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Discovery of a novel series of GPR119 agonists: Design, synthesis, and biological evaluation of *N*-(Piperidin-4-yl)-*N*-(trifluoromethyl) pyrimidin-4-amine derivatives

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ABSTRACT

We undertook an optimization effort involving propan-2-yl 4-({6-[5-(methanesulfonyl)-2,3-dihydro-1*H*-indol-1-yl]pyrimidin-4-yl}oxy)piperidine-1-carboxylate **1**, which we had previously discovered as a novel G protein-coupled receptor 119 (GPR119) agonist. To occupy a presumed hydrophobic space between the pyrimidine and piperidine rings in interaction with GPR119, we replaced the linker oxygen with nitrogen. Subsequently, the introduction of a substituent at the bridging nitrogen atom was explored. We found that the installation of *N*-trifluoromethyl group **10** not only enhanced GPR119 agonist activity but also considerably improved the human ether-à-go-go-related gene (hERG) inhibition profile. These improvements were not observed for non-fluorinated substituents, such as ethyl analog **8b**. The next optimization effort focused on the exploration of a new surrogate structure for the indoline ring and the isosteric replacements of the piperidine *N*-Boc group to improve solubility, metabolic stability, and oral bioavailability. As a result, *N*-{1-[3-(2-fluoropropan-2-yl)-1,2,4-oxadiazol-5-yl] piperidin-4-yl}-6-{[1-(methanesulfonyl)piperidin-4-yl]oxy}-*N*-(trifluoromethyl)pyrimidin-4-amine (**27**) was identified as a potent and orally bioavailable GPR119 agonist. This compound augmented insulin secretion and effectively lowered plasma glucose excursion in a diabetic animal model after oral administration. In this study, we discuss the designs, syntheses, and biological activities of a novel series of *N*-(piperidin-4-yl)-*N*-(trifluoromethyl)pyrimidin-4-amine derivatives as GPR119 agonists, and to determine the distinctive effect of the *N*-trifluoromethyl group on hERG inhibition, we also discuss the conformational preference of representative compounds.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a major public health problem worldwide. Diabetes causes complications such as retinopathy, nephropathy, neurological disorder when left untreated, and blindness. Dialysis treatment may be necessary at the end of the disease. Recently, considerable attention has been paid to incretin-related approaches, such as dipeptidyl peptidase-4 inhibitors and glucagon-like peptide-1 mimetics as medication therapy for T2DM.[1–3] G protein-coupled receptor 119 (GPR119) has also attracted considerable interest as an incretin-related approach for the next generation of antidiabetic drugs.[4–10] To date, several compounds have been investigated under clinical trials, with their results affirming the safety and tolerability of GPR119 agonists in humans.

We have previously reported the lead generation and the subsequent optimization efforts of a novel series of indoline-based GPR119 agonists.[11,12] To address the undesirable human ether-à-go-go-related gene (hERG) issue of lead compound **1**, structural modification was employed for the substituent at the indoline 5-position. This successfully led to the identification of compound **2** as a potent GPR119 agonist with favorable pharmacokinetic profiles and reduced hERG liability (Fig. 1).

In a parallel effort, we conducted structural modification around the

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central pyrimidine core of compound **1**. To induce interaction with the presumed hydrophobic space between the pyrimidine and piperidine rings in GPR119, we introduced a substituent through a nitrogen linkage between the two rings. As a result, the installation of an *N*-trifluoromethyl group at the bridging nitrogen was found to be effective in enhancing GPR119 agonist activity and reducing hERG inhibitory activity. The replacement of the indoline ring and the optimization of the piperidine *N*-substituent resulted in the identification of compound **27**, which exhibited potent GPR119 agonist activity, low risk in hERG inhibition, and favorable pharmacokinetic profiles (Fig. 1). In this study, we report the design, synthesis, and biological activity of *N*-(trifluoromethyl)pyrimidin-4-amine derivatives as a novel series of GPR119 agonists and discuss the cause of improvement in hERG inhibition by the *N*-trifluoromethyl group in terms of conformational preference, basicity, and π-electron density of compounds.

2. Chemistry

The synthetic route was used to prepare compounds **8a**–**e** bearing various alkyl substituents at the nitrogen atom between the pyrimidine and piperidine rings (Scheme 1). The S_NAr of 4,6-dichloropyrimidine with 4-amino-1-Boc-piperidine and the subsequent protection of the resulting anilinic NH with a trifluoroacetyl group yielded compound **5**. The Buchwald–Hartwig reaction of compound **5** with 5-(methylsulfonyl) indoline, followed by the removal of the trifluoroacetyl group under basic conditions, provided key intermediate **6**. Alternatively, compound **6** could be prepared in shorter steps by changing the reaction sequence in reverse order. Notably, the refluxing of 4,6-dichloropyrimidine and 5-(methylsulfonyl)indoline in ethanol (EtOH) gave chloropyrimidine **7**, which was then subjected to the Buchwald–Hartwig reaction with 4-amino-1-Boc-piperidine to attain **6**. The introduction of an alkyl substituent on the nitrogen atom was accomplished by alkylation using sodium hydride (NaH) as a base to give *N*-alkyl derivatives **8a**–**e**.

The syntheses of *N*-trifluoromethyl derivatives **10** are outlined in Scheme 2. According to Hiyama et al.'s method,[13,14] compound **6** was converted to dithiocarbamate **9**, which was subjected to an oxidative desulfurization–fluorination reaction using tetrabutylammonium dihydrogen trifluoride ($TBAH_2F_3$) and *N*-bromosuccinimide (NBS) to give the desired *N*-trifluoromethyl derivative **10** in a moderate yield. After the removal of the *tert*-butyloxycarbonyl protecting group, also known as the Boc group, *N*-cyanation was followed by the construction of a 1,2,4-oxadiazole ring to deliver the final compound **13**.

The synthesis of compounds with various left-hand side substitutes was carried out (Scheme 3). The condensation of 4,6-dichloropyrimidine and 1-Boc-4-aminopiperidine in the same manner described in Scheme 1 yielded compound **14**. Dithiocarbamate was introduced to compound **14** and then trifluorinated with 1,3-dibromo-5,5-dimethylhydantoin and TEA trihydrofluoride to give *N*-trifluoromethylated derivative **15**. Compound **15** was coupled with 1-(methylsulfonyl)piperidin-4-amine using *N,N*-diisopropylethylamine (DIPEA) as a base to afford **16**. Methylation by MeI and NaH gave **17** in a good yield. 1-Methanesulfonyl piperidin-4-ol was installed on **15** using NaH to afford compound **18** in a good yield. Mesylate **19** was prepared by S_NAr of **15** and *cis*-cyclohexane-1,4-diol with NaH, followed by mesylation of the resulting hydroxyl group. Compound **20** was furnished from **19** through the reaction with sodium thiomethoxide, followed by *meta*-chloroperoxybenzoic acid (*m*CPBA) oxidation. Compound **21** was obtained by S_NAr with azetidin-3-ol, followed by sulfonamidation with methanesulfonyl chloride (MsCl). Compound **22** was synthesized under similar conditions used in the synthesis of **18**.

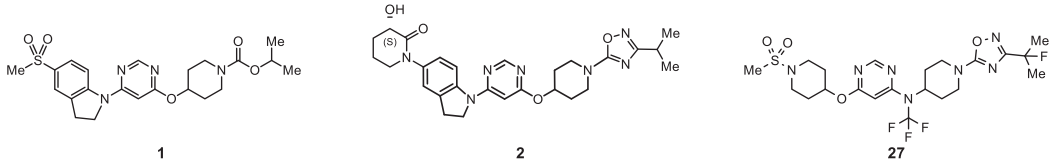
Scheme 4 describes the substitute conversion on the right piperidine nitrogen. The Boc group of compound **18** was deprotected under acidic conditions to give compound **23**. The introduction of oxadiazoles was accomplished via *N*-cyanamide **24**, which was prepared by the reaction of compound **23** with BrCN. The 1,2,4-oxadiazole ring was constructed to afford **25a**, **25b**, and **25c**. By reacting with methyl magnesium chloride (MeMgCl), tertiary alcohol **26** was delivered from ester **25c**. The hydroxyl group of **26** was converted to fluorine atom using Xtal-Fluor-E® as a source of fluorine to give compound **27**. In this reaction, a small amount of dehydrated olefin by-product **28** was observed and could not be separated from the desired compound using silica gel column chromatography. To remove this impurity, ruthenium-catalyzed oxidation was conducted to convert the olefin of the crude mixture to a ketone. Ketone derivative **29** could be easily separated using silica gel column chromatography to give the pure final compound **27**.

3. Results and discussion

The newly synthesized compounds were tested for functional GPR119 agonism using a cAMP reporter assay in Chinese hamster ovary (CHO) cells, stably expressing human or rat GPR119. Inhibitory activity against the hERG channel was measured in vitro using the automated patch clamp assay system at 10 μM concentration of compounds. In vitro metabolic stability was assessed by monitoring the disappearance of the parent compound after incubation with human liver microsomes, which is expressed as CL_{int} (μL/min/mg).

Compound design for the addition of a substituent around the central moiety was based on the structure–activity relationship of the indoline carbamate derivatives, which we had previously reported[9] (Fig. 2). The potency enhancement by the methyl installation on the central spacer (**3a** vs. **3b**) indicated a hydrophobic space around the 4-position of the piperidine ring in binding with GPR119. Based on this observation, we hypothesized that a hydrophobic substituent could be embedded in the indolinylpyrimidine derivatives at the corresponding position to increase GPR119 agonist activity. A flexible alignment of **3b** and **1** using the Molecular Operating Environment software[15] showed that the two molecules overlapped well; it also suggested that the methyl group of compound **3b** could be positioned around the oxygen atom between the pyrimidine and piperidine rings of compound **1** (Fig. 2B). This prompted us to design 6-(indolin-1-yl)-*N*-(piperidin-4-yl)pyrimidin-4-amine derivative **I**, which enabled the introduction of an additional substituent at the bridging point through *N*-alkylation (Fig. 2, A). To investigate the effects of the *N*-substituent on GPR119 agonist activity and hERG inhibitory activity, we planned to introduce alkyl substituents with different sizes or fluorinated alkyl substituents at the bridging nitrogen.

Table 1 shows the structural variations of the nitrogen substituent between the central pyrimidine and piperidine rings with their corresponding GPR119 agonistic and hERG inhibitory activities. The

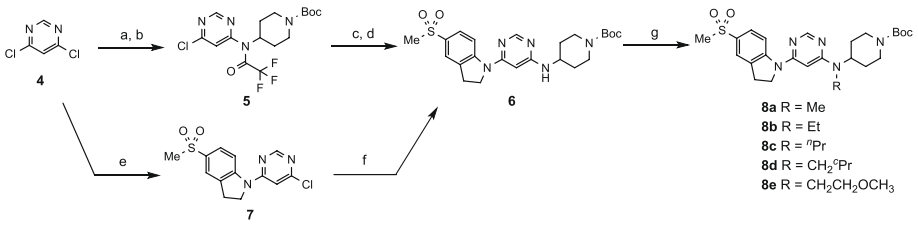


1
hGPR119 EC_{50} = 17 nM
hERG: 84% inhibition at 10 μM

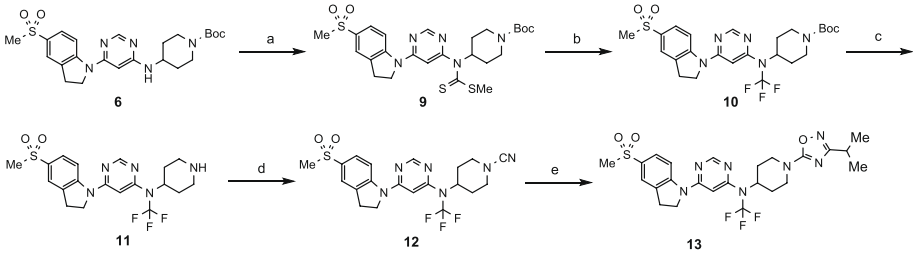
2
hGPR119 EC_{50} = 16 nM
hERG: 31% inhibition at 10 μM
CL_{int} (HLM) < 10 μL/min/mg
F = 24% (rat)

27
hGPR119 EC_{50} = 4.6 nM
hERG: 19% inhibition at 10 μM
CL_{int} (HLM) = 27 μL/min/mg
F = 57% (rat)

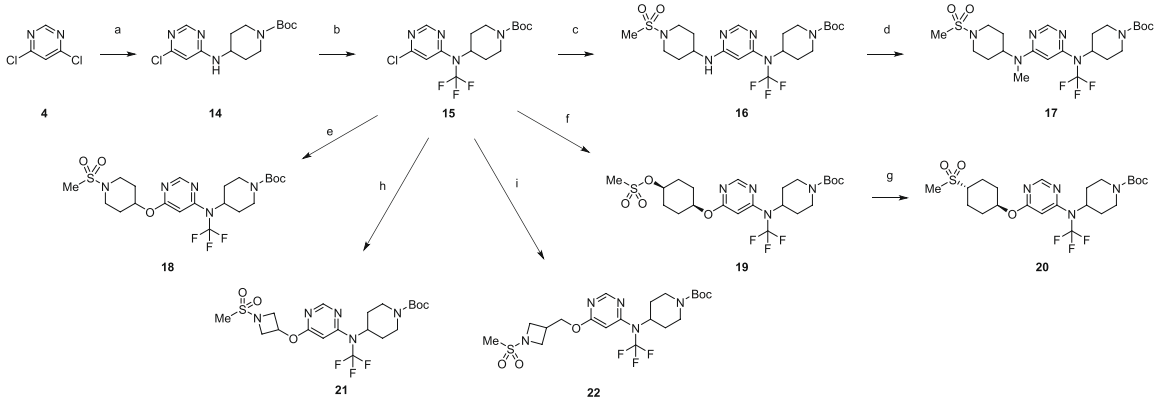
Fig. 1. Pyrimidine-based GPR119 agonists.



