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Discovery and biological evaluation of novel 4-amino-2-phenylpyrimidine derivatives as potent and orally active GPR119 agonists

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ABSTRACT

Novel 4-amino-2-phenylpyrimidine derivatives were synthesized and evaluated as GPR119 agonists. Optimization of the substituents on the phenyl ring at the 2-position and the amino group at the 4-position led to the identification of 3,4-dihalogenated and 2,4,5-trihalogenated phenyl derivatives showing potent GPR119 agonistic activity. The advanced analog (2*R*)-3-{[2-(4-chloro-2,5-difluorophenyl)-6-ethylpyrimidin-4-yl]amino}propane-1,2-diol (**24g**) was found to improve glucose tolerance at 1 mg/kg po in mice and to show excellent pharmacokinetic profiles in mice and monkeys. Compound **24g** also showed an excellent antidiabetic effect in diabetic kk/Ay mice after one week of single daily treatment. These results demonstrate that novel GPR119 agonist **24g** improves glucose tolerance not only by enhancing glucose-dependent insulin secretion but also by preserving pancreatic β-cell function.

1. Introduction

Depletion of glucose-dependent insulin secretion (GDIS) and particularly the loss of the first phase of GDIS are characteristic features in the pathology of type 2 diabetes mellitus (T2DM), which results in postprandial hyperglycemia.[1] For the clinical management of T2DM patients, sulfonylureas (SUs) are the most widely used hypoglycemic agents,[2] despite reports that these compounds can cause severe hypoglycemia due to their glucose-independent mode of action.[3] Recent strategies for promoting normoglycemia have focused on enhancing GDIS through G protein-coupled receptors such as the glucagon-like peptide 1 (GLP-1) receptor. Although GLP-1 analogs such as exendin-4 have been shown to effectively stimulate GDIS and preserve pancreatic β cells, these analogs can cause gastrointestinal side effects and pancreatitis and require parenteral administration.[4] Such limitations for SUs and GLP-1 analogs underscore the need for oral insulin secretagogues that preserve the first phase of GDIS and induce normoglycemia in T2DM patients.

The G-protein-coupled receptor 119 (GPR119) is predominantly expressed in pancreatic β cells and intestinal L-cells, and its activation directly promotes GDIS and indirectly increases GLP-1 levels through the accumulation of the intracellular cAMP, followed by a decrease in blood glucose levels,[5] suggesting GPR119 as an attractive drug target for treating T2DM. To date, several classes of small molecule GPR119 agonists, such as AR231453 (**1**) and PSN632408 (**2**) (Fig. 1), have been reported to show insulinotropic and antiobesity effects,[6] and several clinical trials of GPR119 agonists are currently ongoing.[6]

We have identified distinct structures that function as GPR119 agonists.[7] For example, high-throughput screening of the Astellas corporate compound library revealed the 4-amino-2-phenylpyrimidine derivative **5** as a novel GPR119 agonist. We recently published initial structure–activity relationship (SAR) studies of 4-amino-2-phenylpyrimidine derivatives, finding compound **6** has moderate GPR119 agonistic activity, good potency in an oral glucose tolerance test (OGTT) in vivo and favorable pharmacokinetic (PK) profiles.[8]

Here, we describe further optimization of **6** as a GPR119 agonist including SAR studies and evaluations of in vivo efficacy in diabetic kk/Ay mice.

2. Chemistry

The synthesis of commercially unavailable benzonitrile intermediates is described in Scheme 1. Rosenmund-von Braun reaction of commercially available 4-bromo-2,6-difluoroaniline **7**, followed by Sandmayer reaction in the presence of CuBr gave 4-bromo-3,5-difluorobenzonitrile **9g**[9] in moderate yield. Benzonitrile **9h** was readily prepared from 1,4-dibromo-2,5-difluorobenzene **10** in four steps. Lithium halogen exchange of **10**, followed by trapping with dry ice yielded carboxylic acid **11a**,[10] which was transformed to benzonitrile **9h** via acid chloride and carboxamide intermediates

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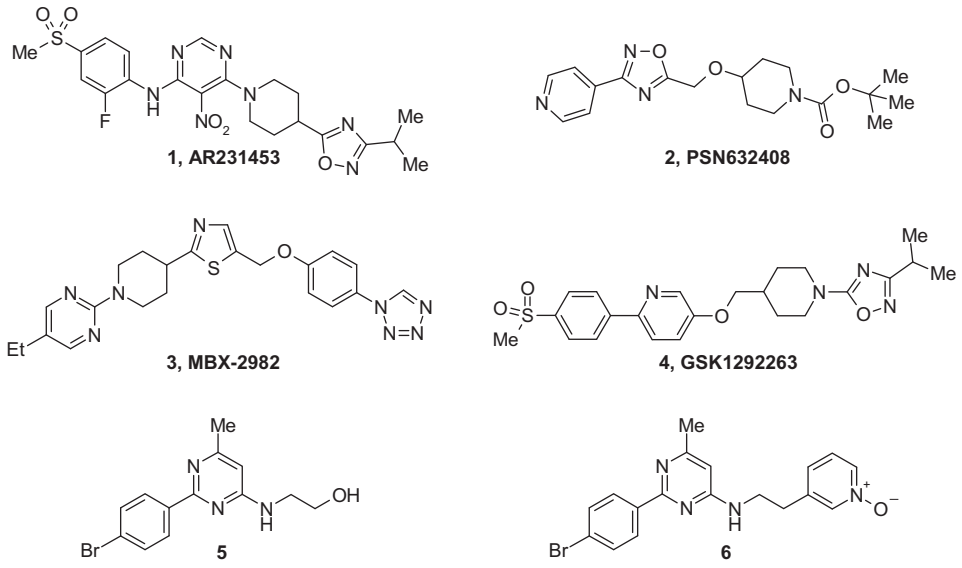
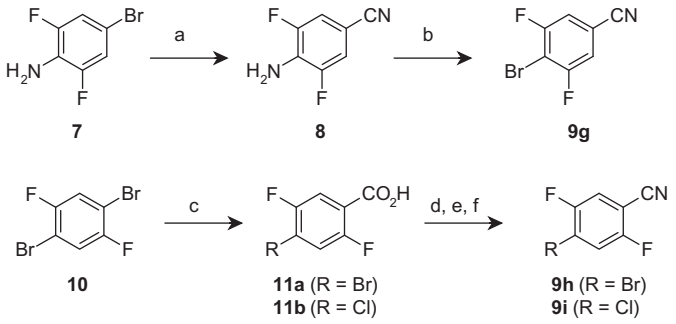


Figure 1. Structures of GPR119 agonists (AR231453 [**1**], PSN632408 [**2**], MBX-2982 [**3**], GSK1292263 [**4**], **5** and **6**).



Scheme 1. Synthesis of benzonitriles **9g–i**. Reagents and conditions: (a) CuCN, NMP, reflux; (b) $NaNO_2$, concd H_2SO_4, 47% HBr, CuBr, AcOH, rt; (c) *n*-BuLi/hexane, Et_2O, dry ice, −78 °C to rt; (d) $SOCl_2$, cat. DMF, 80 °C; (e) 28% aq NH_3, $CHCl_3$, 5 °C; (f) $POCl_3$, 80 °C.

in good yield. In the same manner, 4-chloro-2,5-difluorobenzonitrile **9i** was prepared from the corresponding carboxylic acid **11b** in high yield.

The key intermediates **14b–m** were prepared via a procedure similar to that described in our previous report (Scheme 2). Benzonitriles **9b–i** were first converted into amidines **12b–i** in two steps, and condensation of **12b–i** and the corresponding ketoesters under basic conditions gave pyrimidones **13b–m**, which were treated with phosphorous oxychloride to afford 4-chloropyrimidines **14b–m**.

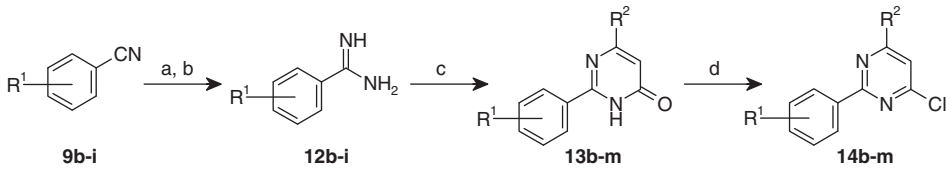
The synthesis of the pyridine N-oxide derivatives **17a–i** listed in Table 1 is shown in Scheme 3. Protection of the amino group in **15** by a Boc group followed by oxidation with *m*-chloroperbenzoic acid (*m*-CPBA) and deprotection of the Boc group gave the intermediate **16** in high yield. The foregoing 4-chloropyrimidines **14a–i** were subjected to substitution with amine **16** to obtain the desired **17a–i**.

The synthesis of the 2-pyridone derivatives **23a–d** is outlined in Scheme 4. The intermediate amine **21** was prepared from methyl 2-chloroisonicotinate **18** in five steps. Substitution of **18** with sodium methoxide, followed by reduction with lithium aluminum hydride gave 2-methoxypyridin-4-ylmethanol **19** in good yield. Chlorination of **19** with thionylchloride, followed by substitution with potassium cyanide and catalytic hydrogenation with Raney®-nickel gave **21**. Substitution of the 4-chroropyrimidines **14d**, **14h**, **14i**, and **14m** with amine **21**, followed by demethylation with hydrobromic acid gave the desired 2-pyridone derivatives **23a–d**.

As outlined in Scheme 5, the diol derivatives **24a–g** were easily prepared from the 4-chroropyrimidines **14d** and **14j–m** by substitution with chiral 3-amino-1,2-propanediols.

