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Discovery of a novel series of indolinylpyrimidine-based GPR119 agonists: Elimination of ether-a-go-go-related gene liability using a hydrogen bond acceptor-focused approach

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ABSTRACT

We previously identified a novel series of indolinylpyrimidine derivatives exemplified by 2 in Figure 1, which is an indoline based derivative, as potent GPR119 agonists. Despite the attractive potency of 2, this compound inhibited the human ether-a-go-go-related gene (hERG) K^+ channel. We elucidated crucial roles of the methylsulfonyl group of 2 in its interaction with the hERG channel and the GPR119 receptor, presumably as a hydrogen bond acceptor (HBA). To remove the undesirable hERG inhibitory activity, a strategy was implemented to arrange an HBA on a less conformationally flexible framework at the indoline 5-position instead of the methylsulfonyl group. This successfully led to the discovery of a piperidinone ring as a desirable motif at the indoline 5-position, which could minimize hERG liability as shown by 24b. Further optimization focused on the reduction of lipophilicity in terms of more favorable drug-like properties. Consequently, the introduction of a hydroxy group at the 3-position of the piperidinone ring effectively reduced lipophilicity without compromising GPR119 potency, resulting in the identification of (3*S*)-3-hydroxy-1-{1-[6-({1-[3-(propan-2-yl)-1,2,4-oxadiazol-5-yl] piperidin-4-yl}oxy)pyrimidin-4-yl]- 2,3-dihydro-1*H*-indol-5-yl}piperidin-2-one ((*S*)-29) as a novel, potent, and orally bioavailable GPR119 agonist with a well-balanced profile. The pharmacological effects of this compound were also confirmed after single and chronic oral administration in diabetic animal models.

1. Introduction

The GPR119 receptor has emerged as an attractive target for the development of novel therapeutics against type 2 diabetes mellitus (T2DM).[1–5] The receptor is a member of class A GPCR, which is predominantly expressed on the pancreatic β-cells and enteroendocrine cells within the gastrointestinal tract. Although several potential endogenous ligands of GPR119, including oleoylethanolamide, have been reported,[6–10] their physiological importance remains unclear due to their relatively low affinity and/or insufficient selectivity over other targets. GPR119 receptor activation leads to insulin secretion from the pancreatic β-cells in a glucose concentration-dependent manner and also promotes secretion of incretins, such as GLP-1 and GIP, from the enteroendocrine cells. As such, GPR119 agonists are expected to act as novel anti-diabetic agents without risk of hypoglycemia and are considered to have potential therapeutic benefits for obesity.[6,11]

Our previous report described the identification of two novel series of indoline-based GPR119 agonists with potent agonist activity, i.e., indoline carbamate derivatives and indolinylpyrimidine derivatives, as exemplified by **1** and **2**, respectively.[12] Of the two series, indolinylpyrimidine derivatives were found to possess favorable metabolic stability and low *in vivo* clearance. Despite its attractive *in vitro* potency and pharmacokinetic profile, however, further assessment revealed that compound **2** potently blocked the human ether-a-go-go-related gene (hERG) K^+ channel current, prohibiting further development of this compound. Accordingly, our subsequent optimization effort was mainly directed at addressing the remaining undesirable hERG issue of indolinylpyrimidine derivatives. We identified the critical roles of the

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hydrogen bond acceptor (HBA) at the indoline 5-position in terms of interaction with the hERG channel and activating the GPR119 receptor. To remove the hERG inhibitory activity, a series of compounds with HBA arranged in a conformationally restricted manner at the indoline 5-position was designed. This led to the successful identification of (*S*)-**29,** characterized by potent GPR119 agonist activity, safe profiles, and favorable pharmacokinetic properties. Herein, we report the design, synthesis, and biological activity of indolinylpyrimidine derivatives as novel GPR119 agonists.

2. Chemistry

Scheme 1 describes the synthetic steps to *N*-thiocarbamate derivative **6**. Compound **4**, which was reported in our previous report, was treated with HCl to remove the *tert*-butyloxycarbonyl (BOC) protecting group, followed by reaction with isopropyl chlorothioformate to give **6** in good yield.

Syntheses of 5-[(2-hydroxyethyl)sulfonyl]indolines and 5-[(2-hydroxyethyl)sulfinyl]indolines were accomplished using the method outlined in Scheme 2. The 5-bromoindoline **10**, which was prepared in a two-step sequence from **7** and **8** in the same manner as **4**, was converted to sulfide **11** by palladium-catalyzed coupling with 2-mercaptoethanol. Oxidation of sulfide **11** by *m*CPBA delivered sulfone **12** and sulfoxide (*RS*)-**14**. After the removal of the BOC group of **12** and (*RS*)-**14**, 1,2,4-oxadiazole ring construction or thiocarbamate formation on the piperidine nitrogen afforded **13**, (*RS*)-**15a**, or (*RS*)-**15b**, respectively. To obtain single enantiomers of (*RS*)-**15b**, chiral preparative HPLC resolution of racemic (*RS*)-**15b** was conducted to yield both enantiomers **15b**-ent1 and **15b**-ent2.

The 5-H-indoline derivative **17** was synthesized *via* the reductive debromination of **10** (Scheme 3)

Scheme 4 describes in detail the syntheses of compounds bearing various heterocycles at the 5-position on the indoline ring *via* aniline intermediate **21**. Lactam rings **23a** and **23b** and morpholinone ring **23c** were constructed by intramolecular cyclization of **22a**–**c**, which were prepared by acylation of **21** with appropriate acyl chlorides. Similarly, the sulfonamidation of **21** with 3-chloropropanesulfonyl chloride and subsequent intramolecular cyclization gave isothiazolidine **23d** in fair yield. To access piperazinone derivatives, the *N*-(2-hydroxyethyl)glycinamide derivative **22e** was prepared by the chloroacetylation of **21**, followed by treatment with 2-aminoethanol. Alcohol **22e** underwent intramolecular cyclization upon Mitsunobu reaction, using the condition $^{n}Bu_3P$/ADDP to afford the desired piperazinone **23e,** after protecting piperazine nitrogen with a trifluoroacetyl group.

Synthesis of 1,2,6-thiadiazinane derivative **23f** included a preparation of sulfamide **22f** using a mild sulfamoylating reagent[SPS:refid::bib1313] adopted by Montero et al. The sulfamide **22f** was subjected to reaction with 1,3-dibromopropane in the presence of potassium carbonate to furnish BOC-protected **23f**. The obtained **23a**–**f** were processed to produce 1,2,4-oxadiazole derivatives **24a**–**f**.

During our research program, Augustine et al. reported an efficient one-pot synthesis of *C*-linked 1,2,4-oxadiazoles from nitrile compounds using a PTSA/$ZnCl_2$ system as a catalyst.[14] We applied this methodology to selected *N*-cyano compounds derived from **23**, which afforded the desired *N*-linked 1,2,4-oxadiazoles **24** in moderate to good yields. Acyclic analog **24 g** was obtained using similar synthetic methods.

(*RS*)-**29** was synthesized similar to piperidinone **24b** (Scheme 5). The requisite carboxylic acid (*RS*)-**26** was prepared from commercially available tetrahydrofuran-2-carboxylic acid ((*RS*)-**25**) in three steps.[15] Following acylation of aniline **21** with the acyl chloride prepared from (*RS*)-**26**, intramolecular cyclization proceeded cleanly upon treatment with potassium carbonate, followed by deacetylation to provide the lactam (*RS*)-**28** in high yield. Protecting group manipulation and construction of a 1,2,4-oxadiazole ring using the same method (described in Scheme 4) furnished the final product, (*RS*)-**29**. To access the enantiomeric pure isomers of (*RS*)-**29**, an identical synthetic procedure for (*RS*)-**29** was followed using chiral starting material (*R*)-**25** or (*S*)-**25**. Consequently, both enantiomers (*R*)-**29** and (*S*)-**29** were obtained without loss of enantiomeric purity.

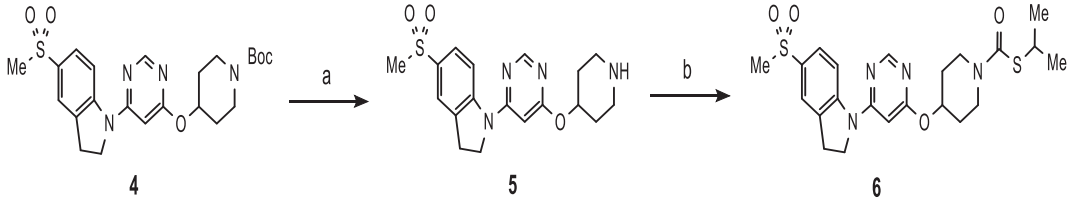
The synthetic schemes of derivatization *via* 5-bromoindoline intermediate **10** are summarized in Scheme 6. Syntheses of racemic 4- and 5-hydroxypiperidinones (*RS*)-**32a** and **32b** were accomplished by the Ullmann–Goldberg type C—N coupling of 5-bromoindoline **10** with hydroxypiperidinones (*RS*)-**30a** or **30b** and subsequent 1,2,4-oxadiazole ring construction at the piperidine nitrogen. The palladium-catalyzed C-arylation[16,17] of cyclohexanone with **10** provided (*RS*)–**33**, a *C*-linked analog of lactam **23b**, which was converted to the final product (*RS*)-**34** as previously described.

3. Results and discussion

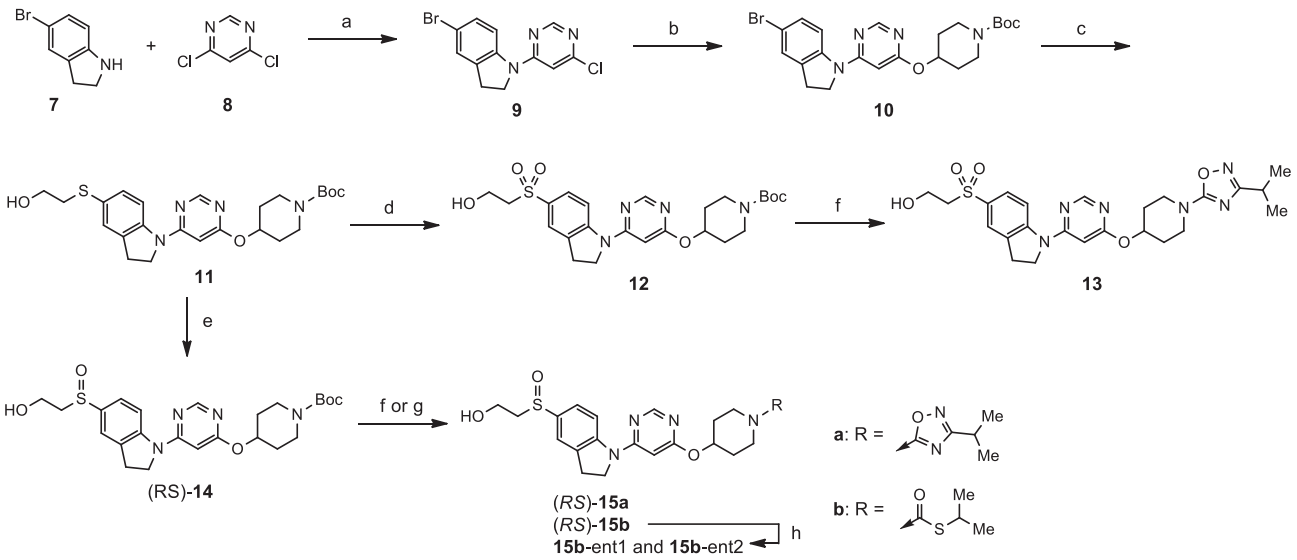
The newly synthesized compounds were tested for functional GPR119 agonism using a cAMP reporter assay in Chinese hamster ovary (CHO) cells stably expressing human GPR119. *In vitro* metabolic stability was assessed by monitoring the disappearance of a parent compound after incubation with human or rat liver microsomes and is expressed as CL_{int} (μL/min/mg). The inhibitory activity against the hERG channel was measured *in vitro* using an automated patch-clamp assay system at 10 or 30 μM compound concentrations.

To obtain in-depth profiles of indoline carbamate derivative **1** and indolinylpyrimidine derivative **2** for the development of novel GPR119 agonists, we conducted further evaluation of these compounds in terms of DMPKTox profiles (Table 1). As a result, indolinylpyrimidine derivative **2** was found to exhibit favorable metabolic stability, whereas indoline carbamate derivative **1** was rapidly cleared by microsomes. These *in vitro* metabolic stabilities were reflected through *in vivo* clearance. That is, compound **2** exhibited much lowered clearance compared with **1** after intravenous administration in rats (CL_{total} = 230 mL/h/kg for **2**; 2166 mL/h/kg for **1**). The same trend of metabolic stability between indolinylpyrimidine and indoline carbamate derivatives was observed for compounds with various piperidine *N*-substituents (data not shown). We assumed that the high microsomal clearance of indoline carbamate derivatives had been caused by metabolic vulnerability of the central aliphatic hydrocarbon chains and/or relatively high flexibility of the central spacer, which may have allowed molecules to easily adopt conformations fit for binding to metabolism enzymes, such as CYPs.

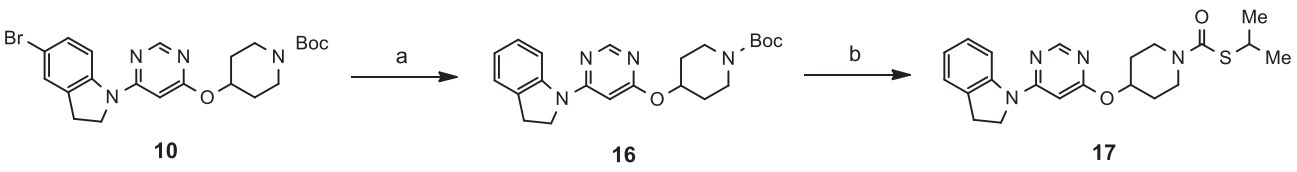
In addition, further assessment revealed that compounds **1** and **2** strongly blocked the hERG channel current by automated patch-clamp assay (95% and 106% inhibition at 10 μM, respectively). Accordingly, our optimization effort was directed at minimizing undesirable hERG activity by focusing on indolinylpyrimidine derivatives, which exhibited better metabolic stability. (See Figure 1).



Scheme 1. Synthesis of indolinylpyrimidine derivative **6**[a]. [a]Reagents and conditions: (a) HCl, AcOEt, MeOH, rt, 94%; (b) ^{i}PrSCOCl, TEA, THF, rt, 94%.



Scheme 2. Synthesis of 2-hydroxyethylsulfone **13** and 2-hydroxyethylsulfoxides **15a**–**b**[a]. [a]Reagents and conditions: (a) EtOH, reflux, 83%; (b) *tert*-butyl 4-hydroxypiperidine-1-carboxylate, NaH, THF, rt, 92%; (c) 2-mercaptoethanol, $Pd_2(dba)_3$, Xantphos, DIEA, toluene, 80 °C, 62%; (d) *m*CPBA (2 eq.), AcOEt, rt, 89%; (e) *m*CPBA (1 eq.), AcOEt, rt, 64%; (f) (1) HCl, AcOEt, rt; (2) BrCN, $NaHCO_3$, THF, H_2O, 0 °C to rt; (3) ^{i}PrCNH(NHOH), $ZnCl_2$, AcOEt, THF, DMSO, 80 °C; (4) conc. HCl, EtOH, 70 °C, 67% (for **13**) and 28% (for (*RS*)-**15a**); (g) (1) HCl, AcOEt, rt; (2) ^{i}PrSCOCl, TEA, THF, rt, 92% (for (*RS*)-**15b**); (h) chiral HPLC separation.