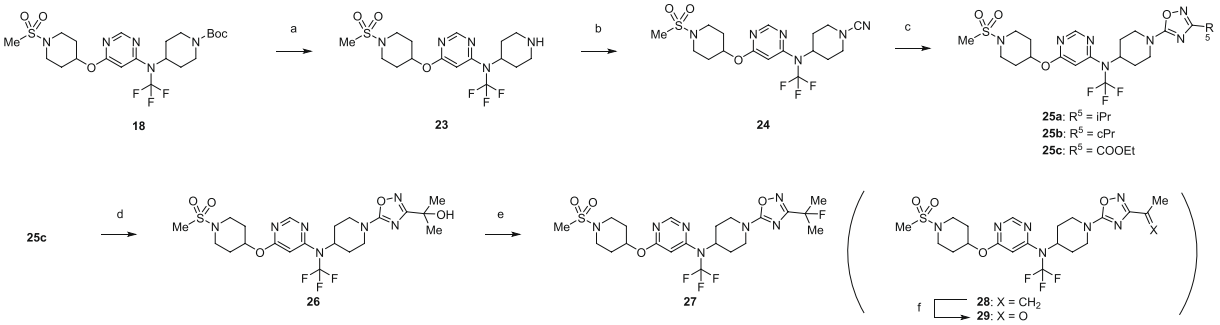
Scheme 1. Synthesis of 4,6-diaminopyrimidine derivatives **6** and **8a**–**e**[a]. Reagents and conditions: (a) 4-amino-1-Boc-piperidine, triethylamine (TEA), EtOH, 80 °C, 84%; (b) trifluoroacetic anhydride (TFAA), TEA, tetrahydrofuran (THF), 0 °C to rt, 88%; (c) 5-(methylsulfonyl)-2,3-dihydro-1*H*-indole, tris(dibenzylideneacetone)dipalladium(0) ($Pd_2(dba)_3$), Xantphos, cesium carbonate (Cs_2CO_3), toluene, 100 °C; (d) sodium hydroxide (NaOH), H_2O, THF, methanol (MeOH), rt, 33% over two steps; (e) 5-(methylsulfonyl)-2,3-dihydro-1*H*-indole, EtOH, reflux, 71%; (f) 4-amino-1-Boc-piperidine, $Pd_2(dba)_3$, X-Phos, Cs_2CO_3, toluene, 110 °C, 42%; (g) RI or RBr, NaH, *N, N*-dimethylformamide (DMF), 0 °C to rt, 9–98%.



Scheme 2. Synthesis of *N*-trifluoromethyl derivatives **10** and **13**[a]. Reagents and conditions: (a) (1) *n*-butyl lithium (^{n}BuLi), THF, 0 °C, (2) carbon disulfide (CS_2), 0 °C to rt, (3) iodomethane (MeI), 0 °C to rt, 60%; (b) $TBAH_2F_3$, NBS, toluene, −78 °C to 0 °C, 41%; (c) hydrochloric acid (HCl), MeOH, ethylacetate (AcOEt), rt, 87%; (d) cyanogen bromide (BrCN), sodium bicarbonate ($NaHCO_3$), THF, H_2O, 0 °C to rt, 97%; (e) ^{i}PrCNH(NHOH), zinc chloride ($ZnCl_2$), *p*-toluenesulfonic acid (*p*-TsOH), DMF, 85 °C, 69%.



Scheme 3. Synthesis of *N*-trifluoromethyl derivatives **16, 17, 18, 20, 21,** and **22**[a]. Reagents and conditions: (a) 4-amino-1-Boc-piperidine, TEA, acetonitrile (MeCN), 85 °C, 75%; (b) (1) NaH, CS_2, MeI, DMF, rt, 80%, (2) TEA trihydrofluoride, 1,3-dibromo-5,5-dimethylhydantoin, chlorobenzene, −20 °C, 33%; (c) 1-(methylsulfonyl) piperidin-4-amine, DIPEA, *N, N*-dimethylacetamide (DMA), 100 °C, 58%; (d) MeI, NaH, DMF, rt, 83%; (e) 1-(methylsulfonyl)piperidin-4-ol, NaH, DMA, rt, 78%; (f) (1) *cis*-cyclohexane-1,4-diol, NaH, DMA, rt, (2) MsCl, 4-dimethylaminopyridine (DMAP), pyridine, rt, 60% over two steps; (g) sodium thiomethoxide, DMF, 80 °C, then *m*CPBA, DMF, rt, 16%; (h) (1) azetidin-3-ol hydrochloride, NaH, DMA, rt, 44%; (2) MsCl, TEA, DMF, 0 °C to rt, 47%; (i) (1-(methylsulfonyl)azetidin-3-yl) methanol, NaH, THF, rt, 54%.



Scheme 4. Synthesis of 1,2,4-oxadiazole derivatives **25a**, **25b**, and **27**[a]. Reagents and conditions: (a) 4 M HCl in AcOEt, MeOH, AcOEt, rt, 98%; (b) BrCN, $NaHCO_3$, THF, H_2O, rt, 88%; (c) *N*-hydroxyisobutyrimidamide, *N*-hydroxycyclopropanecarboximidamide, or ethyl 2-amino-2-(hydroxyimino)acetate, $ZnCl_2$, *p*-TsOH, DMF, 80–85 °C, 69–81%; (d) MeMgCl, THF, 0 °C, 79%; (e) XtalFluor-E®, TEA trihydrofluoride, TEA, toluene, −78 °C to rt; (f) ruthenium(III) chloride, sodium periodate, MeCN, H_2O, 0–80 °C, 72% over two steps.

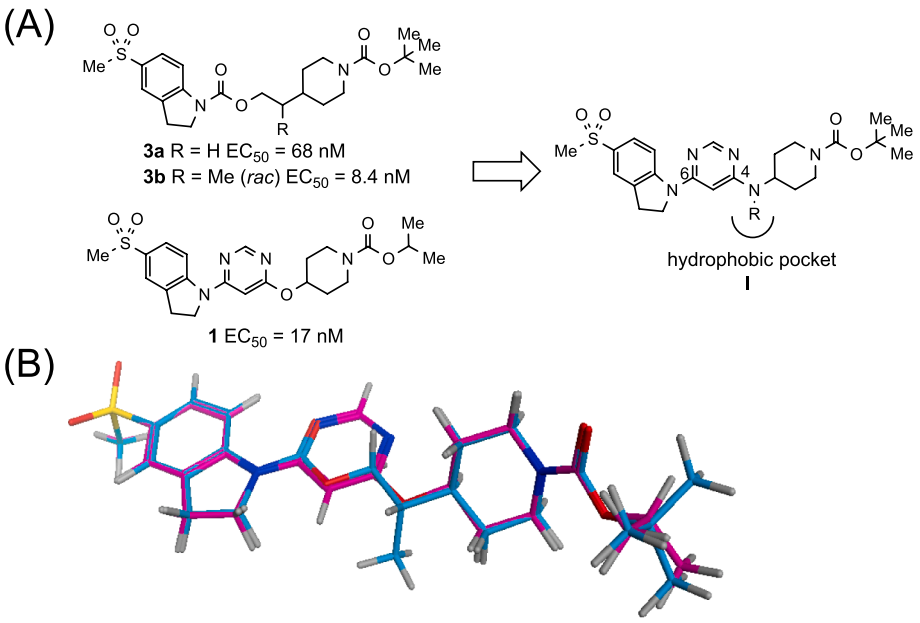
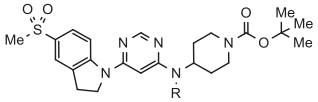


Fig. 2. (A) Filling design of a presumed hydrophobic space between the pyrimidine and piperidine rings through a nitrogen linkage. (B) An alignment of compounds **3b** and **1** obtained by flexible alignment using Molecular Operating Environment software (cyan: **3b**, magenta: **1**). The stereochemistry of the chiral center of **3b** is tentative.

Table 1
GPR119 agonistic and hERG inhibitory activities of 6-(indolin-1-yl)-*N*-(piperidin-4-yl)- pyrimidin-4-amine derivatives: effect of *N*-substituents at the bridging nitrogen.



Compound	R	hGPR119[a] EC_{50} (nM)	hERG[b] % inhibition at 10 μM	$LogD_{7.4}$[d]	LLE[e]
6	H	300	80 ± 3	2.7	3.8
8a	Me	21	85 ± 6	3.2	4.5
8b	Et	14	116 ± 2	3.5	4.4
8c	^{n}Pr	18	94 ± 4	4.0	3.7
8d	$CH_2{}^{c}Pr$	51	100 ± 4	4.1	3.2
8e	$CH_2CH_2OCH_3$	29	120 ± 4	3.4	4.1
10	CF_3	2.5	13 ± 11 (16 ± 1)[c]	4.1	4.5

[a] Agonist activity against human GPR119. EC_{50} values are shown as mean (n = 2).
[b] Automated patch clamp assay. Inhibition percentages are represented as mean ± standard deviation (n = 4).
[c] Data at 30 μM.
[d] LogD value at pH 7.4.
[e] LLE = pEC_{50} − $LogD_{7.4}$.

replacement of the oxygen atom at the bridging position of compound **1** with a nitrogen atom resulted in more than a tenfold loss of activity (**6**). The drop of potency is likely associated with lower lipophilicity ($LogD_{7.4}$ = 3.1 for **1** and 2.7 for **6**) or the presence of the NH proton of **6**. Compared with **6**, the introduction of a methyl group at the bridging nitrogen (**8a**), as we had expected, led to a substantial increase in potency. The ligand–lipophilicity efficiency (LLE) value (pEC_{50} − LogD)[16] increased with a change from 3.8 (**6**) to 4.5 (**8a**), indicating that the *N*-methyl group could make some specific interaction with GPR119. Based on the comparison of **8a** with **3b** ($LogD_{7.4}$ = 3.4), we assumed that increasing lipophilicity would further enhance GPR119 agonist activity.

To investigate the spatial tolerance around the bridging nitrogen and enhance agonist activity, the size of the substituent on the nitrogen atom was increased by elongating the alkyl chain, such as ethyl, *n*-propyl, or cyclopropylmethyl moiety (**8b–d**). All these compounds exhibited similar potency to *N*-methyl analog **8a**. This result suggested that, as expected, GPR119 would have a hydrophobic space between the pyrimidine and piperidine rings in interaction and accommodate a substituent larger than methyl. However, despite an increase in lipophilicity of *N*-substituent, no further enhancement of GPR119 activity was observed for **8b–d**.

To enhance GPR119 agonist activity, another strategy to increase the lipophilicity of an *N*-substituent other than the elongation of carbon atoms was applied. In particular, we designed an *N*-trifluoromethyl analog, for which the entropic cost upon binding to GPR119 might be reduced relative to compounds with flexible *N*-substituents such as **8b–d**.