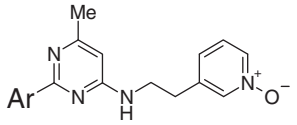
3. Results and discussion

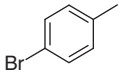
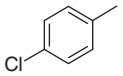
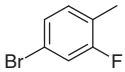
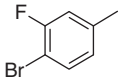
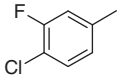
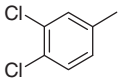
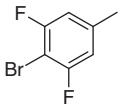
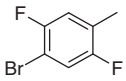
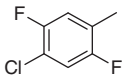
The synthesized compounds were evaluated for their agonistic activities toward GPR119 using a cAMP reporter assay system in which HEK293 cells were transfected with human GPR119 and cAMP responsive element (pCRE)-luciferase expression plasmids.[11] In this assay system, we relatively evaluated our compounds by comparing their EC and IA values with those of lead compound **5**. The EC values refer to the concentration of the tested compounds in this assay system at which they showed as potent efficacy as compound **5** did at 10 μM. The IA values refer to the relative activity (%) of the tested compounds compared to the efficacy of compound **5** at 10 μM in the same assay system.[8,12]



Scheme 2. Synthesis of 4-chloro-2-phenylpyrimidine derivatives **14b–m**. Reagents and conditions: (a) HCl, EtOH, rt; (b) $(NH_4)_2CO_3$, EtOH, rt; (c) $R^2COCH_2CO_2Me$, NaOMe, MeOH, rt; (d) $POCl_3$, 80 °C.

Table 1
In vitro SARs and in vivo antihyperglycemic effects for analogs with substitution of halogen groups in the phenyl moiety



Compound	Ar-	GPR119/pCRE EC[a] (μM)	GPR119/pCRE IA[b] (%)	OGTT (3 mg/kg po)[c] % Decrease
6		1.2	325	NE[f]
17a		NE[d]	ND[e]	(38 at 10 mg/kg po) NT[g]
17b		0.70	376	NT[g]
17c		1.5	268	19
17d		0.22	573	30
17e		0.33	450	25
17f		0.72	430	15
17g		0.34	493	15
17h		0.11	588	23
17i		0.21	536	27

[a] Concentration of tested compounds equipotent to the efficacy of **5** at 10 μM in human recombinant cell-based assays. See Section 5.
[b] Relative efficacy of tested compounds at 10 μM compared with efficacy of **5** at 10 μM. See Section 5.
[c] Antihyperglycemic effects of tested compounds in male ICR mice at 3 mg/kg po. See Section 5.
[d] Not effective at 10 μM.
[e] Not determined.
[f] Not effective at 3 mg/kg po.
[g] Not tested.

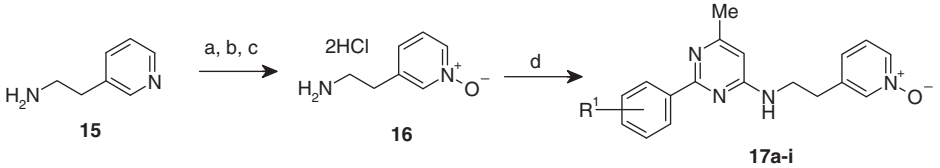
First, we carried out the optimization of substituents in the phenyl moiety of compound **6** (Table 1). Preliminary conversion of the bromo group in **6** indicated that the SARs information in the phenyl moiety obtained by a previous study of lead compound **5**[8] is applicable to the pyridine N-oxide derivative **6**. That is, removal of the bromo group (compound **17a**) resulted in a loss of activity, whereas the chloro derivative **17b** retained potent activity. Because the halogen group at the 4-position in the phenyl moiety was found to be important for agonistic activity, introduction of additional halogen groups was investigated. Introduction of a fluoro group at the 2-position (compound **17c**) was found to have no effect on the agonistic activity. In contrast, the 3-fluoro derivatives **17d** and **17e** showed improved agonistic activity over compounds **6** and **17b**, respectively. These results indicated that the introduction of an additional fluoro group at the 3-position in the phenyl moiety should be particularly preferred for agonistic activity. Introduction of an additional chloro group to compound **17b** at the 3-position (compound **17f**) reduced the agonistic activity, indicating that a small halogen group at the 3-position in the phenyl moiety would be favorable for agonistic activity.

Based on the above results, the trihalogenated compounds **17g** and **17h** were designed and their agonistic activities towards GPR119 were evaluated. The 4-bromo-3,5-difluorophenyl derivative **17g** was found to be slightly less active than the 4-bromo-3-fluorophenyl derivative **17d**. In contrast, a two-fold enhancement in activity was confirmed in the 4-bromo-2,5-difluorophenyl derivative **17h**. From these observations, we concluded that the introduction of 2,5-difluoro groups to compound **6** was more favorable than that of 3,5-difluoro groups, and the 4-bromo-2,5-difluorophenyl derivative **17h** showed an approximately 10-fold improvement in GPR119 agonistic activity over compound **6**. In a similar fashion, the 4-chloro-2,5-difluorophenyl derivative (compound **17i**) was found to show in vitro activity almost equal in potency to that of **17h**.

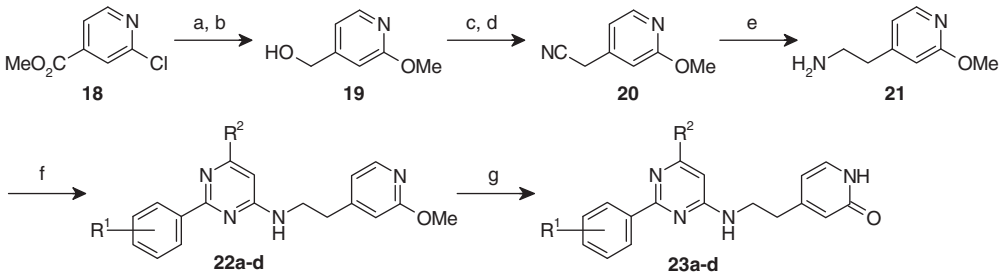
We also evaluated the antihyperglycemic effects of **6** and **17c–i** via OGTT in male ICR mice. Although no antihyperglycemic effects of the parent compound **6** were observed at 3 mg/kg po, the 3,4-dihalogenated (**17d** and **17e**) and the 2,4,5-trihalogenated (**17h** and **17i**) phenyl derivatives all demonstrated clear effects at this dose as a result of the improved in vitro agonistic activities.

As described above, modification of the phenyl moiety in the pyridine N-oxide derivative **6** led to the identification of compounds with potent in vitro and in vivo activities. However, these compounds also showed CYP1A2 inhibitory activity (**17h**/**17i**: IC_{50} = 7.2/3.7 μM). We attributed this inhibition to a 4-amino group[8] and therefore considered replacing the pyridine N-oxide structure of compounds **17d**, **17e**, **17h**, and **17i**, with the goal of reducing the CYP1A2 inhibitory activity but preserving the potent GPR119 agonistic activity.

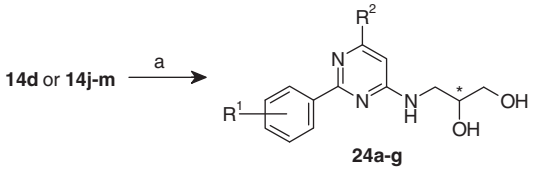
To this end, we introduced a 2-pyridone structure that mimics pyridine N-oxide into the above compounds. As can be seen in Table 2, the 2-(2-oxo-1,2-dihydropyridin-4-yl)ethylamino derivative **23a** showed 10 times more potent activity than corresponding pyridine N-oxide **17d**. The 2,4,5-trihalogenated phenyl derivatives **23b–d** further improved the agonistic activity of **23a**, especially compound **23c**, which had the most potent activity and an EC value in the nano molar range. Compounds **23a–d** were evaluated for their antihyperglycemic effects via OGTT. While we did not observe significant effects for the 3,4-dihalogenated phenyl derivative **23a** at 3 mg/kg po, the 2,4,5-trihalogenated phenyl derivatives **23b–d** all demonstrated antihyperglycemic effects at this dose. These results indicated that the introduction of a fluoro group onto the 2-position in the phenyl moiety is important for instilling in vivo activity and may contribute to the improved PK profiles. In



Scheme 3. Synthesis of compounds **17a–i**. Reagents and conditions: (a) $(Boc)_2O$, THF, rt; (b) *m*-CPBA, $CHCl_3$, rt; (c) 4 M HCl/EtOAc, Et_2O, rt; (d) **14a–i**, K_2CO_3, DMI, 80 °C.



Scheme 4. Synthesis of compounds **23a–d**. Reagents and conditions: (a) NaOMe, dioxane, reflux; (b) $LiAlH_4$, THF, 0 °C; (c) $SOCl_2$, 0 °C; (d) KCN, 18-crown-6, MeCN, rt; (e) 1 atm H_2, Raney®-Ni, 28% aq NH_3, EtOH, rt; (f) **14d**, **14h**, **14i** or **14m**, K_2CO_3, DMI, 80 °C; (g) 48% HBr, 80 °C.



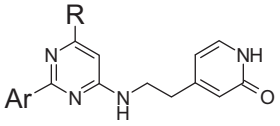
Scheme 5. Synthesis of compounds **24a–g**. Reagents and conditions: (a) (*S* or *R*)-3-amino-1,2-propanediol, *i*-Pr_2NEt, MeCN, 70 °C.

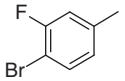
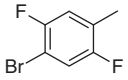
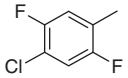
contrast, despite their potent in vitro activity, the 2-pyridone derivatives **23a–d** showed only moderate in vivo activity, comparable to activity achieved with the pyridine N-oxide derivatives **17d**, **17h**, and **17i**. We therefore speculated that **23a–d** have relatively poor PK profiles compared with **17d**, **17h**, and **17i**.

We previously found that 4-(substituted)ethylamino derivatives such as hydroxy, 1-oxidopyridyl and 2-oxo-1,2-dihydropyridyl derivatives show potent GPR119 agonistic activity,[8] implying that some oxy functional group in this region could stimulate potent GPR119 agonistic activity. Therefore, the introduction of a 1,2-diol structure to the 4-ethylamino group was examined (Table 3). Compound **24a** showed about 10-fold less activity than **17d**. However, (*R*)-isomer **24b** showed approximately two times more potent activity than **24a**, indicating that the (*R*)-configuration of the 2,3-dihydroxypropylamino group demonstrated superior GPR119 agonistic activity to the (*S*)-configuration. In addition, the 6-ethyl derivatives **24c** and **24d** showed approximately five-fold improved GPR119 agonistic activity over the 6-methyl derivatives **24a** and **24b**, and (*R*)-isomer **24d** in particular was found to have potent agonistic activity with an EC value in the 10^{-7} molar range. These results indicated that the ethyl group was more favorable than the methyl group as the substituent at the 6-position in the pyrimidine ring for GPR119 agonistic activity. The 4-chloro-3-fluorophenyl derivative **24e** and the 2,4,5-trihalogenated phenyl derivatives **24f** and **24g** showed potent GPR119 agonistic activities with respective EC values of 0.39 μM, 0.22 μM, and 0.28 μM.