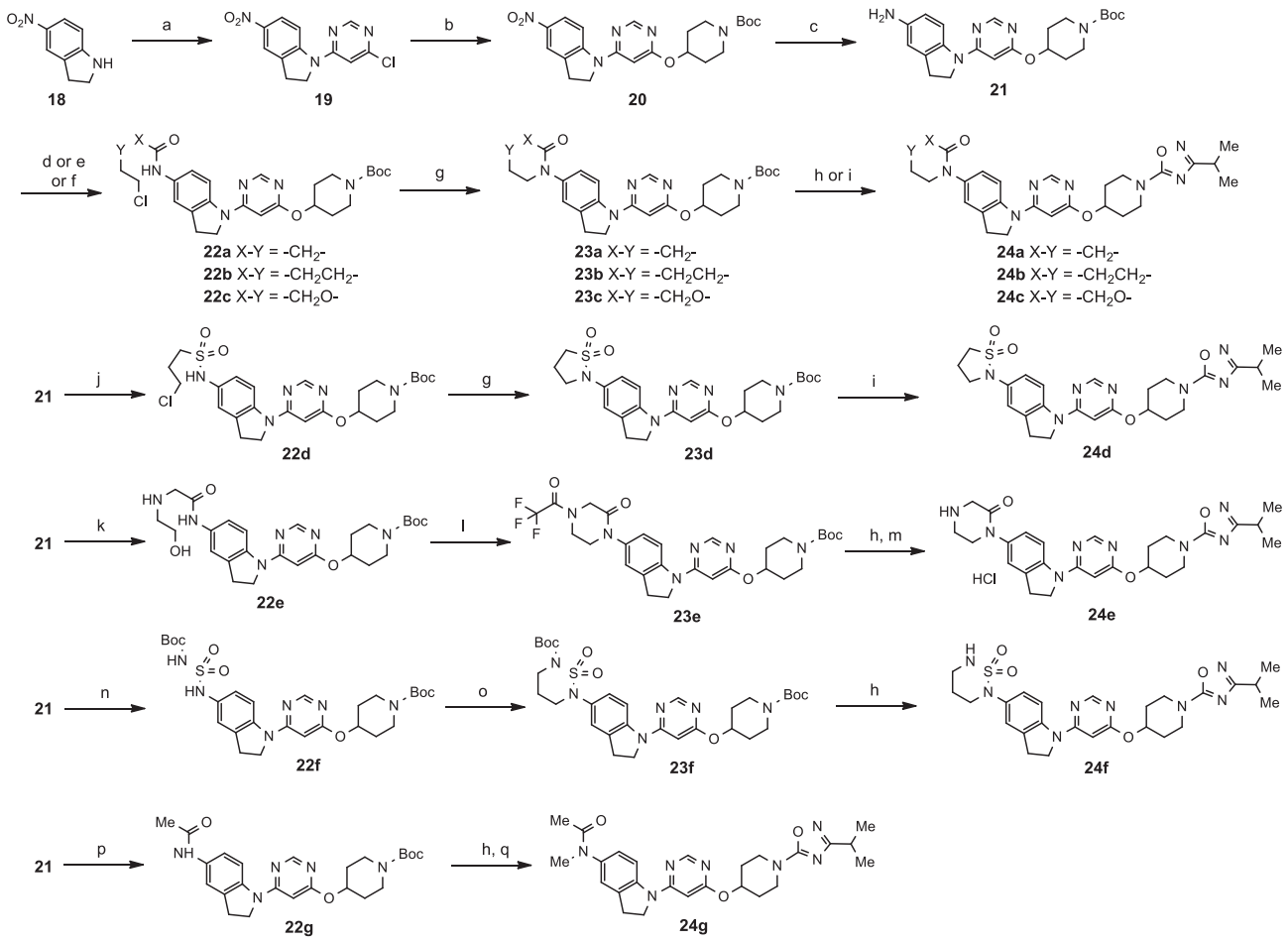
Scheme 3. Synthesis of 5-H-indoline **17**[a]. [a]Reagents and conditions: (a) H_2 (1 atm), Pd on carbon, THF, MeOH, rt, 48%; (b) (1) TFA, rt; (2) ^{i}PrSCOCl, TEA, THF, rt, 90%.

A high degree of hERG inhibitory activities was also observed for *N*-carbamate **3** and *N*-thiocarbamate **6** (Table 2), regardless of the acyclic or cyclic structures of the piperidine *N*-substituent. Interestingly, the deletion of methylsulfonyl group **6** resulted in a complete loss of hERG inhibitory activity, accompanied by a rapid drop in GPR119 potency, despite the substantially increased lipophilicity of compound **17** compared with **6,** with a change in $LogD_{7.4}$ from 3.6 to 5.3. This highlighted the critical roles of the methylsulfonyl group in the interaction with the hERG channel and in the activation of GPR119. Hence, structural modification to address the hERG issue was focused on the substituent at the indoline 5-position. We presumed that the sulfonyl group would make crucial contact with the hERG channel as an HBA since the role of HBA became more prominent in the case of uncharged hERG ligands.[18] It was also assumed that the sulfonyl group functioned as an HBA during GPR119 activation.[19]

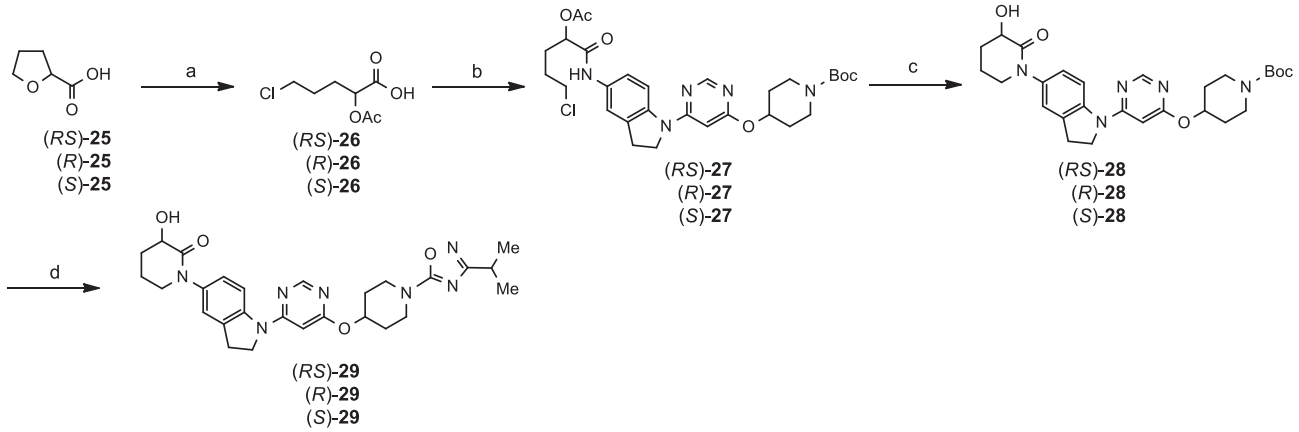
Our first approach for mitigating hERG inhibitory activity included the conversion of the sulfonyl group to a sulfoxide group and reduction of the lipophilicity of compounds.[20,21] Introduction of a polar substituent, such as a (2-hydroxyethyl)sulfonyl group (**13**), maintained strong hERG inhibitory activity and potent GPR119 agonist activity. Transformation of the sulfonyl group into a sulfoxide group afforded racemic (*RS*)-**15a** and (*RS*)-**15b**, for which no beneficial effects on hERG liability were observed. However, one enantiomeric isomer, **15b**-ent1, exhibited improved hERG inhibitory activity compared with antipode **15b**-ent2, while both compounds showed sufficient GPR119 agonist activity. In addition, comparing **13** and **15b**-ent1, the GPR119 agonist activity was equivalent for these 2 compounds, but the hERG inhibitory activity of **15b**-ent1 was improved over that of **13**. Although the right terminal moiety of the molecule is different between **13** and **15b**-ent1, it is unlikely that the right terminal moiety of **15b**-ent1 had a positive impact on hERG inhibitory activity from the comparison of **2** and **6**. These results indicated that the oxygen atom of the sulfonyl or sulfinyl group would be involved in the interaction with the hERG channel, presumably as HBA, and implied that structural modification of a substituent at the indoline 5-position with an appropriate arrangement of HBA could control GPR119/hERG selectivity. Since a sulfoxide group can potentially be susceptible to interconversion to the antipodal sulfoxide *in vivo*,[22] sulfoxide-containing derivatives were not further pursued.

5-position would change the GPR119/hERG selectivity by the combination of the restricted orientation of HBA and steric hindrance of the framework. Accordingly, a series of derivatives with an HBA-containing heterocycle or carbocycle, rather than the alkylsulfonyl group, was designed to identify novel indoline-based GPR119 agonists devoid of hERG liability (Figure 2). We planned to incorporate a carbonyl group and a sulfonyl group as HBA since one oxygen atom at the indoline 5-position was found to be sufficient for activating the GPR119 receptor.

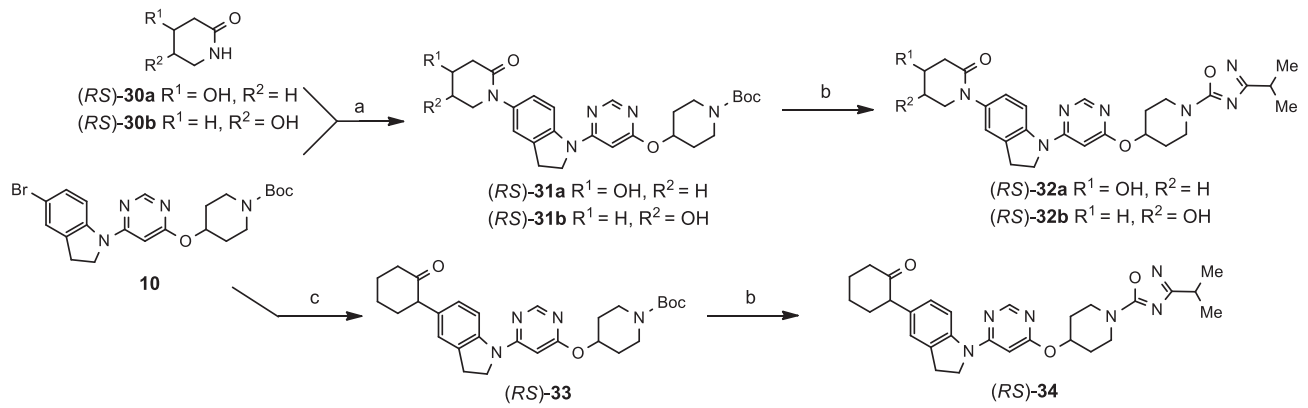
The GPR119 agonist activities and hERG inhibitory activities of compounds with various substituents at the indoline 5-position are presented in Table 3 alongside their microsomal clearances. For the piperidine *N*-substituent, the 3-isopropyl-1,2,4-oxadiazole was selected owing to its balanced potency profile and metabolic stability. Our results indicated that most of the compounds bearing a cyclic substituent exhibited dramatically improved hERG inhibition profiles while maintaining potent GPR119 activity. Conversion of the methylsulfonyl group to a lactam ring conferred an excellent hERG profile, despite the relatively high lipophilicity of **24a** and **24b** ($LogD_{7.4}$ = 3.7 for **24a** and 3.8 for **24b**). Among these, piperidinone **24b** exhibited the desired metabolic stability against both human and rat microsomes. The cyclohexanone derivative (*RS*)-**34** (with greater hydrophobicity) also exhibited a loss of hERG inhibitory activity. However, this compound was



Scheme 4. Synthesis of indolinylpyrimidine derivatives **24a**–**g**[a]. [a]Reagents and conditions: (a) EtOH, reflux, 83%; (b) *tert*-butyl 4-hydroxypiperidine-1-carboxylate, NaH, THF, rt, 69%; (c) H_2 (1 atm), Pd on carbon, THF, MeOH, rt, 100%; (d) 4-chlorobutyryl chloride, TEA, THF, rt (for **22a**); (e) 5-chlorovaleryl chloride, DMA, 0 °C to rt, 86% (for **22b**); (f) (2-chloroethoxy) acetyl chloride, TEA, THF, rt, 96% (for **22c**); (g) NaH, DMF, 0 °C to rt, 81%–97%; (h) (1) HCl/AcOEt or TFA, rt; (2) BrCN, $NaHCO_3$, THF, H_2O, 0 °C to rt, 37%–98% over two steps; (3) ^{i}PrCNH(NHOH), $ZnCl_2$, *p*-TsOH, DMF, 80 °C, 36%–65% (for **24b**–**c**, **24e**, **24f**, and **24 g**); (i) (1) HCl, AcOEt, rt; (2) BrCN, $NaHCO_3$, THF, H_2O, 0 °C to rt; (3) ^{i}PrCNH(NHOH), $ZnCl_2$, AcOEt, THF, reflux; then conc. HCl, EtOH, 70 °C, 31%–34% (for **24a** and **24d**); (j) 3-chloropropanesulfonyl chloride, TEA, THF, rt; (k) (1) chloroacetyl chloride, DMA, rt, 99%; (2) 2-aminoethanol, THF, ^{i}PrOAc, reflux, 98%, (l) (1) ADDP, $^{n}Bu_3P$, THF, rt to 60 °C; (2) TFAA, TEA, AcOEt, rt, 46% over two steps; (m) (1) NaOH, H_2O, MeOH, THF, rt; (2) HCl, MeOH, rt, 95%; (n) (*tert*-butoxycarbonyl){[4-(dimethyliminio)pyridin-1(4*H*)-yl]sulfonyl}azanide, THF, 50 °C, 97%; (o) 1,3-dibromopropane, K_2CO_3, acetone, DMA, 70 °C, 100%; (p) AcCl, TEA, THF, 0 °C to rt, 95%; (q) NaH, MeI, DMF, rt, 78%.

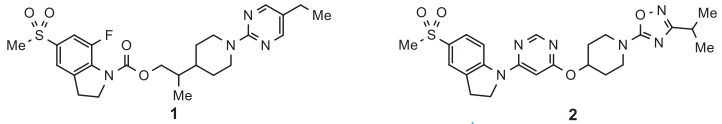


Scheme 5. Synthesis of 3-hydroxylpiperidinones (*RS*)-**29**, (*R*)-**29**, and (*S*)-**29**[a]. [a]Reagents and conditions: (a) (1) $(COCl)_2$, BnOH, pyridine, Et_2O, rt, 63%–69%; (2) $ZnCl_2$, AcCl, reflux, 64%–83%; (3) H_2 (1 atm), Pd on carbon, AcOEt, rt, 99%–100%; (b) $(COCl)_2$, rt, then **21**, DMA, rt, 81%–85%; (c) (1) K_2CO_3, DMF, 50 °C; (2) K_2CO_3, MeOH, THF, rt, 86%–90% over two steps; (d) (1) HCl, AcOEt, rt; (2) BrCN, $NaHCO_3$, THF, H_2O, rt, 70%–94% over two steps; (3) ^{i}PrCNH(NHOH), $ZnCl_2$, *p*-TsOH, DMF, 85 °C, 29%–52%.



Scheme 6. Synthesis of 4- and 5-hydroxypiperidinones (*RS*)-**32a**–**b** and cyclohexanone (*RS*)-**34**[a]. [a]Reagents and conditions: (a) CuI, *trans*-*N*,*N*'-dimethylcyclohexane-1,2-diamine, K_2CO_3, toluene, 120 °C, 4%–10%; (b) (1) HCl, AcOEt, rt; (2) BrCN, $NaHCO_3$, THF, H_2O, 0 °C to rt, 83%–87% over two steps; (3) ^{i}PrCNH(NHOH), $ZnCl_2$, *p*-TsOH, DMF, 80 °C, 11%–67%; (c) cyclohexanone, $Pd_2(dba)_3$, Xantphos, Cs_2CO_3, DME, 80 °C, 24%.

Table 1
Profiles of indoline-based GPR119 agonists **1** and **2**.



Compound	GPR119[a] EC_{50} (nM)	CL_{int}[b] (μL/min/mg) HLM	RLM	CL_{total}[c] (mL/h/kg)	hERG[d] %inhibition at 10 μM	$LogD_{7.4}$[e]
1	3.9	329	365	2,861	75 ± 10	3.6
2	7.7	69	ND	230	106 ± 9	3.1

[a] Agonist activity against human GPR119; EC_{50} values are expressed as means (n = 2).
[b] HLM/RLM: Human/rat liver microsomal clearance; ND: a decrease in compound concentration was not observed.
[c] Rat, 0.1 mg/kg, iv.
[d] Automated patch-clamp assay; percentages of inhibition are expressed as means ± standard deviation (n = 4). [e]LogD value at pH 7.4.

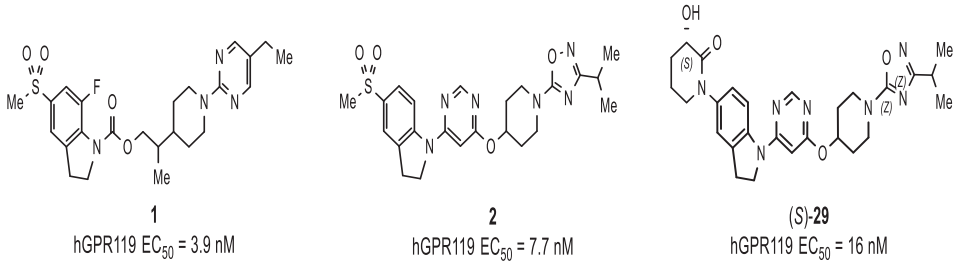


Fig. 1. Indoline-based GPR119 agonists.

metabolically more vulnerable than piperidinone **24b**, presumably due to its more lipophilic nature. Contrary to expectations based on lipophilicity, embedding an additional heteroatom in the piperidinone ring had a somewhat negative impact on hERG inhibition and/or metabolic stability (**24c** and **24e**).

