/pCRE EC[a] (nM)	GPR119/pCRE IA[b] (%)
12a		350	405
12b		770	313
12c		4100	205
12d		150	467
12e		970	258
12f		57	426
14a		28	668

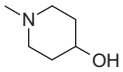
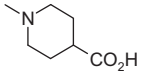
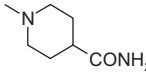
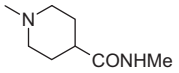
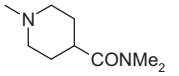
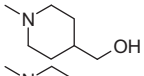
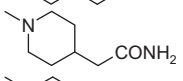
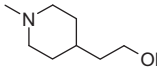
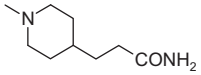
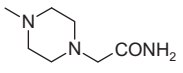
[a] Concentration of tested compounds showing equipotent efficacy to that of **5** at 10 μM in human recombinant cell-based assay. See Section 5.
[b] Relative efficacy of tested compounds at 10 μM compared to efficacy of **5** at 10 μM. See Section 5.



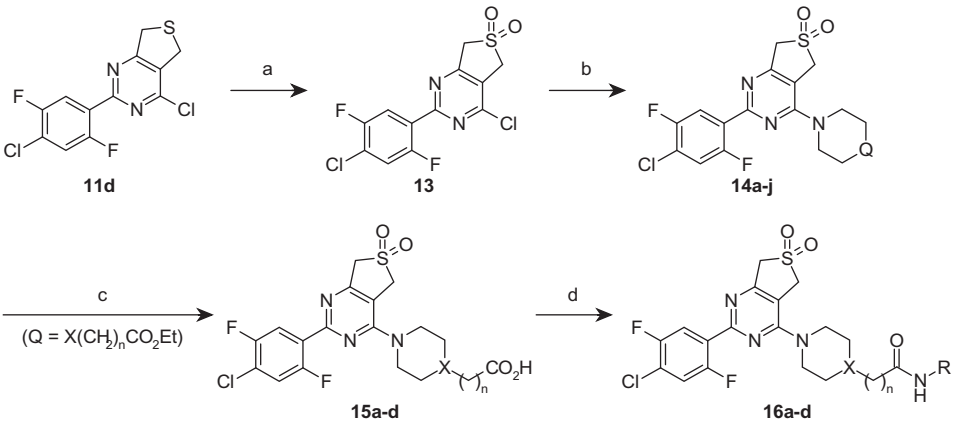
Scheme 1. Synthesis of the fused-pyrimidine derivatives **12a–f**. Reagents and conditions: (a) MeOH, reflux; (b) NaOMe, MeOH, rt to 50 °C; (c) $POCl_3$, reflux; (d) morpholine, MeCN, rt.

Table 2
In vitro SARs for analogs with replacement of the 4-morpholino group in compound **14a**



Compound	–R	GPR119/pCRE		OGTT/ICR mice		Solubility
		EC[a] (nM)	IA[b] (%)	% Decrease[c] (1 mg/kg po)	MED[d] (mg/kg po)	pH 6.8[e] (μM)
14a		28	668	30	1	<1
14b		14	575	32	1	91
15a		560	528	NT[f] (15 at 3 mg/kg po)	>3	NT[f]
14c		21	294	12	3	40
16a		51	457	NE[g]	3	11
14d		350	292	32	1	>100
14e		3.9	501	29	0.3	16
16b		8.3	483	35 (27 at 0.1 mg/kg po)	0.1	15
14f		2.8	506	29	1	23
16c		18	627	NT[f] (30 at 0.3 mg/kg po)	0.3	8
16d		44	737	17	3	>100

[a,b] See the corresponding footnotes to Table 1.
[c] Antihyperglycemic effects of tested compounds in male ICR mice at 1 mg/kg po. See Section 5.
[d] Minimum effective dose (MED) of tested compounds.
[e] Solubility of tested compounds in the buffer solution of pH 6.8. See Section 5.
[f] Not tested.
[g] Not effective at 1 mg/kg po.



Scheme 2. Synthesis of the 5,7-dihydrothieno[3,4-*d*]pyrimidine 6,6-dioxide derivatives. Reagents and conditions: (a) *m*-CPBA, $CHCl_3$, rt; (b) amine, *i*-Pr_2NEt, MeCN, 70 °C; (c) 1 M NaOH, EtOH, THF, rt; (d) NH_4Cl (R = H) or 1 M $MeNH_2$/THF (R = Me), Et_3N, EDAC·HCl, HOBt, DMF, rt.

We first designed and synthesized several fused-pyrimidine derivatives. As shown in Table 1, the cyclopentane fused-pyrimidine derivative **12a** showed agonistic activity comparable to that of compound **6**, indicating that introduction of 4-chloro and 2,5-difluoro substituents into the phenyl moiety of the fused-pyrimidine derivative **7** was effective in improving GPR119 agonistic activity. Ring expansion of **12a** (compound **12b**) resulted in reduced agonistic activity, suggesting that the five-membered ring was preferable for agonistic activity compared to the six-membered ring. Replacement of the methylene linker at the 6-position in compound **12a** with the ether linker (compound **12c**) led to a 10-fold loss of activity compared to **12a**. In contrast, a two-fold increase in activity was observed for the thioether derivative **12d**. These results suggested that the introduction of a hetero atom on the fused-ring was tolerated, and that the hydrophobicity in this region was particularly favorable for agonistic activity. The 5,6-dihydrothieno[2,3-*d*]pyrimidine derivative **12e** was found to be less potent than the 5,7-dihydrothieno[3,4-*d*]pyrimidine derivative **12d**; however, the 6,7-dihydrothieno[3,2-*d*]pyrimidine derivative **12f** showed potent activity, with an EC value in the subnano molar range, indicating that introduction of a sulfur atom at the 5- or 6-position might be preferable to that at the 7-position. Oxidation of the thioether linker in compound **12d** (compound **14a**; EC = 28 nM) resulted in a five-fold improvement of GPR119 agonistic activity over compound **12d**, suggesting that the 5,7-dihydrothieno[3,4-*d*]pyrimidine 6,6-dioxide structure might be suitable for showing potent agonistic activity.[18] Meanwhile, the low solubility in water of compound **14a** (<1 μM in pH 6.8 buffer solution) highlighted the need to improve the physicochemical properties of this compound. Nevertheless, we selected the 5,7-dihydrothieno[3,4-*d*]pyrimidine 6,6-dioxide derivative **14a** as a template for further investigation.