We then evaluated the antihyperglycemic effects at a lower dose of the five compounds **24c–g** that showed potent agonistic activities. Surprisingly, the 2,3-dihydroxypropylamino derivatives **24d**, **24e**, and **24g** demonstrated excellent potency in vivo as assessed via OGTT at 1 mg/kg po, and their activity was found to be more potent than that of pyridine N-oxide derivatives despite showing equipotent in vitro activity. From these observations, we speculated that the improved PK profiles of **24d**, **24e,** and **24g** could result in potent in vivo activity compared with those of pyridine N-oxide derivatives. On evaluation of the CYP1A2 inhibitory activities of the compounds listed in Table 3, the 3,4-dihalogenated phenyl derivatives **24b–e** inhibited CYP1A2 with IC_{50} values in the 10^{-6} molar range. In contrast, the 2,4,5-trihalogenated phenyl derivatives **24f** and **24g** were found to be 10- to 20 times less potent than the 3,4-dihalogenated phenyl derivatives for CYP1A2 inhibitory activity. Examination of the in vitro metabolic stability for the 2,3-dihydroxypropylamino derivatives **24d**, **24e** and **24g** found that the 3,4-dihalogenated phenyl derivatives **24d** and **24e** were relatively unstable in human liver microsomes (168 and 236 mL/min/kg, respectively). Introducing a 2,4,5-trihalogenated phenyl group (compound **24g**), however, significantly improved

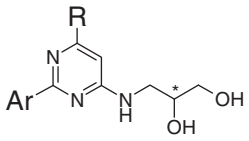
Table 2
In vitro SARs and in vivo antihyperglycemic effects for 2-(2-oxo-1,2-dihydropyridin-4-yl)ethylamino derivatives

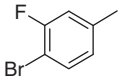
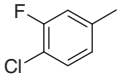
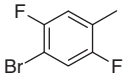
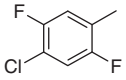


Compound	Ar-	R-	GPR119/pCRE EC[a] (μM)	GPR119/pCRE IA[b] (%)	OGTT (3 mg/kg po) [c] % Decrease
23a		Me-	0.021	617	10
23b		Me-	0.017	1077	23
23c		Me-	0.0069	722	21
23d		Et-	0.013	578	25

[a–c] See the corresponding footnotes to Table 1.

Table 3
In vitro SARs, in vivo antihyperglycemic effects and CYP1A2 inhibitory activities for 2,3-dihydroxypropylamino derivatives



Compound	Ar-	R-	Configuration[e]	GPR119/pCRE		OGTT (1 mg/kg po)[c]	CYP 1A2
				EC[a] (μM)	IA[b] (%)	% Decrease	IC_{50} (μM)
24a		Me-	*S*	2.3	257	NT[d]	NT[d]
24b		Me-	*R*	1.2	346	NT[d]	1.7
24c		Et-	*S*	0.59	283	16	1.6
24d		Et-	*R*	0.19	284	23	1.5
24e		Et-	*R*	0.39	266	24	2.1
24f		Et-	*R*	0.22	391	19	25
24g		Et-	*R*	0.28	497	25	35

[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 experimental section.
[d] Not tested.
[e] Configuration of the chiral 2,3-dihydroxypropylamino group.

Table 4
Pharmacokinetic data for compound **24g** in mice and monkeys

Species	Route	Dose (mg/kg)	T_{max} (h)	$t_{1/2}$ (h)	V_{dss} (L/kg)	CL_{tot} (mL/min/kg)	Bioavailability (%)
Mice	iv	1		2.2	6.9	35.9	
	po	3	0.1				86
Monkeys	iv	1		4.6	4.7	12.0	
	po	3	2.2				51

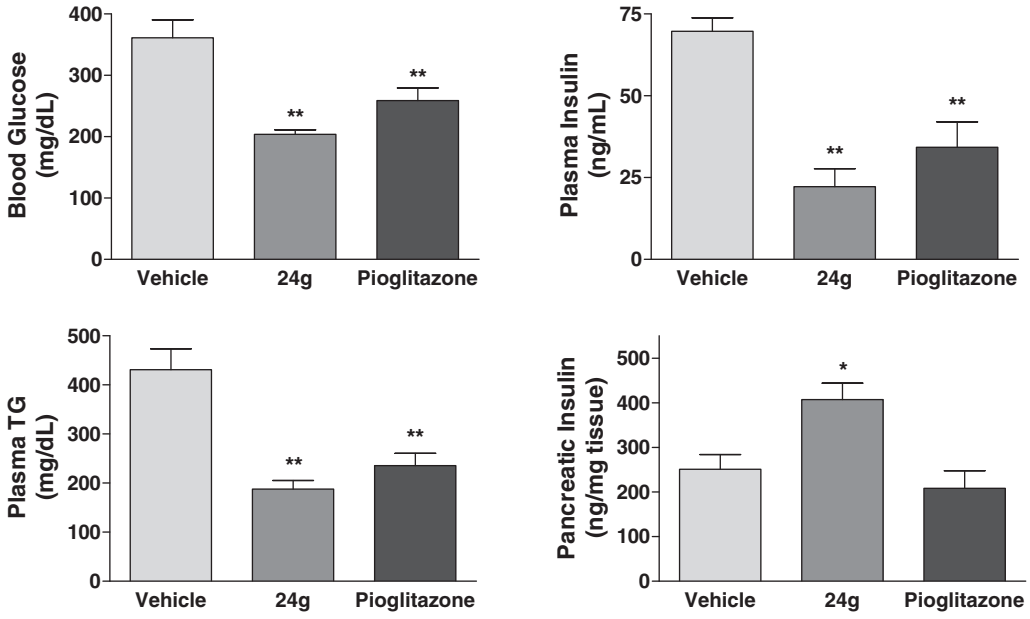


Figure 2. Effect of repeated administration in male kk/Ay mice (3 mg/kg po, 1 week once daily treatment, $n = 8$). Data are presented as the mean ± SE. $^{*}p < 0.05$, $^{**}p < 0.01$ versus vehicle group, as determined using the Dunnett's multiple comparison test.

the metabolic stability (<20 mL/min/kg). From these findings, we speculated that the phenyl moiety at the 2-position in the pyrimidine ring played important roles in both the recognition and metabolism of compounds by CYP enzymes, and lowering the electron density of this phenyl moiety contributed to the reduction in CYP1A2 inhibitory activity and improvement of the metabolic stability of the given compounds. In addition, the improved in vitro GPR119 agonistic activity and metabolic stability of compound **24g** was considered to contribute to its potent antihyperglycemic effect in vivo in the OGTT.

The PK profiles of compound **24g** in mice and monkeys are shown in Table 4. This compound showed moderate total clearance, high volume of distribution, good half-life after oral dosing, and excellent oral bioavailability in both animals, and was therefore selected for further in vivo studies using animal models of T2DM.

To evaluate the effects of chronic treatment with GPR119 agonist, eight-week-old male diabetic kk/Ay mice were treated once daily at 3 mg/kg with compound **24g**, pioglitazone and vehicle for a week. After the treatment period, the blood glucose, plasma insulin, plasma triglyceride (TG) and pancreatic insulin levels were measured (Fig. 2). Compound **24g** significantly reduced blood glucose, plasma insulin and TG levels at potencies equal to or exceeding that of pioglitazone. Further, unlike pioglitazone, compound **24g** also improved pancreatic insulin levels. These results demonstrate that novel GPR119 agonist **24g** not only effectively controls glucose and lipid levels to a similar degree as pioglitazone, the compound also preserves pancreatic β-cell function in diabetic kk/Ay mice.

4. Conclusion

We optimized the 4-amino-2-phenylpyrimidine derivative **6** to develop a novel and potent GPR119 agonist for use in treating T2DM. Optimization of the substituents in the phenyl moiety of compound **6** led to the identification of the 2,4,5-trihalogenated phenyl derivatives and an approximately 10-fold improvement in GPR119 agonistic activity. Subsequent replacement of the amino group at the 4-position in the pyrimidine ring gave novel compound **23c**, which contains a 2-(2-oxo-1,2-dihydropyridin-4-yl)ethylamino group and possesses highly potent agonistic activity with an EC value of 6.9 nM. Further replacement of the amino group with a 2,3-dihydroxypropylamino group led to the identification of (2*R*)-3-{[2-(4-chloro-2,5-difluorophenyl)-6-ethylpyrimidin-4-yl]amino}propane-1,2-diol (**24g**) as an advanced analog. Compound **24g** was found to improve glucose tolerance at 1 mg/kg po in mice, showed excellent PK profiles in mice and monkeys, and improved blood glucose, plasma insulin, triglyceride levels and pancreatic insulin content in diabetic kk/Ay mice after one week of single daily treatment. These results suggest that novel GPR119 agonist **24g** not only improves glucose tolerance through enhancing GDIS, but also preserves pancreatic β-cell function. We therefore propose that **24g** represents a new type of antihyperglycemic agent with promising potential for the effective treatment of T2DM.