N-trifluoromethyl analog **10** gratifyingly exhibited an enhanced agonist activity with an EC_{50} value of 2.5 nM and also maintained a comparable LLE value to that of *N*-methyl analog **8a** (LLE = 4.5 for **10** and 4.5 for **8a**). The comparison between **10** and **8d** with similar lipophilicity also illustrated the usefulness of the trifluoromethyl group in terms of GPR119 agonist activity.

We examined the effect of *N*-substituents on hERG inhibition. Unfortunately, NH derivative **6** and *N*-alkyl derivatives **8a–d** were found to potently block the hERG channel current with almost complete inhibition at 10 μM, independently of the size of *N*-substituent. The insertion of an oxygen atom in the *N*-substituent to lower lipophilicity of the compound had no clear beneficial effect on hERG inhibition profile **8e**. However, the introduction of the trifluoromethyl group had a positive impact on hERG inhibition, and compound **10** exhibited low hERG inhibitory activity even at 30 μM.

This result might be assumed to be attributed to the lowered basicity of the aminopyrimidine moiety by the high electronegativity of fluorine atoms.[17] To examine this assumption, we tested the pKa values of the conjugate acids of *N*-methyl analog **8a** and *N*-trifluoromethyl analog **10** using an ultraviolet (UV) spectrophotometric method. Indeed, the basicity of compound **10** was altered, reducing pKa to 0.8 (**10**) from 3.7 (**8a**). However, since the basicity of *N*-methyl analog **8a** was not essentially very high and oxygen-linked analog **1** with a pKa value of 1.9

displayed a potent hERG inhibitory activity, it is unlikely that the lowering of basicity by electron withdrawing property of the trifluoromethyl group would be the only cause of the dramatic effect on hERG inhibition. In effect, it can be presumed that the reduced π-electron density of the pyrimidine ring by the trifluoromethyl group is associated with the improved hERG inhibition profile of **10,** because π-stacking is considered a major determinant of hERG–drug interaction.[17]

Mindful of the distinctive conformational preference of trifluoromethyl aryl ethers,[18] we anticipated that the introduction of the trifluoromethyl group would affect conformational orientation around the bridging nitrogen, leading to a different propensity for hERG inhibition. To verify this hypothesis, X-ray analyses of single-crystal structures of *N*-methyl, ethyl, and trifluoromethyl analogs (**8a**, **8b**, and **10**) were conducted, mainly because the conformational preference of *N*-trifluoromethyl anilines has not been reported (Table 2). In general, anilines prefer planar conformation to achieve π-conjugation of the anilinic nitrogen atom with the aryl system. It has been reported that anilines are often slightly pyramidal and that dimethyl aniline, which is the simplest *N*,*N*-disubstituted aniline, has nonsymmetrical conformation with one methyl group in plane with the ring and the other one out of plane deviating 14° from the ring.[19] Our X-ray examination illustrated the same tendency for *N*-methyl analog **8a** and *N*-ethyl analog **8b** with similar conformations. As shown by the distance between the nitrogen atom and the plane formed by nitrogen's three substituents (C4, C4′, and C1″), nitrogen atoms at the bridging point exhibit slight inclination to pyramidal conformation. The carbon atoms at the 4-position of the piperidine ring (C4′) of **8a** and **8b** are located in plane, whereas the other carbon atom (C1″) deviates 10–15°from the pyrimidine plane. For *N*-trifluoromethyl analog **10**, however, both substituents on the nitrogen atom are out of plane, with a larger dihedral angle of 26° (τ1). There was hardly any difference in the degree of pyramidalization observed for these three compounds. Since the trifluoromethyl group has a similar size to the ethyl group, which was supported by the analysis of van der Waals volumes and experimental assessment using a protease probe,[20,21] the electron withdrawing nature of fluorine atoms of **10** likely reduces the degree of π-conjugation between the nitrogen atom and the pyrimidine ring to further deviate the *N*-substituents from the plane. We assumed that the improved hERG profiles of the *N*-trifluoromethyl derivatives would be driven by this conformational preference together with the reduced π-electron density of the pyrimidine ring by the trifluoromethyl group.

We discovered that the *N*-trifluoromethyl group was a key motif of the central moiety to attain high GPR119/hERG selectivity and focused on further optimization based on **10**. The acid-labile Boc group of **10** was replaced with 3-isopropyl-1,2,4-oxadiazole, which had been found as a surrogate moiety of the Boc group in this series of our previous work.[11] As expected, oxadiazole derivative **13** displayed potent GPR119 agonist activity similar to **10** (Fig. 3). Our further profiling of **13** illustrated the low solubility of this compound. We thought that the cause of low solubility was linked to the planarity of the molecule and hypothesized that the conversion of the aromatic ring to a nonaromatic ring would enable the escape of the planar structure and improvement in solubility.

In our previous report, we identified that the hydrogen bond acceptor (HBA) existing on the left-hand side of the molecule is important for exhibiting GPR119 agonistic activity.[11,12] We assumed that the indoline ring would not be essential and could be replaced with another motif if the HBA existed in a proper arrangement. Based on this hypothesis, instead of the indoline ring, we tried to introduce various nonaromatic rings (Fig. 3).

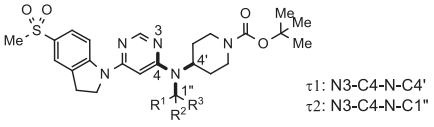
The results of compounds with a non-aromatic ring system on the left-hand side are shown in Table 3. To confirm whether the activity was retained after each modification, the substituent on the nitrogen atom of the right piperidine ring was fixed with the Boc group. 4-Amino piperidine derivative **16** exhibited an EC_{50} value of 43 nM, which was attenuated nearly 20-fold relative to indoline derivative **10**. *N*-Methylated compound **17** showed loss of activity, whereas the derivative in which a linker atom was converted to an oxygen atom (**18**) showed tenfold improved activity compared with **16**, exhibiting the agonist activity equivalent to that of indoline derivative **10**. The activity of *trans*-cyclohexane **20** was 70-fold weaker than that of **18**. Moreover, azetidine derivatives **21** and **22** exhibited further attenuated potency (7100 nM and 960 nM, respectively). These results supported our hypothesis that the indoline ring can be replaced with other saturated rings and a proper arrangement of the HBA would be important for the expression of potent agonistic activity.

We selected **18** as the new lead compound, and the Boc group was replaced with the 3-isopropyl 1,2,4-oxadiazole ring to give **25a** as before. Compared with **13** and consistent with our expectation, compound **25a** displayed potent GPR119 agonist activity and dramatically improved solubility (Table 4, <0.22–29 μg/mL). This compound displayed good metabolic stability in rat microsomes but was rapidly metabolized by human microsomes. The metabolic vulnerability of **25a** in human microsomes may be due to the isopropyl moiety susceptible to metabolic oxidation. Based on this assumption, structural modification was employed for the isopropyl group of **25a** to improve its metabolic stability (Table 4). Consequently, the cyclization of the isopropyl moiety (**25b**) or the introduction of a fluorine atom at the 2-position of the isopropyl moiety (**27**) remarkably improved human microsomal stability and retained equivalent potency for **25a**. This remarkable improvement in microsomal stability illustrates the oxidation of the isopropyl moiety of **25a** as a major route of metabolism by human microsomes.

We selected **27** as a representative compound for further evaluation. First, we confirmed the rat GPR119 agonist potency of compound **27**, with compound **27** displaying strong agonistic activity (EC_{50} = 24 nM). We then evaluated the compound's effects on hormone secretion in some in vitro cell lines, because GPR119 is involved in glucose-dependent insulin secretion and gastrointestinal hormone release (e.g., GLP-1). Compound **27** augmented GLP-1 secretion in mouse intestinal L cell line (i.e., GLUTag cells) and insulin secretion in hamster pancreatic beta cell line (i.e., HIT-T15) (Table 5). These results demonstrated the strong activities of in vitro hormone secretion of compound **27**. Next, we investigated hERG inhibitory activity and pharmacokinetic parameters in rats for **27** (Table 6). This compound displayed low hERG inhibitory activity as with *N*-trifluoromethyl derivatives thus far. In vivo low clearance illustrated the in vitro low clearance of **27**, with the compound displaying favorable area under the curve, prolonged mean residence time, and good oral bioavailability (F = 57%).

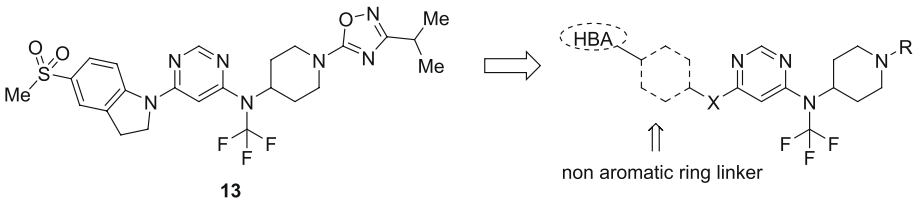
The antidiabetic effects of **27** were assessed by an oral glucose

Table 2
Analysis of the single-crystal X-ray structures of 6-(indolin-1-yl)-*N*-(piperidin-4-yl)pyrimidin- 4-amine derivatives.



Compound	R^1 R^2R^3	Distance *N* plane[b] (angstrom)	Dihedral angle (degree)	
			τ1	τ12
8a[a]	Me	0.089 (0.0021)	1.8	169.3
		0.077 (0.0020)	1.3	170.6
8b[a]	Et	0.102 (0.0021)	−1.2	164.7
		0.065 (0.0020)	−0.9	170.4
10	CF_3	0.097 (0.0018)	−26.5	166.9

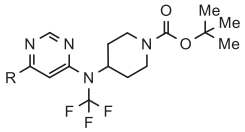
[a] Two crystallographically independent X-ray structures were observed.
[b] Distance between the nitrogen atom at the 4-position of the pyrimidine ring and the triangle formed by nitrogen's three substituents: C4, C4′, and C1″. Mean values and standard deviations are presented in parentheses.

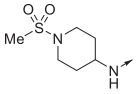
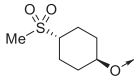
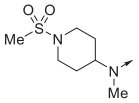
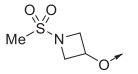
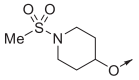
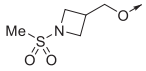


13
human GPR119 EC_{50} = 2.9 nM
Solubility(pH6.8) = <0.22 μg/mL
logD(pH7.4) = 3.79

Fig. 3. Profile of **13** and design of compounds for better solubility while retaining potency.

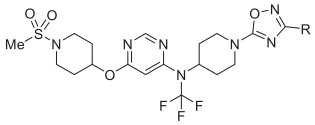
Table 3
Replacement of the indoline ring with various nonaromatic rings.



Compound	R	hGPR119[a] EC_{50}(nM)	Compound	R	hGPR119[a] EC_{50}(nM)
16		43	**20**		270
17		>10000	**21**		7100
18		3.6	**22**		960

[a] Agonist activity against human GPR119. EC_{50} values are shown as mean (n = 2).

Table 4
Exploration of the substitute on 1,2,4-oxadiazole.



Compound	R	hGPR119[a] EC_{50} (nM)	$CL_{int.}$[b] (μL/min/mg)		Solubility[c] (μg/mL)
			HLM	RLM	
25a	^{i}Pr	2.7	108	36	29
25b	^{c}Pr	5.4	31	13	15
27		4.6	27	<1	21

[a] Agonist activity against human GPR119. EC_{50} values are shown as mean (n = 2).
[b] Human or rat hepatic microsomal clearance.
[c] pH6.8.

Table 5
Rat GPR119 agonist activity, in vitro hormone-secretion activity on HIT-T15, and GLUTag cell line of **27.**

Compound	rGPR119[a] EC_{50} (nM)	Insulin secretion in HIT-T15[b] EC_{50} (nM)	GLP-1 secretion in GLUTag[c] EC_{50} (nM)
27	24	4.8	3.8

[a] Agonist activity against rat GPR119. EC_{50} values are shown as mean (n = 2).
[b] Insulin secretion assay in HIT-T15 cell (n = 2).
[c] GLP-1 secretion assay in GLUTag cell (n = 2).

Table 6
hERG inhibitory activity and pharmacokinetic profiles of **27** in rats.