Having identified a favorable scaffold which showed highly potent GPR119 agonistic activity, we then shifted our focus to optimization of the morpholine moiety of compound **14a** to improve water solubility and agonistic activity. As described in our previous reports,[15] introduction of the oxy functional groups onto the amino group at the 4-position in the pyrimidine ring was effective in inducing potent GPR119 agonistic activity. These groups could be considered to influence the water solubility of the compounds. Therefore, replacement of the morpholino group in compound **14a** with other cyclic amines which have oxy functional groups, such as alcohols, amides and carboxylic acids, was examined (Table 2). Compound **14b**, which had a 4-hydroxypiperidino group, showed a two-fold improvement in in vitro agonistic activity over the morpholino derivative **14a**, implying that introduction of a hydrogen-bond donating group in this region was favorable for agonistic activity compared to that of a hydrogen-bond accepting group. In addition, compound **14b** was found to have dramatically improved water solubility over compound **14a**. Replacement of the hydroxy group in compound **14b** with a carboxyl group (compound **15a**) led to 40-fold reduction in agonistic activity compared to compound **14b**, indicating that introduction of the highly acidic group in this region was unfavorable for agonistic activity. The carbamoyl derivative **14c** was found to be equipotent to the hydroxy derivative **14b** and to have improved water solubility over compound **14a**. The N-methylated analog **16a** was slightly less potent than the carbamoyl derivative **14c** and the N,N-dimethylated analog **14d** showed approximately 15-fold less potent activity compared to compound **14c**, suggesting that hydrogen-bond donation in this region was important for showing potent agonistic activity. One carbon elongated analogs of **14b** and **14c** (compound **14e** and **16b**) showed extremely potent agonistic activity with EC values in the nano molar range, which were approximately three-fold more potent than the corresponding non-elongated analogs **14b** and **14c**, respectively. Two carbon elongated analogs of **14b** (compound **14f**) was found to show slightly more potent agonistic activity than the corresponding one carbon elongated analog **14e**. In contrast, two carbon elongated analogs of **14c** (compound **16c**) was found to show two-fold less potent agonistic activity than the corresponding one carbon elongated analog **16b**. These results indicated that the introduction of a hydrogen-bond donating group to the two-carbon-chain length away from the 4-position of the piperidine ring might be most preferable for agonistic activity. Replacement of the piperidine ring in compound **16b** with the piperazine ring (compound **16d**) resulted in an improvement in water solubility, but also led to a five-fold reduction in agonistic activity compared to compound **16b**. These results revealed that the piperidine ring was more favorable than the piperazine ring as a cyclic amine at the 4-position in the pyrimidine ring for GPR119 agonistic activity.

We then evaluated the antihyperglycemic effects of the all compounds listed in Table 2 via the oral glucose tolerance test (OGTT) in male ICR mice by measuring the blood glucose-lowering ratio (%) at 30 min after glucose loading in comparison with animals in the vehicle group. In each evaluation dose of the tested compounds, minimum effective dose (MED) was defined as the lowest dose showing a greater than 20% decrease in blood glucose-lowering ratio. The morpholino (**14a**) and hydroxypiperidino (**14b**, **14e** and **14f**) derivatives demonstrated excellent potency in vivo at 1 mg/kg po, whereas the carboxypiperidino derivative **15a** did not show a significant glucose-lowering effect at 3 mg/kg po. The carbamoyl derivatives **14c**, **14d** and **16a**–**d** all demonstrated antihyperglycemic effects at a dose of 3 mg/kg po or lower. In particular, the 4-carbamoylmethylpiperidino derivative **16b** was found to show the lowest MED among the compounds listed in Table 2, with

a MED of 0.1 mg/kg. We speculated that compound **16b** might show an excellent in vivo antihyperglycemic effect as a result of both its potent GPR119 agonistic activity and good pharmacokinetic profile. In addition, compound **16b** was found to have low clearance in human liver microsomes (58 mL/min/kg) and no appreciable inhibition of the CYP_{450} enzyme isoforms tested up to concentrations of 50 μM.

A homology model of hGPR119 based on an agonist-bound $hA_{2A}AR$ X-ray structure (PDB code 3QAK)[19] was used to study the putative interactions between compound **16b** and GPR119. A docking study of **16b** to the GPR119 homology model suggested that **16b** exerts its GPR119 agonistic activity by effectively utilizing a number of different interactions (Fig. 2). In this docking model, the 4-chloro-2,5-difluorophenyl group extends toward the hydrophobic region sandwiched between Pro140 (TM4) and Met178, Val182 (TM5), and the chloro group at the 4-position of the phenyl moiety forms a halogen bond[20] with Pro144 (TM4). These results might explain why the 4-chloro-2,5-difluorophenyl group increased activity. The sulfone linker in the fused-ring moiety of compound **16b** forms hydrophobic interactions with Val85 (TM3), Trp265 (TM7) and Phe241 (TM6), suggesting that the sulfone linker would be recognized as the hydrophobic element.[20] In addition, the amino group at the 4-position in the pyrimidine ring extends to the narrow space formed by TM5, TM6 and TM3, and the carbamoyl moiety forms a hydrogen-bond with Cys186 (TM5). This information also afforded the opportunity to investigate the effect of the substituent in the amino group.

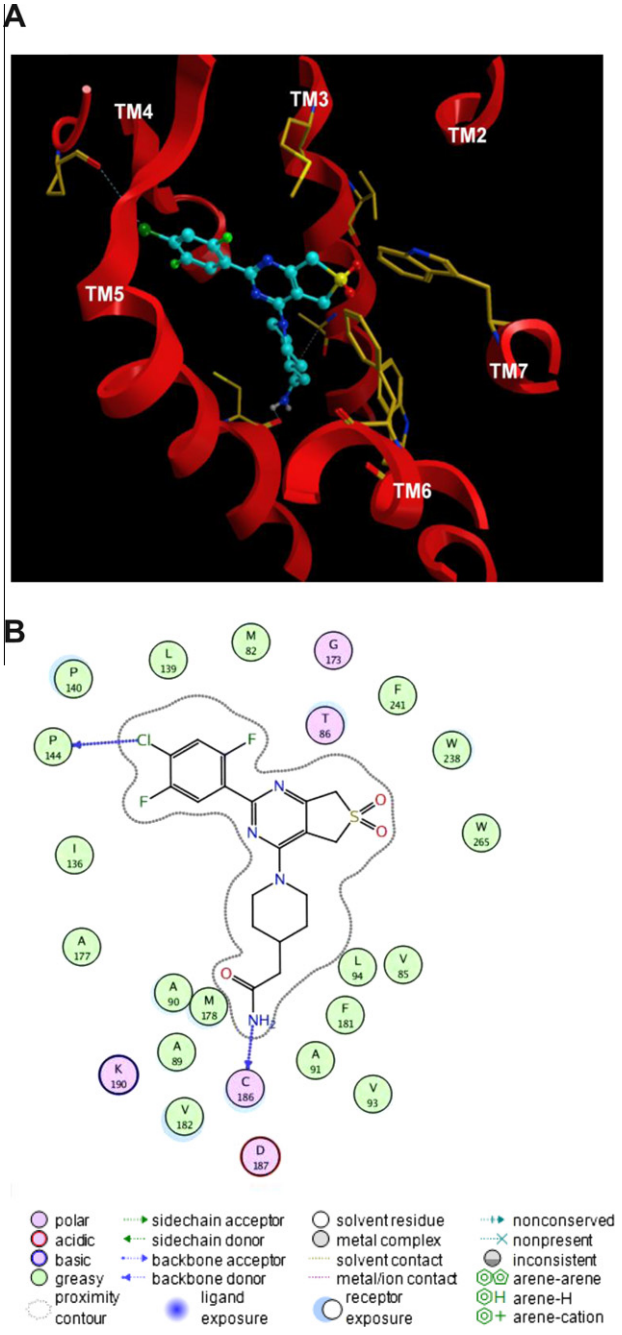


Figure 2. (A) Binding mode of compound **16b** to the hGPR119 homology model. The template was built based on the crystal structure of an agonist-bound $hA_{2A}AR$ (PDB code 3QAK); (B) two-dimensional diagram showing the interactions of **16b** with hGPR119 prepared using the ligand interactions application in MOE.