5. Experimental

5.1. Chemistry

^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL JNM-LA300 or a JEOL JNM-EX400 spectrometer and were referenced to an internal standard, tetramethylsilane. The abbreviations of ^{1}H NMR 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, a Hitachi M-80 or a Waters ZQ-2000 mass spectrometer. The elemental analyses were performed with a Yanako MT-5 microanalyzer (C, H, N) and a Yokogawa IC-7000S ion chromatographic analyzer (halogens). 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. 4-Amino-3,5-difluorobenzonitrile (8)

A mixture suspension of 4-bromo-2,6-difluoroaniline (**7**, 25.85 g) and copper(I) cyanide (16.70 g) in NMP (60 mL) was stirred at reflux temperature for 1.5 h and then cooled down to room temperature. To the mixture was added 1,2-diaminoethane (23 mL) and the mixture was poured into water (150 mL). The mixture was extracted with ethyl acetate and the organic layer was washed with 10 wt % 1,2-diaminoethane solution in water and water, and then 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 **8** (16.54 g, 86%) as a pale yellow solid: ^{1}H NMR (DMSO-d_6) δ 6.36 (2H, s), 7.50 (2H, dd, J = 2.7, 6.7 Hz); EI-MS m/z 154 [$(M)^+$].

5.3. 4-Bromo-3,5-difluorobenzonitrile (9g)

To concentrated sulfuric acid (25 mL) was added sodium nitrite (3.20 g) portionwise at 5 °C, and the mixture was stirred at room temperature for 0.5 h. The mixture was cooled down to 5 °C, and then acetic acid (40 mL) was added dropwise to the mixture. The mixture was stirred at 5 °C for 5 min. To the mixture was added 4-amino-3,5-difluorobenzonitrile (**8**, 6.50 g) portionwise, and then the mixture was stirred at room temperature for 1 h. The mixture was transferred into a dropping funnel, and was added dropwise to a solution of copper(I) bromide (9.07 g) in 47 wt % hydrobromic acid (25 mL) over 0.5 h. The mixture was stirred at room temperature for 13 h. Water (300 mL) was added to the mixture, and the mixture was extracted with ethyl acetate. The organic layer was washed with brine, and then 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 **9g** (5.00 g, 54%) as a white solid: ^{1}H NMR (DMSO-d_6) δ 7.98 (2H, d, J = 6.3 Hz); EI-MS m/z 217, 219 [$(M)^+$].

5.4. 4-Bromo-2,5-difluorobenzoic acid (11a)

Under an argon atmosphere, to a solution of 1,4-dibromo-2,5-difluorobenzene (**10**, 70.00 g) in diethyl ether (500 mL) was added dropwise 1.58 M *n*-butyl lithium solution in hexane (171 mL) at −78 °C, and the mixture was stirred at same temperature for 2 min. The mixture was added quickly to the mixture of dry ice (about 300 g) and diethyl ether (600 mL), and the mixture was warmed up to room temperature. The precipitate was collected by filtration, and washed with diethyl ether. The obtained solid was treated with water (100 mL) and 1 M hydrochloric acid (500 mL), and extracted with diethyl ether. The organic layer was washed with brine and dried. The desiccant was removed by filtration and the filtrate was evaporated in vacuo. The resulting residue was washed with hexane, dried in vacuo to obtain **11a** (53.29 g, 83%) as a pale yellow solid: ^{1}H NMR (DMSO-d_6) δ 7.78 (1H, dd, J = 6.4, 8.3 Hz), 7.89 (1H, dd, J = 5.9, 9.8 Hz); FAB-MS m/z 235, 237 [$(M-H)^-$].

5.5. 4-Bromo-2,5-difluorobenzonitrile (9h)

A mixture of 4-bromo-2,5-difluorobenzoic acid (**11a**, 53.28 g), thionyl chloride (165 mL) and DMF (0.87 mL) was stirred at 80 °C for 1.5 h, and cooled down to room temperature. The mixture was evaporated in vacuo, and the resulting residue was dissolved in chloroform (300 mL). To the solution was added dropwise 28 wt % aqueous ammonia (300 mL) at 5 °C, and the mixture was stirred at 5 °C for 0.5 h. 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 to obtain a pale yellow solid. The mixture of the obtained solid and phosphoryl chloride (195 mL) was stirred at 80 °C for 2 h, and cooled down to room temperature. The mixture was evaporated in vacuo, and the resulting residue was treated with diethyl ether (500 mL)

and ice-water (300 mL), then stirred at room temperature for 0.5 h. The mixture was extracted with diethyl ether and the organic layer was washed with saturated aqueous sodium hydrogen carbonate and brine, and then dried. The desiccant was removed by filtration and the filtrate was evaporated in vacuo to obtain **9h** (41.57 g, 85%) as a pale yellow solid: ^{1}H NMR (DMSO-d_6) δ 8.15–8.25 (2H, m); EI-MS *m/z* 217, 219 [(M)$^+$].

5.6. 4-Chloro-2,5-difluorobenzonitrile (9i)

This compound was prepared from commercially available 4-chloro-2,5-difluorobenzoic acid **11b** by a procedure similar to that described for **9h**.

Light brown solid (yield 98%); ^{1}H NMR (DMSO-d_6) δ 8.08 (1H, dd, *J* = 6.3, 8.8 Hz), 8.25 (1H, dd, *J* = 5.6, 8.6 Hz); EI-MS *m/z* 173, 175 [(M)$^+$].

5.7. 4-Chlorobenzamidine hydrochloride (12b)

Hydrogen chloride gas was passed through a solution of 4-chlorobenzonitrile (**9b**, 25.0 g) in chloroform (350 mL) and ethanol (100 mL) at −78 °C for 0.5 h. Then the solution was warmed up to room temperature, and stirred at room temperature overnight. The solution was evaporated in vacuo, and the resulting residue was dissolved with ethanol (500 mL). To the solution was added ammonium carbonate (90.0 g), and the reaction mixture was stirred at room temperature for 3 days. To the mixture was added water (300 mL), and ethanol was removed by concentration in vacuo. The resulting solid was collected by filtration, washed with water and dried in vacuo to give **12b** (25.4 g, 71%) as a white solid: ^{1}H NMR (DMSO-d_6) δ 2.60–4.80 (2H, br), 7.53 (2H, d, *J* = 8.8 Hz), 7.81 (2H, d, *J* = 8.8 Hz), 7.50–9.50 (2H, br); FAB-MS *m/z* 155, 157 [(M+H)$^+$].

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

5.8. 4-Bromo-2-fluorobenzamidine (12c)

White solid (yield 48%); ^{1}H NMR (DMSO-d_6) δ 3.17 (1H, s), 6.38 (2H, s), 7.40–7.55 (2H, m), 7.58 (1H, dd, *J* = 1.6, 10.0 Hz); FAB-MS *m/z* 217,219 [(M)$^+$].

5.9. 4-Bromo-3-fluorobenzamidine (12d)

White solid (yield 60%); ^{1}H NMR (DMSO-d_6) δ 6.00–7.00 (3H, br), 7.60 (1H, dd, *J* = 1.5, 8.3 Hz), 7.70–7.80 (2H, m); EI-MS *m/z* 216, 218 [(M)$^+$].

5.10. 4-Chloro-3-fluorobenzamidine (12e)

Brown solid (yield 65%); ^{1}H NMR (DMSO-d_6) δ 6.10–7.10 (3H, br), 7.58–7.72 (2H, m), 7.79 (1H, dd, *J* = 2.0, 10.8 Hz); FAB-MS *m/z* 173, 175 [(M+H)$^+$].

5.11. 3,4-Dichlorobenzamidine (12f)

White solid (yield 68%); ^{1}H NMR (DMSO-d_6) δ 6.20–7.00 (3H, br), 7.67 (1H, d, *J* = 8.8 Hz), 7.80 (1H, dd, *J* = 2.0, 8.8 Hz), 8.03 (1H, d, *J* = 2.0 Hz); EI-MS *m/z* 188, 190 [(M)$^+$].

5.12. 4-Bromo-3,5-difluorobenzamidine (12g)

Pale yellow solid (yield 98%); ^{1}H NMR (DMSO-d_6) δ 6.20–6.90 (3H, br), 7.63–7.71 (2H, m); EI-MS *m/z* 234, 236 [(M)$^+$].

5.13. 4-Bromo-2,5-difluorobenzamidine (12h)

Pale yellow solid (yield 47%); ^{1}H NMR (DMSO-d_6) δ 5.80–7.20 (3H, br), 7.53 (1H, dd, *J* = 6.1, 9.0 Hz), 7.77 (1H, dd, *J* = 5.4, 9.3 Hz); EI-MS *m/z* 234, 236 [(M)$^+$].

5.14. 4-Chloro-2,5-difluorobenzamidine (12i)

Pale yellow solid (yield 35%); ^{1}H NMR (DMSO-d_6) δ 6.00–7.00 (3H, br), 7.58 (1H, dd, *J* = 6.1, 9.5 Hz), 7.69 (1H, dd, *J* = 6.1, 9.5 Hz); FAB-MS *m/z* 191, 193 [(M+H)$^+$].

5.15. 2-(4-Chlorophenyl)-6-methylpyrimidin-4-one (13b)

To a solution of 4-chlorobenzamidine hydrochloride (**12b**, 1.12 g) in methanol (15 mL) were added sodium methoxide (0.95 g) and methyl acetoacetate (0.73 g), and the reaction mixture was stirred at room temperature for 5 days. To the mixture was added 1 M hydrochloric acid (20 mL). The resulting precipitate was collected by filtration, washed with water, and dried in vacuo at 50 °C overnight to give **13b** (0.72 g, 56%) as a white solid: ^{1}H NMR (DMSO-d_6) δ 2.29 (3H, s), 6.26 (1H, s), 7.59 (2H, d, *J* = 7.8 Hz), 8.14 (2H, d, *J* = 7.8 Hz), 12.40–12.90 (1H, br); FAB-MS *m/z* 221, 223 [(M+H)$^+$].

The following compounds (**13c–m**) were prepared by a procedure similar to that described for **13b**.

5.16. 2-(4-Bromo-2-fluorophenyl)-6-methylpyrimidin-4-one (13c)

White solid (yield 83%); ^{1}H NMR (DMSO-d_6) δ 2.25 (3H, s), 6.26 (1H, s), 7.57 (1H, dd, *J* = 1.8, 8.2 Hz), 7.67 (1H, t, *J* = 8.0 Hz), 7.75 (1H, dd, *J* = 1.8, 10.2 Hz); FAB-MS *m/z* 283, 285 [(M)$^+$].