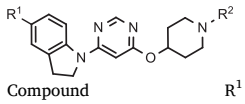
Interestingly, isothiazolidine **24d** and 1,2,6-thidiazinane **24f** exhibited significantly reduced hERG liability, regardless of bearing a sulfonyl group. These improvements in hERG profiles indicated that the introduction of a cyclic structure at the indoline 5-position would have effectively interfered with the interaction between the compound and the hERG channel as expected. A direct comparison between **24b** and the corresponding acyclic analog **24 g** underscored the advantageous effect of cyclization on GPR119/hERG selectivity. Taken together, a lactam ring, particularly a piperidinone ring, emerged as a favorable motif at the indoline 5-position, which suggested a well-balanced profile with low hERG liability.

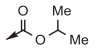
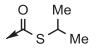
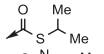
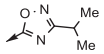
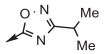
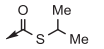
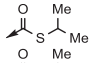
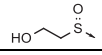
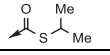
To understand the conformational preference of **24b**, X-ray crystallographic analysis was conducted (Figure 3). The piperidinone ring of **24b** adopted a nearly perpendicular orientation to the indoline ring. Considering the conformational similarity to aryl–sulfonyl systems, which prefer orthogonal conformations with torsion angles of the C═C—S—C unit between 60° and 120°,[23] we assumed that the carbonyl group of the lactam ring of **24b** would be able to function as a good surrogate (as an HBA) for the sulfonyl group in terms of GPR119 agonism.

We also assumed that the decrease in hERG inhibitory activity of **24b** may have been driven by an unfavorable orientation of the carbonyl group for interaction with the hERG channel. In addition, it was presumed that the steric bulk of the alkylene moiety of the lactam ring disrupted the interaction within the spatially limited hERG channel binding site.

Although the high GPR119/hERG selectivity of piperidinone **24b** was attractive, the relatively high LogD value of this compound motivated us to reduce lipophilicity from the perspective of providing a

Table 2
The GPR119 agonist activity and hERG inhibitory activity of indolinylpyrimidine derivatives: the effect of piperidine *N*-substituents and substituents at the indoline 5-position.



Compound	R^1	R^2	GPR119[a] EC_{50} (nM)	hERG[b] %inhibition at 10 μM	$LogD_{7.4}$[c]
3			17	84 ± 14	3.1
6			3.2	89 ± 17	3.6
17	H		> 10,000	14 ± 5(16 ± 6)[d]	5.3
13			41	102 ± 12	2.7
(RS)-**15a**			48	107 ± 2	2.7
(RS)-**15b**			14	95 ± 7	3.3
15b-ent1			57	44 ± 10	3.3
15b-ent2			9.7	101 ± 5	3.3

[a] Agonist activity against human GPR119; EC_{50} values are expressed as means (n = 2).
[b] Automated patch-clamp assay; percentages of inhibition are expressed as means ± standard deviation (n = 4).
[c] LogD value at pH 7.4.
[d] Data at 30 μM.

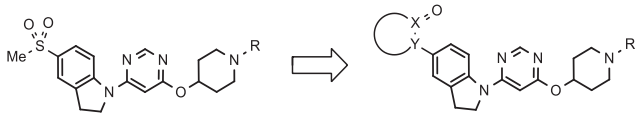


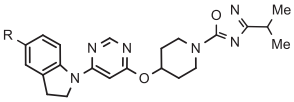
Fig. 2. The design of a novel GPR119 agonists with a cyclic LHS motif.

better opportunity for successful development.[24]

Furthermore, we predicted that reducing lipophilicity could further improve metabolic stability in **24b** rats. Based on our SAR data, it was assumed that the introduction of a hydroxy group in the substituent at the indoline 5-position would be tolerated for GPR119 activity. Thus, 3-, 4-, and 5-hydroxy piperidinones were designed and synthesized (Table 4). As expected, the installation of a hydroxy group effectively reduced the lipophilicity of compounds, and the lowered degree was distinct, depending on the position of OH-substitution. The 3-OH derivative (*RS*)-**29** was of comparable potency to the parent **24b** and was superior to the 4-OH (*RS*)-**32a** and 5-OH isomer (*RS*)-**32b**. In terms of lipophilic efficiency,[25] (*RS*)-**29** exhibited the highest LLE value ($pEC_{50} - LogD_{7.4}$) among the three regioisomers (LLE = 4.0 for **24b**, 4.5 for (*RS*)-**29**, 4.3 for (*RS*)-**32a**, and 4.4 for (*RS*)-**32b**). All hydroxylated compounds were also found to exhibit desirable hERG inhibition profiles and good stability in both human and rat liver microsomes. To fully investigate the 3-OH derivative (*RS*)-**29** with a high LLE value, the enantiomeric pure isomers of this compound, (*R*)-**29** and (*S*)-**29**, were prepared. These isomers exhibited almost the same GPR119 agonist activity and a clean hERG profile, and good clearance in human/rat liver microsome. Both isomers also showed good *in vivo* clearance when dosed intravenously in rats. Among them, the (*S*)-isomer demonstrated a lower *in vivo* clearance (148 mL/h/kg) than the (*R*)-isomer (375 mL/h/kg), which made this compound an attractive candidate for further evaluation.

Prior to *in vivo* evaluation, we prepared CHO cells stably expressing rat GPR119 and evaluated the agonist activity of selected compounds. Compound (*S*)-**29** effectively increased intracellular cAMP level in rat GPR119, expressing cells with an EC_{50} value of 120 nM (Table 5). To further assess functional agonism for GPR119, an insulin-secretion assay using HIT-T15 cells and a GLP-1-secretion assay using GLUTag cells were conducted. Compound (*S*)-**29** exerted potent hormone secretion

Table 3
The GPR119 agonist activity, hERG inhibitory activity, and microsomal clearance of indolinylpyrimidine derivatives: introduction of cyclic substituents at the indoline 5-position.



Compound	R	GPR119[a] EC_{50} (nM)	hERG[b] %inhibition at 10 μM	CL_{int}[d] (μL/min/mg) HLM	RLM	$LogD_{7.4}$[e]
24a		33	20 ± 3	ND	138	3.7
24b		17	31 ± 4 (21 ± 2)[c]	ND	13	3.8
(RS)-**34**		48	13 ± 6	78	141	4.7
24c		15	54 ± 7	27	46	3.1
24e		36	15 ± 2	ND	98	2.7
24d		9.7	19 ± 2	18	1	3.2
24f		24	20 ± 7	79	21	3.2
24 g		75	66 ± 15 (108 ± 3)[c]	173	17	3.7

[a] Agonist activity against human GPR119; EC_{50} values are expressed as means (n = 2).
[b] Automated patch-clamp assay; percentages of inhibition are expressed as means ± standard deviation (n = 4).
[c] Data at 30 μM.
[d] HLM/RLM: Human/rat liver microsomal clearance; ND: decrease in compound concentration was not observed.
[e] LogD value at pH 7.4.

activities with EC_{50} values of nearly 10 nM in both assays. We observed a good correlation between cAMP-based GPR119 agonism and these hormone secretion activities (data not shown), confirming that our indoline-based chemotype could secrete several hormones by activating

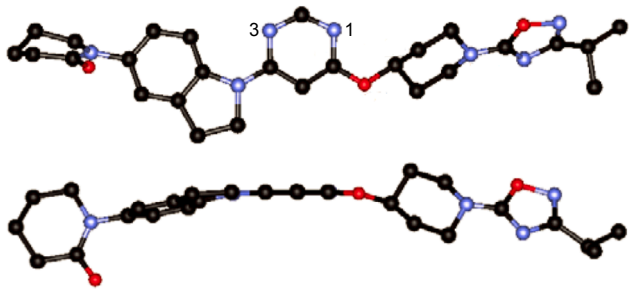


Fig. 3. Single-crystal X-ray structure of compound **24b**: (A) frontal view to the central pyrimidine ring; (B) side view to the central pyrimidine ring.

GPR119.

Next, we investigated pharmacokinetic parameters when compounds were dosed orally in rats at a dose of 1 mg/kg. Pharmacokinetic studies revealed that compound (*S*)-**29** had favorable bioavailability ($F = 24\%$). The low clearance of compound (*S*)-**29** was reflected in a high area under the curve (AUC) (1670 ng·h/mL) and a prolonged MRT (3.8 h). This pharmacokinetic data promised potent *in vivo* pharmacological effects and encouraged us to evaluate their efficacy in animal models.

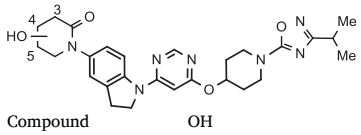
The anti-diabetic effects of compounds by single administration were assessed by an oral glucose tolerance test in the *N*-STZ-1.5 rats. Candidate compounds and vehicle were administered 1 h before the oral glucose load, and plasma glucose and insulin concentrations were monitored over 2 h. As presented in Figure 4, (*S*)-**29** effectively lowered plasma glucose levels after oral administration accompanied by insulin secretion in a glucose-dependent manner. The glucose-lowering effect of compound (*S*)-**29** was dose-dependent and significant ($p < 0.025$) at a dose of 1 mg/kg. To further evaluate *in vivo* pharmacological effects, the effect on glycosylated hemoglobin (GHb) levels after chronic treatment in the *N*-STZ-1.5 rats was examined (Figure 5). Compound (*S*)-**29** significantly lowered GHb levels after a 4- week treatment in a dose-dependent manner. These results strongly supported the usefulness of GPR119 agonists for the treatment of T2DM.

The results from the *in vitro* safety assessment of compound (*S*)-**29**, including the inhibition of the CYP isoforms, induction of CYP3A4, ATP as a marker for cell viability, phototoxicity, time-dependent CYP inhibition, phospholipidosis, and mutagenic side effects were also promising. In addition to the potent *in vivo* pharmacological effects, these safety profiles warrant further development of (*S*)-**29** as a useful anti-diabetic agent.

4. Conclusion

It important that a new anti-diabetic agent without side effects be made available, particularly as it relates to cardiovascular concerns. To develop a novel class of GPR119 agonists with desirable DMPKTox profiles, we continued our optimization campaign of indolinylpyrimidine derivatives to primarily address potential hERG liability. Structural modification based on an HBA-focused approach proved effective in removing undesirable hERG inhibitory activity; however, reducing the lipophilicity of compounds had a marginal impact on the hERG profiles for this class of derivatives. We designed a series of compounds with an HBA-containing heterocycle or carbocycle to restrict HBA orientation, together with the introduction of steric bulk. Using this approach, we found that a lactam ring served as an excellent substituent at the indoline 5-position, which may offer an improved hERG profile coupled with high GPR119 potency. Furthermore, we pursued more desirable physicochemical properties for a successful drug development, particularly with a focus on lipophilicity reduction. Consequently, the appropriate installation of a hydroxy group on the lactam ring resulted in favorable lipophilicity without compromising GPR119 potency. A representative compound (*S*)-**29** demonstrated sufficient *in vivo* pharmacological effects after single and chronic oral administration in diabetic animal models, alongside excellent DMPKTox profiles.

Table 4
Profiles of indolinylpyrimidine derivatives: the effect of a hydroxy group on the piperidinone ring.



Compound	OH	GPR119[a] EC_{50} (nM)	hERG[b] %inhibition at 10 μM	CL_{int}[c] (μL/min/mg) HLM	RLM	CL_{total}[d] (mL/h/kg)	$LogD_{7.4}$[e]
(RS)-**29**	3-OH (*Rac*)	17	20 ± 2	ND	4	–	3.3
(RS)-**32a**	4-OH (*Rac*)	89	18 ± 4	4	13	–	2.8
(RS)-**32b**	5-OH (*Rac*)	90	18 ± 4	1	7	–	2.6
(R)-**29**	3-OH (*R*)	25	23 ± 4	ND	ND	375	3.3
(S)-**29**	3-OH (*S*)	16	31 ± 4	9	1	148	3.3

[a] Agonist activity against human GPR119; EC_{50} values are expressed as means (n = 2).
[b] Automated patch-clamp assay; percentages of inhibition are expressed as means ± standard deviation (n = 4).
[c] HLM/RLM: Human/rat liver microsomal clearance; ND: a decrease in the compound concentration was not observed.
[d] Rat, 0.1 mg/kg, iv.
[e] LogD value at pH 7.4.

Table 5
Profiles of compound (*S*)-**29**.

Rat GPR119[a] EC_{50} (nM)	Insulin secretion[b] EC_{50} (nM)	GLP-1 secretion[c] EC_{50} (nM)	Pharmacokinetic profiles in rats[d, e] Vdss[d] (mL/kg)	CL_{total}[d] (mL/h/kg)	AUC[e] (ng·h/mL)	MRT[e] (h)	F[e] (%)
120	25	19	332	148	1670	3.8	24

[a] Agonist activity against rat GPR119; EC_{50} values are expressed as means (n = 2).
[b] Insulin secretion assay in HIT-T15 cells (n = 2).
[c] GLP-1 secretion assay in GLUTag cells (n = 2).
[d] Rat, 0.1 mg/kg, iv.
[e] Rat, 1 mg/kg, po.

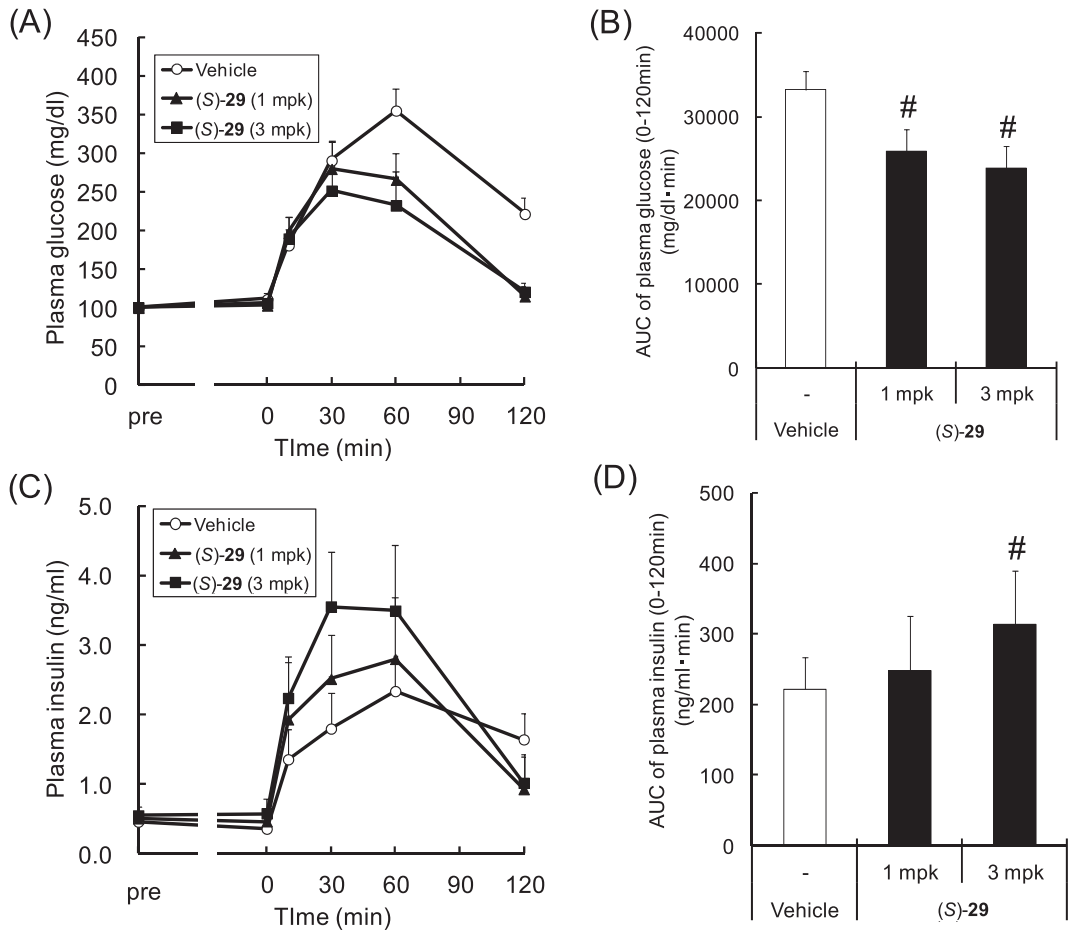


Fig. 4. Acute effects of (*S*)-**29** on glucose excursion and insulin secretion during an oral glucose tolerance test in type 2 diabetic rats. Male *N*-STZ-1.5 rats were fasted overnight and orally given vehicle or (*S*)-**29** (1 and 3 mpk); (A) and (C) show time-dependent changes in plasma glucose and insulin levels after a 1.5 g/kg glucose challenge, respectively. Data in (B) and (D) represents the AUC of plasma glucose shown in (A) and AUC of plasma insulin shown in (C). The data are expressed as mean ± SD (n = 6) #, $p \leq 0.025$ vs vehicle by one-tailed Williams' test.