4. Conclusion

A series of fused-pyrimidine derivatives have been prepared and evaluated in order to create potent and orally active GPR119 agonists. A combination of the cyclopentane ring fused-pyrimidine structure and 4-chloro-2,5-difluorophenyl group provided a potent GPR119 agonist **12a** with an original scaffold. Optimization of the fused-pyrimidine moiety in compound **12a** led to identification of the 5,7-dihydrothieno[3,4-*d*]pyrimidine 6,6-dioxide derivative **14a** and an approximately 10-fold improvement in GPR119 agonistic activity. Subsequent replacement of the amino group at the 4-position in the pyrimidine ring to improve activity and water solubility led to the identification of 2-{1-[2-(4-chloro-2,5-difluorophenyl)-6,6-dioxido-5,7-dihydrothieno[3,4-*d*]pyrimidin-4-yl]piperidin-4-yl}acetamide (**16b**) as an advanced analog. Compound **16b** was found to have extremely potent agonistic activity, with an EC value of 8.3 nM, and to improve glucose tolerance at 0.1 mg/kg po in mice. In addition, a docking study of **16b** to the GPR119 homology model suggested that **16b** exerts its GPR119 agonistic activity by effectively utilizing a number of different interactions. We consider that compound **16b** and its analogs have clear utility in exploring the practicality of GPR119 agonists as potential therapeutic agents for the treatment of T2DM.

5. Experimental

5.1. Chemistry

^{1}H NMR spectra were recorded on a JEOL JNM-EX400 spectrometer and were referenced to an internal standard, tetramethylsilane. The abbreviations used for the signal patterns are as follows: s, singlet; br, broad; d, doublet; t, triplet; q, quartet; dd, double doublet; m, multiplet. Mass spectra were recorded on a JEOL LX-2000 or Waters ZQ-2000 mass spectrometer. Elemental analyses were performed with a Yanako MT-5 microanalyzer (C, H, N) and a Yokogawa IC-7000S ion chromatographic analyzer (halogens, S). Where analyses are indicated by symbols, the analytical results are within ±0.4% of the theoretical values. Drying of organic solutions during workup was done over anhydrous $MgSO_4$. Column chromatography was performed with Wakogel C-200 or Merck silica gel 60.

5.2. 2-(4-Chloro-2,5-difluorophenyl)-3,5,6,7-tetrahydro-4*H*-cyclopenta[*d*]pyrimidin-4-one (10a)

To a solution of 4-chloro-2,5-difluorobenzamidine (**8**[15], 800 mg) in methanol (10 mL) was added methyl 2-oxocyclopentanecarboxylate (**9a**, 700 mg), and the reaction mixture was stirred at reflux temperature for 5 h. To the mixture was added 2-oxocyclopentan-

ecarboxylate (150 mg), and the reaction mixture was stirred at reflux temperature for 21 h. The mixture was then evaporated in vacuo, and the resulting residue was washed with diethyl ether and dried in vacuo to give **10a** (810 mg, 68%) as a white solid: ^{1}H NMR (DMSO-d_6) δ 1.92–2.10 (2H, m), 2.69 (2H, t, J = 7.7 Hz), 2.80 (2H, t, J = 7.7 Hz), 7.77 (1H, dd, J = 6.4, 9.3 Hz), 7.83 (1H, dd, J = 6.1, 9.5 Hz), 10.90–12.10 (1H, br); FAB-MS m/z 283, 285 [(M+H)$^+$].

The following compound (**10b**) was prepared by a procedure similar to that described for **10a**.

5.3. 2-(4-Chloro-2,5-difluorophenyl)-5,6,7,8-tetrahydroquinazolin-4(3*H*)-one (10b)

Yellow solid (yield 96%); ^{1}H NMR (DMSO-d_6) δ 1.62–1.80 (4H, m), 2.39 (2H, t, J = 5.9 Hz), 2.57 (2H, t, J = 6.2 Hz), 7.76 (1H, dd, J = 6.3, 9.3 Hz), 7.85 (1H, dd, J = 6.2, 9.6 Hz), 12.30–12.80 (1H, br); FAB-MS m/z 297, 299 [(M+H)$^+$].

5.4. 2-(4-Chloro-2,5-difluorophenyl)-5,7-dihydrofuro[3,4-*d*]pyrimidin-4(3*H*)-one (10c)

To a solution of methyl 4-oxotetrahydrofuran-3-carboxylate (**9c**[16a], 1.36 g) in methanol (15 mL) were added 4-chloro-2,5-difluorobenzamidine (**8**[15], 1.50 g) and sodium methoxide (420 mg), and the reaction mixture was stirred at room temperature overnight. The mixture was then stirred at 50 °C for 9 h and cooled to room temperature. To the mixture were added 1 M hydrochloric acid (10 mL) and water (40 mL) at 5 °C, and the reaction mixture was stirred at room temperature for 4 h. The resulting precipitates were collected by filtration, washed with water and dried in vacuo at 50 °C to give **10c** (756 mg, 68%) as a brown solid: ^{1}H NMR (DMSO-d_6) δ 4.85–4.93 (2H, m), 4.93–5.00 (2H, m), 7.82 (1H, dd, J = 6.4, 9.3 Hz), 7,89 (1H, d, J = 6.1, 9.5 Hz), 12.50–13.50 (1H, br); ESI-MS m/z 285, 287 [(M+H)$^+$].

The following compound (**10d**–**f**) was prepared by a procedure similar to that described for **10c**.

5.5. 2-(4-Chloro-2,5-difluorophenyl)-5,7-dihydrothieno[3,4-*d*]pyrimidin-4(3*H*)-one (10d)

Compound **10d** was prepared from **8** and methyl 4-oxotetrahydrothiophene-3-carboxylate **9d**.[16a]

Brown solid (yield 69%); ^{1}H NMR (DMSO-d_6) δ 3.95–4.10 (2H, m), 4.15–4.25 (2H, m), 7.81 (1H, dd, J = 6.3, 9.2 Hz), 7,89 (1H, d, J = 5.9, 9.6 Hz), 12.70–13.30 (1H, br); FAB-MS m/z 301, 303 [(M+H)$^+$].