5.17. 2-(4-Bromo-3-fluorophenyl)-6-methylpyrimidin-4-one (13d)

White solid (yield 92%); ^{1}H NMR (DMSO-d_6) δ 2.31 (3H, s), 6.33 (1H, s), 7.86 (1H, dd, *J* = 7.3, 8.3 Hz), 7.95 (1H, dd, *J* = 2.0, 8.3 Hz), 8.07 (1H, dd, *J* = 2.0, 10.3 Hz); FAB-MS *m/z* 283, 285 [(M+H)$^+$].

5.18. 2-(4-Chloro-3-fluorophenyl)-6-methylpyrimidin-4-one (13e)

White solid (yield 75%); ^{1}H NMR (DMSO-d_6) δ 2.31 (3H, s), 6.32 (1H, s), 7.70–7.79 (1H, m), 7.99–8.07 (1H, m), 8.12 (1H, dd, *J* = 2.0, 10.3 Hz), 11.60–13.20 (1H, br); FAB-MS *m/z* 239, 241 [(M+H)$^+$].

5.19. 2-(3,4-Dichlorophenyl)-6-methylpyrimidin-4-one (13f)

White solid (yield 55%); ^{1}H NMR ($CDCl_3$) δ 2.40 (3H, s), 3.30–3.80 (1H, br), 6.32 (1H, s), 7.62 (1H, d, *J* = 8.3 Hz), 7.98 (1H, dd, *J* = 2.0, 8.3 Hz), 8.31 (1H, d, *J* = 2.0 Hz); FAB-MS *m/z* 255, 257 [(M+H)$^+$].

5.20. 2-(4-Bromo-3,5-difluorophenyl)-6-methylpyrimidin-4-one (13g)

White solid (yield 88%); ^{1}H NMR (DMSO-d_6) δ 2.33 (3H, s), 2.70–4.50 (1H, br), 6.40 (1H, s), 7.97 (2H, d, *J* = 7.8 Hz); FAB-MS *m/z* 301, 303 [(M+H)$^+$].

5.21. 2-(4-Bromo-2,5-difluorophenyl)-6-methylpyrimidin-4-one (13h)

Pale yellow solid (yield 97%); ^{1}H NMR (DMSO-d_6) δ 2.27 (3H, s), 6.30 (1H, s), 7.76 (1H, dd, *J* = 5.9, 8.8 Hz), 7.94 (1H, dd, *J* = 5.6, 9.5 Hz), 12.20–13.10 (1H, br); FAB-MS *m/z* 301, 303 [(M)$^+$].

5.22. 2-(4-Chloro-2,5-difluorophenyl)-6-methylpyrimidin-4-one (13i)

White solid (yield 91%); ^{1}H NMR (DMSO-d_6) δ 2.27 (3H, s), 6.30 (1H, s), 7.81 (1H, dd, J = 6.4, 9.3 Hz), 7.86 (1H, dd, J = 6.1, 9.7 Hz), 12.10–13.20 (1H, br); FAB-MS m/z 257, 259 [(M+H)$^+$].

5.23. 2-(4-Bromo-3-fluorophenyl)-6-ethylpyrimidin-4-one (13j)

White solid (yield 91%); ^{1}H NMR (DMSO-d_6) δ 1.21 (3H, t, J = 7.9 Hz), 2.59 (2H, q, J = 7.9 Hz), 6.30 (1H, s), 7.82–7.91 (1H, m), 7.92–8.02 (1H, m), 8.09 (1H, dd, J = 1.7, 10.0 Hz), 11.90–13.00 (1H, br); FAB-MS m/z 297, 299 [(M+H)$^+$].

5.24. 2-(4-Chloro-3-fluorophenyl)-6-ethylpyrimidin-4-one (13k)

White solid (yield 107%); ^{1}H NMR (DMSO-d_6) δ 1.21 (3H, t, J = 7.5 Hz), 2.59 (2H, q, J = 7.5 Hz), 6.30 (1H, s), 7.70–7.80 (1H, m), 7.98–8.09 (1H, m), 8.14 (1H, dd, J = 2.0, 10.8 Hz), 11.70–13.10 (1H, br); FAB-MS m/z 253, 255 [(M+H)$^+$].

5.25. 2-(4-Bromo-2,5-difluorophenyl)-6-ethylpyrimidin-4-one (13l)

Pale yellow solid (yield 82%); ^{1}H NMR (DMSO-d_6) δ 1.18 (3H, t, J = 7.6 Hz), 2.54 (2H, q, J = 7.6 Hz), 6.28 (1H, s), 7.77 (1H, d, J = 6.3, 8.8 Hz), 7.94 (1H, dd, J = 5.6, 9.5 Hz), 12.20–13.10 (1H, br); FAB-MS m/z 315, 317 [(M)$^+$].

5.26. 2-(4-Chloro-2,5-difluorophenyl)-6-ethylpyrimidin-4-one (13m)

Light brown solid (yield 67%); ^{1}H NMR (DMSO-d_6) δ 1.18 (3H, t, J = 7.6 Hz), 2.55 (2H, q, J = 7.6 Hz), 6.28 (1H, s), 7.82 (1H, d, J = 6.4, 9.3 Hz), 7.85 (1H, dd, J = 5.9, 9.8 Hz), 12.30–13.20 (1H, br); FAB-MS m/z 271, 273 [(M+H)$^+$].

5.27. 4-Chloro-2-(4-chlorophenyl)-6-methylpyrimidine (14b)

A mixture of 2-(4-chlorophenyl)-6-methylpyrimidin-4-one (**13b**, 0.71 g) and phosphorus oxychloride (10 mL) was stirred at 80 °C for 2.5 h. The mixture was cooled down to room temperature, and evaporated in vacuo. To the resulting residue was added water, and the solid was collected by filtration, washed with water and dried in vacuo to give **14b** (0.81 g, 105%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 2.57 (3H, s), 7.11 (1H, s), 7.44 (2H, d, J = 8.8 Hz), 8.40 (2H, d, J = 8.8 Hz); FAB-MS m/z 239, 241 [(M+H)$^+$].

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

5.28. 2-(4-Bromo-2-fluorophenyl)-4-chloro-6-methylpyrimidine (14c)

White solid (yield 99%); ^{1}H NMR (DMSO-d_6) δ 2.55 (3H, s), 7.59 (1H, d, J = 8.4 Hz), 7.64 (1H, s), 7.72 (1H, d, J = 10.8 Hz), 7.98 (1H, t, J = 8.4 Hz); FAB-MS m/z 301, 303 [(M)$^+$].

5.29. 2-(4-Bromo-3-fluorophenyl)-4-chloro-6-methylpyrimidine (14d)

Pale yellow solid (yield 111%); ^{1}H NMR (DMSO-d_6) δ 2.55 (3H, s), 7.60 (1H, s), 7.85 (1H, dd, J = 7.4, 8.6 Hz), 8.02–8.11 (2H, m); FAB-MS m/z 301, 303 [(M+H)$^+$].

5.30. 4-Chloro-2-(4-chloro-3-fluorophenyl)-6-methylpyrimidine (14e)

Pale yellow solid (yield 104%); ^{1}H NMR (DMSO-d_6) δ 2.56 (3H, s), 7.61 (1H, s), 7.71–7.79 (1H, m), 8.11–8.18 (2H, m); FAB-MS m/z 257, 259 [(M+H)$^+$].

5.31. 4-Chloro-2-(3,4-dichlorophenyl)-6-methylpyrimidine (14f)

White solid (yield 100%); ^{1}H NMR ($CDCl_3$) δ 2.58 (3H, s), 7.13 (1H, s), 7.54 (1H, d, J = 8.3 Hz), 8.29 (1H, dd, J = 2.1, 8.3 Hz), 8.56 (1H, d, J = 2.1 Hz); FAB-MS m/z 273, 275 [(M+H)$^+$].

5.32. 2-(4-Bromo-3,5-difluorophenyl)-4-chloro-6-methylpyrimidine (14g)

Pale yellow solid (yield 73%); ^{1}H NMR (DMSO-d_6) δ 2.58 (3H, s), 7.68 (1H, s), 7.97–8.05 (2H, m); FAB-MS m/z 319, 321 [(M+H)$^+$].

5.33. 2-(4-Bromo-2,5-difluorophenyl)-4-chloro-6-methylpyrimidine (14h)

Pale yellow solid (yield 96%); ^{1}H NMR (DMSO-d_6) δ 2.56 (3H, s), 7.67 (1H, s), 7.85–7.98 (2H, m); FAB-MS m/z 319, 321 [(M)$^+$].

5.34. 4-Chloro-2-(4-chloro-2,5-difluorophenyl)-6-methylpyrimidine (14i)

Pale yellow solid (yield 100%); ^{1}H NMR (DMSO-d_6) δ 2.56 (3H, s), 7.68 (1H, s), 7.74–7.89 (1H, m), 7.90–8.04 (1H, m); FAB-MS m/z 275, 277 [(M)$^+$].

5.35. 2-(4-Bromo-3-fluorophenyl)-4-chloro-6-ethylpyrimidine (14j)

Light brown solid (yield 103%); ^{1}H NMR (DMSO-d_6) δ 1.30 (3H, t, J = 7.6 Hz), 2.84 (2H, q, J = 7.6 Hz), 7.60 (1H, s), 7.87 (1H, dd, J = 7.3, 8.5 Hz), 8.04–8.14 (2H, m); FAB-MS m/z 315, 317 [(M+H)$^+$].

5.36. 4-Chloro-2-(4-chloro-3-fluorophenyl)-6-ethylpyrimidine (14k)

Brown solid (yield 106%); ^{1}H NMR NMR (DMSO-d_6) δ 1.30 (3H, t, J = 7.6 Hz), 2.83 (2H, q, J = 7.6 Hz), 7.57 (1H, s), 7.66–7.75 (1H, m), 8.06–8.15 (2H, m); FAB-MS m/z 271, 273 [(M+H)$^+$].