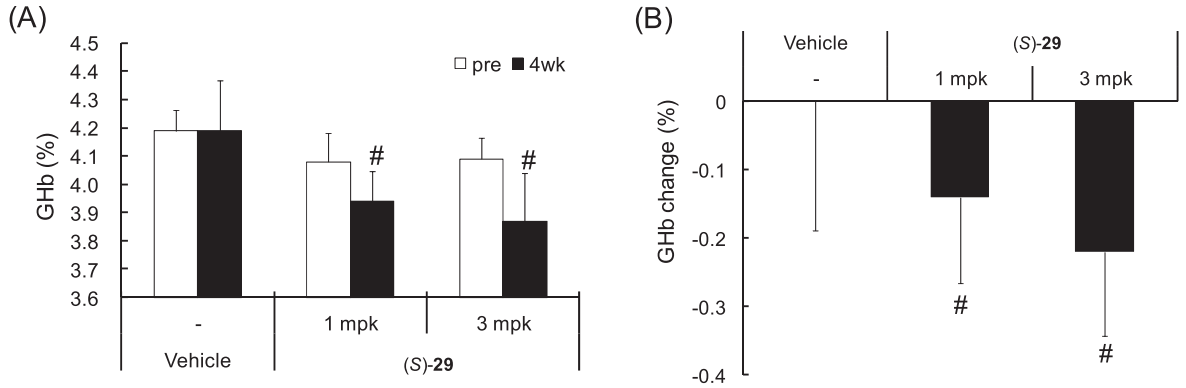


Fig. 5. The chronic effects of (*S*)-**29** on GHb in male *N*-STZ-1.5 rats under time-restricted feeding conditions; (A) GHb levels before and after a 4-week treatment of (*S*)-**29**; (B) the percentage of GHb change after a 4-week treatment of (*S*)-**29**. Data are expressed as mean ± SD (n = 10) #, $p \leq 0.025$ versus vehicle by one-tailed Williams' test.

5. Experimental section

5.1. Chemistry

^{1}H NMR spectra were recorded on Bruker AVANCE III (300 MHz), Bruker AVANCE 300 (300 MHz), or Bruker Advance III plus (400 MHz) spectrometer. 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 doublets, dt = doublet of triplets, 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-(Methylsulfonyl)-1-[6-(piperidin-4-yloxy)pyrimidin-4-yl]-2,3-dihydro-1*H*-indole (5).

To a mixture of *tert*-butyl 4-({6-[5-(methylsulfonyl)-2,3-dihydro-1*H*-indol-1-yl]pyrimidin-4-yl}oxy)piperidine-1-carboxylate (**4**, 6.17 g, 13.0 mmol), AcOEt (100 mL), and MeOH (100 mL) was added 4 M HCl in AcOEt (15 mL). The mixture was stirred at room temperature for 16 h. After the mixture was concentrated under reduced pressure, the residue was diluted with AcOEt and basified with 1 M aqueous NaOH solution. The organic layer was separated, washed with water and brine, and dried over $MgSO_4$. The solvent was removed by evaporation to give the title compound as a white solid (4.56 g, 94%). This product was used for the next step without further purification. MS (ESI/APCI) *m/z* 375 $[M + H]^+$.

***S*-Propan-2-yl 4-({6-[5-(methylsulfonyl)-2,3-dihydro-1*H*-indol-1-yl]pyrimidin-4-yl}oxy)piperidine-1-carbothioate (6).**

To a mixture of compound **5** (150 mg, 0.401 mmol) and triethylamine (0.139 mL, 0.997 mmol) in THF (10 mL) was added *S*-isopropyl chlorothioformate (111 mg, 0.801 mmol). The mixture was stirred at room temperature for 16 h. The reaction mixture was diluted with AcOEt, washed with water and brine, and dried over $MgSO_4$. The solvent was removed by evaporation to give the title compound as a white solid (180 mg, 94%). MS (ESI/APCI) *m/z* 477 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.28 (6H, d, J = 6.8 Hz), 1.53–1.71 (2H, m), 1.94–2.10 (2H, m), 3.15 (3H, s), 3.22–3.40 (4H, m), 3.48 (1H, q, J = 6.9 Hz), 3.61–3.98 (2H, m), 4.08 (2H, t, J = 8.9 Hz), 5.25–5.40 (1H, m), 6.23 (1H, s), 7.67–7.79 (2H, m), 8.54 (2H, t, J = 4.7 Hz). Anal. Calcd for $C_{22}H_{28}N_4O_4S_2$: C, 55.44; H, 5.92; N, 11.76. Found: C, 55.55; H, 5.97; N, 11.66.

5-Bromo-1-(6-chloropyrimidin-4-yl)-2,3-dihydro-1*H*-indole (9).

A mixture of 4,6-dichloropyrimidine (17.3 g, 116 mmol), 5-bromo-2,3-dihydro-1*H*-indole (20.0 g, 101 mmol), and EtOH (500 mL) was refluxed for 16 h. After the mixture was concentrated under reduced pressure, the residual solid was successively washed with 1 M aqueous NaOH solution, H_2O, and Et_2O and then dried under reduced pressure to give the title compound as a white solid (26.0 g, 83%). MS (ESI/APCI) *m/z* 312 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.24 (2H, t, J = 8.1 Hz), 4.07 (2H, t, J = 8.3 Hz), 6.97 (1H, s), 7.30–7.58 (2H, m), 8.35 (1H, d, J = 8.7 Hz), 8.60 (1H, s).

***tert*-Butyl 4-{[6-(5-bromo-2,3-dihydro-1*H*-indol-1-yl)pyrimidin-4-yl]oxy}piperidine-1-carboxylate (10).**

To a stirred solution of *tert*-butyl 4-hydroxypiperidine-1-carboxylate (75.0 g, 373 mmol) in THF (1 L) was added sodium hydride (60% oil dispersion, 14.9 g, 373 mmol) at 0 °C. After the mixture was stirred at room temperature for 1.5 h, compound **9** (37.0 g, 119 mmol) was added to the mixture. The resulting mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with water and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was triturated with ^{i}Pr$_2$O and the precipitated solid was collected to give the title compound as a white solid (52.0 g, 92%). MS (ESI/APCI) *m/z* 475 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.41 (9H, s), 1.47–1.65 (2H, m), 1.88–2.05 (2H, m), 3.05–3.27 (4H, m), 3.64–3.80 (2H, m), 3.98 (2H, t, J = 8.7 Hz), 5.16–5.30 (1H, m), 6.09 (1H, s), 7.33 (1H, dd, J = 8.7, 2.3 Hz), 7.40 (1H, s), 8.32 (1H, d, J = 8.7 Hz), 8.46 (1H, s).

***tert*-Butyl 4-[(6-{5-[(2-hydroxyethyl)sulfanyl]-2,3-dihydro-1*H*-indol-1-yl}pyrimidin-4-yl)oxy]piperidine-1-carboxylate (11).**

A mixture of compound **10** (10.0 g, 21.0 mmol), 2-mercaptoethanol (1.80 g, 23.0 mmol), $Pd_2(dba)_3$ (1.83 g, 2.00 mmol), Xantphos (2.30 g, 4.00 mmol), and *N*,*N*-diisopropylethylamine (8.14 g, 63.0 mmol) in toluene (100 mL) was stirred at 80 °C under Ar atmosphere for 16 h. The mixture was partitioned between with AcOEt and water. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 80/20 to 50/50) to give the title compound as a solid (6.20 g, 62%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.41 (9H, s), 1.46–1.65 (2H, m), 1.87–2.05 (2H, m), 2.94 (2H, t, J = 7.0 Hz), 3.05–3.26 (4H, m), 3.46–3.59 (2H, m), 3.63–3.83 (2H, m), 3.97 (2H, t, J = 8.7 Hz), 4.86 (1H, t, J = 5.7 Hz), 5.17–5.31 (1H, m), 6.08 (1H, s), 7.19 (1H, dd, J = 8.3, 1.9 Hz), 7.27 (1H, s), 8.30 (1H, d, J = 8.3 Hz), 8.45 (1H, s).

***tert*-Butyl 4-[(6-{5-[(2-hydroxyethyl)sulfonyl]-2,3-dihydro-1*H*-indol-1-yl}pyrimidin-4-yl)oxy]piperidine-1-carboxylate (12).**

To a mixture of compound **11** (800 mg, 1.69 mmol) in AcOEt (100 mL) was added *m*CPBA (70%, 868 mg, 3.50 mmol), and the mixture was stirred at room temperature for 2 h. The mixture was passed through silica gel (NH, AcOEt) to give the title compound as a white solid (760 mg, 89%). MS (ESI/APCI) *m/z* 505 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.41 (9H, s), 1.49–1.66 (2H, m), 1.89–2.07 (2H, m), 3.08–3.31 (4H, m), 3.34–3.43 (2H, m), 3.58–3.84 (4H, m), 4.09 (2H, t, J = 8.9 Hz), 4.86 (1H, t, J = 5.7 Hz), 5.16–5.37 (1H, m), 6.23 (1H, s), 7.64–7.76 (2H, m), 8.48–8.60 (2H, m). Anal. Calcd for $C_{24}H_{32}N_4O_6S$: C, 57.13; H, 6.39; N, 11.10. Found: C, 57.21; H, 6.39; N, 11.05.

2-({1-[6-({1-[3-(Propan-2-yl)-1,2,4-oxadiazol-5-yl]piperidin-4-yl}oxy)pyrimidin-4-yl]-2,3-dihydro-1*H*-indol-5-yl}sulfonyl)ethanol (13).

Step A. A mixture of compound **12** (2.10 g, 4.16 mmol), 4 M HCl in AcOEt (10 mL), and AcOEt (250 mL) was stirred at room temperature for 16 h. The solvent was removed by evaporation and dried to give 2-({1-[6-(piperidin-4-yloxy)pyrimidin-4-yl]-2,3-dihydro-1*H*-indol-5-yl}sulfonyl)ethanol hydrochloride as a solid (1.83 g, 100%). MS (ESI/APCI) *m/z* 405 $[M + H]^+$.

Step B. To a mixture of 2-({1-[6-(piperidin-4-yloxy)pyrimidin-4-yl]-2,3-dihydro-1H-indol-5-yl}sulfonyl)ethanol hydrochloride (1.83 g, 4.15 mmol), $NaHCO_3$ (2.10 g, 25.0 mmol), THF (90 mL), and water (30 mL) was added cyanogen bromide (595 mg, 5.62 mmol) at 0 °C. The mixture was stirred at room temperature for 16 h. Aqueous $NaHCO_3$ solution was added and the mixture was extracted with a mixed solvent of AcOEt and THF. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to give 4-[(6-{5-[(2-hydroxyethyl)sulfonyl]-2,3-dihydro-1*H*-indol-1-yl}pyrimidin-4-yl)oxy]piperidine-1-carbonitrile as a solid. This product was used for the next step without further purification. MS (ESI/APCI) *m/z* 430 $[M + H]^+$.

Step C. The solid obtained in step B was dissolved in AcOEt/THF/DMSO (5:5:1, 220 mL), and then *N*-hydroxy-2-methylpropanimidamide (623 mg, 6.10 mmol) and zinc chloride (1 M Et_2O solution, 6.1 mL, 6.1 mmol) were added. The mixture was stirred at 80 °C for 2 h. After the mixture was concentrated under reduced pressure, EtOH (100 mL) and concentrated hydrochloric acid (5 mL) were added to the residue. The resulting mixture was stirred at 70 °C for 3 h. The reaction mixture was concentrated under reduced pressure and partitioned between AcOEt and aqueous NaOH solution. 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 (AcOEt 100%) to give the title compound as a white solid (**13**, 1.42 g, 67% over 2 steps). MS (ESI/APCI) *m/z* 515 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.19 (6H, d, J = 6.8 Hz), 1.64–1.87 (2H, m), 2.02–2.19 (2H, m), 2.70–2.96 (1H, m), 3.28 (2H, t, J = 8.7 Hz), 3.34–3.42 (2H, m), 3.43–3.57 (2H, m), 3.66 (2H, q, J = 6.2 Hz), 3.75–3.91 (2H, m), 4.09 (2H, t, J = 8.9 Hz), 4.86 (1H, t, J = 5.5 Hz), 5.27–5.42 (1H, m), 6.25 (1H, s), 7.65–7.75 (2H, m), 8.51–8.62 (2H, m). Anal. Calcd for $C_{24}H_{30}N_6O_5S$: C, 56.02; H, 5.88; N, 16.33. Found: C, 56.04; H, 5.93; N, 16.07.

***tert*-Butyl 4-[(6-{5-[(2-hydroxyethyl)sulfinyl]-2,3-dihydro-1*H*-indol-1-yl}pyrimidin-4-yl)oxy] piperidine-1-carboxylate ((*RS*)-14).**

To a mixture of compound **11** (800 mg, 1.69 mmol) in AcOEt (100 mL) was added *m*CPBA (70%, 419 mg, 1.69 mmol), and the mixture was stirred at room temperature for 1 h. The mixture was passed through silica gel (NH, AcOEt) to give the title compound as a white solid (530 mg, 64%). MS (ESI/APCI) *m/z* 489 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.41 (9H, s), 1.48–1.69 (2H, m), 1.87–2.06 (2H, m), 2.78–3.02 (2H, m), 3.07–3.31 (4H, m), 3.54–3.89 (4H, m), 4.05 (2H, t, J = 8.7 Hz), 5.02 (1H, t, J = 5.1 Hz), 5.17–5.36 (1H, m), 6.16 (1H, s), 7.45 (1H, dd, J = 8.7, 1.9 Hz), 7.52 (1H, s), 8.44–8.62 (2H, m). Anal. Calcd for $C_{24}H_{32}N_4O_5S$: C, 59.00; H, 6.60; N, 11.47. Found: C, 58.72; H, 6.60; N, 11.36.

2-({1-[6-({1-[3-(Propan-2-yl)-1,2,4-oxadiazol-5-yl] piperidin-4-yl}oxy)pyrimidin-4-yl]-2,3-dihydro-1*H*-indol-5-yl}sulfinyl) ethanol ((*RS*)-15a).

Compound (*RS*)-**15a** was prepared from (*RS*)-**14** in a manner similar to that described for compounds **13**. White solid. Yield 28% over 3 steps. MS (ESI/APCI) *m/z* 499 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.19 (6H, d, J = 6.8 Hz), 1.65–1.88 (2H, m), 2.00–2.20 (2H, m), 2.72–3.04 (3H, m), 3.26 (2H, t, J = 8.5 Hz), 3.42–3.93 (6H, m), 4.05 (2H, t, J = 8.7 Hz), 4.79–5.09 (1H, m), 5.25–5.43 (1H, m), 6.06–6.31 (1H, m), 7.14–7.61 (2H, m), 8.43–8.62 (2H, m). Anal. Calcd for $C_{24}H_{30}N_6O_4S{\cdot}0.2H_2O$: C, 57.40; H, 6.10; N, 16.73. Found: C, 57.28; H, 6.15; N, 16.53.