5.6. 2-(4-Chloro-2,5-difluorophenyl)-5,6-dihydrothieno[2,3-*d*]pyrimidin-4(3*H*)-one (10e)

Compound **10e** was prepared from **8**[15] and ethyl 2-oxotetrahydrothiophene-3-carboxylate **9e**.[16b]

Pale yellow solid (yield 86%); ^{1}H NMR (DMSO-d_6) δ 2.50–3.05 (4H, m), 3.60–3.75 (1H, m), 7.80 (1H, dd, J = 6.4, 9.3 Hz), 7.86 (1H, dd, J = 6.1, 9.5 Hz), 11.00–12.60 (1H, br); FAB-MS m/z 319, 321 [(M+H_2O+H)$^+$].

5.7. 2-(4-Chloro-2,5-difluorophenyl)-6,7-dihydrothieno[3,2-*d*]pyrimidin-4(3*H*)-one (10f)

Compound **10f** was prepared from **8**[15] and methyl 3-oxotetrahydrothiophene-2-carboxylate **9f**.[16a]

Brown solid (yield 18%); ^{1}H NMR (DMSO-d_6) δ 3.25–3.80 (4H, m), 7.78 (1H, dd, J = 6.4, 9.3 Hz), 7.86 (1H, dd, J = 5.9, 9.8 Hz), 12.50–13.20 (1H, br); FAB-MS m/z 301, 303 [(M+H)$^+$].

5.8. 4-Chloro-2-(4-chloro-2,5-difluorophenyl)-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidine (11a)

A mixture of 2-(4-chloro-2,5-difluorophenyl)-3,5,6,7-tetrahydro-4*H*-cyclopenta[*d*]pyrimidin-4-one (**10a**, 0.80 g) and phosphorus oxychloride (10 mL) was stirred at reflux temperature for 3 h. The mixture was cooled to room temperature, and evaporated in vacuo. To the resulting residue was added water, and extracted with ethyl acetate. The organic layer was dried, filtered and evaporated in vacuo to give **11a** (0.75 g, 88%) as a light brown solid: ^{1}H NMR (DMSO-d_6) δ 2.10–2.21 (2H, m), 3.01 (2H, t, J = 7.7 Hz), 3.10 (2H, t, J = 7.7 Hz), 7.81 (1H, dd, J = 6.4, 10.3 Hz), 7.96 (1H, dd, J = 6.4, 9.8 Hz); FAB-MS m/z 301, 303 [(M+H)$^+$].

The following compounds (**11b**–**f**) were prepared by a procedure similar to that described for **11a**.

5.9. 4-Chloro-2-(4-chloro-2,5-difluorophenyl)-5,6,7,8-tetrahydroquinazoline (11b)

White solid (yield 94%); ^{1}H NMR (DMSO-d_6) δ 1.78–1.90 (4H, m), 2.70–2.80 (2H, m), 2.85–2.95 (2H, m), 7.81 (1H, dd, J = 6.4, 10.3 Hz), 7.95 (1H, dd, J = 6.6, 9.5 Hz); FAB-MS m/z 315, 317 [(M+H)$^+$].

5.10. 4-Chloro-2-(4-chloro-2,5-difluorophenyl)-5,7-dihydrofuro[3,4-*d*]pyrimidine (11c)

Brown solid (yield 19%); ^{1}H NMR (DMSO-d_6) δ 5.13 (2H, t, J = 2.0 Hz), 5.20 (2H, t, J = 2.0 Hz), 7.87 (1H, dd, J = 5.9, 10.3 Hz), 8.03 (1H, dd, J = 6.9, 9.8 Hz); FAB-MS m/z 303, 305 [(M+H)$^+$].

5.11. 4-Chloro-2-(4-chloro-2,5-difluorophenyl)-5,7-dihydrothieno[3,4-*d*]pyrimidine (11d)

Pale red solid (yield 97%); ^{1}H NMR (DMSO-d_6) δ 4.33 (2H, t, J = 2.0 Hz), 4.44 (2H, t, J = 2.0 Hz), 7.85 (1H, dd, J = 6.4, 10.3 Hz), 8.01 (1H, dd, J = 6.4, 9.8 Hz); FAB-MS m/z 319, 321 [(M+H)$^+$].

5.12. 4-Chloro-2-(4-chloro-2,5-difluorophenyl)-5,6-dihydrothieno[2,3-*d*]pyrimidine (11e)

Brown oil (yield 83%); ^{1}H NMR (DMSO-d_6) δ 3.35–3.50 (2H, m), 3.50–3.65 (2H, m), 7.82 (1H, dd, J = 5.9, 10.3 Hz), 7.95 (1H, dd, J = 6.4, 9.8 Hz); FAB-MS m/z 319, 321 [(M+H)$^+$].

5.13. 4-Chloro-2-(4-chloro-2,5-difluorophenyl)-6,7-dihydrothieno[3,2-*d*]pyrimidine (11f)

Brown solid (yield 76%); ^{1}H NMR (DMSO-d_6) δ 3.52–3.58 (4H, m), 7.81 (1H, dd, J = 6.4, 10.3 Hz), 7.96 (1H, dd, J = 6.9, 9.8 Hz); FAB-MS m/z 319, 321 [(M+H)$^+$].

5.14. 2-(4-Chloro-2,5-difluorophenyl)-4-(morpholin-4-yl)-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidine hydrochloride (12a)

To a solution of 4-chloro-2-(4-chloro-2,5-difluorophenyl)-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidine (**11a**, 730 mg) in THF (5 mL) was added morpholine (5 mL), and the mixture was stirred at room temperature for 12 h. The mixture was evaporated in vacuo and to the resulting residue was added water (30 mL). The solid was collected by filtration, washed with water and then suspended with methanol (30 mL). To the suspension was added 4 M hydrochloride solution in ethyl acetate (5 mL), and the mixture was evaporated in vacuo. The resulting solid was recrystallized from methanol and diethyl ether to give **12a** (581 mg, 62%) as a colorless crystal: ^{1}H

NMR (DMSO-d_6) δ 2.00–2.15 (2H, m), 2.95 (2H, t, J = 7.6 Hz), 3.12 (2H, t, J = 7.6 Hz), 3.65–3.78 (4H, m), 3.80–3.90 (4H, m), 3.90–6.00 (1H, br), 7.89 (1H, dd, J = 6.4, 9.8 Hz), 8.04 (1H, dd, J = 6.3, 9.8 Hz); FAB-MS m/z 352, 354 [$(M+H)^+$]. Anal. ($C_{17}H_{16}N_3OClF_2$·HCl): C, H, N, Cl, F.

The following compounds (**12b**–**f**) were prepared by a procedure similar to that described for **12a**.