Compound	hERG[a]% inhibitionat 10 μM	V_{dss}[b](mL/h/kg)	CL_{total}[b] (mL/h/kg)	AUC[c] (ng*h/kg)	MRT[c] (h)	F[c] (%)
27	19	1270	373	1540	4.05	57

[a] Automated patch clamp assay. Percentages of inhibition are represented as mean ± standard deviation (n = 4).
[b] Rat cassette dosing, 0.1 mg/kg, iv.
[c] Rat cassette dosing, 1 mg/kg, po.

tolerance test (OGTT) in *N*-STZ-1.5 rats (Fig. 4). Compound **27** and vehicle were administered 1 h before the oral glucose load, and plasma glucose and insulin concentrations were monitored over 2 h. As shown in Fig. 4, compound **27** effectively lowered the plasma glucose levels after oral administration, which was accompanied by dose-dependent insulin secretion. The glucose-lowering effect of compound **27** was significant ($p < 0.025$) at a dose of 0.3 mg/kg.

4. Conclusion

We described the optimization of an initial lead **1** to **27**. The novel potent GPR119 agonist **27** with excellent ADME-Tox profiles showed a remarkable blood glucose-lowering effect from the dosing of 0.3 mg/kg in the OGTT in *N*-STZ-1.5 rats. Through the optimization effort, we discovered that the *N*-trifluoromethyl group was a key motif of the central moiety; it not only increased agonistic activity but also yielded high GPR119/hERG selectivity. Improvement of hERG profiles may be ascribed to conformational preference, with reduced π-electron density of the pyrimidine ring linked to the trifluoromethyl group. A second key

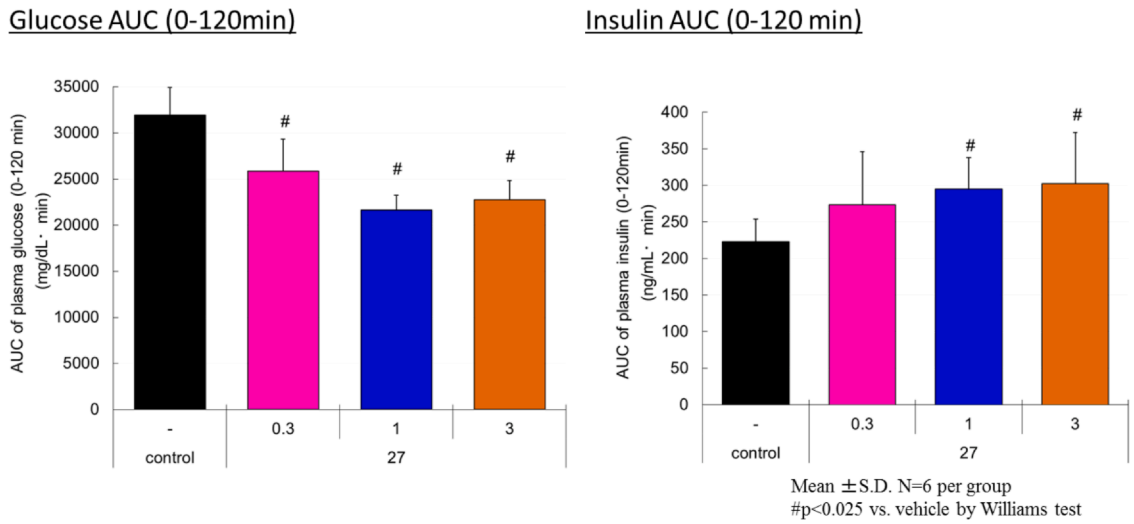


Fig. 4. OGTT results using **27** in *N*-STZ-1.5 rats. Glucose-lowering and insulinotropic effects of compound **27** during OGTT by a single dose in *N*-STZ-1.5 rats. (A) $AUC_{0–120min}$ of plasma glucose levels and (C) plasma insulin levels. Values are mean ± standard deviation ($n = 6$): (#) $p \leq 0.025$ vs. control by one-tailed Williams' test.

challenge in this program was the weak solubility of the new lead **13**. We focused on the number of aromatic rings and succeeded in improving solubility by replacing the indoline ring with a piperidin-4-yl-oxy moiety. Subsequent modification to the potential metabolic site of **25a**, which was the alkyl moiety on the 1,2,4-oxadiazole ring, led to the identification of **27** having a fluorine atom introduced at the branching site of the isopropyl group. More follow-up findings will be reported in the near future.

5. Experimental section

5.1. Chemistry

NMR spectra were recorded on Bruker AVANCE III or Bruker AVANCE 300 spectrometer (^{1}H at 300 MHz and ^{19}F at 282 MHz). Chemical shifts for ^{1}H NMR are given in parts per million (ppm) downfield from tetramethysilane (δ) as the internal standard in deuterated solvent and coupling constants (J) are in Hertz (Hz). Data are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, td = triplet of doublets, tt = triplet of triplets, dtd = doublet of triplet of doublets, ddd = doublet of doublet of doublets, bs = broad singlet), and coupling constants. All solvents and reagents were obtained from commercial suppliers and used without further purification. Thin-layer chromatography (TLC) was performed on Merck silica gel plates 60F254. Column chromatography was performed on silica gel 60 (0.063–0.200 or 0.040–0.063 mm, E. Merck), basic silica gel (Chromatorex NH, 100–200 mesh, Fuji Silysia Chemical Ltd.) or Purif-Pack (Si or NH, Moritex Corporation). LC–MS analysis was performed on a Waters, Agilent, or Shimadzu Liquid Chromatography–Mass Spectrometer System, operating in APCI (+or −) or ESI (+or −) ionization mode. Analytes were eluted using a linear gradient of 0.05% TFA containing water/acetonitrile or 5 mM ammonium acetate containing water/acetonitrile mobile phase. Determination of chemical purity by HPLC (detection at 220 nm) was conducted using a Shimadzu Liquid Chromatography System with 0.05% TFA containing water/acetonitrile mobile phase. Elemental analyses were performed by Takeda Analytical Research Laboratories, Ltd. Yields are not optimized.

5.1.1. tert-Butyl 4-[(6-chloropyrimidin-4-yl)(trifluoroacetyl)amino]piperidine-1-carboxylate (5)

Step A. A mixture of 4,6-dichloropyrimidine (8.93 g, 59.9 mmol), *tert*-butyl 4-aminopiperidine-1-carboxylate (10.0 g, 49.9 mmol), and TEA (10.5 mL, 75.3 mmol) in EtOH was stirred at 80 °C for 16 h. After the mixture was concentrated under reduced pressure, the residue was diluted with AcOEt, washed with water and brine, and dried over magnesium sulfate ($MgSO_4$). The solvent was removed by evaporation to give *tert*-butyl 4-[(6-chloropyrimidin-4-yl)amino]piperidine-1-carboxylate as a solid (13.2 g, 84%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30–1.55 (11*H*, m), 1.93–2.11 (2H, m), 2.81–3.06 (2H, m), 3.86 (1H, bs), 3.98–4.23 (2H, m), 5.11 (1H, bs), 6.34 (1H, s), 8.34 (1H, s).

Step B. To a mixture of *tert*-butyl 4-[(6-chloropyrimidin-4-yl)amino] piperidine-1-carboxylate (8.00 g, 25.6 mmol) and TEA (5.44 mL, 39.0 mmol) in THF was added TFAA (6.45 g, 30.7 mmol) at 0 °C, and the mixture was stirred at room temperature for 16 h. The reaction was quenched by addition of water and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. After removal of the solvent, the residue was purified by silica gel column chromatography (hexane/AcOEt = 95/5 to 60/40) to give the title compound as an oil (**5**, 9.22 g, 88%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 (9H, s), 1.48–1.71 (2H, m), 1.87 (2H, d, $J = 12.1$ Hz), 2.78 (2H, t, $J = 12.6$ Hz), 4.06–4.36 (2H, m), 4.45–4.67 (1H, m), 7.27 (1H, s), 9.01 (1H, s).

5.1.2. 1-(6-Chloropyrimidin-4-yl)-5-(methylsulfonyl)-2,3-dihydro-1H-indole (7)

A mixture of 4,6-dichloropyrimidine (4.10 g, 27.5 mmol), 5-(methylsulfonyl)-2,3-dihydro-1*H*-indole (5.00 g, 25.0 mmol), and EtOH (160 mL) was refluxed for 4 h. After the mixture was concentrated under reduced pressure, saturated aqueous $NaHCO_3$ solution was added to the residue. The precipitated solid was collected by filtration, washed with water, and dried under reduced pressure to give the title compound as a colorless solid (5.50 g, 71%). MS (ESI/APCI) *m/z* 310 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.17 (3H, s), 3.24–3.37 (2H, m), 4.16 (2H, t, $J =$ 8.7 Hz), 7.09 (1H, s), 7.73–7.85 (2H, m), 8.57 (1H, d, $J = 9.4$ Hz), 8.68 (1H, s).

5.1.3. tert-Butyl 4-({6-[5-(methylsulfonyl)-2,3-dihydro-1H-indol-1-yl] pyrimidin-4-yl}amino)piperidine-1-carboxylate (6)

Method A (from compound **5**). A mixture of compound **5** (9.00 g, 22.0 mmol), 5-(methylsulfonyl)-2,3-dihydro-1*H*-indole (3.00 g, 15.2 mmol), $Pd_2(dba)_3$ (641 mg, 0.700 mmol), Xantphos (810 mg, 1.40 mmol), and Cs_2CO_3 (8.10 g, 24.9 mmol) in toluene (120 mL) was stirred at 100 °C under Argon (Ar) atmosphere for 16 h. The reaction mixture was partitioned between AcOEt and water. The organic layer was

washed with brine and dried over $MgSO_4$. The solvent was removed by evaporation to give an oil. The oil was dissolved in THF/MeOH (1:1 v/v, 200 mL), and then 1 M aqueous NaOH solution (15 mL) was added. The resulting mixture was stirred at room temperature for 2 h. After removal of THF and MeOH, the residue was extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to give a crude material, which was purified by silica gel column chromatography (hexane/AcOEt = 50/50 to 10/90) to give the title compound as a white solid (2.40 g, 33% over 2 steps). MS (ESI/APCI) *m/z* 474 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.14–1.38 (2H, m), 1.41 (9H, s), 1.78–1.95 (2H, m), 2.80–3.03 (2H, m), 3.12 (3H, s), 3.25 (2H, t, J = 8.7 Hz), 3.79–4.13 (5H, m), 5.78 (1H, s), 7.14 (1H, d, J = 7.5 Hz), 7.59–7.75 (2H, m), 8.25 (1H, s), 8.47 (1H, d, J = 9.0 Hz). Anal. Calcd for $C_{23}H_{31}N_5O_4S$: C, 58.33; H, 6.60; N, 14.79. Found: C, 58.33; H, 6.71; N, 14.74.

Method B (from compound **7**). A mixture of compound **7** (11.0 g, 35.6 mmol), *tert*-butyl 4-aminopiperidine-1-carboxylate (10.0 g, 49.9 mmol), $Pd_2(dba)_3$ (1.83 g, 2.00 mmol), XPhos (1.91 g, 4.01 mmol), and Cs_2CO_3 (23.4 g, 71.8 mmol) in toluene (150 mL) was stirred at 110 °C under nitrogen (N_2) atmosphere for 16 h. The reaction mixture was partitioned between AcOEt and water. The organic layer was washed with brine and dried over $MgSO_4$. After removal of the solvent, the residue was purified by silica gel column chromatography (hexane/AcOEt = 70/30 to 20/80) to give the title compound as a white solid (7.10 g, 42%).

5.1.4. tert-Butyl 4-(methyl{6-[5-(methylsulfonyl)-2,3-dihydro-1H-indol-1-yl]pyrimidin-4-yl}amino)piperidine-1-carboxylate (8a)

Compound **8a** was prepared from compound **6** and iodomethane in a manner similar to that described for compound **8b**. White solid. Yield 88%. MS (ESI/APCI) *m/z* 488 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 1.48 (9H, s), 1.57–1.77 (4H, m), 2.78–2.94 (5H, m), 3.02 (3H, s), 3.27 (2H, t, J = 8.7 Hz), 3.98–4.17 (2H, m), 4.17–4.39 (2H, m), 4.81–5.04 (1H, m), 5.59 (1H, s), 7.68 (1H, s), 7.75 (1H, dd, J = 8.7, 1.9 Hz), 8.39 (1H, s), 8.53 (1H, d, J = 8.7 Hz). Anal. Calcd for $C_{24}H_{33}N_5O_4S$: C, 59.12; H, 6.82; N, 14.36. Found: C, 58.95; H, 6.90; N, 14.63.