5.37. 2-(4-Bromo-2,5-difluorophenyl)-4-chloro-6-ethylpyrimidine (14l)

Pale yellow oil (yield 99%); ^{1}H NMR (DMSO-d_6) δ 1.29 (3H, t, J = 7.5 Hz), 2.84 (2H, q, J = 7.5 Hz), 7.65 (1H, s), 7.87 (1H, d, J = 5.7, 10.1 Hz), 7.92 (1H, dd, J = 6.3, 9.3 Hz); FAB-MS m/z 333, 335 [(M)$^+$].

5.38. 4-Chloro-2-(4-chloro-2,5-difluorophenyl)-6-ethylpyrimidine (14m)

Dark brown oil (yield 97%); ^{1}H NMR (DMSO-d_6) δ 1.28 (3H, t, J = 7.5 Hz), 2.84 (2H, q, J = 7.5 Hz), 7.66 (1H, s), 7.80 (1H, d, J = 6.1, 10.0 Hz), 7.98 (1H, dd, J = 6.9, 9.8 Hz); FAB-MS m/z 289, 291 [(M)$^+$].

5.39. 2-(1-oxidopyridin-3-yl)ethylamine hydrochloride (16)

To a solution of 2-(3-pyridyl)ethylamine (**15**, 50.22 g) in THF (500 mL) was added di-*tert*-butyl dicarbonate (94.20 g) portionwise at 5 °C, and then the mixture was stirred at room temperature

for 3 h. The mixture was diluted with ethyl acetate, and then water and 1 M aqueous sodium hydroxide were added. The mixture was extracted with ethyl acetate, and the organic layer was washed with brine and dried. The desiccant was removed by filtration and the filtrate was evaporated in vacuo to obtain a pale yellow oil. The obtained oil was dissolved in chloroform (1000 mL) and to the solution was added *m*-CPBA (about 65% purity, 111.47 g), and the mixture was stirred at room temperature for 2.5 h. To the reaction mixture were added a solution of sodium thiosulfate (20.00 g) in water (100 mL) and 1 M aqueous sodium hydroxide (600 mL), and extracted with a mixture of chloroform and 2-propanol. The organic layer was dried and the desiccant was removed by filtration, and then the solvent was evaporated in vacuo. The resulting residue was dissolved with diethyl ether (500 mL), and to the solution was added 4 M hydrochloride solution in ethyl acetate (500 mL) at 5 °C. Then the mixture was stirred at room temperature for 8 h. The precipitate was collected by filtration, and dried in vacuo to obtain **16** (76.82 g, 89%) as a white solid: ^{1}H NMR (DMSO-d_6) δ 3.00–3.24 (4H, m), 7.72–7.83 (1H, m), 7.88–7.98 (1H, m), 8.10–8.50 (3H, m), 8.60–8.70 (1H, m), 8.74 (1H, s), 9.50–11.50 (1H, br); FAB-MS *m/z* 139 [(M+H)$^+$].

5.40. 6-Methyl-*N*-[2-(1-oxidopyridin-3-yl)ethyl]-2-phenylpyrimidin-4-amine oxalate (17a)

To a solution of 4-chloro-6-methyl-2-phenylpyrimidine (**14a**, 160 mg) in DMI (5 mL) were added 2-(1-oxidopyridin-3-yl)ethylamine hydrochloride (**16**, 480 mg) and potassium carbonate (540 mg), and the mixture was stirred at 80 °C for 24 h. The reaction mixture was cooled down to room temperature and treated with water, then extracted with ethyl acetate. The organic layer was washed with brine, dried, filtered, and then the solvent was evaporated in vacuo. The resulting residue was purified by silica gel column chromatography (chloroform–methanol). The obtained solid (90 mg) was treated with ethanol (5 mL) and oxalic acid (53 mg), and the mixture was evaporated in vacuo and the resulting residue was washed with diethyl ether to give **17a** (70 mg, 19%) as a white solid: ^{1}H NMR (DMSO-d_6) δ 2.31 (3H, s), 2.88 (2H, t, *J* = 6.6 Hz), 3.50–4.00 (2H, m), 6.30 (1H, s), 7.20–7.30 (1H, m), 7.30–7.40 (1H, m), 7.40–7.55 (3H, m), 7.55–7.75 (1H, br), 8.07 (1H, d, *J* = 5.8 Hz), 8.19 (1H, s), 8.24–8.35 (2H, m); FAB-MS *m/z* 307 [(M)$^+$]. Anal. ($C_{18}H_{18}N_4O\cdot1.8C_2H_2O_4\cdot0.4H_2O$): C, H, N, Br, F.

The following compounds (**17b–i**) were prepared by a procedure similar to that described for **17a**.

5.41. 2-(4-Chlorophenyl)-6-methyl-*N*-[2-(1-oxidopyridin-3-yl)ethyl]pyrimidin-4-amine oxalate (17b)

White solid (yield 63%); ^{1}H NMR (DMSO-d_6) δ 2.29 (3H, s), 2.87 (2H, t, *J* = 6.9 Hz), 3.00–4.50 (2H, m), 6.29 (1H, s), 7.26 (1H, d, *J* = 7.8 Hz), 7.33 (1H, t, *J* = 7.1 Hz), 7.45–7.70 (3H, m), 8.06 (1H, d, *J* = 6.4 Hz), 8.19 (1H, s), 8.31 (2H, d, *J* = 8.3 Hz); FAB-MS *m/z* 341, 343 [(M+H)$^+$]. Anal. ($C_{18}H_{17}N_4OCl\cdot1.5C_2H_2O_4\cdot H_2O$): C, H, N, Cl.

5.42. 2-(4-Bromo-2-fluorophenyl)-6-methyl-*N*-[2-(1-oxidopyridin-3-yl)ethyl]pyrimidin-4-amine oxalate (17c)

Colorless crystal (yield 32%); ^{1}H NMR NMR (DMSO-d_6) δ 2.27 (3H, s), 2.85 (2H, t, *J* = 6.9 Hz), 3.00–4.50 (1H, m), 6.30 (1H, s), 7.15–7.26 (1H, m), 7.32 (1H, dd, *J* = 6.5, 7.5 Hz), 7.46–7.54 (1H, m), 7.53–7.60 (1H, m), 7.61 (1H, dd, *J* = 1.9, 10.5 Hz), 7.78–7.96 (1H, m), 8.02–8.09 (1H, m), 8.09–8.23 (1H, br); FAB-MS *m/z* 403, 405 [(M)$^+$]. Anal. ($C_{18}H_{16}N_4OBrF\cdot C_2H_2O_4$): C, H, N, Br, F.

5.43. 2-(4-Bromo-3-fluorophenyl)-6-methyl-*N*-[2-(1-oxidopyridin-3-yl)ethyl]pyrimidin-4-amine oxalate (17d)

White solid (yield 56%); ^{1}H NMR (DMSO-d_6) δ 2.35–2.56 (5H, m), 2.80–3.00 (2H, m), 3.40–4.00 (2H, m), 6.51 (1H, s), 7.30–7.45 (2H, m), 7.94 (1H, t, *J* = 7.6 Hz), 8.00–8.19 (2H, m), 8.20–8.38 (2H, m), 8.50–9.20 (1H, br); FAB-MS *m/z* 403, 405 [(M+H)$^+$]. Anal. ($C_{18}H_{16}N_4OBrF\cdot1.5C_2H_2O_4$): C, H, N, Br, F.

5.44. 2-(4-Chloro-3-fluorophenyl)-6-methyl-*N*-[2-(1-oxidopyridin-3-yl)ethyl]pyrimidin-4-amine hydrochloride (17e)

Pale yellow solid (yield 52%); ^{1}H NMR (DMSO-d_6) δ 2.45–2.60 (5H, m), 2.95–3.10 (2H, m), 3.80–4.00 (2H, m), 6.64 (1H, s), 7.62 (1H, t, *J* = 7.1 Hz), 7.70–7.83 (1H, m), 7.83–7.93 (1H, m), 8.21 (1H, d, *J* = 7.9 Hz), 8.37–8.51 (2H, m), 8.63 (1H, s), 9.50–10.00 (1H, br); FAB-MS *m/z* 359, 361 [(M+H)$^+$]. Anal. ($C_{18}H_{16}N_4OClF\cdot2HCl\cdot0.25H_2O$): C, H, N, Cl, F.

5.45. 2-(3,4-Dichlorophenyl)-6-methyl-*N*-[2-(1-oxidopyridin-3-yl)ethyl]pyrimidin-4-amine oxalate (17f)

White solid (yield 76%); ^{1}H NMR (DMSO-d_6) δ 2.30 (3H, s), 2.87 (2H, t, *J* = 6.6 Hz), 3.50–4.00 (2H, m), 6.30 (1H, s), 7.27 (1H, d, *J* = 7.8 Hz), 7.33 (1H, t, *J* = 7.1 Hz), 7.50–7.68 (1H, br), 7.74 (1H, d, *J* = 8.4 Hz), 8.07 (1H, d, *J* = 6.3 Hz), 8.19 (1H, s), 8.25 (1H, d, *J* = 8.4 Hz), 8.44 (1H, d, *J* = 2.0 Hz); FAB-MS *m/z* 341, 343 [(M+H)$^+$]. Anal. ($C_{18}H_{16}N_4OCl_2\cdot1.7C_2H_2O_4$): C, H, N, Cl.

5.46. 2-(4-Bromo-3,5-difluorophenyl)-6-methyl-*N*-[2-(1-oxidopyridin-3-yl)ethyl]pyrimidin-4-amine oxalate (17g)

Pale yellow solid (yield 69%); ^{1}H NMR (DMSO-d_6) δ 2.29 (3H, s), 2.87 (2H, t, *J* = 6.6 Hz), 3.30–4.00 (3H, m), 6.32 (1H, s), 7.24–7.40 (2H, m), 7.50–7.70 (1H, br), 7.92–8.13 (3H, m), 8.21 (1H, s); FAB-MS *m/z* 421, 423 [(M+H)$^+$]. Anal. ($C_{18}H_{15}N_4OBrF_2\cdot1.5C_2H_2O_4\cdot0.3C_2H_5O$): C, H, N, Br, F.