5.15. 2-(4-Chloro-2,5-difluorophenyl)-4-(morpholin-4-yl)-5,6,7,8-tetrahydroquinazoline hydrochloride (12b)

White solid (yield 85%); ^{1}H NMR (DMSO-d_6) δ 1.55–1.75 (2H, m), 1.75–1.95 (2H, m), 2.60–2.72 (2H, m), 2.80–2.95 (2H, m), 3.50–4.00 (8H, m), 7.92 (1H, dd, J = 6.1, 10.0 Hz), 8.04 (1H, dd, J = 6.4, 9.3 Hz); FAB-MS m/z 366, 368 [$(M+H)^+$]. Anal. ($C_{18}H_{18}N_3OClF_2$·HCl·0.9H_2O): C, H, N, Cl, F.

5.16. 2-(4-Chloro-2,5-difluorophenyl)-4-(morpholin-4-yl)-5,7-dihydrofuro[3,4-*d*]pyrimidine hydrochloride (12c)

Light brown solid (yield 61%); ^{1}H NMR (DMSO-d_6) δ 3.50–3.75 (8H, m), 4.00–4.80 (1H, br), 4.87 (2H, t, J = 2.4 Hz), 5.26 (2H, t, J = 2.4 Hz), 7.75 (1H, dd, J = 5.9, 10.3 Hz), 8.00 (2H, dd, J = 6.6, 10.0 Hz); FAB-MS m/z 354, 356 [$(M+H)^+$]. Anal. ($C_{16}H_{14}N_3O_2ClF_2$·0.7HCl·0.5H_2O): C, H, N, Cl, F.

5.17. 2-(4-Chloro-2,5-difluorophenyl)-4-(morpholin-4-yl)-5,7-dihydrothieno[3,4-*d*]pyrimidine hydrochloride (12d)

Pink solid (yield 84%); ^{1}H NMR (DMSO-d_6) δ 3.65–3.78 (8H, m), 3.80–4.12 (1H, br), 4.15 (2H, t, J = 2.2 Hz), 4.39 (2H, t, J = 2.2 Hz), 7.75 (1H, dd, J = 5.9, 10.3 Hz), 7.99 (2H, dd, J = 6.6, 10.0 Hz); FAB-MS m/z 370, 372 [$(M+H)^+$]. Anal. ($C_{16}H_{14}N_3OSClF_2$·HCl·0.5C_2H_6O): C, H, N, S, Cl, F.

5.18. 2-(4-Chloro-2,5-difluorophenyl)-4-(morpholin-4-yl)-5,6-dihydrothieno[2,3-*d*]pyrimidine hydrochloride (12e)

Brown solid (yield 32%); ^{1}H NMR (DMSO-d_6) δ 3.30–3.50 (4H, m), 3.58–3.75 (8H, m), 4.20–4.80 (1H, br), 4.39 (2H, t, J = 2.2 Hz), 7.73 (1H, dd, J = 6.4, 10.3 Hz), 7.94 (2H, dd, J = 6.8, 9.8 Hz); FAB-MS m/z 370, 372 [$(M+H)^+$]. Anal. ($C_{16}H_{14}N_3OSClF_2$·0.95HCl·0.25H_2O): C, H, N, S, Cl, F.

5.19. 2-(4-Chloro-2,5-difluorophenyl)-4-(morpholin-4-yl)-6,7-dihydrothieno[3,2-*d*]pyrimidine hydrochloride (12f)

Yellow solid (yield 72%); ^{1}H NMR (DMSO-d_6) δ 3.22–3.42 (4H, m), 3.65–3.75 (8H, m), 4.00–5.25 (1H, br), 4.39 (2H, t, J = 2.2 Hz), 7.73 (1H, dd, J = 6.4, 10.3 Hz), 7.98 (2H, dd, J = 6.9, 9.8 Hz); FAB-MS m/z 370, 372 [$(M+H)^+$]. Anal. ($C_{16}H_{14}N_3OSClF_2$·HCl): C, H, N, S, Cl, F.

5.20. 4-Chloro-2-(4-chloro-2,5-difluorophenyl)-5,7-dihydrothieno[3,4-*d*]pyrimidine 6,6-dioxide (13)

To a mixture of 4-chloro-2-(4-chloro-2,5-difluorophenyl)-5,7-dihydrothieno[3,4-*d*]pyrimidine (**11d**, 8.00 g) and chloroform (200 mL) was added *m*-CPBA (20.19 g), and then the mixture was stirred at room temperature for 1 h. To the mixture was added sat. aqueous sodium hydrogen carbonate. The mixture was extracted with chloroform, and the organic layer was dried. The desiccant was removed by filtration and the filtrate was evaporated in vacuo. The resulting residue was purified by silica gel column chromatography (hexane–ethyl acetate) to obtain a pale yellow solid. The obtained solid was recrystallized from ethyl acetate and hexane to give **13** (6.10 g, 69%) as a slightly yellow crystal: ^{1}H NMR (DMSO-d_6) δ 4.81 (2H, s), 4.93 (2H, s), 7.88 (1H, dd, J = 6.4, 10.3 Hz), 8.03 (1H, dd, J = 6.6, 9.6 Hz); FAB-MS m/z 351, 353 [$(M+H)^+$].

5.21. 2-(4-Chloro-2,5-difluorophenyl)-4-(morpholin-4-yl)-5,7-dihydrothieno[3,4-*d*]pyrimidine 6,6-dioxide (14a)

A mixture of 4-chloro-2-(4-chloro-2,5-difluorophenyl)-5,7-dihydrothieno[3,4-*d*]pyrimidine 6,6-dioxide (**13**, 250 mg), morpholine (80 μL), *N*,*N*-diisopropylethylamine (0.37 mL) and acetonitrile (5 mL) was stirred at 70 °C for 15 h and then cooled to room temperature. To the mixture was added water (20 mL). The solid was collected by filtration, washed with water and dried in vacuo at 50 °C to give a pale yellow solid. The obtained solid was dissolved with THF (10 mL) and methanol (5 mL), and to the solution was added 4 M hydrochloride solution in dioxane (2 mL). The mixture was evaporated in vacuo and the resulting residue was recrystallized from ethanol and ethyl acetate to obtain **14a** (234 mg, 82%) as a colorless crystal: ^{1}H NMR (DMSO-d_6) δ 3.60–3.75 (8H, m), 4.54 (2H, s), 4.74 (2H, s), 7.76 (1H, dd, J = 6.4, 10.3 Hz), 7.99 (1H, dd, J = 6.9, 9.8 Hz); FAB-MS m/z 402, 404 [$(M+H)^+$]. Anal. ($C_{16}H_{14}N_3O_3SClF_2$·0.08HCl): C, H, N, S, Cl, F.

The following compounds (**14b**–**j**) were prepared by a procedure similar to that described for **14a**.