5.1.5. tert-Butyl 4-(ethyl{6-[5-(methylsulfonyl)-2,3-dihydro-1H-indol-1-yl]pyrimidin-4-yl}amino)piperidine-1-carboxylate (8b)

To a mixture of compound **6** (1.30 g, 2.75 mmol) and iodoethane (0.328 mL, 4.10 mmol) in DMF (20 mL) was added sodium hydride (60% oil dispersion, 164 mg, 4.1 mmol) at 0 °C, and the mixture was stirred at room temperature for 16 h. The reaction was quenched by addition of water and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. After removal of the solvent, the residue was purified by silica gel column chromatography (hexane/AcOEt = 90/10 to 50/50) to give the title compound as a white solid (903 mg, 65%). MS (ESI/APCI) *m/z* 502 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 1.22 (3H, t, J = 7.0 Hz), 1.48 (9H, s), 1.56–1.84 (4H, m), 2.76–2.95 (2H, m), 3.03 (3H, s), 3.19–3.48 (4H, m), 4.09 (2H, t, J = 8.7 Hz), 4.22 (2H, bs), 4.87 (1H, bs), 5.61 (1H, s), 7.68 (1H, s), 7.75 (1H, dd, J = 8.7, 1.9 Hz), 8.39 (1H, s), 8.50 (1H, d, J = 8.7 Hz). Anal. Calcd for $C_{25}H_{35}N_5O_4S$: C, 59.86; H, 7.03; N, 13.96. Found: C, 59.57; H, 6.98; N, 13.78.

5.1.6. tert-Butyl 4-[{6-[5-(methylsulfonyl)-2,3-dihydro-1H-indol-1-yl]pyrimidin-4-yl}(propyl)amino]piperidine-1-carboxylate (8c)

Compound **8c** was prepared from compound **6** and 1-iodopropane in a manner similar to that described for compound **8b**. White solid. Yield 66%. MS (ESI/APCI) *m/z* 516 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 0.89–1.06 (3H, m), 1.49 (9H, s), 1.59–1.82 (6H, m), 2.76–2.94 (2H, m), 3.03 (3H, s), 3.11–3.23 (2H, m), 3.28 (2H, t, J = 8.7 Hz), 4.08 (2H, t, J = 8.7 Hz), 4.15–4.38 (2H, m), 4.85 (1H, bs), 5.58 (1H, s), 7.69 (1H, s), 7.75 (1H, dd, J = 8.7, 1.9 Hz), 8.39 (1H, s), 8.48 (1H, d, J = 8.7 Hz). Anal. Calcd for $C_{26}H_{37}N_5O_4S$: C, 60.56; H, 7.23; N, 13.58. Found: C, 60.52; H, 7.21; N, 13.50.

5.1.7. tert-Butyl 4-[(cyclopropylmethyl){6-[5-(methylsulfonyl)-2,3-dihydro-1H-indol-1-yl]pyrimidin-4-yl}amino]piperidine-1-carboxylate (8d)

Compound **8d** was prepared from compound **6** and (bromomethyl) cyclopropane in a manner similar to that described for compound **8b**. White solid. Yield 52%. MS (ESI/APCI) *m/z* 528 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 0.25–0.39 (2H, m), 0.57–0.70 (2H, m), 0.91–1.07 (1H, m), 1.48 (9H, s), 1.55–1.85 (4H, m), 2.78–2.93 (2H, m), 3.03 (3H, s), 3.21 (2H, d, J = 5.7 Hz), 3.28 (2H, t, J = 8.7 Hz), 4.10 (2H, t, J = 8.9 Hz), 4.16–4.40 (2H, m), 4.66–4.90 (1H, m), 5.79 (1H, s), 7.68 (1H, s), 7.75 (1H, dd, J = 8.5, 2.1 Hz), 8.40 (1H, s), 8.51 (1H, d, J = 8.7 Hz). Anal. Calcd for $C_{27}H_{37}N_5O_4S$: C, 61.46; H, 7.07; N, 13.27. Found: C, 61.16; H, 7.25; N, 13.37.

5.1.8. tert-Butyl 4-[(2-methoxyethyl){6-[5-(methylsulfonyl)-2,3-dihydro-1H-indol-1-yl]pyrimidin-4-yl}amino]piperidine-1-carboxylate (8e)

Compound **8e** was prepared from compound **6** and 2-bromoethyl methyl ether in a manner similar to that described for compound **8b**. White solid. Yield 9%. MS (ESI/APCI) *m/z* 532 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 1.48 (9H, s), 1.53–1.84 (4H, m), 2.75–2.94 (2H, m), 3.03 (3H, s), 3.28 (2H, t, J = 8.7 Hz), 3.38 (3H, s), 3.51 (4H, s), 4.00–4.38 (4H, m), 4.65–4.87 (1H, m), 5.83 (1H, s), 7.64–7.80 (2H, m), 8.40 (1H, s), 8.49 (1H, d, J = 8.7 Hz). Anal. Calcd for $C_{26}H_{37}N_5O_5S$: C, 58.74; H, 7.01; N, 13.17. Found: C, 59.13; H, 7.11; N, 12.77.

5.1.9. tert-Butyl 4-([(methylsulfanyl)carbonothioyl]{6-[5-(methylsulfonyl)-2,3-dihydro-1H-indol-1-yl]pyrimidin-4-yl}amino) piperidine-1-carboxylate (9)

To a mixture of compound **6** (8.32 g, 17.6 mmol) in THF (500 mL) was added dropwise ^{n}BuLi (1.6 M hexane solution, 13.2 mL, 21.1 mmol) at 0 °C under N_2 atmosphere, and the mixture was stirred at 0 °C for 1 h. Carbon disulfide (1.59 mL, 26.4 mmol) was added to the mixture at 0 °C. The mixture was allowed to warm to room temperature followed by stirring for 7 h. After cooling to 0 °C, MeI (3.28 mL, 52.7 mmol) was added, and the resulting mixture was stirred at room temperature for 63 h. The reaction was quenched by addition of water and extracted with AcOEt. The organic layer was washed with brine and dried over sodium sulfate (Na_2SO_4). After removal of the solvent, the residue was purified by silica gel column chromatography (hexane/AcOEt = 60/40 to 0/100) to give the title compound as a pale yellow amorphous solid (5.91 g, 60%). ^1H NMR (300 MHz, $CDCl_3$) δ 1.41 (9H, s), 1.49 (2H, dd, J = 12.5, 4.5 Hz), 2.10 (2H, d, J = 12.1 Hz), 2.60 (3H, s), 2.82 (2H, t, J = 13.0 Hz), 3.07 (3H, s), 3.38 (2H, t, J = 8.5 Hz), 4.07–4.29 (4H, m), 5.54 (1H, tt, J = 11.9, 3.6 Hz), 6.57 (1H, d, J = 0.8 Hz), 7.79 (1H, d, J = 1.5 Hz), 7.86 (1H, dd, J = 8.5, 2.1 Hz), 8.65 (1H, d, J = 8.7 Hz), 8.90 (1H, d, J = 1.1 Hz).

5.1.10. tert-Butyl 4-[{6-[5-(methylsulfonyl)-2,3-dihydro-1H-indol-1-yl]pyrimidin-4-yl}(trifluoromethyl)amino]piperidine-1-carboxylate (10)

Condition A. To a mixture of compound **9** (170 mg, 0.302 mmol) and $TBAH_2F_3$ (455 mg, 1.51 mmol) in toluene (20 mL) was added NBS (215 mg, 1.21 mmol) at room temperature. The mixture was stirred at room temperature for 6 h. The reaction mixture was diluted with AcOEt and quenched by addition of aqueous NaOH solution. The organic layer was separated, washed with water and brine, and dried over Na_2SO_4. After removal of the solvent, the residue was purified by silica gel column chromatography (hexane/AcOEt = 70/30 to 0/100) to give a solid. The obtained solid was suspended in diisopropylether (IPE) and the precipitated solid was collected to give the title compound as a white solid (34 mg, 21%). MS (ESI/APCI) *m/z* 542 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 1.48 (9H, s), 1.80–1.93 (2H, m), 1.99–2.14 (2H, m), 2.79 (2H, t, J = 12.1 Hz), 3.04 (3H, s), 3.27–3.39 (2H, m), 4.06–4.33 (4H, m), 4.57–4.75 (1H, m), 6.26 (1H, d, J = 1.5 Hz), 7.73 (1H, d, J = 1.5 Hz), 7.80 (1H, dd, J = 8.7, 1.9 Hz), 8.56 (1H, d, J = 8.7 Hz), 8.62 (1H, s). ^{19}F NMR (282 MHz, DMSO-d_6) δ − 51.16 (s). Anal. Calcd for $C_{24}H_{30}F_3N_5O_4S$: C, 53.22; H, 5.58; N, 12.93. Found: C, 53.22; H, 5.47; N, 12.93.

Condition B. To a mixture of compound **9** (564 mg, 1.00 mmol) and

$TBAH_2F_3$ (1.51 g, 5.00 mmol) in toluene (10 mL) was added NBS (712 mg, 4.00 mmol) at −78 °C. After stirring at −78 °C for 30 min, the mixture was allowed to warm to at 0 °C followed by stirring at 0 °C for 7 h. The reaction mixture was diluted with AcOEt and quenched by addition of aqueous NaOH solution. The organic layer was separated, washed with water and brine, and dried over Na_2SO_4. After removal of the solvent, the residue was purified by silica gel column chromatography ((hexane/AcOEt = 70/30 = 0/100) to give the title compound as a white solid (220 mg, 41%).

5.1.11. 6-[5-(Methylsulfonyl)-2,3-dihydro-1H-indol-1-yl]-N-(piperidin-4-yl)-N-(trifluoromethyl)pyrimidin-4-amine (11)

A mixture of compound **10** (220 mg, 0.405 mmol), 4 M HCl in AcOEt (2 mL), AcOEt (10 mL), and MeOH (10 mL) was stirred at room temperature for 20 h. After the reaction mixture was concentrated under reduced pressure, the residue was diluted with AcOEt and water, and then basified with aqueous NaOH solution. The organic layer was separated and dried over Na_2SO_4. The solvent was removed by evaporation and dried to give the title compound as a white solid (156 mg, 87%). MS (ESI/APCI) *m/z* 442 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 1.83–1.96 (2H, m), 1.98–2.16 (2H, m), 2.72 (3H, td, J = 12.6, 2.8 Hz), 3.04 (3H, s), 3.19 (2H, dd, J = 10.6, 2.6 Hz), 3.32 (2H, t, J = 8.5 Hz), 4.12 (2H, t, J = 8.7 Hz), 4.48–4.66 (1H, m), 6.27 (1H, d, J = 1.1 Hz), 7.73 (1H, d, J = 1.1 Hz), 7.80 (1H, dd, J = 8.7, 2.3 Hz), 8.56 (1H, d, J = 8.7 Hz), 8.63 (1H, d, J = 0.8 Hz).

5.1.12. 4-[{6-[5-(Methylsulfonyl)-2,3-dihydro-1H-indol-1-yl]pyrimidin-4-yl}(trifluoromethyl)amino]piperidine-1-carbonitrile (12)

To a mixture of compound **11** (330 mg, 0.748 mmol), $NaHCO_3$ (190 mg, 2.26 mmol), THF (15 mL), and water (5 mL) was added cyanogen bromide (105 mg, 0.991 mmol) at 0 °C, and the mixture was stirred at room temperature for 16 h. The reaction mixture was diluted with saturated aqueous $NaHCO_3$ solution and extracted with a mixed solvent of AcOEt and THF. The organic layer was separated, washed with brine, and dried over $MgSO_4$. The solvent was removed by evaporation and dried to give the title compound as a white solid (335 mg, 96%). MS (ESI/APCI) *m/z* 467 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.84–2.12 (4H, m), 3.14–3.30 (7H, m), 3.40–3.54 (2H, m), 4.12–4.21 (2H, m), 4.26–4.42 (1H, m), 6.38 (1H, s), 7.73–7.82 (2H, m), 8.56 (1H, d, J = 9.0 Hz), 8.70 (1H, s).