***S*-Propan-2-yl 4-[(6-{5-[(2-hydroxyethyl)sulfinyl]-2,3-dihydro-1*H*-indol-1-yl}pyrimidin-4-yl)oxy] piperidine-1-carbothioate ((*RS*)-15b).**

Compound (*RS*)-**15b** was prepared from (*RS*)-**14** in a manner similar to that described for compounds **5** and **6**. White solid. Yield 92% over 2 steps. MS (ESI/APCI) *m/z* 491 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.28 (6H, d, J = 7.2 Hz), 1.50–1.71 (2H, m), 1.91–2.09 (2H, m), 2.78–3.00 (2H, m), 3.15–3.40 (5H, m), 3.41–3.56 (1H, m), 3.56–3.93 (3H, m), 3.98–4.15 (2H, m), 5.03 (1H, t, J = 5.1 Hz), 5.23–5.40 (1H, m), 6.17 (1H, s), 7.46 (1H, dd, J = 8.3, 1.9 Hz), 7.52 (1H, s), 8.47–8.59 (2H, m). Anal. Calcd for $C_{23}H_{30}N_4O_4S_2{\cdot}0.1H_2O$: C, 56.10; H, 6.18; N, 11.38. Found: C, 55.85; H, 6.20; N, 11.16.

***S*-Propan-2-yl 4-[(6-{5-[(2-hydroxyethyl)sulfinyl]-2,3-dihydro-1*H*-indol-1-yl}pyrimidin-4-yl)oxy] piperidine-1-carbothioate (15b-ent1 and 15b-ent2).**

Compound (*RS*)-**15b** (racemate, 97.7 mg) was subjected to chiral HPLC separation (CHIRALPAK AD LF001 (50 mmID × 500 mmL), eluting with EtOH at 60 mL/min flow rate) to afford two enantiomers, **15**-ent1 (the first eluted enantiomer, white solid, 40.7 mg) and **15**-ent2 (the second eluted enantiomer, white solid, 44.6 mg). **15b**-ent1: Retention time 20.1 min (CHIRALPAK AD-3 NC002 (4.6 mmID × 250 mmL), eluting with EtOH at 0.7 mL/min flow rate). > 99.9%ee. MS (ESI) *m/z* 491 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.29 (6H, d, J = 6.9 Hz), 1.54–1.70 (2H, m), 1.95–2.07 (2H, m), 2.81–2.99 (2H, m), 3.19–3.39 (5H, m), 3.42–3.54 (1H, m), 3.57–3.88 (3H, m), 3.99–4.11 (2H, m), 4.97–5.05 (1H, m), 5.26–5.37 (1H, m), 6.18 (1H, s), 7.46 (1H, dd, J = 8.5, 1.9 Hz), 7.53 (1H, s), 8.48–8.56 (2H, m). Anal. Calcd for $C_{23}H_{30}N_4O_4S_2{\cdot}0.2H_2O$: C, 55.89; H, 6.20; N, 11.34. Found: C, 55.96; H, 6.24; N, 11.10. **15b**-ent2: Retention time 24.5 min (CHIRALPAK AD-3 NC002 (4.6 mmID × 250 mmL), eluting with EtOH at 0.7 mL/min flow rate). 96.8%ee. MS (ESI) *m/z* 491 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.29 (6H, d, J = 6.9 Hz), 1.54–1.68 (2H, m), 1.95–2.06 (2H, m), 2.83–2.98 (2H, m), 3.22–3.39 (5H, m), 3.43–3.54 (1H, m), 3.56–3.87 (3H, m), 4.01–4.10 (2H, m), 4.98–5.03 (1H, m), 5.26–5.36 (1H, m), 6.18 (1H, s), 7.46 (1H, dd, J = 8.5, 1.9 Hz), 7.53 (1H, s), 8.49–8.55 (2H, m). Anal. Calcd for $C_{23}H_{30}N_4O_4S_2$: C, 56.30; H, 6.16; N, 11.42. Found: C, 56.12; H, 6.24; N, 11.24.

***tert*-Butyl 4-{[6-(2,3-dihydro-1*H*-indol-1-yl)pyrimidin-4-yl] oxy}piperidine-1-carboxylate (16).**

A mixture of compound **10** (1.00 g, 2.10 mmol), 10% Pd on carbon (200 mg), THF (20 mL), and MeOH (10 mL) was stirred at room temperature under H_2 atmosphere (1 atm, balloon) for 3 days. The catalyst was removed by filtration through a PTFE membrane filter and the filtrate was concentrated under reduced pressure. The residue was partitioned between AcOEt and aqueous $NaHCO_3$ solution. The organic layer was washed with brine and dried over Na_2SO_4. After removal of the solvent, the residue was purified by silica gel column chromatography (hexane/AcOEt = 80/20 to 65/35) to give the title compound as a white solid (400 mg, 48%). MS (ESI/APCI) *m/z* 397 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.41 (9H, s), 1.48–1.63 (2H, m), 1.89–2.02 (2H, m), 3.09–3.24 (4H, m), 3.71 (2H, dt, J = 13.5, 4.8 Hz), 3.97 (2H, t, J = 8.7 Hz), 5.18–5.29 (1H, m), 6.08 (1H, s), 6.88–6.96 (1H, m), 7.16 (1H, t, J = 7.8 Hz), 7.23 (1H, d, J = 7.2 Hz), 8.37 (1H, d, J = 8.0 Hz), 8.45 (1H, s).

***S*-Propan-2-yl 4-{[6-(2,3-dihydro-1*H*-indol-1-yl)pyrimidin-4-yl] oxy}piperidine-1-carbothioate (17).**

Compound **17** was prepared from compound **16** in a manner similar to that described for compounds **5** and **6**. White solid. Yield 90% over 2 steps. MS (ESI/APCI) *m/z* 399 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.28 (6H, d, J = 6.8 Hz), 1.54–1.69 (2H, m), 1.93–2.08 (2H, m), 3.14–3.26 (2H, m), 3.28–3.41 (2H, m), 3.42–3.55 (1H, m), 3.64–3.89 (2H, m), 3.97 (2H, t, J = 8.7 Hz), 5.25–5.36 (1H, m), 6.10 (1H, s), 6.89–6.98 (1H, m), 7.12–7.29 (2H, m), 8.37 (1H, d, J = 8.3 Hz), 8.45 (1H, s). Anal. Calcd for $C_{21}H_{26}N_4O_2S$: C, 63.29; H, 6.58; N, 14.06. Found: C, 63.31; H, 6.71; N, 13.94.

1-(6-Chloropyrimidin-4-yl)-5-nitro-2,3-dihydro-1*H*-indole (19).

Compound **19** was prepared from 5-nitro-2,3-dihydro-1*H*-indole in a manner similar to that described for compound **9**. Pale yellow solid. Yield 83%. MS (ESI/APCI) *m/z* 277 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.33 (2H, t, J = 8.5 Hz), 3.96–4.34 (2H, m), 7.16 (1H, s), 8.09–8.28 (2H, m), 8.56 (1H, d, J = 9.0 Hz), 8.72 (1H, s).

***tert*-Butyl 4-{[6-(5-nitro-2,3-dihydro-1*H*-indol-1-yl)pyrimidin-4-yl] oxy}piperidine-1-carboxylate (20).**

Compound **20** was prepared from compound **19** in a manner similar to that described for compound **10**. Pale yellow solid. Yield 69%. MS (ESI/APCI) *m/z* 442 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.41 (9H, s), 1.49–1.66 (2H, m), 1.90–2.06 (2H, m), 3.08–3.24 (2H, m), 3.24–3.36 (2H, m), 3.64–3.82 (2H, m), 4.12 (2H, t, J = 8.7 Hz), 5.21–5.37 (1H, m), 6.28 (1H, s), 8.04–8.10 (1H, m), 8.10–8.19 (1H, m), 8.51 (1H, d, J = 9.1 Hz), 8.56 (1H, s). Anal. Calcd for $C_{22}H_{27}N_5O_5$: C, 59.85; H, 6.16; N, 15.86. Found: C, 59.83; H, 6.12; N, 15.90.

***tert*-Butyl 4-{[6-(5-amino-2,3-dihydro-1*H*-indol-1-yl)pyrimidin-4-yl] oxy}piperidine-1-carboxylate (21).**

A mixture of compound **20** (8.80 g, 19.9 mmol), 10% Pd on carbon (500 mg), THF (150 mL), and MeOH (300 mL) was stirred at room temperature under H_2 atmosphere (1 atm, balloon) for 16 h. The catalyst was removed by filtration through a PTFE membrane filter and the filtrate was concentrated under reduced pressure to give the title compound as a white solid (8.44 g, quant.). MS (ESI/APCI) *m/z* 412 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.41 (9H, s), 1.46–1.65 (2H, m), 1.84–2.03 (2H, m), 2.98–3.25 (4H, m), 3.63–3.78 (2H, m), 3.86 (2H, t, J = 8.5 Hz), 4.79 (2H, s), 5.11–5.31 (1H, m), 5.91 (1H, s), 6.38 (1H, dd, J = 8.5, 2.5 Hz), 6.49 (1H, d, J = 2.3 Hz), 8.03 (1H, d, J = 8.3 Hz), 8.34 (1H, s). Anal. Calcd for $C_{22}H_{29}N_5O_3$: C, 64.21; H, 7.10; N, 17.02. Found: C, 64.09; H, 7.23; N, 16.73.

***tert*-Butyl 4-({6-[5-(2-oxopyrrolidin-1-yl)-2,3-dihydro-1*H*-indol-1-yl] pyrimidin-4-yl}oxy)piperidine-1-carboxylate (23a).**

Compound **23a** was prepared from compound **21** in a manner similar to that described for compounds **22b** and **23b**. Pale yellow solid. Yield 90% over 2 steps. MS (ESI/APCI) *m/z* 494 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.41 (9H, s), 1.46–1.67 (2H, m), 1.89–2.13 (4H, m), 2.42–2.50 (2H, m), 3.07–3.26 (4H, m), 3.65–3.76 (2H, m), 3.80 (2H, t, J = 7.0 Hz), 3.97 (2H, t, J = 8.5 Hz), 5.16–5.33 (1H, m), 6.06 (1H, s), 7.35 (1H, dd, J = 9.1, 2.3 Hz), 7.61 (1H, d, J = 1.9 Hz), 8.32 (1H, d, J = 9.1 Hz), 8.44 (1H, s).

1-{1-[6-({1-[3-(Propan-2-yl)-1,2,4-oxadiazol-5-yl] piperidin-4-yl}oxy)pyrimidin-4-yl]-2,3-dihydro-1*H*-indol-5-yl}pyrrolidin-2-

one (24a).

Compound **24a** was prepared from compound **23a** in a manner similar to that described for compounds **13**. White solid. Yield 34% over 3 steps. MS (ESI/APCI) *m/z* 490 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.19 (6H, d, $J = 6.8$ Hz), 1.65–1.84 (2H, m), 1.96–2.18 (4H, m), 2.41–2.50 (2H, m), 2.82 (1H, q, $J = 6.9$ Hz), 3.20 (2H, t, $J = 8.5$ Hz), 3.41–3.59 (2H, m), 3.73–3.90 (4H, m), 3.98 (2H, t, $J = 8.7$ Hz), 5.23–5.39 (1H, m), 6.09 (1H, s), 7.35 (1H, dd, $J = 8.7$, 2.3 Hz), 7.61 (1H, d, $J = 2.3$ Hz), 8.32 (1H, d, $J = 9.0$ Hz), 8.46 (1H, s). Anal. Calcd for $C_{26}H_{31}N_7O_3 \cdot 0.1H_2O$: C, 63.55; H, 6.40; N, 19.95. Found: C, 63.39; H, 6.52; N, 19.70.

***tert*-Butyl 4-[(6-{5-[(5-chloropentanoyl)amino]-2,3-dihydro-1*H*-indol-1-yl}pyrimidin-4-yl)oxy] piperidine-1-carboxylate (22b).**

To a solution of compound **21** (20.0 g, 48.6 mmol) in DMA (200 mL) was added dropwise 5-chloropentanoyl chloride (7.76 g, 50.1 mmol) at 0 °C, and the mixture was stirred at room temperature for 1 h. The mixture was diluted with AcOEt, successively washed with water, saturated aqueous $NaHCO_3$ solution and brine, and dried over Na_2SO_4. The solvent was evaporated to give the title compound as a gray powder (22.2 g, 86%). MS (ESI/APCI) *m/z* 530 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.41 (9H, s), 1.47–1.63 (2H, m), 1.64–1.82 (4H, m), 1.89–2.02 (2H, m), 2.31 (2H, t, $J = 7.0$ Hz), 3.07–3.24 (4H, m), 3.62–3.78 (4H, m), 3.95 (2H, t, $J = 8.5$ Hz), 5.17–5.30 (1H, m), 6.04 (1H, s), 7.27 (1H, dd, $J = 8.9$, 2.1 Hz), 7.58 (1H, d, $J = 1.5$ Hz), 8.25 (1H, d, $J = 8.7$ Hz), 8.42 (1H, s), 9.80 (1H, s).

***tert*-Butyl 4-({6-[5-(2-oxopiperidin-1-yl)-2,3-dihydro-1*H*-indol-1-yl] pyrimidin-4-yl}oxy)piperidine-1-carboxylate (23b).**

To a solution of compound **22b** (22.1 g, 41.7 mmol) in anhydrous DMF (300 mL) was added sodium hydride (60% oil dispersion, 2.00 g, 50.0 mmol) at 0 °C, and the mixture was stirred at room temperature for 3 h. After the mixture was cooled to at 0 °C again, water (300 mL) was added dropwise to the reaction mixture. The precipitated solid was collected, washed with water and Et_2O, and dried to give the title compound as a pale yellow powder (19.0 g, 92%). MS (ESI/APCI) *m/z* 494 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.41 (9H, s), 1.47–1.63 (2H, m), 1.77–2.02 (6H, m), 2.36 (2H, t, $J = 6.0$ Hz), 3.09–3.23 (4H, m), 3.55 (2H, t, $J = 5.3$ Hz), 3.65–3.78 (2H, m), 3.99 (2H, t, $J = 8.7$ Hz), 5.19–5.30 (1H, m), 6.09 (1H, s), 7.03 (1H, dd, $J = 8.7$, 2.3 Hz), 7.12 (1H, d, $J = 1.9$ Hz), 8.33 (1H, d, $J = 8.7$ Hz), 8.46 (1H, s).

1-{1-[6-({1-[3-(Propan-2-yl)-1,2,4-oxadiazol-5-yl] piperidin-4-yl}oxy)pyrimidin-4-yl]-2,3-dihydro-1*H*-indol-5-yl}piperidin-2-one (24b).

Step A. A mixture of compound **23b** (18.9 g, 38.3 mmol), 4 N HCl in AcOEt (77 mL), AcOEt (200 mL), and MeOH (100 mL) was stirred at room temperature for 15 h. After the mixture was concentrated under reduced pressure, the residue was suspended with AcOEt (100 mL). The precipitated solid was collected and washed with AcOEt. The solid was dissolved in a mixed solvent of THF (270 mL) and water (126 mL), and then sodium bicarbonate (16.1 g, 191 mmol) and cyanogen bromide (5.27 g, 49.8 mmol) were added to the mixture. The resulting mixture was stirred at room temperature for 3 h. The reaction mixture was diluted with AcOEt, washed with brine, and dried over Na_2SO_4. The solvent was removed by evaporation to give 4-({6-[5-(2-oxopiperidin-1-yl)-2,3-dihydro-1*H*-indol-1-yl]pyrimidin-4-yl}oxy)piperidine-1-carbonitrile as a pale yellow solid (15.7 g, 98%). MS (ESI/APCI) *m/z* 419 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.66–1.90 (6H, m), 1.98–2.11 (2H, m), 2.36 (2H, t, $J = 5.9$ Hz), 3.13–3.47 (6H, m), 3.55 (2H, t, $J = 5.3$ Hz), 4.00 (2H, t, $J = 8.5$ Hz), 5.15–5.28 (1H, m), 6.11 (1H, s), 7.03 (1H, dd, $J = 8.5$, 2.1 Hz), 7.12 (1H, d, $J = 1.9$ Hz), 8.33 (1H, d, $J = 8.7$ Hz), 8.45 (1H, s).