5.22. 1-[2-(4-Chloro-2,5-difluorophenyl)-6,6-dioxido-5,7-dihydrothieno[3,4-*d*]pyrimidin-4-yl]piperidin-4-ol hydrochloride (14b)

Ivory crystal (yield 85%); ^{1}H NMR (DMSO-d_6) δ 1.35–1.55 (2H, m), 1.73–1.90 (2H, m), 3.25–3.43 (2H, m), 3.70–3.85 (1H, m), 3.90–4.05 (2H, m), 4.52 (2H, s), 4.69 (2H, s), 5.20–6.10 (1H, br), 7.76 (1H, dd, J = 6.1, 10.0 Hz), 7.97 (1H, dd, J = 6.9, 9.8 Hz); FAB-MS m/z 416, 418 [$(M+H)^+$]. Anal. ($C_{17}H_{16}N_3O_3SClF_2$·HCl): C, H, N, S, Cl, F.

5.23. 1-[2-(4-Chloro-2,5-difluorophenyl)-6,6-dioxido-5,7-dihydrothieno[3,4-*d*]pyrimidin-4-yl]piperidine-4-carboxamide (14c)

White solid (yield 78%); ^{1}H NMR (DMSO-d_6) δ 1.50–1.70 (2H, m), 1.70–1.88 (2H, m), 2.35–2.50 (1H, m), 3.00–3.17 (2H, m), 3.20–3.50 (2H, m), 4.20–4.35 (2H, m), 4.52 (2H, s), 4.70 (2H, s), 6.81 (1H, s), 7.31 (1H, s), 7.76 (1H, dd, J = 6.4, 10.3 Hz), 7.98 (1H, dd, J = 6.6, 10.0 Hz); FAB-MS m/z 443, 445 [$(M+H)^+$]. Anal. ($C_{18}H_{17}N_4O_3SClF_2$): C, H, N, S, Cl, F.

5.24. 1-[2-(4-Chloro-2,5-difluorophenyl)-6,6-dioxido-5,7-dihydrothieno[3,4-*d*]pyrimidin-4-yl]-*N*,*N*-dimethylpiperidine-4-carboxamide hydrochloride (14d)

Pale yellow solid (yield 41%); ^{1}H NMR (DMSO-d_6) δ 1.50–1.80 (4H, m), 2.81 (3H, s), 2.95–3.25 (6H, m), 4.20–4.40 (2H, m), 4.52 (2H, s), 4.71 (2H, s), 4.75–5.80 (1H, br), 7.76 (1H, dd, J = 5.9, 10.3 Hz), 7.98 (1H, dd, J = 6.4, 9.8 Hz); FAB-MS m/z 471, 473 [$(M+H)^+$]. Anal. ($C_{20}H_{21}N_4O_3SClF_2$·0.6HCl·0.75H_2O·0.2$C_4H_8O_2$): C, H, N, S, Cl, F.

5.25. {1-[2-(4-Chloro-2,5-difluorophenyl)-6,6-dioxido-5,7-dihydrothieno[3,4-*d*]pyrimidin-4-yl]piperidin-4-yl}methanol hydrochloride (14e)

Pale yellow crystal (yield 87%); ^{1}H NMR (DMSO-d_6) δ 1.10–1.30 (2H, m), 1.60–1.82 (3H, m), 2.90–3.12 (2H, m), 3.28 (2H, d, J = 5.9 Hz), 4.20–4.40 (2H, m), 4.52 (2H, s), 4.69 (2H, s), 6.30–7.40

(1H, br), 7.76 (1H, dd, *J* = 5.9, 10.3 Hz), 7.97 (1H, dd, *J* = 6.9, 9.8 Hz); FAB-MS *m*/*z* 430, 432 [(M+H)$^+$]. Anal. ($C_{18}H_{18}N_3O_3SClF_2$·HCl): C, H, N, S, Cl, F.

5.26. 2-{1-[2-(4-Chloro-2,5-difluorophenyl)-6,6-dioxido-5,7-dihydrothieno[3,4-*d*]pyrimidin-4-yl]piperidin-4-yl}ethanol hydrochloride (14f)

Yellow crystal (yield 84%); ^{1}H NMR (DMSO-d_6) δ 1.09–1.27 (2H, m), 1.30–1.48 (2H, m), 1.63–1.83 (3H, m), 2.92–3.10 (2H, m), 3.46 (2H, t, *J* = 6.6 Hz), 4.21–4.35 (2H, m), 4.51 (2H, s), 4.68 (2H, s), 5.10–6.00 (1H, br), 7.76 (1H, dd, *J* = 6.4, 10.3 Hz), 7.96 (1H, dd, *J* = 6.3, 9.8 Hz); FAB-MS *m*/*z* 444, 446 [(M+H)$^+$]. Anal. ($C_{19}H_{20}N_3O_3SClF_2$·HCl): C, H, N, S, Cl, F.

5.27. Ethyl 1-[2-(4-chloro-2,5-difluorophenyl)-6,6-dioxido-5,7-dihydrothieno[3,4-*d*]pyrimidin-4-yl]piperidine-4-carboxylate (14g)

White solid (yield 83%); ^{1}H NMR (DMSO-d_6) δ 1.19 (3H, t, *J* = 7.1 Hz), 1.55–1.70 (2H, m), 1.85–1.98 (2H, m), 2.63–2.76 (1H, m), 3.10–3.25 (2H, m), 4.08 (2H, q, *J* = 7.1 Hz), 4.13–4.25 (2H, m), 4.52 (2H, s), 4.70 (2H, s), 7.77 (1H, dd, *J* = 6.1, 10.0 Hz), 7.98 (1H, dd, *J* = 6.9, 9.8 Hz); FAB-MS *m*/*z* 470, 472 [(M+H)$^+$].

5.28. Ethyl {1-[2-(4-chloro-2,5-difluorophenyl)-6,6-dioxido-5,7-dihydrothieno[3,4-*d*]pyrimidin-4-yl]piperidin-4-yl}acetate (14h)

White solid (yield 98%); ^{1}H NMR (DMSO-d_6) δ 1.18 (3H, t, *J* = 7.1 Hz), 1.20–1.33 (2H, m), 1.68–1.81 (2H, m), 1.95–2.10 (1H, m), 2.26 (2H, d, *J* = 7.4 Hz), 2.98–3.13 (2H, m), 4.07 (2H, q, *J* = 7.1 Hz), 4.20–4.35 (2H, m), 4.51 (2H, s), 4.67 (2H, s), 7.76 (1H, dd, *J* = 6.2, 10.1 Hz), 7.97 (1H, dd, *J* = 6.9, 9.8 Hz); FAB-MS *m*/*z* 486, 488 [(M+H)$^+$].

5.29. Ethyl 3-{1-[2-(4-chloro-2,5-difluorophenyl)-6,6-dioxido-5,7-dihydrothieno[3,4-*d*]pyrimidin-4-yl]piperidin-4-yl}propanoate (14i)

Pale yellow solid (yield 96%); ^{1}H NMR (DMSO-d_6) δ 1.08–1.26 (5H, m), 1.42–1.65 (3H, m), 1.65–1.82 (2H, m), 2.32 (2H, t, *J* = 7.6 Hz), 2.90–3.07 (2H, m), 4.05 (2H, q, *J* = 7.2 Hz), 4.20–4.36 (2H, m), 4.50 (2H, s), 4.67 (2H, s), 7.76 (1H, dd, *J* = 6.1, 10.0 Hz), 7.96 (1H, dd, *J* = 6.9, 9.8 Hz); FAB-MS *m*/*z* 500, 502 [(M+H)$^+$].