5.1.13. 6-[5-(Methylsulfonyl)-2,3-dihydro-1H-indol-1-yl]-N-{1-[3-(propan-2-yl)-1,2,4-oxadiazol-5-yl]piperidin-4-yl}-N-(trifluoromethyl)pyrimidin-4-amine (13)

To a mixture of compound **12** (500 mg, 1.07 mmol) and *N*-hydroxy-2-methylpropanimidamide (131 mg, 1.29 mmol) in anhydrous DMF (20 mL) were added ZnCl (1.0 M Et_2O solution, 0.640 mL, 0.640 mmol) and *p*-TsOH monohydrate (122 mg, 0.641 mmol) at room temperature, and the mixture was stirred at 85 °C for 10 h. The reaction mixture was diluted with AcOEt successively washed with 0.03 M hydrochloric acid, 0.03 M aqueous NaOH solution, and brine, and dried over Na_2SO_4. After removal of the solvent, the residue was purified by silica gel column chromatography (hexane/AcOEt = 50/50 to 20/80) to give the title compound as a white powder (410 mg, 69%). MS (ESI/APCI) *m/z* 552 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.18 (6H, d, J = 6.8 Hz), 1.94–2.07 (4H, m), 2.74–2.89 (1H, m), 3.16 (3H, s), 3.19–3.39 (4H, m), 4.01–4.11 (2H, m), 4.17 (2H, t, J = 8.5 Hz), 4.36–4.52 (1H, m), 6.40 (1H, s), 7.73–7.80 (2H, m), 8.51–8.59 (1H, m), 8.69 (1H, s). ^{19}F NMR (282 MHz, DMSO-d_6) δ − 51.34 (s). Anal. Calcd for $C_{24}H_{28}F_3N_7O_3S{\cdot}0.1AcOEt$: C, 52.30; H, 5.18; N, 17.50. Found: C, 52.49; H, 5.28; N, 17.11.

5.1.14. tert-Butyl 4-(6-chloropyrimidin-4-ylamino)piperidine-1-carboxylate (14)

A mixture of 4,6-dichloropyrimidine (**4**, 18.5 g, 124 mmol), *tert*-butyl 4-aminopiperidine-1-carboxylate (24.8 g, 124 mmol), and TEA (34.5 mL, 248 mmol) in MeCN (250 mL) was stirred at 85 °C for 10 h. After the reaction mixture was concentrated in vacuo, the residue was diluted with AcOEt and THF, washed with 0.2 M aqueous NaOH solution and brine, and dried over Na_2SO_4. The solvent was removed by evaporation to give a pale yellow solid. The solid was suspended in AcOEt and the precipitated solid was collected to give the title compound as a white solid (29.0 g, 74.7%). MS (ESI/APCI) *m/z* 313 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.16–1.47 (11*H*, m), 1.74–1.92 (2H, m), 2.78–3.01 (2H, m), 3.77–4.12 (3H, m), 6.48 (1H, bs), 7.71 (1H, d, J = 7.6 Hz), 8.27 (1H, s).

5.1.15. tert-Butyl 4-[(6-chloropyrimidin-4-yl)(trifluoromethyl)amino]piperidine-1-carboxylate (15)

Step A. To a solution of **14** (30.0 g, 95.9 mmol) in DMF(dry) (450 mL) was added sodium hydride (60% oil dispersion, 3.84 g, 95.9 mmol) at room temperature. After the mixture was stirred at room temperature for 1 h, carbon disulfide (9.81 mL, 163 mmol) was added dropwise followed by stirring at room temperature for further 2 h. MeI (10.2 mL, 163 mmol) was added and the resulting mixture was stirred at room temperature for 1 h. The reaction was quenched with water. The mixture was extracted with AcOEt, washed with water (3 times) and brine, and dried over Na_2SO_4. After removal of the solvent, the residue was purified by column chromatography (hexane/AcOEt = 90/10 to 80/20) to give *tert*-butyl 4-((6-chloropyrimidin-4-yl)(methylthiocarbonothioyl)amino) piperidine-1-carboxylate as a yellow oil (30.8 g, 80%). MS (ESI/APCI) *m/z* 403 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.22–1.43 (11*H*, m), 1.94–2.06 (2H, m), 2.56 (3H, s), 2.69–2.93 (2H, m), 3.91–4.07 (2H, m), 5.30 (1H, tt, J = 12.0, 3.5 Hz), 8.07 (1H, s), 9.19 (1H, s).

Step B. To a solution of *tert*-butyl 4-((6-chloropyrimidin-4-yl) (methylthiocarbonothioyl)amino)piperidine-1-carboxylate (1.80 g, 4.47 mmol) in PhCl (36 mL) were added triethylamine trihydrofluoride (3.60 g, 22.3 mmol) and 1,3-dibromo-5,5-dimethylimidazolidine-2,4-dione (3.96 g, 13.85 mmol)) at −20 °C. The mixture was stirred at −20 °C under N_2 for 20 min. The reaction was quenched with saturated aqueous $NaHCO_3$ solution at the same temperature. The mixture was extracted with AcOEt, successively washed saturated aqueous $NaHCO_3$ solution, aqueous $Na_2S_2O_3$ solution and brine, and dried over Na_2SO_4. After removal of the solvent, the residue was purified by column chromatography (NH, hexane/AcOEt = 88/12) to give the title compound as a white solid (560 mg, 32.9%). MS (ESI/APCI) *m/z* 381 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.40 (9H, s), 1.75–1.94 (4H, m), 2.74–2.94 (2H, m), 3.95–4.09 (2H, m), 4.42–4.56 (1H, m), 7.31 (1H, s), 8.81 (1H, s).

5.1.16. tert-Butyl 4-[(6-{[1-(methanesulfonyl)piperidin-4-yl]amino}pyrimidin-4-yl)(trifluoromethyl)amino]piperidine-1-carboxylate (16)

A mixture of **15** (300 mg, 0.790 mmol), 1-(methylsulfonyl)piperidin-4-amine (211 mg, 1.18 mmol) and DIPEA (0.274 mL, 1.58 mmol) in DMA (6 mL) was stirred at 100 °C for 15 h. The mixture was stirred at room temperature for 15 h. The mixture was extracted with AcOEt, washed water (3 times) and brine, and dried over Na_2SO_4. After removal of the solvent, the residue was purified by column chromatography (hexane/AcOEt = 50/50 to 20/80) and crystallization from diethyl ether (Et_2O) to give the title compound as a white solid (240 mg, 58.3%). MS (ESI/APCI) *m/z* 523 $[M+H]^+$.^1H NMR (300 MHz, DMSO-d_6) δ 1.33–1.54 (11*H*, m), 1.67–1.85 (4H, m), 1.90–2.03 (2H, m), 2.67–2.97 (7H, m), 3.44–3.55 (2H, m), 3.85–4.08 (3H, m), 4.17–4.31 (1H, m), 6.15 (1H, bs), 7.50 (1H, d, J = 7.6 Hz), 8.29 (1H, s). Anal. Calcd for $C_{21}H_{33}N_6O_4SF_3$:C,48.26;H,6.36;N,16.08;S,6.14;F,10.91.Found:C, 48.12;H,6.41;N,15.89.

5.1.17. tert-Butyl 4-[(6-{[1-(methanesulfonyl)piperidin-4-yl](methyl)amino}pyrimidin-4-yl)(trifluoromethyl)amino]piperidine-1-carboxylate (17)

To a mixture of **16** (140 mg, 0.270 mmol) in DMF(dry) (6 mL) was added NaH (12.9 mg, 0.320 mmol) at room temperature. After the

mixture was stirred at room temperature for 30 min, MeI (0.0250 mL, 0.400 mmol) was added. The resulting mixture was stirred at room temperature for 1 h. The mixture was quenched with water, extracted with AcOEt, washed with water (3 times) and brine, and dried over Na_2SO_4. After removal of the solvent, the residue was purified by column chromatography (Hexane/AcOEt = 60/40 to 30/70) to give the title compound as a pale yellow solid (120 mg, 83%). MS (ESI/APCI) *m/z* 537 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.38 (9H, s), 1.60–1.92 (8H, m), 2.69–2.97 (10*H*, m), 3.58–3.73 (2H, m), 3.91–4.06 (2H, m), 4.10–4.23 (1H, m), 4.53–4.83 (1H, m), 6.15 (1H, s), 8.37 (1H, s). Anal. Calcd for $C_{22}H_{35}N_6O_4SF_3$:C,49.24;H,6.57;N,15.66;S,5.98;F,10.62. Found:C,49.10;H,6.63;N,15.46.

5.1.18. *tert*-Butyl 4-[(6-{[1-(methanesulfonyl)piperidin-4-yl]oxy}pyrimidin-4-yl)(trifluoromethyl)amino]piperidine-1-carboxylate (18)

To a solution of 1-(methylsulfonyl)piperidin-4-ol (21.2 g, 118 mmol) in DMA (450 mL) was added sodium hydride (4.73 g, 118 mmol) at room temperature, and the mixture was stirred at room temperature for 1.5 h. **15** (30.0 g, 78.8 mmol) was added to the mixture at room temperature in one portion. The resulting mixture was stirred at room temperature for 5 h. The reaction was quenched with water. The mixture was extracted with AcOEt, successively washed with water (3 times) and brine, and dried over Na_2SO_4. After removal of the solvent, the residue was dissolved in AcOEt (150 mL) at 80 °C. IPE (300 mL) was added to the mixture at 80 °C and then the resulting mixture was allowed to cool to room temperature. After 15 h, the precipitated solid was collected and washed with IPE to give the title compound as a white powder (32.1 g, 78%). MS (ESI/APCI) *m/z* 524 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.40 (9H, s), 1.69–1.89 (6H, m), 1.99–2.13 (2H, m), 2.71–2.88 (2H, m), 2.90 (3H, s), 3.06–3.19 (2H, m), 3.28–3.43 (2H, m), 3.94–4.07 (2H, m), 4.32–4.47 (1H, m), 5.19–5.30 (1H, m), 6.45 (1H, s), 8.61 (1H, d, $J = 0.8$ Hz). Anal. Calcd for $C_{21}H_{32}N_5O_5SF_3$:C,48.17;H,6.16;N,13.38;S,6.12;F,10.89.Found:C,48.26;H,6.21;N,13.25.

5.1.19. *tert*-Butyl 4-{[6-({(cis)-4-[(methanesulfonyl)oxy]cyclohexyl}oxy)pyrimidin-4-yl](trifluoromethyl)amino}piperidine-1-carboxylate (19)

To a solution of *tert*-butyl 4-((6-((*trans*)-4-hydroxycyclohexyloxy)pyrimidin-4-yl)(trifluoromethyl)amino)piperidine-1-carboxylate (1.28 g, crude, as 2.63 mmol) in pyridine (15 mL) was added methanesulfonyl chloride (0.447 mL, 5.78 mmol) at room temperature. The mixture was stirred at room temperature for 2 h. After the reaction mixture was concentrated in vacuo, the residue was diluted with AcOEt, successively washed with water, 10% aqueous $KHSO_4$ solution, water and brine, and dried over Na_2SO_4. After removal of the solvent, the residue was purified by column chromatography (hexane/AcOEt = 70/30 to 50/50) to give the title compound as a colorless amorphous solid(0.850 g, 60.1%). MS (ESI/APCI) *m/z* 539 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.40 (9H, s), 1.71–1.97 (12H, m), 2.71–2.92 (2H, m), 3.19 (3H, s), 3.93–4.09 (2H, m), 4.31–4.47 (1H, m), 4.73–4.85 (1H, m), 5.13–5.23 (1H, m), 6.45 (1H, s), 8.59 (1H, s).

5.1.20. *tert*-Butyl 4-[(6-{[(trans)-4-(methanesulfonyl)cyclohexyl]oxy}pyrimidin-4-yl)(trifluoromethyl)amino]piperidine-1-carboxylate (20)

A mixture of **19** (450 mg, 0.840 mmol) and sodium thiomethoxide (88.0 mg, 1.25 mmol) in DMF(dry) (10 mL) was stirred at 80 °C for 2 h. The mixture was diluted with AcOEt, washed with water (3 times) and brine, and dried over Na_2SO_4. The solvent was removed by evaporation to give *tert*-butyl 4-((6-((*trans*)-4-(methylthio)cyclohexyloxy)pyrimidin-4-yl)(trifluoromethyl)amino)piperidine-1-carboxylate as a crude material (pale yellow oil).