Step B. To a mixture of 4-({6-[5-(2-oxopiperidin-1-yl)-2,3-dihydro-1*H*-indol-1-yl]pyrimidin-4-yl}oxy)piperidine-1-carbonitrile (34.0 g, 81.2 mmol) and *N*-hydroxy-2-methylpropanimidamide (10.8 g, 106 mmol) in anhydrous DMF (510 mL) were added zinc chloride (1.0 M Et_2O solution, 24.4 mL, 24.4 mmol) and toluenesulfonic acid monohydrate (4.64 g, 24.4 mmol) at room temperature, and the mixture was stirred at 80 °C for 8 h. The reaction mixture was diluted with AcOEt and THF, washed with 0.03 N hydrochloric acid, 0.1 N aqueous NaOH solution, water and brine, and dried over Na_2SO_4. The filtrate was passed through a pad of silica gel (NH, AcOEt) and concentrated under reduced pressure to give a pale yellow solid. The solid was suspended in AcOEt-EtOH (10:1 v/v, 165 mL) and stirred at 80 °C for 1 h. After the mixture was cooled to rt, the precipitated solid was collected to give the title compound as a white powder (**24b**, 25.5 g, 62%). MS (ESI/APCI) *m/z* 504 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.19 (6H, d, $J = 6.8$ Hz), 1.67–1.92 (6H, m), 2.02–2.15 (2H, m), 2.36 (2H, t, $J = 6.0$ Hz), 2.74–2.91 (1H, m), 3.19 (2H, t, $J = 8.5$ Hz), 3.42–3.59 (4H, m), 3.76–3.88 (2H, m), 4.00 (2H, t, $J = 8.7$ Hz), 5.26–5.38 (1H, m), 6.12 (1H, s), 7.03 (1H, dd, $J = 8.7$, 2.3 Hz), 7.12 (1H, d, $J = 1.9$ Hz), 8.33 (1H, d, $J = 8.7$ Hz), 8.47 (1H, s). Anal. Calcd for $C_{27}H_{33}N_7O_3$: C, 64.39; H, 6.60; N, 19.47. Found: C, 64.27; H, 6.63; N, 19.41.

***tert*-Butyl 4-{[6-(5-{[(2-chloroethoxy)acetyl]amino}-2,3-dihydro-1*H*-indol-1-yl)pyrimidin-4-yl]oxy}piperidine-1-carboxylate (22c).**

Compound **22c** was prepared from compound **21** in a manner similar to that described for compound **22b**. Brown oil. Yield 96%. MS (ESI/APCI) *m/z* 532 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.41 (9H, s), 1.46–1.63 (2H, m), 1.88–2.02 (2H, m), 3.11–3.23 (4H, m), 3.62–3.77 (2H, m), 3.81(4H, s), 3.96 (2H, t, $J = 8.7$ Hz), 4.11 (2H, s), 5.15–5.29 (1H, m), 6.05 (1H, s), 7.35 (1H, dd, $J = 8.7$, 1.9 Hz), 7.60 (1H, s), 8.28 (1H, d, $J = 8.7$ Hz), 8.43 (1H,s), 9.58 (1H, s).

***tert*-Butyl 4-({6-[5-(3-oxomorpholin-4-yl)-2,3-dihydro-1*H*-indol-1-yl] pyrimidin-4-yl}oxy)piperidine-1-carboxylate (23c).**

Compound **23c** was prepared from compound **22c** in a manner similar to that described for compound **23b**. Brown solid. Yield 97%. MS (ESI/APCI) *m/z* 496 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.41 (9H, s), 1.47–1.64 (2H, m), 1.86–2.03 (2H, m), 3.08–3.26 (4H, m), 3.63–3.78 (4H, m), 3.89–4.05 (4H, m), 4.18 (2H, s), 5.17–5.32 (1H, m), 6.10 (1H, s), 7.15 (1H, dd, $J = 8.5$, 2.1 Hz), 7.24 (1H, d, $J = 2.3$ Hz), 8.36 (1H, d, $J = 8.7$ Hz), 8.46 (1H, s).

4-{1-[6-({1-[3-(Propan-2-yl)-1,2,4-oxadiazol-5-yl] piperidin-4-yl}oxy)pyrimidin-4-yl]-2,3-dihydro-1*H*-indol-5-yl}morpholin-3-one (24c).

Compound **24c** was prepared from compound **23c** in a manner similar to that described for compound **24b**. White solid. Yield 24% over 3 steps. MS (ESI/APCI) *m/z* 506 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.19 (6H, d, $J = 7.2$ Hz), 1.66–1.83 (2H, m, $J = 12.9$, 8.7, 8.7, 4.0 Hz), 2.01–2.15 (2H, m), 2.82 (1H, q, $J = 6.9$ Hz), 3.20 (2H, t, $J = 8.5$ Hz), 3.49 (2H, ddd, $J = 13.2$, 9.2, 3.6 Hz), 3.64–3.73 (2H, m), 3.76–3.88 (2H, m), 3.91–4.07 (4H, m), 4.18 (2H, s), 5.32 (1H, dt, $J = 7.9$, 4.3 Hz), 6.12 (1H, s), 7.15 (1H, dd, $J = 8.7$, 2.3 Hz), 7.24 (1H, d, $J = 1.9$ Hz), 8.36 (1H, d, $J = 8.7$ Hz), 8.48 (1H, s). Anal. Calcd for $C_{26}H_{31}N_7O_4$: C, 61.77; H, 6.18; N, 19.39. Found: C, 61.69; H, 6.21; N, 19.32.

***tert*-Butyl 4-({6-[5-(1,1-dioxido-1,2-thiazolidin-2-yl)-2,3-dihydro-1*H*-indol-1-yl] pyrimidin-4-yl}oxy)piperidine-1-carboxylate (23d).**

To a solution of compound **21** (5.00 g, 12.2 mmol) and triethylamine (2.79 mL, 20.0 mmol) in THF (220 mL) was added dropwise 3-chloropropanesulfonyl chloride (2.58 g, 14.6 mmol), and the mixture was stirred at room temperature for 16 h. The mixture was diluted with AcOEt, successively washed with water and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residual solid was dissolved in DMF (100 mL). Sodium hydride (60% oil dispersion, 583 mg, 14.6 mmol) was added to the mixture at 0 °C, and the mixture was stirred at room temperature for 3 h. The reaction was quenched by addition of water and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. The solvent was removed by evaporation to give the title compound as a brown solid (5.10 g, 81% over 2 steps). MS (ESI/APCI) *m/z* 516 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.41 (9H, s), 1.47–1.69 (2H, m), 1.87–2.06 (2H, m), 2.30–2.45 (2H, m), 3.06–3.27 (4H, m), 3.46 (2H, t, $J = 7.4$ Hz),

3.62–3.80 (4H, m), 3.98 (2H, t, J = 8.7 Hz), 5.13–5.35 (1H, m), 6.07 (1H, s), 7.02 (1H, dd, J = 8.9, 2.5 Hz), 7.17 (1H, d, J = 2.3 Hz), 8.33 (1H, d, J = 8.7 Hz), 8.45 (1H, s). Anal. Calcd for $C_{25}H_{33}N_5O_5S$: C, 58.23; H, 6.45; N, 13.58. Found: C, 58.12; H, 6.43; N, 13.68.

5-(1,1-Dioxido-1,2-thiazolidin-2-yl)-1-[6-({1-[3-(propan-2-yl)-1,2,4-oxadiazol-5-yl] piperidin-4-yl}oxy)pyrimidin-4-yl] -2,3-dihydro-1*H*-indole (24d).

Compound **24d** was prepared from compound **23d** in a manner similar to that described for compound **13**. White solid. Yield 31% over 3 steps. MS (ESI/APCI) *m/z* 526 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.19 (6H, d, J = 6.8 Hz), 1.66–1.84 (2H, m), 1.99–2.18 (2H, m), 2.30–2.45 (2H, m), 2.68–2.93 (1H, m), 3.21 (2H, t, J = 8.5 Hz), 3.40–3.59 (4H, m), 3.69 (2H, t, J = 6.6 Hz), 3.76–3.90 (2H, m), 3.98 (2H, t, J = 8.7 Hz), 5.22–5.40 (1H, m), 6.10 (1H, s), 7.02 (1H, dd, J = 8.9, 2.5 Hz), 7.17 (1H, d, J = 2.3 Hz), 8.34 (1H, d, J = 9.1 Hz), 8.46 (1H, s). Anal. Calcd for $C_{25}H_{31}N_7O_4S$: C, 57.13; H, 5.94; N, 18.65. Found: C, 56.89; H, 5.84; N, 18.47.

***tert*-Butyl 4-{[6-(5-{[*N*-(2-hydroxyethyl)glycyl] amino}-2,3-dihydro-1*H*-indol-1-yl)pyrimidin-4-yl] oxy}piperidine-1-carboxylate (22e).**

Step A. To a mixture of compound **21** (2.00 g, 4.86 mmol) in DMA (20 mL) was added chloracetyl chloride (0.426 mL, 5.35 mmol) at 0 °C, and the mixture was stirred at room temperature for 3 h. The reaction mixture was diluted with AcOEt, washed with water and brine, and dried over Na_2SO_4. The solvent was removed by evaporation to give *tert*-butyl 4-[(6-{5-[(chloroacetyl)amino]-2,3-dihydro-1*H*-indol-1-yl}pyrimidin-4-yl)oxy]piperidine-1-carboxylate as a white powder (2.35, 99%). MS (ESI/APCI) *m/z* 488 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.41 (9H, s), 1.47–1.64 (2H, m), 1.89–2.02 (2H, m), 3.08–3.26 (4H, m), 3.65–3.77 (2H, m), 3.97 (2H, t, J = 8.5 Hz), 4.22 (2H, s), 5.16–5.30 (1H, m), 6.06 (1H, s), 7.29 (1H, dd, J = 8.9, 2.1 Hz), 7.57 (1H, d, J = 1.5 Hz), 8.30 (1H, d, J = 8.7 Hz), 8.44 (1H, s), 10.21 (1H, s).

Step B. A mixture of *tert*-butyl 4-[(6-{5-[(chloroacetyl)amino]-2,3-dihydro-1*H*-indol-1-yl}pyrimidin-4-yl)oxy]piperidine-1-carboxylate (1.00 g, 2.05 mmol) and 2-aminoethanol (0.618 mL, 10.3 mmol) in a mixed solvent of THF (20 mL) and AcO^{i}Pr (20 mL) was stirred at 70 °C for 8 h and then refluxed for further 3 h. The reaction mixture was diluted with AcOEt, washed with saturated aqueous $NaHCO_3$ solution and brine, and dried over Na_2SO_4. The solvent was removed by evaporation to give the title compound as a pale yellow powder (**22e**, 1.03 g, 98%). MS (ESI/APCI) *m/z* 513 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.41 (9H, s), 1.47–1.64 (2H, m), 1.89–2.03 (2H, m), 2.44 (1H, bs), 2.61 (2H, t, J = 5.5 Hz), 3.08–3.23 (4H, m), 3.26 (2H, s), 3.42–3.52 (2H, m), 3.66–3.77 (2H, m), 3.96 (2H, t, J = 8.5 Hz), 4.63 (1H, t, J = 5.3 Hz), 5.17–5.29 (1H, m), 6.04 (1H, s), 7.34 (1H, dd, J = 8.7, 1.9 Hz), 7.61 (1H, d, J = 1.9 Hz), 8.27 (1H, d, J = 8.7 Hz), 8.43 (1H, s), 9.80 (1H, bs).

***tert*-Butyl 4-[(6-{5-[2-oxo-4-(trifluoroacetyl)piperazin-1-yl] -2,3-dihydro-1*H*-indol-1-yl}pyrimidin-4-yl)oxy] piperidine-1-carboxylate (23e).**

To a mixture of compound **22e** (1.00 g, 1.95 mmol) and tri-*n*-butylphosphine (0.729 mL, 2.93 mmol) in THF (20 mL) was added 1,1′-(azodicarbonyl)dipiperidine (0.738 g, 2.93 mmol) at 0 °C, and the mixture was stirred at room temperature for 15 h. Tributylphosphine (0.729 mL, 2.93 mmol) and 1,1′-(azodicarbonyl)dipiperidine (0.738 g, 2.93 mmol) were added again and the resulting mixture was stirred at 60 °C for 4 h. After the mixture was cooled to room temperature, AcOEt (20 mL) was added and the insoluble materials were filtered off. To the filtrate were added triethylamine (1.63 mL, 11.7 mmol) and trifluoroacetic anhydride (0.542 mL, 3.91 mmol). After being stirred for 6 h, the reaction mixture was diluted with AcOEt, washed with water, aqueous $NaHCO_3$ solution and brine, and dried over Na_2SO_4. After removal of the solvent, the residue was purified by crystallization from toluene to give the title compound as a white powder (0.530 g, 46%). MS (ESI/APCI) *m/z* 591 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.41 (9H, s), 1.48–1.64 (2H, m), 1.89–2.04 (2H, m), 3.09–3.26 (4H, m), 3.65–3.83 (4H, m), 3.90–4.08 (4H, m), 4.24–4.39 (2H, m), 5.17–5.32 (1H, m), 6.11 (1H, s), 7.09–7.18 (1H, m), 7.23 (1H, s), 8.37 (1H, d, J = 8.7 Hz), 8.47 (1H, s).

1-{1-[6-({1-[3-(Propan-2-yl)-1,2,4-oxadiazol-5-yl] piperidin-4-yl}oxy)pyrimidin-4-yl] -2,3-dihydro-1*H*-indol-5-yl}piperazin-2-one hydrochloride (24e).

Step A. 1-{1-[6-({1-[3-(Propan-2-yl)-1,2,4-oxadiazol-5-yl]piperidin-4-yl}oxy)pyrimidin-4-yl]-2,3-dihydro-1*H*-indol-5-yl}-4-(trifluoroacetyl)piperazin-2-one was prepared from compound **23e** in a manner similar to that described for compound **24b**. White solid. Yield 63% over 3 steps. MS (ESI/APCI) *m/z* 601 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.19 (6H, d), 1.67–1.83 (2H, m), 2.02–2.15 (2H, m), 2.74–2.91 (1H, m), 3.20 (2H, t, J = 8.3 Hz), 3.43–3.56 (2H, m), 3.73–3.88 (4H, m), 3.90–4.07 (4H, m), 4.23–4.38 (2H, m), 5.26–5.38 (1H, m), 6.14 (1H, s), 7.13 (1H, dd, J = 8.7, 2.3 Hz), 7.23 (1H, s), 8.37 (1H, d, J = 8.7 Hz), 8.48 (1H, s).

Step B. A mixture of 1-{1-[6-({1-[3-(propan-2-yl)-1,2,4-oxadiazol-5-yl]piperidin-4-yl}oxy)pyrimidin-4-yl]-2,3-dihydro-1*H*-indol-5-yl}-4-(trifluoroacetyl)piperazin-2-one (225 mg, 0.37 mmol), 1 M aqueous NaOH solution (1.12 mL, 1.12 mmol), MeOH (4 mL) and THF (4 mL) was stirred at room temperature for 1 h. The reaction mixture was diluted with AcOEt, washed with brine, and dried over Na_2SO_4. The solvent was removed by evaporation to give a pale yellow solid. The solid was suspended in MeOH (4 mL) and a solution of HCl in MeOH (0.37 mmol, prepared from acetyl chloride (0.027 mL, 0.37 mmol) and MeOH (4 mL) was added. The mixture was concentrated under reduced pressure and triturated with Et_2O. The precipitated solid was collected to give the title compound as a pale yellow powder (**24e**, 193 mg, 95%). MS (ESI/APCI) *m/z* 505 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.19 (6H, d, J = 6.8 Hz), 1.65–1.85 (2H, m, J = 12.9, 8.6, 8.6, 3.8 Hz), 2.00–2.17 (2H, m), 2.74–2.91 (1H, m), 3.22 (2H, t, J = 8.7 Hz), 3.40–3.57 (4H, m), 3.75–3.91 (6H, m), 4.02 (2H, t, J = 8.7 Hz), 5.25–5.40 (1H, m), 6.15 (1H, s), 7.10 (1H, dd, J = 8.7, 2.3 Hz), 7.17 (1H, s), 8.40 (1H, d, J = 8.7 Hz), 8.49 (1H, s), 9.70 (2H, bs). Anal. Calcd for $C_{26}H_{33}ClN_8O_3 \cdot 0.9H_2O$: C, 56.04; H, 6.29; N, 20.11. Found: C, 56.21; H, 6.25; N, 19.81.