5.30. Ethyl {4-[2-(4-chloro-2,5-difluorophenyl)-6,6-dioxido-5,7-dihydrothieno[3,4-*d*]pyrimidin-4-yl]piperazin-1-yl}acetate (14j)

Pale gray solid (yield 99%); ^{1}H NMR (DMSO-d_6) δ 1.19 (3H, t, *J* = 7.1 Hz), 2.57–2.68 (4H, m), 3.29 (2H, s), 3.62–3.75 (4H, m), 4.09 (2H, q, *J* = 7.1 Hz), 4.53 (2H, s), 4.71 (2H, s), 7.76 (1H, dd, *J* = 5.8, 10.2 Hz), 7.98 (1H, dd, *J* = 6.4, 9.6 Hz); FAB-MS *m*/*z* 487, 489 [(M+H)$^+$].

5.31. 1-[2-(4-Chloro-2,5-difluorophenyl)-6,6-dioxido-5,7-dihydrothieno[3,4-*d*]pyrimidin-4-yl]piperidine-4-carboxylic acid hydrochloride (15a)

To a solution of 1-[2-(4-chloro-2,5-difluorophenyl)-6,6-dioxido-5,7-dihydrothieno[3,4-*d*]pyrimidin-4-yl]piperidine-4-carboxylate (**14g**, 1.11 g) in THF (12 mL) and ethanol (12 mL) was added 1 M aqueous sodium hydroxide (5 mL), and the mixture was stirred at room temperature for 8 h. To the mixture were added 1 M hydrochloric acid (5 mL) and water. The mixture was extracted with diethyl ether and the organic layer was washed with water and water, and then dried. The desiccant was removed by filtration and the filtrate was evaporated in vacuo to give a solid (500 mg, 44%). The obtained solid (100 mg) was dissolved with THF (10 mL), and to the solution was added 4 M hydrochloride solution in dioxane (1 mL). The mixture was evaporated in vacuo and the resulting residue was washed with diethyl ether to obtain **15a** (95 mg, 39%) as a white solid: ^{1}H NMR (DMSO-d_6) δ 1.50–1.70 (2H, m), 1.80–2.00 (2H, m), 2.51–2.70 (1H, m), 3.10–3.25 (2H, m), 3.60–5.20 (3H, m), 4.52 (2H, s), 4.70 (2H, s), 7.77 (1H, dd, *J* = 6.1, 10.0 Hz), 7.98 (1H, dd, *J* = 6.9, 9.8 Hz); ESI-MS *m*/*z* 444 [(M+H)$^+$]. Anal. ($C_{18}H_{16}N_3O_4SClF_2$·0.4HCl·1.25H_2O·0.2$C_4H_{10}O$): C, H, N, S, Cl, F.

The following compounds (**15b**–**d**) were prepared by a procedure similar to that described for **15a**.

5.32. {1-[2-(4-Chloro-2,5-difluorophenyl)-6,6-dioxido-5,7-dihydrothieno[3,4-*d*]pyrimidin-4-yl]piperidin-4-yl}aceticacid hydrochloride (15b)

Brown crystal (yield 65%); ^{1}H NMR (DMSO-d_6) δ 1.15–1.34 (2H, m), 1.70–1.84 (2H, m), 1.90–2.10 (1H, m), 2.19 (2H, d, *J* = 6.8 Hz), 2.98–3.15 (2H, m), 4.22–4.35 (2H, m), 4.52 (2H, s), 4.68 (2H, s), 7.76 (1H, dd, *J* = 6.4, 10.3 Hz), 7.97 (1H, dd, *J* = 6.6, 10.0 Hz); FAB-MS *m*/*z* 458, 460 [(M+H)$^+$]. Anal. ($C_{19}H_{18}N_3O_4SClF_2$·0.9HCl·0.25H_2O·0.15$C_4H_{10}O$·0.1C_2H_3N): C, H, N, S, Cl, F.

5.33. 3-{1-[2-(4-Chloro-2,5-difluorophenyl)-6,6-dioxido-5,7-dihydrothieno[3,4-*d*]pyrimidin-4-yl]piperidin-4-yl}propanoic acid (15c)

Pale yellow solid (yield 96%); ^{1}H NMR (DMSO-d_6) δ 1.08–1.26 (2H, m), 1.40–1.65 (3H, m), 1.66–1.82 (2H, m), 2.25 (2H, t, *J* = 7.4 Hz), 2.90–3.10 (2H, m), 4.20–4.40 (2H, m), 4.50 (2H, s), 4.68 (2H, s), 7.76 (1H, dd, *J* = 6.4, 10.0 Hz), 7.96 (1H, dd, *J* = 6.6, 10.2 Hz), 11.90–12.20 (1H, br); FAB-MS *m*/*z* 472, 474 [(M+H)$^+$].

5.34. 4-[2-(4-Chloro-2,5-difluorophenyl)-6,6-dioxido-5,7-dihydrothieno[3,4-*d*]pyrimidin-4-yl]piperazin-1-yl}acetic acid (15d)

Pale yellow solid (yield 98%); ^{1}H NMR (DMSO-d_6) δ 2.58–2.70 (4H, m), 3.21 (2H, s), 3.60–3.80 (4H, m), 2.70–4.00 (1H, br), 4.53 (2H, s), 4.71 (2H, s), 7.77 (1H, dd, *J* = 6.2, 10.2 Hz), 7.99 (1H, dd, *J* = 6.8, 10.0 Hz); FAB-MS *m*/*z* 459 [(M+H)$^+$].

5.35. 1-[2-(4-Chloro-2,5-difluorophenyl)-6,6-dioxido-5,7-dihydrothieno[3,4-*d*]pyrimidin-4-yl]-*N*-methylpiperidine-4-carboxamide (16a)

To a solution of 1-[2-(4-chloro-2,5-difluorophenyl)-6,6-dioxido-5,7-dihydrothieno[3,4-*d*]pyrimidin-4-yl]piperidine-4-carboxylic acid (free form of **15a**, 256 mg) in DMF (5 mL) were added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) hydrochloride (0.17 g), 1-hydroxybenzotriazole (HOBt, 0.12 g) and 2 M methylamine solution in THF (0.43 mL), and the mixture was stirred at room temperature for two days. To the mixture was added water and the solid was collected by filtration, washed with chloroform and ethyl acetate and dried in vacuo to obtain **16a** (205 mg, 78%) as a white solid: ^{1}H NMR (DMSO-d_6) δ 1.50–1.90 (2H, m), 2.35–2.55 (2H, m), 2.34–2.48 (1H, m), 2.57 (3H, d, *J* = 4.4 Hz), 3.00–3.20 (2H, m), 4.20–4.40 (2H, m), 4.52 (2H, s), 4.70 (2H, s), 7.70–7.81 (2H, m), 7.98 (1H, dd, *J* = 6.6, 10.0 Hz); FAB-MS *m*/*z* 457, 459 [(M+H)$^+$]. Anal. ($C_{19}H_{19}N_4O_3SClF_2$·0.4H_2O·0.01$CHCl_3$): C, H, N, S, Cl, F.