The oil was dissolved in DMF(dry) (10 mL), and *m*CPBA(70%) (618 mg, 2.51 mmol) was added at room temperature. The resulting mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with AcOEt, successively washed with water (3 times) and brine, and dried over Na_2SO_4. After removal of the solvent, the residue was purified by column chromatography (hexane/AcOEt = 60/40 to 40/60) to give the title compound as a white powder (70.0 mg, 16.0%). MS (ESI/APCI) *m/z* 523 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.31–1.69 (13H, m), 1.72–1.90 (4H, m), 2.09–2.29 (4H, m), 2.70–2.90 (2H, m), 2.94 (3H, s), 3.05–3.21 (1H, m), 3.92–4.09 (2H, m), 4.28–4.45 (1H, m), 4.96–5.11 (1H, m), 6.41 (1H, s), 8.60 (1H, s). Anal. Calcd for $C_{22}H_{33}N_4O_5SF_3$:C,50.56;H,6.36;N,10.72;S,6.14;F,10.91.Found:C,50.60;H,6.41;N,10.50.

5.1.21. *tert*-Butyl 4-[(6-{[1-(methanesulfonyl)azetidin-3-yl]oxy}pyrimidin-4-yl)(trifluoromethyl)amino]piperidine-1-carboxylate (21)

MsCl (0.0980 mL, 1.26 mmol) was added to a solution of **15** (0.48 g, 1.15 mmol) and TEA (0.176 mL, 1.26 mmol) in DMF(dry) (10 mL) at 0 °C. The mixture was stirred at room temperature under N_2 overnight. The mixture was poured into water and extracted with AcOEt. The organic layer was separated, washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 50/50 to 0/100) to give the title compound as a beige solid (0.268 g, 47.0%). MS (ESI/APCI) *m/z* 496 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.39 (9H, s), 1.69–1.86 (4H, m), 2.80 (2H, bs), 3.29 (3H, s), 3.99 (2H, d, $J = 11.7$ Hz), 4.14 (2H, dd, $J = 10.4$, 3.6 Hz), 4.19–4.32 (1H, m), 4.44 (2H, dd, $J = 10.2$, 6.4 Hz), 5.32–5.56 (1H, m), 5.95 (1H, s), 8.37 (1H, s). Anal. Calcd for $C_{19}H_{28}N_5O_5SF_3$:C,46.05;H,5.70;N,14.13;S,6.47;F,11.50.Found:C,46.28;H,5.90;N,13.87.

5.1.22. *tert*-Butyl 4-[(6-{[1-(methanesulfonyl)azetidin-3-yl]methoxy}pyrimidin-4-yl)(trifluoromethyl)amino]piperidine-1-carboxylate (22)

Sodium hydride (13.0 mg, 0.330 mmol) was added to a solution of (1-(methylsulfonyl)azetidin-3-yl)methanol (44.8 mg, 0.27 mmol) in THF(dry) (3 mL) at room temperature. The mixture was stirred at the same temperature under N_2 for 1 h. **15** (103 mg, 0.27 mmol) was added to the mixture. The mixture was stirred at room temperature for 3 h. The mixture was quenched with ice at room temperature and extracted with AcOEt. The organic layer was separated, washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 50/50 to 0/100) to give the title compound as a white solid (74.8 mg, 54.1%). MS (ESI/APCI) *m/z* 454 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.40 (9H, s), 1.71–1.91 (4H, m), 2.68–2.90 (2H, m), 2.93–3.08 (4H, m), 3.72–3.80 (2H, m), 3.89–4.08 (4H, m), 4.29–4.44 (1H, m), 4.49 (2H, d, $J = 6.4$ Hz), 6.48 (1H, s), 8.62 (1H, s). Anal. Calcd for $C_{20}H_{30}N_5O_5SF_3$:C,47.14;H,5.93;N,13.74;S,6.29;F,11.19.Found:C,47.29;H,6.11;N,13.43.

5.1.23. 6-{[1-(Methanesulfonyl)piperidin-4-yl]oxy}-*N*-(piperidin-4-yl)-*N*-(trifluoromethyl)pyrimidin-4-amine dihydrochloride (23)

To a mixture of **18** (20.4 g, 38.96 mmol), AcOEt (115 mL) and MeOH (70 mL) was added 4 M HCl in AcOEt (117 mL, 468 mmol) at room temperature, and the mixture was stirred at room temperature for 3 h. IPE (250 mL) was added to the mixture. The precipitated solid was collected and washed with IPE to the title compound as a white powder (18.9 g, 98%). MS (ESI/APCI) *m/z* 424 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.70–1.86 (2H, m), 1.95–2.12 (4H, m), 2.14–2.31 (2H, m), 2.91 (3H, s), 2.94–3.19 (4H, m), 3.25–3.45 (4H, m), 4.37–4.54 (1H, m), 5.18–5.31 (1H, m), 6.55 (1H, s), 8.62 (1H, s), 8.75–8.95 (1H, m), 9.10–9.26 (1H, m), 1H was not observed.

5.1.24. 4-[(6-{[1-(Methanesulfonyl)piperidin-4-yl]oxy}pyrimidin-4-yl)(trifluoromethyl)amino]piperidine-1-carbonitrile (24)

Cyanogen bromide (16.3 g, 154 mmol) was added to a solution of **23** (63.5 g, 128 mmol) and $NaHCO_3$ (53.7 g, 640 mmol) in THF (200 mL) and water (200 mL) at room temperature. The mixture was stirred at room temperature for 1 h. The mixture was poured into water and extracted with AcOEt and THF. The organic layer was separated, washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was suspended in IPE and the mixture was stirred for 1 h at room temperature. The precipitate was filtered to obtain the title compound as

a pale yellow solid(50.2 g, 88%). MS (ESI/APCI) *m/z* 449 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.68–1.94 (4H, m), 1.96–2.19 (4H, m), 2.90 (3H, s), 3.03–3.26 (4H, m), 3.32–3.51 (4H, m), 4.34 (1H, t, J = 12.1 Hz), 5.25 (1H, tt, J = 7.8, 3.7 Hz), 6.48 (1H, s), 8.63 (1H, d, J = 0.8 Hz).

5.1.25. 6-{[1-(Methanesulfonyl)piperidin-4-yl]oxy}-N-{1-[3-(propan-2-yl)-1,2,4-oxadiazol-5-yl]piperidin-4-yl}-N-(trifluoromethyl)pyrimidin-4-amine (25a)

To a mixture of **24** (125 mg, 0.280 mmol) and *N*-hydroxyisobutyrimidamide (34.2 mg, 0.330 mmol) in DMF(dry) (10 mL) were added $ZnCl_2$ (1 M Et_2O solution, 0.167 mL, 0.167 mmol) and *p*-TsOH monohydrate (31.8 mg, 0.167 mmol) at room temperature, and the mixture was stirred at 85 °C for 6 h. The mixture was diluted with AcOEt, successively washed 0.03 M HCl solution., water, 0.03 M aqueous NaOH solution and brine, and dried over Na_2SO_4. After removal of the solvent, the residue was purified by column chromatography (hexane/AcOEt = 55/45 to 30/70) and crystallized from Et_2O-hexane to give the title compound as a white solid(103 mg, 69.3%). MS (ESI/APCI) *m/z* 534 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.18 (6H, d, J = 7.2 Hz), 1.71–1.85 (2H, m), 1.90–2.13 (6H, m), 2.75–2.87 (1H, m), 2.90 (3H, s), 3.07–3.43 (6H, m), 3.98–4.10 (2H, m), 4.38–4.53 (1H, m), 5.19–5.30 (1H, m), 6.49 (1H, s), 8.62 (1H, s). Anal. Calcd for $C_{21}H_{30}N_7O_4SF_3$: C,47.27;H,5.67;N,18.38;S,6.01;F,10.68.Found:C,47.14;H,5.69;N,18.31.

5.1.26. N-[1-(3-Cyclopropyl-1,2,4-oxadiazol-5-yl)piperidin-4-yl]-6-{[1-(methanesulfonyl)piperidin-4-yl]oxy}-N-(trifluoromethyl)pyrimidin-4-amine (25b)

To a mixture of **24** (1.00 g, 2.23 mmol) and *N*-hydroxycyclopropanecarboximidamide (0.268 g, 2.68 mmol) in DMF(dry) (20 mL) were added $ZnCl_2$ (1 M Et_2O solution, 1.34 mL, 1.34 mmol) and *p*-TsOH monohydrate (0.254 g, 1.34 mmol) at room temperature, and the mixture was stirred at 85 °C for 10 h. The mixture was diluted with AcOEt, successively washed 0.03 M HCl solution., water, 0.03 M aqueous NaOH solution and brine, and dried over Na_2SO_4. After removal of the solvent, the residue was purified by column chromatography (hexane/AcOEt = 60/40 to 20/80) and crystallized from Et_2O-hexane to give the title compound as a white solid (870 mg, 73.4%). MS (ESI/APCI) *m/z* 532 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 0.75–0.98 (4H, m), 1.71–2.12 (9H, m), 2.90 (3H, s), 3.07–3.43 (6H, m), 3.93–4.07 (2H, m), 4.38–4.51 (1H, m), 5.19–5.31 (1H, m), 6.48 (1H, s), 8.62 (1H, d, J = 0.8 Hz). Anal. Calcd for $C_{21}H_{28}N_7O_4SF_3$:C,47.45;H,5.31;N,18.45;S,6.03;F,10.72.Found:C,47.55;H,5.48;N,18.23.

5.1.27. Ethyl 5-{4-[(6-{[1-(methanesulfonyl)piperidin-4-yl]oxy}pyrimidin-4-yl)(trifluoromethyl)amino]piperidin-1-yl}-1,2,4-oxadiazole-3-carboxylate (25c)

$ZnCl_2$ (1 M Et_2O solution, 111 mL, 111 mmol) was added to a solution of **24** (25 g, 55.8 mmol) and ethyl 2-amino-2-(hydroxyimino)acetate (14.7 g, 111 mmol) in DMF(dry) (250 mL) at room temperature. The mixture was warmed to 80 °C with stirring. *p*-TsOH monohydrate (15.9 g, 83.6 mmol) was added at the same temperature and the mixture was stirred for 4 h. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (300 mL) then water (200 mL) was added. The mixture was stirred at room temperature for 1 h. The emerged solid was filtered and the filtrate was suspended in IPE. The suspension was stirred for 1 h. The water layer was extracted with AcOEt. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered and the precipitate was added. The suspension was passed to silica gel pad. The fraction was concentrated. The residue was suspended in IPE and the mixture was stirred for 4 h and then filtered to obtain the title compound as a white solid (25.5 g, 81%). MS (ESI/APCI) *m/z* 564 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.29 (3H, t, J = 7.2 Hz), 1.79 (2H, dtd, J = 12.6, 8.5, 3.8 Hz), 1.92–2.16 (6H, m), 2.90 (3H, s), 3.13 (2H, ddd, J = 12.0, 8.4, 3.4 Hz), 3.25–3.46 (4H, m), 4.10 (2H, d, J = 13.3 Hz), 4.34 (2H, q, J = 7.1 Hz), 4.41–4.58 (1H, m), 5.25 (1H, tt, J = 7.6, 3.5 Hz), 6.50 (1H, s), 8.62 (1H, s).

5.1.28. 2-(5-{4-[(6-{[1-(Methanesulfonyl)piperidin-4-yl]oxy}pyrimidin-4-yl)(trifluoromethyl)amino]piperidin-1-yl}-1,2,4-oxadiazol-3-yl)propan-2-ol (26)

MeMgCl (45.2 mL, 135 mmol) was added to a solution of **25c** (25.5 g, 45.2 mmol) in THF(dry) (250 mL) at 0 °C. The mixture was stirred at room temperature overnight. The mixture was quenched with saturated aqueous ammonium chloride solution at 0 °C and extracted with AcOEt. The organic layer was separated, washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (AcOEt) to give the title compound as a white solid (19.7 g, 79%). MS (ESI/APCI) *m/z* 550 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.40 (6H, s), 1.79 (2H, dtd, J = 12.4, 8.3, 3.8 Hz), 1.91–2.13 (6H, m), 2.90 (3H, s), 3.07–3.18 (2H, m), 3.25 (2H, t, J = 10.7 Hz), 3.32–3.44 (2H, m), 4.00–4.13 (2H, m), 4.37–4.56 (1H, m), 5.17–5.34 (2H, m), 6.49 (1H, s), 8.63 (1H, s).