***tert*-Butyl 4-{[6-(5-{[(*tert*-butoxycarbonyl)sulfamoyl] amino}-2,3-dihydro-1*H*-indol-1-yl)pyrimidin-4-yl] oxy}piperidine-1-carboxylate (22f).**

A mixture of compound **21** (2.18 g, 5.30 mmol) and (*tert*-butoxycarbonyl){[4-(dimethyliminio)pyridin-1(4*H*)-yl]sulfonyl}azanide (1.92 g, 6.36 mmol) in THF (20 mL) was stirred at 50 °C for 5 h. The reaction mixture was diluted with AcOEt, successively washed with 0.1 M hydrochloric acid, water and brine, and dried over Na_2SO_4. The solvent was removed by evaporation to give the title compound as a pale yellow amorphous solid (3.05 g, 97%) MS (ESI/APCI) *m/z* 591 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.38 (9H, s), 1.41 (9H, s), 1.47–1.63 (2H, m), 1.88–2.05 (2H, m), 3.06–3.26 (4H, m), 3.65–3.78 (2H, m), 3.88–4.10 (2H, m), 5.17–5.31 (1H, m), 6.07 (1H, s), 6.92–7.00 (1H, m), 7.05 (1H, s), 8.28 (1H, d, J = 8.7 Hz), 8.44 (1H, s), 9.95 (1H, s), 11.01 (1H, bs).

***tert*-Butyl 6-[1-(6-{[1-(*tert*-butoxycarbonyl)piperidin-4-yl] oxy} pyrimidin-4-yl)-2,3-dihydro-1*H*-indol-5-yl] -1,2,6-thiadiazinane-2-carboxylate 1,1-dioxide (23f).**

A mixture of compound **22f** (460 mg, 0.779 mmol), 1,3-dibromopropane (0.120 mL, 1.17 mmol) and potassium carbonate (323 mg, 2.34 mmol) in a mixed solvent of acetone (15 mL) and DMA (5 mL) was stirred at 70 °C for 6 h. The reaction mixture was diluted with AcOEt, washed with water and brine, and dried over Na_2SO_4. The solvent was removed by evaporation to give the title compound as a pale yellow amorphous solid (490 mg, 100%). MS (ESI/APCI) *m/z* 631 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.41 (9H, s), 1.43 (9H, s), 1.48–1.63 (2H, m), 1.88–2.01 (4H, m), 3.09–3.26 (4H, m), 3.66–3.76 (2H, m), 3.81 (2H, t, J = 5.8 Hz), 3.95–4.06 (4H, m), 5.18–5.31 (1H, m), 6.12 (1H, s), 7.15 (1H, dd, J = 8.9, 2.4 Hz), 7.22 (1H, d, J = 1.9 Hz), 8.36 (1H, d, J = 8.7 Hz), 8.47 (1H, s).

5-(1,1-Dioxido-1,2,6-thiadiazinan-2-yl)-1-[6-({1-[3-(propan-2-yl)-1,2,4-oxadiazol-5-yl] piperidin-4-yl}oxy)pyrimidin-4-yl] -2,3-dihydro-1*H*-indole (24f).

Compound **24f** was prepared from compound **23f** in a manner similar to that described for compound **24b**. Pale yellow amorphous solid. Yield 47% over 3 steps. MS (ESI/APCI) *m/z* 541 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.19 (6H, d, $J = 7.2$ Hz), 1.67–1.88 (4H, m), 2.01–2.15 (2H, m), 2.73–2.90 (1H, m), 3.20 (2H, t, $J = 8.5$ Hz), 3.31–3.40 (2H, m), 3.42–3.61 (4H, m), 3.76–3.87 (2H, m), 4.00 (2H, t, $J = 8.7$ Hz), 5.25–5.37 (1H, m), 6.13 (1H, s), 7.13 (1H, dd, $J = 8.7$, 2.3 Hz), 7.17–7.25 (2H, m), 8.34 (1H, d, $J = 8.7$ Hz), 8.48 (1H, s). Anal. Calcd for $C_{25}H_{32}N_8O_4S{\cdot}0.1$AcOEt: C, 55.52; H, 6.02; N, 20.39. Found: C, 55.26; H, 5.91; N, 20.20.

***tert*-Butyl 4-({6-[5-(acetylamino)-2,3-dihydro-1*H*-indol-1-yl] pyrimidin-4-yl}oxy)piperidine-1-carboxylate (22 g).**

To a mixture of compound **21** (1.00 g, 2.43 mmol) and triethylamine (1.01 mL, 7.29 mmol) in THF (20 mL) was added acetyl chloride (0.225 mL, 3.16 mmol) dropwise at 0 °C. The mixture was stirred at room temperature for 30 min. The reaction mixture was partitioned between AcOEt and water. The organic layer was successively 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 = 40/60 to 0/100) to give the title compound as a brown amorphous solid (1.05 g, 95%). MS (ESI/APCI) *m/z* 454 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.41 (9H, s), 1.47–1.63 (2H, m), 1.90–2.04 (5H, m), 3.07–3.23 (4H, m), 3.62–3.77 (2H, m), 3.95 (2H, t, $J = 8.5$ Hz), 5.17–5.29 (1H, m), 6.03 (1H, s), 7.21–7.29 (1H, m), 7.57 (1H, d, $J = 1.5$ Hz), 8.25 (1H, d, $J = 9.1$ Hz), 8.42 (1H, d, $J = 0.8$ Hz), 9.81 (1H, s).

***N*-Methyl-*N*-{1-[6-({1-[3-(propan-2-yl)-1,2,4-oxadiazol-5-yl] piperidin-4-yl}oxy)pyrimidin-4-yl]-2,3-dihydro-1*H*-indol-5-yl} acetamide (24 g).**

Step A. *N*-{1-[6-({1-[3-(Propan-2-yl)-1,2,4-oxadiazol-5-yl]piperidin-4-yl}oxy)pyrimidin-4-yl]-2,3-dihydro-1*H*-indol-5-yl}acetamide was prepared from compound **22 g** in a manner similar to that described for compound **24b**. White solid. Yield 37% over 3 steps. MS (ESI/APCI) *m/z* 464 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.19 (6H, d, $J = 6.8$ Hz), 1.66–1.82 (2H, m), 1.97–2.14 (5H, m), 2.76–2.90 (1H, m), 3.17 (2H, t, $J = 8.3$ Hz), 3.49 (2H, ddd, $J = 13.2$, 9.3, 3.6 Hz), 3.76–3.87 (2H, m), 3.96 (2H, t, $J = 8.5$ Hz), 5.25–5.35 (1H, m), 6.06 (1H, s), 7.26 (1H, dd, $J = 8.9$, 2.1 Hz), 7.57 (1H, d, $J = 1.9$ Hz), 8.25 (1H, d, $J = 8.7$ Hz), 8.44 (1H, d, $J = 0.8$ Hz), 9.81 (1H, s).

Step B. To a mixture of *N*-{1-[6-({1-[3-(propan-2-yl)-1,2,4-oxadiazol-5-yl]piperidin-4-yl}oxy)pyrimidin-4-yl]-2,3-dihydro-1*H*-indol-5-yl} acetamide (300 mg, 0.65 mmol) in anhydrous DMF (15 mL) was added sodium hydride (60% oil dispersion, 38.8 mg, 0.970 mmol) at room temperature, and the mixture was stirred at room temperature for 1 h. Iodomethane (0.081 mL, 1.3 mmol) was added followed by stirring at room temperature for 3 h. The mixture was diluted with AcOEt, successively washed with water and brine, and dried over Na_2SO_4. After removal of the solvent, the residue was suspended in Et_2O. The precipitated solid was collected to give the title compound as a white solid (**24 g**, 240 mg, 78%). MS (ESI/APCI) *m/z* 478 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.19 (6H, d, $J = 7.2$ Hz), 1.67–1.84 (5H, m), 2.02–2.14 (2H, m), 2.74–2.91 (1H, m), 3.11 (3H, s), 3.22 (2H, t, $J = 8.5$ Hz), 3.49 (2H, ddd, $J = 13.2$, 9.2, 3.6 Hz), 3.76–3.88 (2H, m), 4.02 (2H, t, $J = 8.7$ Hz), 5.32 (1H, dt, $J = 7.9$, 4.3 Hz), 6.13 (1H, s), 7.11 (1H, d, $J = 8.3$ Hz), 7.20 (1H, s), 8.40 (1H, d, $J = 8.7$ Hz), 8.48 (1H, s). Anal. Calcd for $C_{25}H_{31}N_7O_3$: C, 62.88; H, 6.54; N, 20.53. Found: C, 62.88; H, 6.59; N, 20.29.

2-(Acetyloxy)-5-chloropentanoic acid ((*RS*)-26).

Compound (*RS*)-**26** was prepared from tetrahydrofuran-2-carboxylic acid in a manner similar to that described for compound (*S*)-**26**. Pale yellow oil. Yield 47% over 3 steps. ^1H NMR (300 MHz, $CDCl_3$) δ 1.88–2.12 (4H, m), 2.15 (3H, s), 3.58 (2H, t, $J = 6.2$ Hz), 5.06 (1H, dd, $J = 7.3$, 4.7 Hz), 6.52 (1H, bs).

***tert*-Butyl 4-{[6-(5-{[2-(acetyloxy)-5-chloropentanoyl] amino}-2,3-dihydro-1*H*-indol-1-yl)pyrimidin-4-yl] oxy}piperidine-1-carboxylate ((*RS*)-27).**

Compound (*RS*)-**27** was prepared from (*RS*)-**26** in a manner similar to that described for compound (*S*)-**27**. White amorphous solid. Yield 81%. MS (ESI/APCI) *m/z* 588 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.41 (9H, s), 1.47–1.63 (2H, m), 1.76–2.00 (6H, m), 2.10 (3H, s), 3.10–3.23 (4H, m), 3.66–3.76 (4H, m), 3.92–4.00 (2H, m), 4.94–4.99 (1H, m), 5.19–5.28 (1H, m), 6.05 (1H, s), 7.28 (1H, dd, $J = 8.7$, 2.3 Hz), 7.56 (1H, d, $J = 1.9$ Hz), 8.29 (1H, d, $J = 9.1$ Hz), 8.44 (1H, d, $J = 0.8$ Hz), 10.01 (1H, s).

***tert*-Butyl 4-({6-[5-(3-hydroxy-2-oxopiperidin-1-yl)-2,3-dihydro-1*H*-indol-1-yl] pyrimidin-4-yl}oxy)piperidine-1-carboxylate ((*RS*)-28).**

Compound (*RS*)-**28** was prepared from (*RS*)-**27** in a manner similar to that described for compound (*S*)-**28**. Pale brown solid. Yield 86% over 2 steps. MS (ESI/APCI) *m/z* 510 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.41 (9H, s), 1.49–1.63 (2H, m), 1.67–2.02 (5H, m), 2.04–2.15 (1H, m), 3.10–3.23 (4H, m), 3.45–3.55 (1H, m), 3.56–3.66 (1H, m), 3.67–3.76 (2H, m), 3.95–4.08 (3H, m), 5.18 (1H, d, $J = 3.8$ Hz), 5.21–5.30 (1H, m), 6.09 (1H, s), 7.05 (1H, dd, $J = 8.7$, 1.9 Hz), 7.13 (1H, d, $J = 1.9$ Hz), 8.34 (1H, d, $J = 8.7$ Hz), 8.46 (1H, s).

3-Hydroxy-1-{1-[6-({1-[3-(propan-2-yl)-1,2,4-oxadiazol-5-yl] piperidin-4-yl}oxy)pyrimidin-4-yl]-2,3-dihydro-1*H*-indol-5-yl} piperidin-2-one ((*RS*)-29).

Compound (*RS*)-**29** was prepared from (*RS*)-**28** in a manner similar to that described for compound **24b**. White solid. Yield 20% over 3 steps. MS (ESI/APCI) *m/z* 520 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.19 (6H, d), 1.67–1.81 (3H, m), 1.82–2.00 (2H, m), 2.03–2.14 (3H, m), 2.77–2.87 (1H, m), 3.19 (2H, t, $J = 8.5$ Hz), 3.43–3.55 (3H, m), 3.57–3.67 (1H, m), 3.77–3.88 (2H, m), 3.96–4.09 (3H, m), 5.19 (1H, d, $J = 3.4$ Hz), 5.27–5.37 (1H, m), 6.12 (1H, s), 7.05 (1H, d, $J = 8.7$ Hz), 7.13 (1H, s), 8.35 (1H, d, $J = 8.3$ Hz), 8.48 (1H, s). Anal. Calcd for $C_{27}H_{33}N_7O_4$: C, 62.41; H, 6.40; N, 18.87. Found: C, 62.24; H, 6.42; N, 18.59.

(2*R*)-2-(Acetyloxy)-5-chloropentanoic acid ((*R*)-26).

Compound (*R*)-**26** was prepared from (2*R*)-tetrahydrofuran-2-carboxylic acid in a manner similar to that described for compound (*S*)-**26**. Colorless oil. Yield 39% over 3 steps. ^1H NMR (300 MHz, $CDCl_3$) δ 1.86–2.13 (4H, m), 2.15 (3H, s), 3.49–3.74 (2H, m), 4.86–5.18 (1H, m), 6.51 (1H, bs).

***tert*-Butyl 4-{[6-(5-{[(2*R*)-2-(acetyloxy)-5-chloropentanoyl] amino}-2,3-dihydro-1*H*-indol-1-yl)pyrimidin-4-yl] oxy}piperidine-1-carboxylate ((*R*)-27).**

Compound (*R*)-**27** was prepared from (*R*)-**26** in a manner similar to that described for compound (*S*)-**27**. White amorphous solid. Yield 83%. MS (ESI/APCI) *m/z* 588 $[M + H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 1.47 (9H, s), 1.65–1.73 (2H, m), 1.86–2.00 (4H, m), 2.08–2.16 (2H, m), 2.23 (3H, s), 3.16–3.42 (4H, m), 3.53–3.65 (2H, m), 3.72–3.88 (2H, m), 3.96 (2H, t, $J = 8.7$ Hz), 5.21–5.42 (2H, m), 5.89 (1H, s), 7.00–7.18 (1H, m), 7.64 (1H, s), 7.76 (1H, s), 8.31 (1H, d, $J = 8.7$ Hz), 8.44 (1H, s).

***tert*-Butyl 4-[(6-{5-[(3*R*)-3-hydroxy-2-oxopiperidin-1-yl]-2,3-dihydro-1*H*-indol-1-yl}pyrimidin-4-yl)oxy] piperidine-1-carboxylate ((*R*)-28).**

Compound (*R*)-**28** was prepared from (*R*)-**27** in a manner similar to that described for compound (*S*)-**28**. White solid. Yield 86% over 2 steps. MS (ESI/APCI) *m/z* 510 $[M + H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 1.47 (9H, s), 1.65–1.91 (3H, m), 1.93–2.10 (4H, m), 2.36–2.47 (1H, m), 3.19–3.34 (4H, m), 3.56–3.86 (5H, m), 3.99 (2H, t, $J = 8.5$ Hz), 4.15–4.27 (1H, m), 5.22–5.34 (1H, m), 5.91 (1H, s), 7.04 (1H, dd, $J = 8.7$, 2.3 Hz), 7.12 (1H, s), 8.38 (1H, d, $J = 8.7$ Hz), 8.45 (1H, s).

(3*R*)-3-Hydroxy-1-{1-[6-({1-[3-(propan-2-yl)-1,2,4-oxadiazol-5-yl] piperidin-4-yl}oxy)pyrimidin-4-yl]-2,3-dihydro-1*H*-indol-5-yl} piperidin-2-one ((*R*)-29).

Compound (*R*)-**29** was prepared from (*R*)-**28** in a manner similar to that described for compound **24b**. White solid. Yield 43% over 3 steps. Retention time 15.5 min (CHIRALCEL OD3 (4.6 mmID × 250 mmL), eluting with MeOH at 1 mL/min flow rate). 99.9%ee. $[\alpha]_D^{25}$ + 17.1 (*c* 1.014, $CHCl_3$). MS (ESI/APCI) *m/z* 520 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.19 (6H, d, $J = 6.8$ Hz), 1.68–2.01 (5H, m), 2.03–2.15 (3H,

m), 2.76–2.89 (1H, m), 3.19 (2H, t, J = 8.3 Hz), 3.43–3.67 (4H, m), 3.76–3.88 (2H, m), 3.96–4.08 (3H, m), 5.16 (1H, d, J = 3.0 Hz), 5.27–5.37 (1H, m), 6.12 (1H, s), 7.05 (1H, dd, J = 8.7, 1.9 Hz), 7.14 (1H, d, J = 1.9 Hz), 8.34 (1H, d, J = 8.7 Hz), 8.48 (1H, s). Anal. Calcd for $C_{27}H_{33}N_7O_4$: C, 62.41; H, 6.40; N, 18.87. Found: C, 62.40; H, 6.51; N, 18.81.

(2*S*)-2-(Acetyloxy)-5-chloropentanoic acid ((*S*)-26).