5.47. 2-(4-Bromo-2,5-difluorophenyl)-6-methyl-*N*-[2-(1-oxidopyridin-3-yl)ethyl]pyrimidin-4-amine oxalate (17h)

White solid (yield 50%); ^{1}H NMR NMR (DMSO-d_6) δ 2.28 (3H, s), 2.85 (2H, t, *J* = 6.8 Hz), 3.00–5.50 (3H, m), 6.31 (1H, s), 7.15–7.28 (1H, m), 7.28–7.38 (1H, m), 7.61 (1H, s), 7.80 (1H, dd, *J* = 5.9, 9.8 Hz), 7.81–8.00 (1H, br), 8.07 (1H, d, *J* = 6.4 Hz), 8.15 (1H, s); FAB-MS *m/z* 421, 423 [(M+H)$^+$]. Anal. ($C_{18}H_{15}N_4OBrF_2\cdot C_2H_2O_4$): C, H, N, Br, F.

5.48. 2-(4-Chloro-2,5-difluorophenyl)-6-methyl-*N*-[2-(1-oxidopyridin-3-yl)ethyl]pyrimidin-4-amine oxalate (17i)

Pale yellow solid (yield 28%); ^{1}H NMR (DMSO-d_6) δ 2.28 (3H, s), 2.85 (2H, t, *J* = 6.8 Hz), 3.40–3.70 (2H, m), 6.31 (1H, s), 7.15–7.28 (1H, m), 7.29–7.37 (1H, m), 7.52–7.66 (1H, m), 7.71 (1H, dd, *J* = 6.4, 10.3 Hz), 7.82–8.00 (1H, m), 8.08 (1H, d, *J* = 6.4 Hz), 8.10–8.25 (1H, br); FAB-MS *m/z* 377, 379 [(M+H)$^+$]. Anal. ($C_{18}H_{15}N_4OClF_2\cdot C_2H_2O_4$): C, H, N, Cl, F.

5.49. (2-Methoxypyridin-4-yl)methanol (19)

To methyl 2-chloroisonicotinate (**18**, 53.63 g) were added dioxane (150 mL) and sodium methoxide (25.32 g), and the mixture was stirred at reflux temperature for 26 h and then cooled down to room temperature. To the mixture was added acetic acid (28 mL), and the mixture was evaporated in vacuo. To the resulting residue were added ethyl acetate and water, and the mixture was extracted with ethyl acetate. The organic layer was washed with

water and brine, dried, filtered, and the filtrate was evaporated in vacuo. The resulting residue was dissolved in THF (300 mL). Under an argon atmosphere, the solution was added dropwise to a suspension of lithium aluminum hydride (11.88 g) in THF (500 mL) at 0 °C over 1 h. Then the reaction mixture was stirred at 0 °C for 2 h. To the mixture was added water (30 mL) dropwise at 0 °C, and then THF (270 mL) was added. The mixture was filtered over celite, and the filtrate was evaporated in vacuo to obtain **19** (33.55 g, 75%) as a orange oil: ^{1}H NMR (DMSO-d_6) δ 3.83 (3H, s), 4.49 (2H, d, J = 5.3 Hz), 4.70 (1H, t, J = 5.3 Hz), 6.73 (1H, s), 6.90 (1H, d, J = 5.4 Hz), 8.07 (1H, d, J = 5.4 Hz); EI-MS m/z 139 [(M)$^+$].

5.50. (2-Methoxypyridin-4-yl)acetonitrile (20)

Thionyl chloride (170 mL) was added dropwise to (2-methoxypyridin-4-yl)methanol (**19**, 34.53 g) at 0 °C over 15 min. The mixture was stirred at 0 °C for 2 h and evaporated in vacuo. The residue was dissolved in chloroform (400 mL) and treated with sat. aqueous sodium hydrogen carbonate (500 mL). The mixture was extracted with chloroform. The organic layer was dried, filtered, and the filtrate was evaporated in vacuo. To the residue were added acetonitrile (700 mL), potassium cyanide (30.00 g) and 18-crown-6 ether (61.03 g), and the reaction mixture was stirred at room temperature for 13 h. The mixture was evaporated in vacuo, and to the residue was added water (300 mL). The mixture was extracted with ethyl acetate, and the organic layer was washed with brine, dried, filtered, and the filtrate was evaporated in vacuo. The resulting residue was purified by silica gel column chromatography (hexane–ethyl acetate) to obtain **20** (12.12 g, 33%) as a pale yellow solid: ^{1}H NMR NMR (DMSO-d_6) δ 3.86 (3H, s), 4.09 (2H, s), 6.79 (1H, s), 6.97 (1H, d, J = 5.4 Hz), 8.18 (1H, d, J = 5.4 Hz); FAB-MS m/z 149 [(M+H)$^+$].

5.51. 2-(2-Methoxypyridin-4-yl)ethylamine (21)

A mixture of (2-methoxypyridin-4-yl)acetonitrile (**20**, 12.12 g), ethanol (100 mL), 28 wt % aqueous ammonia (25 mL) and Raney®-nickel (25 mL) was stirred at room temperature under hydrogen atmosphere for 22 h. The mixture was filtered over celite, and the filtrate was evaporated in vacuo to obtain **21** (12.73 g, 102%) as a brown oil.: ^{1}H NMR (DMSO-d_6) δ 2.10–3.75 (6H, m), 3.82 (3H, s), 6.65 (1H, s), 6.84 (1H, d, J = 5.4 Hz), 8.04 (1H, d, J = 5.4 Hz); EI-MS m/z 152 [(M)$^+$].

5.52. 2-(4-Bromo-3-fluorophenyl)-*N*-[2-(6-methoxypyridin-2-yl)ethyl]-6-methylpyrimidin-4-amine (22a)

A mixture of 2-(4-bromo-3-fluorophenyl)-4-chloro-6-methylpyrimidine (**14d**, 198 mg), DMI (2 mL), potassium carbonate (454 mg) and 2-(6-methoxypyridin-2-yl)ethylamine (**21**, 200 mg) was stirred at 95 °C overnight. The mixture was cooled down to room temperature, and water was added to the mixture. The mixture was extracted with toluene and the organic layer was dried, filtered and evaporated in vacuo to obtain **22a** (220 mg, 80%) as a yellow oil: ^{1}H NMR (DMSO-d_6) δ 2.28 (3H, s), 2.87 (2H, t, J = 6.8 Hz), 3.50–3.75 (2H, m), 3.81 (3H, s), 6.30 (1H, s), 6.72 (1H, s), 6.90 (1H, d, J = 5.3 Hz), 7.49 (1H, s), 7.74–7.85 (1H, m), 7.98–8.20 (3H, m); FAB-MS m/z 417, 419 [(M+H)$^+$].

The following compounds (**22b–d**) were prepared by a procedure similar to that described for **22a**.

5.53. 2-(4-Bromo-2,5-difluorophenyl)-*N*-[2-(2-methoxypyridin-4-yl)ethyl]-6-methylpyrimidin-4-amine (22b)

This compound was synthesized from **14h** and **21**, and used to next reaction without purification by silica gel column chromatography.

5.54. 2-(4-Chloro-2,5-difluorophenyl)-*N*-[2-(2-methoxypyridin-4-yl)ethyl]-6-methylpyrimidin-4-amine (22c)

This compound was synthesized from **14i** and **21**, and used to next reaction without purification by silica gel column chromatography.

5.55. 2-(4-Chloro-2,5-difluorophenyl)-6-ethyl-*N*-[2-(2-methoxypyridin-4-yl)ethyl]pyrimidin-4-amine (22d)

This compound was synthesized from **14m** and **21**, and used to next reaction without purification by silica gel column chromatography.

5.56. 4-(2-{[2-(4-Bromo-3-fluorophenyl)-6-methylpyrimidin-4-yl]amino}ethyl)pyridin-2(1*H*)-one (23a)

A mixture of 2-(4-bromo-3-fluorophenyl)-*N*-[2-(6-methoxypyridin-2-yl)ethyl]-6-methylpyrimidin-4-amine (**22a**, 206 mg) and 48 wt % hydrobromic acid (2.0 mL) was stirred at 80 °C for 3 days. The mixture was cooled down to room temperature and treated with saturated aqueous sodium hydrogen carbonate and ethyl acetate. The organic layer was dried, filtered and evaporated in vacuo. The resulting residue was washed with diethyl ether and dried in vacuo to obtain **23a** (82 mg, 41%) as a white solid: ^{1}H NMR (DMSO-d_6) δ 2.29 (3H, s), 2.70 (2H, t, J = 6.9 Hz), 3.50–3.80 (2H, m), 6.12 (1H, dd, J = 1.5, 6.9 Hz), 6.18 (1H, s), 6.30 (1H, s), 7.27 (1H, d, J = 6.3 Hz), 7.36–7.52 (1H, br), 7.74–7.85 (1H, m), 8.00–8.20 (2H, m), 11.10–11.50 (1H, br); ESI-MS m/z 403, 405 [(M+H)$^+$]. Anal. ($C_{18}H_{16}N_4OBrF\cdot0.35H_2O$): C, H, N, Br, F.

The following compounds (**23b–d**) were prepared by a procedure similar to that described for **23a**.

5.57. 4-(2-{[2-(4-Bromo-2,5-difluorophenyl)-6-methylpyrimidin-4-yl]amino}ethyl)pyridin-2(1*H*)-one hydrochloride (23b)

White solid (yield 39% from **14h**); ^{1}H NMR (DMSO-d_6) δ 2.52 (3H, s), 2.87 (2H, t, J = 6.6 Hz), 3.76–3.95 (2H, m), 5.00–7.50 (2H, m), 6.53 (1H, d, J = 6.4 Hz), 6.56 (1H, s), 6.66 (1H, s), 7.55 (1H, d, J = 6.4 Hz), 8.24–8.35 (2H, m), 9.60–9.90 (1H, br); FAB-MS m/z 421, 423 [(M+H)$^+$]. Anal. ($C_{18}H_{15}N_4OBrF_2\cdot1.8HCl\cdot0.5H_2O$): C, H, N, Br, Cl, F.