5.1.29. N-{1-[3-(2-fluoropropan-2-yl)-1,2,4-oxadiazol-5-yl]piperidin-4-yl}-6-{[1-(methanesulfonyl)piperidin-4-yl]oxy}-N-(trifluoromethyl)pyrimidin-4-amine (27)

Xtalfluor-E® (12.3 g, 53.7 mmol) was added to a solution of **26** (19.7 g, 35.8 mmol), TEA (4.99 mL, 35.8 mmol) and TEA trihydrogen fluoride (11.6 mL, 71.6 mmol) in toluene (200 mL) at −78 °C. The mixture was stirred overnight (-78 °C to room temperature). The mixture was quenched with saturated aqueous $NaHCO_3$ solution at 0 °C and extracted with AcOEt. The organic layer was separated, washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (NH, hexane/AcOEt = 100/0 to 50/50) to obtain the crude title compound as a white solid (18.8 g, 95%). The crude was dissolved in MeCN (100 mL) and water (100 mL). Sodium periodate (3.46 g, 16.2 mmol) and ruthenium(III) chloride (0.390 g, 1.73 mmol) were added at 0 °C. The mixture was stirred at 80 °C overnight. The mixture was diluted with water at room temperature and extracted with AcOEt. The organic layer was separated, washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (NH, AcOEt) to give the title compound as a white solid, which was crystallized from Et_2O/hexane (12.9 g, 72.2%). MS (ESI/APCI) *m/z* 552 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.61 (6H, s), 1.79 (2H, dtd, J = 12.6, 8.4, 4.0 Hz), 1.91–2.14 (6H, m), 2.90 (3H, s), 3.13 (2H, ddd, J = 12.0, 8.4, 3.4 Hz), 3.22–3.44 (4H, m), 4.07 (2H, d, J = 13.3 Hz), 4.40–4.55 (1H, m), 5.25 (1H, tt, J = 7.8, 3.6 Hz), 6.50 (1H, s), 8.63 (1H, s). Anal. Calcd for $C_{21}H_{29}N_7O_4SF_4$:C,45.73;H,5.30;N,17.78;S,5.81;F,13.78.Found:C,45.77;H,5.38;N,17.64;S,5.81;F,13.60.

5.2. Estimation of LogD at pH 7.4

$LogD_{7.4}$, which is a partion coefficient between 1-octanol and aqueous buffer pH 7.4, of the compounds was measured on the chromatographic procedure whose condition was developed based on a published method.[22, 23]

5.3. Solubility determination

Small volumes of the compound DMSO solutions were added to the aqueous buffer solution (pH 6.8). After incubation, precipitates were separated by filtration. The solubility was determined by HPLC analysis of each filtrate.

5.4. In vitro GPR119 agonist activity

GPR119 agonist activities were evaluated in the reporter gene assay using CHO cells stably co-expressing cyclic AMP response element (CRE)–luciferase reporter gene (Promega) and GPR119. Cells were seeded at 10,000 cells/well in Minimum essential medium (MEM) α

containing 10% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 500 μg/mL Geneticin in 384-well white opaque plates, and cultured at 37 °C under 5% CO_2 with saturated humidity overnight. The cells were washed once with assay buffer (MEMα, 20 mmol/L HEPES, 0.1% bovine serum albumin, 100 U/mL penicillin, 100 μg/mL streptomycin), and incubated with various concentrations of test compounds diluted in assay buffer for 2 h. After removal of culture supernatant, cAMP-induced luciferase activities were measured with Steady-Glo reagent (Promega) and EnVision Multilabel Plate Reader (PerkinElmer). Agonist activities of test compounds on GPR119 were expressed as [(A − B)/(C − B)] × 100 (luciferase activities (A) in test compounds-treated cells, (B) in vehicle-treated cells, and (C) in cells treated with 10 μM *N*-[4-(methylsulfonyl)phenyl]-5-nitro-6-{4-[4-(trifluoromethoxy)phenoxy]piperidin-1-yl}pyrimidin-4-amine29). EC_{50} values were obtained with XLfit software (ID Business Solutions).

5.5. hERG inhibition assay

hERG/CHO cells stably expressing hERG channel were purchased from Millipore (UK) Ltd. (cat. # CYL3038). Cells were cultured at 32 °C, 5% CO_2 in Ham's F-12 medium supplemented with 10% fetal bovine serum, 500 μg/mL Geneticin (Invitrogen). The hERG inhibition assay was performed on the IonWorks Quattro (Molecular Devices) system in population patch clamp (PPC) mode. The extracellular solution was phosphate-buffered salines (PBS) with calcium and magnesium. The intracellular solution contained 140 mM KCl, 2 mM $MgCl_2$, 1 mM EGTA and 20 mM HEPES, pH 7.3 with KOH. After perforation using 100 μg/mL amphotericin B, hERG current was measured under the potential-clamp protocol (Holding potential −80 mV, the first voltage 40 mV: 2 *sec*, the second voltage −50 mV: 2 *sec*). The peaktail current before addition of the compounds was measured as the pre hERG current. Test compounds were incubated on the cells for a period of 5 min. The peaktail current after addition of the compounds was measured as the post hERG current. %hERG inhibition was calculated (n = 4) to the following.

$$\%\text{hERG inhibition} = 100 - (\text{post hERG current}/\text{pre hERG current}) \times 100$$

5.6. In vitro metabolic clearance in human and rat hepatic microsomes

Human and mouse liver microsomes were purchased from Xenotech, LLC (Lenexa, KS). An incubation mixture consisted of microsomal protein in 50 mM KH_2PO_4–K_2HPO_4 phosphate buffer (pH 7.4) and 1 μM test compound. The concentration of microsomal protein was 0.2 mg/mL. An NADPH-generating system containing 5 mM $MgCl_2$, 5 mM glucose-6-phosphate, 0.5 mM β-$NADP^+$, and 1.5 units/mL glucose-6-phosphate dehydrogenase was added to the incubation mixture to initiate the enzyme reaction. The reaction was terminated 15 and 30 min after the initiation of the reaction by mixing the reaction mixture with acetonitrile, followed by centrifugation. The supernatant was subjected to LC/MS/MS analysis. The metabolic velocity was calculated as the slope of the concentration–time plot.

5.7. Single-crystal X-ray structure analysis

Crystal data for compound **8a**: C24H33N5O4S *MW* = 487.62; crystal size, 0.37 × 0.19 × 0.14 mm; colorless, block; monoclinic, space group *P*21/*c*, *a* = 11.0270(3) Å, *b* = 22.6674(5) Å, *c* = 20.4331(6) Å, *α* = *γ* = 90°, *β* = 100.9880(17)°, *V* = 5013.7(2) Å3, *Z* = 8, *Dx* = 1.292 g/cm3, *T* = 298 K, *μ* = 1.473 mm-1, *λ* = 1.54184 Å, *R*1 = 0.0554, w*R*2 = 0.1500, *S* = 1.045. *Crystal data for* compound **8b**: C25H35N5O4S *MW* = 501.64; crystal size, 0.25 × 0.15 × 0.14 mm; colorless, block; triclinic, space group *P*-1, *a* = 10.5215(2) Å, *b* = 14.3969(3) Å, *c* = 17.9065(3) Å, *α* = 91.9556(7)°, *β* = 96.7813(7)°, *γ* = 109.6530(7)°, *V* = 2528.63(8) Å3, *Z* = 4, *Dx* = 1.318 g/cm3, *T* = 100 K, *μ* = 1.475 mm-1, *λ* = 1.54184 Å, *R*1 = 0.0611, w*R*2 = 0.1729, *S* = 1.069. *Crystal data for* compound **10**: C24H30F3N5O4S *MW* = 541.59; crystal size, 0.61 × 0.37 × 0.25 mm; colorless, block; triclinic, space group *P*-1, *a* = 7.09110(10) Å, *b* = 12.6309(2) Å, *c* = 14.7884(3) Å, *α* = 99.5130(10)°, *β* = 90.0550(10)°, *γ* = 94.3820(10)°,*V* = 1302.39(4) Å3, *Z* = 2, *Dx* = 1.381 g/cm3, *T* = 298 K, *μ* = 1.647 mm-1, *λ* = 1.54184 Å, *R*1 = 0.0544, w*R*2 = 0.1503, *S* = 1.075.

All measurements were made on a Rigaku R-AXIS RAPID diffractometer using graphite monochromated Cu-Kα radiation. The structure was solved by direct methods with SHELXT-2018/21) and was refined using full-matrix least-squares on *F*2 with SHELXL-2018/3.2) All non-H atoms were refined with anisotropic displacement parameters.[24, 25]

CCDC 2,063,007 for compound **8a**, CCDC 2,063,008 for compound **8b**, and CCDC 2,063,009 for compound **10** the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/structures.

5.8. In vitro GLP-1 secretion assay

GLUTag cells, the murine L cell line, were cultured in Dulbecco's modified Eagle's medium (DMEM, high glucose) containing 10% heat-inactivated FBS, 100 IU/mL penicillin and 100 μg/mL streptomycin. GLUTag cells were seeded at density of 1 × 104 cells/well in a 96 well poly-L-lysine coated plate. The following day, the medium was replaced with DMEM (low glucose) containing 10% heat-inactivated FBS, 100 IU/mL penicillin and 100 μg/mL streptomycin, and the cells were incubated overnight before experiments. After washing with Hank's balanced salt solution, Krebs-Ringer-bicarbonate HEPES buffer containing 0.2% fatty acid free BSA, 10 mmol/L glucose, and compounds was added, and the cells were incubated for 2 h at 37 °C. After incubation, supernatants from each well were collected, and secreted active GLP-1 concentration was measured using active GLP-1 ELISA kit (Millipore, EGLP-35 K) according to the manufacture's instruction.

5.9. In vitro insulin secretion assay

HIT-T15 cells, the hamster pancreatic beta cell line, were cultured in Ham's F12 containing 10% heat-inactivated FBS, 100 IU/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-Glutamine. HIT-T15 cells were seeded at density of 5 × 104 cells/well in a 96 well plate. The following day, the medium was replaced with Krebs-Ringer-bicarbonate HEPES (KRBH) buffer (116 mM NaCl, 4.7 mM KCl, 1.17 mM KH_2PO_4, 1.17 mM $MgSO_4 \cdot 7H_2O$, 25 mM $NaHCO_3$, 2.52 mM $CaCl_2 \cdot 2H_2O$, 24 mM HEPES, 0.2% BSA) and the cells were pre-incubated 2 h before experiments. After pre-incubation, KRBH buffer containing 0.2% fatty acid free BSA, 10 mmol/L glucose, and compounds was added, and the cells were incubated for 2 h at 37 °C. After incubation, supernatants from each well were collected, and secreted insulin concentration was measured using AlphaLISA insulin kit (Perkin Elmer) according to the manufacture's instruction.

5.10. Pharmacokinetic analysis in rat cassette dosing

Test compounds were administered intravenously (0.1 mg/kg) or orally (1 mg/kg, solvent: 0.5% methylcellulose aqueous solution) by cassette dosing to non-fasted mice. After administration, blood samples were collected and centrifuged to obtain the plasma fraction. The plasma samples were deproteinized followed by centrifugation. The compound concentrations in the supernatant were measured by LC/MS/MS.

5.11. Oral glucose tolerance test

The care and use of the animals and the experimental protocols used in this research were approved by the Experimental Animal Care and Use Committee of Takeda Pharmaceutical Company Limited. Male N-STZ1.5 rats were obtained from Takeda Rabics, Ltd. (Hikari, Japan).

They were fed a commercial diet CE-2 (Clea Japan Co.) and tap water ad libitum. Male N-STZ1.5 rats (25–30 weeks of age) were fasted overnight and orally given vehicle (0.5% methylcellulose) or compounds. Sixty minutes later, all animals were received an oral glucose load (1.5 g/kg). Blood samples were collected from tail vein before drug administration (pre), and just before glucose load (time 0), and 10, 30, 60, and 120 min after glucose load. Plasma glucose and plasma insulin levels were measured by Autoanalyzer 7080 (Hitachi, Japan) and radioimmunoassay (Millipore, USA), respectively. Differences between two groups were analyzed by one-tailed Williams test.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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