The following compounds (**16b**–**d**) were prepared by a procedure similar to that described for **16a**.

5.36. 2-{1-[2-(4-Chloro-2,5-difluorophenyl)-6,6-dioxido-5,7-dihydrothieno[3,4-*d*]pyrimidin-4-yl]piperidin-4-yl}acetamide (16b)

Colorless crystal (yield 60%); ^{1}H NMR (DMSO-d_6) δ 1.10–1.30 (2H, m), 1.65–1.80 (2H, m), 1.90–2.05 (3H, m), 2.95–3.10 (2H, m), 4.20–4.35 (2H, m), 4.51 (2H, s), 4.67 (2H, s), 6.76 (1H, s), 7.26 (1H, s), 7.76 (1H, dd, J = 6.0, 10.4 Hz), 7.97 (1H, dd, J = 6.8, 9.6 Hz); FAB-MS m/z 457 [(M+H)$^+$]. Anal. ($C_{19}H_{19}N_4O_3SClF_2\cdot H_2O$): C, H, N, S, Cl, F.

5.37. 3-{1-[2-(4-Chloro-2,5-difluorophenyl)-6,6-dioxido-5,7-dihydrothieno[3,4-*d*]pyrimidin-4-yl]piperidin-4-yl}propanamide hydrochloride (16c)

Pale yellow crystal (yield 94%); ^{1}H NMR (DMSO-d_6) δ 1.00–1.30 (2H, m), 1.37–1.64 (3H, m), 1.68–1.82 (2H, m), 2.02–2.15 (2H, m), 2.92–3.10 (2H, m), 4.20–4.40 (2H, m), 4.51 (2H, s), 4.68 (2H, s), 4.90–6.00 (1H, br), 6.50–7.00 (1H, br), 7.00–7.60 (1H, br), 7.76 (1H, dd, J = 6.2, 9.8 Hz), 7.97 (1H, dd, J = 6.8, 9.6 Hz); FAB-MS m/z 471, 473 [(M+H)$^+$]. Anal. ($C_{20}H_{21}N_4O_3SClF_2\cdot HCl\cdot 0.3H_2O\cdot 0.2C_4H_{10}O$): C, H, N, S, Cl, F.

5.38. 2-{4-[2-(4-Chloro-2,5-difluorophenyl)-6,6-dioxido-5,7-dihydrothieno[3,4-*d*]pyrimidin-4-yl]piperazin-1-yl}acetamide hydrochloride (16d)

Slightly yellow crystal (yield 91%); ^{1}H NMR (DMSO-d_6) δ 3.20–3.80 (6H, m), 3.99 (2H, s), 4.25–4.55 (2H, m), 4.61 (2H, s), 4.79 (2H, s), 5.90–7.00 (1H, br), 7.71 (1H, s), 7.79 (1H, dd, J = 6.2, 10.2 Hz), 8.05 (1H, dd, J = 6.6, 9.8 Hz), 8.13 (1H, s), 10.40–11.30 (1H, br); FAB-MS m/z 458, 460 [(M+H)$^+$]. Anal. ($C_{18}H_{18}N_5O_3SClF_2\cdot 1.6HCl\cdot 0.25H_2O$): C, H, N, S, Cl, F.

5.39. Human GPR119 cAMP reporter assay

Human GPR119 agonist activity was evaluated in HEK293 cells stably expressing human GPR119 and pCRE-Luc. HEK293-hGPR119 cells were seeded in 96-well plates at 2.5×10^4 cells/well, incubated overnight at 37 °C in 5% CO_2, and then exposed to the test compound dissolved in DMSO at concentrations ranging from 0.01 to 10 μM. After 6 h incubation, cells were harvested using 0.2% Triton X-100 in phosphate-buffered saline (pH 7.4). Luciferase activity was measured using a model ML-3000 Luminometer (Dynex Tech, VA, USA). Three replicates for each concentration were performed.

5.40. Oral glucose tolerance test (OGTT)

Eight-week-old male ICR mice were fasted overnight and then orally administered 0.5% methyl-cellulose (vehicle) or 10 mg/kg test compounds. After 10 min, glucose was given orally at a dose of 2 g/kg/10 mL, and a blood sample was collected from a tail vein after 30 min. Blood glucose levels were determined using the Glucose CII test (Wako, Osaka, Japan).

5.41. Solubility test in pH 6.8 buffer solution with the precipitation method

To 13 μL of a 10 mM DMSO solution of a test compound that had been prepared in advance was added exactly 1 mL of a second liquid (pH 6.8) for a disintegration test of Japanese Pharmacopoeia, followed by shaking at 25 °C for 20 h, thereby giving a sample stock solution. Next, using a filter impregnated with 200 μL of the sample stock solution, 200 μL of a fresh sample stock solution was added for filtration to obtain a liquid, which was taken as a sample solution. Separately to this, to 10 μL of the 10 mM DMSO solution of the test compound was added accurately 1 mL of methanol, followed by stirring, thereby giving a standard solution. 10 μL portions each of the sample solution and standard solution were tested by liquid chromatography, and the ratio of the peak area of the sample solution to the peak area of the standard solution was determined, thereby calculating the solubility.

5.42. Homology modeling and ligand docking

Crystal structures of GPCRs are now available for rhodopsin, adrenergic, and adenosine receptors in both inactive and activated forms, as well as for chemokine, dopamine, and histamine receptors in inactive conformations.[21] Homology between the hGPR119 sequence and transmembrane domain sequence of these seven families were compared using MOE,[22] during which human A_{2A} adenosine receptor ($hA_{2A}AR$) was found to be the highest. A homology model of hGPR119 was constructed using the crystal structure of agonist-bound $hA_{2A}AR$ (PDB code 3QAK),[19] obtained from the RCSB Protein Data Bank, as a structural template. Sequence alignment between hGPR119 and $hA_{2A}AR$ was created using PRIME,[23] with the ICL3 loop of $hA_{2A}AR$ excluded and manual revision. Homology modeling was performed using PRIME.[23]

The ligand molecule was sketched in Maestro[24] and energy-minimized using Confgen[25] with the OPLS_2005 force field.

A docking study was performed using GOLD.[26] The ligand binding pocket was defined using the O from Thr86 as a central atom with radius of 10 Å. The ligand molecule was docked 10 times. The top scoring pose, as assessed by goldscore, was employed for discussions. The two-dimensional diagram was prepared using the ligand interactions application in MOE.[22]

Acknowledgments

We are grateful to Dr. Masakazu Imamura for his useful advice, and to the staff of the Division of Analysis & Pharmacokinetics Research Laboratories for the elemental analysis, spectral measurements and solubility test.

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