5.58. 4-(2-{[2-(4-Chloro-2,5-difluorophenyl)-6-methylpyrimidin-4-yl]amino}ethyl)pyridin-2(1*H*)-one hydrochloride (23c)

White solid (yield 58% from **14i**); ^{1}H NMR (DMSO-d_6) δ 2.46 (3H, s), 2.72–2.87 (2H, m), 3.50–3.80 (2H, m), 6.20–6.60 (2H, m), 6.68 (1H, s), 7.30–7.60 (1H, m), 7.90–8.10 (2H, m), 9.40–10.00 (2H, m); FAB-MS m/z 377, 379 [(M+H)$^+$]. Anal. ($C_{18}H_{15}N_4OClF_2\cdot2HCl\cdot0.25H_2O$): C, H, N, Cl, F.

5.59. 4-(2-{[2-(4-Chloro-2,5-difluorophenyl)-6-ethylpyrimidin-4-yl]amino}ethyl)pyridin-2(1*H*)-one hydrochloride (23d)

Pale yellow solid (yield 78% from **14m**); ^{1}H NMR (DMSO-d_6) δ 1.24 (3H, t, J = 7.5 Hz), 2.70–2.87 (4H, m), 3.50–3.90 (2H, m), 6.20–6.60 (2H, m), 6.69 (1H, s), 7.35–7.55 (1H, m), 7.90–8.10 (2H, m), 9.40–10.00 (2H, m); FAB-MS m/z 391, 393 [(M+H)$^+$]. Anal. ($C_{19}H_{17}N_4OClF_2\cdot2HCl\cdot H_2O$): C, H, N, Cl, F.

5.60. (2*S*)-3-{[2-(4-Bromo-3-fluorophenyl)-6-methylpyrimidin-4-yl]amino}propane-1,2-diol oxalate (24a)

A mixture of 2-(4-bromo-3-fluorophenyl)-4-chloro-6-methylpyrimidine (**14d**, 241 mg), (*S*)-3-amino-1,2-propanediol (366 mg),

N,*N*-diisopropylethylamine (0.14 mL) and acetonitrile (4 mL) was stirred at 70 °C for 4 days. The reaction mixture was cooled down to room temperature. To the mixture was added water, and extracted with chloroform. The organic layer was dried, and the desiccant was removed by filtration, and then the solvent was evaporated in vacuo. The resulting residue was dissolved in ethanol (5 mL), and to the solution was added oxalic acid (108 mg). The mixture was evaporated in vacuo and the residue was recrystallized from acetonitrile and ethanol, and the crystal was dried in vacuo to obtain **24a** (228 mg, 64%) as a colorless crystal: ^{1}H NMR (DMSO-d_6) δ 2.29 (3H, s), 3.20–3.75 (5H, m), 4.00–7.00 (4H, m), 6.38 (1H, s), 7.30–7.55 (1H, br), 7.60–7.95 (1H, m), 8.00–8.22 (2H, m); FAB-MS *m/z* 356, 358 [(M+H)$^+$]. Anal. ($C_{14}H_{15}N_3O_2BrF\cdot1.5C_2H_2O_4$): C, H, N, Br, F.

The following compounds (**24b–g**) were prepared by a procedure similar to that described for **24a**.

5.61. (2*R*)-3-{[2-(4-Bromo-3-fluorophenyl)-6-methylpyrimidin-4-yl]amino}propane-1,2-diol oxalate (24b)

Colorless crystal (yield 41% from **14d**); ^{1}H NMR (DMSO-d_6) δ 2.29 (3H, s), 2.70–3.50 (3H, m), 3.50–3.80 (2H, m), 3.80–6.00 (3H, m), 6.38 (1H, s), 7.00–7.60 (1H, br), 7.75–7.85 (1H, m), 8.09 (1H, d, J = 8.3 Hz), 8.15 (1H, d, J = 10.3 Hz); FAB-MS *m/z* 356, 358 [(M+H)$^+$]. Anal. ($C_{14}H_{15}N_3O_2BrF\cdot C_2H_2O_4\cdot0.25H_2O$): C, H, N, Br, F.

5.62. (2*S*)-3-{[2-(4-Bromo-3-fluorophenyl)-6-ethylpyrimidin-4-yl]amino}propane-1,2-diol oxalate (24c)

Colorless crystal (yield 61% from **14j**); ^{1}H NMR (DMSO-d_6) δ 1.21 (3H, t, J = 7.4 Hz), 2.57 (2H, q, J = 7.4 Hz), 2.70–3.50 (3H, m), 3.50–3.90 (2H, m), 3.90–6.00 (2H, br), 6.39 (1H, s), 7.00–7.70 (1H, br), 7.75–7.88 (1H, m), 8.00–8.25 (2H, m); FAB-MS *m/z* 370, 372 [(M)$^+$]. Anal. ($C_{15}H_{17}N_3O_2BrF\cdot C_2H_2O_4\cdot0.25H_2O$): C, H, N, Br, F.

5.63. (2*R*)-3-{[2-(4-Bromo-3-fluorophenyl)-6-ethylpyrimidin-4-yl]amino}propane-1,2-diol oxalate (24d)

Colorless crystal (yield 62% from **14j**); ^{1}H NMR (DMSO-d_6) δ 1.22 (3H, t, J = 7.4 Hz), 2.58 (2H, q, J = 7.4 Hz), 2.70–3.50 (3H, m), 3.50–3.90 (2H, m), 4.50–6.35 (2H, br), 6.40 (1H, s), 7.00–7.70 (1H, br), 7.75–7.88 (1H, m), 8.00–8.25 (2H, m); FAB-MS *m/z* 370, 372 [(M)$^+$]. Anal. ($C_{15}H_{17}N_3O_2BrF\cdot C_2H_2O_4\cdot0.25H_2O$): C, H, N, Br, F.

5.64. (2*R*)-3-{[2-(4-Chloro-3-fluorophenyl)-6-ethylpyrimidin-4-yl]amino}propane-1,2-diol oxalate (24e)

Colorless crystal (yield 64% from **14k**); 1.22 (3H, t, J = 7.4 Hz), 2.58 (2H, q, J = 7.4 Hz), 2.70–3.50 (3H, m), 3.50–3.80 (2H, m), 6.39 (1H, s), 7.00–7.64 (1H, br), 7.64–7.74 (1H, m), 8.10–8.25 (2H, m); FAB-MS *m/z* 326, 328 [(M+H)$^+$]. Anal. ($C_{15}H_{17}N_3O_2ClF\cdot$-$C_2H_2O_4\cdot0.5H_2O$): C, H, N, Cl, F.

5.65. (2*R*)-3-{[2-(4-Bromo-2,5-difluorophenyl)-6-ethylpyrimidin-4-yl]amino}propane-1,2-diol oxalate (24f)

Slightly yellow crystal (yield 54% from **14l**); ^{1}H NMR (DMSO-d_6) δ 1.19 (3H, t, J = 7.5 Hz), 2.55 (2H, q, J = 7.5 Hz), 2.80–3.75 (5H, m), 3.75–6.30 (3H, br), 6.40 (1H, s), 7.00–7.70 (1H, br), 7.79 (1H, dd, J = 5.9, 9.8 Hz), 7.82–8.00 (1H, m); FAB-MS *m/z* 388, 390 [(M)$^+$]. Anal. ($C_{15}H_{16}N_3O_2BrF_2\cdot C_2H_2O_4$): C, H, N, Br, F.

5.66. (2*R*)-3-{[2-(4-Chloro-2,5-difluorophenyl)-6-ethylpyrimidin-4-yl]amino}propane-1,2-diol oxalate (24g)

Colorless crystal (yield 26% from **14m**); ^{1}H NMR (DMSO-d_6) δ 1.19 (3H, t, J = 7.6 Hz), 2.55 (2H, q, J = 7.6 Hz), 2.70–3.75 (5H, m), 3.75–6.30 (3H, br), 6.40 (1H, s), 7.00–7.68 (1H, br), 7.71 (1H, dd, J = 6.1, 10.0 Hz), 7.80–8.05 (1H, m); ^{13}C NMR (DMSO-d_6) δ 17.05, 30.75, 51.21, 57.67, 76.45, 105.49, 105.74, 106.31, 106.60, 139.63, 142.05, 146.66, 148.52, 152.02, 168.39, 183.80, 191.34; FAB-MS *m/z* 344, 346 [(M+H)$^+$]. Anal. ($C_{15}H_{16}N_3O_2ClF_2\cdot C_2H_2O_4$): C, H, N, Cl, F.

5.67. 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 × 104 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.

5.68. Oral glucose tolerance test (OGTT)

Eight-week-old male ICR mice were fasted overnight and then orally administered 0.5% methyl-cellulose (vehicle) or 1–3 mg/kg test compounds. After 10 min, glucose was given orally at a dose of 2 g/kg/10 mL, and blood sample were collected from tail veins after 30 min. Blood glucose levels were determined using the Glucose CII test (Wako, Osaka, Japan).

5.69. Efficacy of repeated administration in male kk/Ay mice

Male kk/Ay mice were purchased from Japan CLEA Inc. (Kanagawa, Japan). Eight-week-old male kk/Ay mice (n = 8) were orally administered 0.5% methyl-cellulose (vehicle), 3 mg/kg compound **24g** or 3 mg/kg Pioglitazone hydrochloride once daily for a week. Blood samples were collected from tail veins under feeding conditions for the measurement of blood glucose, insulin, and triglyceride (TG) levels. Pancreatic tissue samples were also collected, weighed, homogenized, extracted with 2 mL acid–ethanol solution (concentrated HCl: ethanol: distilled H_2O = 1.5: 75: 23.5), centrifuged for 10 min at 3000 g, and the insulin concentration in the supernatant was then determined. Blood glucose levels were determined using the Glucose CII test (Wako, Osaka, Japan). Plasma triglyceride level was determined using the Triglyceride E-test (Wako). Data are expressed as the mean ± SE. Significant differences between two groups and multiple groups were determined using the Dunnett's multiple comparison test. A value of p <0.05 was considered to represent statistical significance. All statistical analyses were performed using the SAS 8.2 software package (SAS Institute Japan, Ltd, Tokyo, Japan).

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