Step A. A mixture of (2*S*)-tetrahydrofuran-2-carboxylic acid (1.16 g, 9.99 mmol) and oxalyl dichloride (3 mL, 34.4 mmol) was stirred at room temperature for 20 h. After the mixture was concentrated under reduced pressure, the residual oil was added to a solution of phenylmethanol (0.973 g, 9.00 mmol) and pyridine (2.14 g, 27.0 mmol) in Et_2O ($_2$0 mL) at 0 °C. The resulting mixture was allowed to warm to room temperature followed by stirring for 2 h. The reaction mixture was quenched with water and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/AcOEt = 100/0 to 95/5) to give benzyl (2*S*)-tetrahydrofuran-2-carboxylate as colorless oil (1.17 g, 63%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.82–2.11 (3H, m), 2.14–2.35 (1H, m), 3.82–4.08 (2H, m), 4.44–4.55 (1H, m), 5.09–5.26 (2H, m), 7.29–7.44 (5H, m).

Step B. A mixture of benzyl (2*S*)-tetrahydrofuran-2-carboxylate (1.15 g, 5.58 mmol), acetyl chloride (2.63 g, 33.5 mmol) and zinc chloride (3.8 mg, 0.028 mmol) was stirred under reflux overnight. The reaction mixture was concentrated under reduced pressure, diluted with Et_2O and saturated aqueous $NaHCO_3$ solution, and extracted with Et_2O. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/AcOEt = 100/0 to 85/15) to give benzyl (2*S*)-2-(acetyloxy)-5-chloropentanoate as colorless oil (1.01 g, 64%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.76–1.91 (2H, m), 1.96–2.08 (2H, m), 2.14 (3H, s), 3.53 (2H, t, J = 6.2 Hz), 5.01–5.11 (1H, m), 5.12–5.28 (2H, m), 7.28–7.49 (5H, m).

Step C. A mixture of benzyl (2*S*)-2-(acetyloxy)-5-chloropentanoate (1.01 g, 3.55 mmol) and 10% Pd on carbon (80 mg) in AcOEt was stirred at room temperature under H_2 atmosphere (1 atm, baloon) overnight. After the catalyst was removed by filtration, the filtrate was concentrated under reduced pressure to give the title compound as a colorless oil ((*S*)-**26**, 0.690 g, 100%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.87–2.11 (4H, m), 2.13–2.24 (3H, m), 3.51–3.73 (2H, m), 4.93–5.19 (1H, m), 6.40 (1H, bs).

***tert*-Butyl 4-{[6-(5-{[(2*S*)-2-(acetyloxy)-5-chloropentanoyl]amino}-2,3-dihydro-1*H*-indol-1-yl)pyrimidin-4-yl]oxy}piperidine-1-carboxylate ((*S*)-27).**

A mixture of compound (*S*)-**26** (680 mg, 3.49 mmol) and oxalyl dichloride (1.50 mL, 17.2 mmol) was stirred at room temperature for 20 h and concentrated under reduced pressure. The residual oil was added to a mixture of compound **21** (1.25 g, 3.04 mmol) in DMA at room temperature. The resulting mixture was stirred for 4 h, quenched with water, and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/AcOEt = 100/0 to 50/50) to give the title compound as a pale yellow amorphous solid (1.74 g, 85%). MS (ESI/APCI) m/z 588 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.47 (9H, s), 1.69–1.79 (2H, m), 1.85–2.02 (4H, m), 2.07–2.18 (2H, m), 2.23 (3H, s), 3.15–3.36 (4H, m), 3.57 (2H, t, J = 6.4 Hz), 3.69–3.87 (2H, m), 3.96 (2H, t, J = 8.7 Hz), 5.16–5.38 (2H, m), 5.89 (1H, s), 7.09 (1H, dd, J = 8.7, 2.3 Hz), 7.64 (1H, s), 7.75 (1H, s), 8.31 (1H, d, J = 8.7 Hz), 8.44 (1H, s).

***tert*-Butyl 4-[(6-{5-[(3*S*)-3-hydroxy-2-oxopiperidin-1-yl]-2,3-dihydro-1*H*-indol-1-yl}pyrimidin-4-yl)oxy]piperidine-1-carboxylate ((*S*)-28).**

Step A. A mixture of compound (*S*)-**27** (1.41 g, 2.40 mmol) and potassium carbonate (0.663 g, 4.80 mmol) in DMF (30 mL) was stirred at 50 °C for 8 h. After the mixture was cooled to 0 °C, 0.1 M hydrochloric acid was added. The precipitated solid was collected by filtration, washed, and dried under reduced pressure to give *tert*-butyl 4-[(6-{5-[(3*S*)-3-(acetyloxy)-2-oxopiperidin-1-yl]-2,3-dihydro-1*H*-indol-1-yl}pyrimidin-4-yl)oxy]piperidine-1-carboxylate as a white powder (1.19 g, 90%). MS (ESI/APCI) m/z 552 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.47 (9H, s), 1.96–2.12 (6H, m), 3.17–3.35 (7H, m), 3.56–3.85 (6H, m), 3.94–4.04 (2H, m), 5.23–5.46 (2H, m), 5.91 (1H, s), 7.04 (1H, dd, J = 8.5, 2.1 Hz), 7.14 (1H, s), 8.34 (1H, d, J = 8.7 Hz), 8.44 (1H, s).

Step B. A mixture of *tert*-butyl 4-[(6-{5-[(3*S*)-3-(acetyloxy)-2-oxopiperidin-1-yl]-2,3-dihydro-1*H*-indol-1-yl}pyrimidin-4-yl)oxy]piperidine-1-carboxylate (16.3 g, 29.6 mmol), potassium carbonate (8.17 g, 59.1 mmol), THF (100 mL) and MeOH (100 mL) was stirred at room temperature for 3 h. After cooling to 0 °C, 0.45 M hydrochloric acid (250 mL) was added to the reaction mixture followed by stirring at room temperature for 1 h. The precipitated solid was collected by filtration and washed with water to give a pale yellow solid. The solid was dissolved in THF and dried over Na_2SO_4. The solvent was removed by evaporation to give the title compound as a pale yellow solid ((*S*)-**28**, 15.0 g, 100%). MS (ESI/APCI) m/z 510 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.41 (9H, s), 1.48–1.63 (2H, m), 1.65–2.15 (6H, m), 3.10–3.24 (4H, m), 3.46–3.77 (4H, m), 3.95–4.09 (3H, m), 5.15 (1H, d, J = 3.8 Hz), 5.20–5.31 (1H, m), 6.09 (1H, s), 7.05 (1H, dd, J = 8.7, 2.3 Hz), 7.13 (1H, d, J = 1.9 Hz), 8.34 (1H, d, J = 8.7 Hz), 8.46 (1H, s).

(3*S*)-3-Hydroxy-1-{1-[6-({1-[3-(propan-2-yl)-1,2,4-oxadiazol-5-yl]piperidin-4-yl}oxy)pyrimidin-4-yl]-2,3-dihydro-1*H*-indol-5-yl}piperidin-2-one ((*S*)-29).

Compound (*S*)-**29** was prepared from (*S*)-**28** in a manner similar to that described for compound **24b**. White solid. Yield 49% over 3 steps. Retention time 13.6 min (CHIRALCEL OD3 (4.6 mmID × 250 mmL), eluting with MeOH at 1 mL/min flow rate). 99.9%ee. $[\alpha]_D^{25}$ − 16.2 (c 1.027, $CHCl_3$). MS (ESI/APCI) m/z 520 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.19 (6H, d, J = 6.8 Hz), 1.67–2.01 (5H, m), 2.03–2.16 (3H, m), 2.74–2.91 (1H, m), 3.14–3.25 (2H, m), 3.43–3.68 (4H, m), 3.76–3.90 (2H, m), 3.96–4.10 (3H, m), 5.16 (1H, d, J = 3.8 Hz), 5.26–5.39 (1H, m), 6.12 (1H, s), 7.05 (1H, d, J = 8.7 Hz), 7.14 (1H, s), 8.34 (1H, d, J = 8.7 Hz), 8.47 (1H, s). Anal. Calcd for $C_{27}H_{33}N_7O_4$: C, 62.41; H, 6.40; N, 18.87. Found: C, 62.41; H, 6.55; N, 18.79.

***tert*-Butyl 4-({6-[5-(4-hydroxy-2-oxopiperidin-1-yl)-2,3-dihydro-1*H*-indol-1-yl]pyrimidin-4-yl}oxy)piperidine-1-carboxylate ((*RS*)-31a).**

A mixture of compound **10** (240 mg, 0.505 mmol), (*RS*)-4-hydroxypiperidin-2-one (174 mg, 1.51 mmol), *trans*-*N*,*N*′-dimethylcyclohexane-1,2-diamine (57.4 mg, 0.404 mmol), copper(I) iodide (38.5 mg, 0.202 mmol) and potassium carbonate (349 mg, 2.52 mmol) in toluene (10 mL) was stirred at 120 °C for 2 days. The reaction was quenched with water and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (AcOEt/MeOH = 100/0 to 90/10) gave the title compound as a white solid (27.0 mg, 10%). MS (ESI/APCI) m/z 510 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.47 (9H, s), 1.84–1.87 (2H, m), 1.95–2.00 (2H, m), 2.09–2.23 (2H, m), 2.53–2.64 (1H, m), 2.80–2.92 (1H, m), 3.19–3.34 (4H, m), 3.51–3.61 (1H, m), 3.71–3.87 (4H, m), 3.99 (2H, t, J = 8.7 Hz), 4.29–4.42 (1H, m), 5.24–5.36 (1H, m), 5.92 (1H, s), 7.00–7.14 (2H, m), 8.35 (1H, d, J = 8.7 Hz), 8.45 (1H, s).

***tert*-Butyl 4-({6-[5-(5-hydroxy-2-oxopiperidin-1-yl)-2,3-dihydro-1*H*-indol-1-yl]pyrimidin-4-yl}oxy)piperidine-1-carboxylate ((*RS*)-31b).**

Compound (*RS*)-**31b** was prepared from compound **10** and (*RS*)-5-hydroxypiperidin-2-one in a manner similar to that described for compound (*RS*)-**31a**. White solid. Yield 4%. MS (ESI/APCI) m/z 510 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.47 (9H, s), 1.66–1.81 (3H, m), 1.92–2.22 (4H, m), 2.48–2.64 (1H, m), 2.70–2.84 (1H, m), 3.18–3.35 (4H, m), 3.51–3.64 (1H, m), 3.73–3.85 (3H, m), 3.98 (2H, t, J = 8.7 Hz), 4.26–4.36 (1H, m), 5.22–5.34 (1H, m), 5.91 (1H, s), 7.04 (1H, dd, J = 8.7, 2.3 Hz), 7.11 (1H, s), 8.36 (1H, d, J = 8.7 Hz), 8.45 (1H, s).

4-Hydroxy-1-{1-[6-({1-[3-(propan-2-yl)-1,2,4-oxadiazol-5-yl]piperidin-4-yl}oxy)pyrimidin-4-yl]-2,3-dihydro-1*H*-indol-5-yl}

piperidin-2-one ((*RS*)-32a).

Compound (*RS*)-**32a** was prepared from (*RS*)-**31a** in a manner similar to that described for compound **24b**. Pale yellow solid. Yield 58% over 3 steps. MS (ESI/APCI) *m/z* 520 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (6H, d, $J = 6.8$ Hz), 1.82–1.97 (2H, m), 1.98–2.09 (2H, m), 2.08–2.24 (2H, m), 2.52–2.65 (1H, m), 2.81–2.87 (2H, m), 3.24 (2H, t, $J = 8.5$ Hz), 3.52–3.66 (3H, m), 3.77–4.05 (6H, m), 4.32–4.39 (1H, m), 5.32–5.43 (1H, m), 5.93 (1H, s), 7.05 (1H, dd, $J = 8.5$, 2.1 Hz), 7.11 (1H, s), 8.36(1H, d, $J = 8.3$ Hz), 8.45 (1H, s). Anal. Calcd for $C_{27}H_{33}N_7O_4$: C, 62.41; H, 6.40; N, 18.87. Found: C, 62.31; H, 6.29; N, 18.72.

5-Hydroxy-1-{1-[6-({1-[3-(propan-2-yl)-1,2,4-oxadiazol-5-yl] piperidin-4-yl}oxy)pyrimidin-4-yl] -2,3-dihydro-1*H*-indol-5-yl} piperidin-2-one ((*RS*)-32b).

Compound (*RS*)-**32b** was prepared from (*RS*)-**31b** in a manner similar to that described for compound **24b**. White solid. Yield 36% over 3 steps. MS (ESI/APCI) *m/z* 520 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (6H, d, $J = 6.8$ Hz), 1.86–1.97 (2H, m), 2.00–2.20 (5H, m), 2.47–2.61 (1H, m), 2.67–2.95 (2H, m), 3.15–3.30 (2H, m), 3.52–3.62 (3H, m), 3.75–3.82 (1H, m), 3.83–3.93 (2H, m), 3.98 (2H, t, $J = 8.7$ Hz), 4.25–4.37 (1H, m), 5.29–5.45 (1H, m), 5.93 (1H, s), 7.05 (1H, d, $J = 8.7$ Hz), 7.12 (1H, s), 8.36 (1H, d, $J = 8.3$ Hz), 8.45 (1H, s). Anal. Calcd for $C_{27}H_{33}N_7O_4$: C, 62.41; H, 6.40; N, 18.87. Found: C, 62.26; H, 6.43; N, 18.59.

***tert*-Butyl 4-({6-[5-(2-oxocyclohexyl)-2,3-dihydro-1*H*-indol-1-yl] pyrimidin-4-yl}oxy)piperidine-1-carboxylate ((*RS*)–33).**

To a solution of compound **10** (1.00 g, 2.10 mmol) and cyclohexanone (0.436 mL, 4.21 mmol) in DME (10 mL) were added $Pd_2(dba)_3$ (0.096 g, 0.11 mmol), Xantphos (0.122 g, 0.211 mmol), and cesium carbonate (2.06 g, 6.31 mmol) at room temperature. The mixture was stirred at 80 °C under N_2 atmosphere overnight. The reaction mixture was diluted with water, and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/AcOEt = 85/15 to 50/50) to give a yellow solid. The obtained solid was crystallized from AcOEt–hexane to give the title compound as a white solid (0.244 g, 24%). MS (ESI/APCI) *m/z* 493 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.41 (9H, s), 1.48–1.62 (2H, m), 1.68–2.00 (6H, m), 2.02–2.15 (2H, m), 2.25–2.33 (1H, m), 2.45–2.58 (1H, m), 3.10–3.22 (4H, m), 3.65–3.76 (3H, m), 3.96 (2H, t, $J = 8.7$ Hz), 5.18–5.29 (1H, m), 6.06 (1H, s), 6.90 (1H, dd, $J = 8.3$, 1.5 Hz), 6.99 (1H, s), 8.25 (1H, d, $J = 8.3$ Hz), 8.44 (1H, d, $J = 0.8$ Hz).

2-{1-[6-({1-[3-(Propan-2-yl)-1,2,4-oxadiazol-5-yl] piperidin-4-yl}oxy)pyrimidin-4-yl] -2,3-dihydro-1*H*-indol-5-yl}cyclohexanone ((*RS*)-34).

Compound (*RS*)-**34** was prepared from (*RS*)–**33** in a manner similar to that described for compound **24b**. Pale yellow solid. Yield 9% over 3 steps. MS (ESI/APCI) *m/z* 503 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.19 (6H, d, $J = 7.2$ Hz), 1.68–1.97 (6H, m), 2.02–2.14 (4H, m), 2.25–2.33 (1H, m), 2.44–2.59 (1H, m), 2.77–2.87 (1H, m), 3.17 (2H, t, $J = 8.7$ Hz), 3.44–3.55 (2H, m), 3.69 (1H, dd, $J = 12.3$, 5.5 Hz), 3.77–3.87 (2H, m), 3.97 (2H, t, $J = 8.5$ Hz), 5.26–5.37 (1H, m), 6.09 (1H, s), 6.91 (1H, dd, $J = 8.3$, 1.5 Hz), 6.99 (1H, s), 8.25 (1H, d, $J = 8.3$ Hz), 8.46 (1H, d, $J = 0.8$ Hz). Anal. Calcd for $C_{28}H_{34}N_6O_3$: C, 66.91; H, 6.82; N, 16.72. Found: C, 66.75; H, 6.57; N, 16.44.

5.2. 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-amine[26]). EC_{50} values were obtained with XLfit software (ID Business Solutions).

5.3. 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 (In vitrogen). 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 (Cat. #14040, In vitrogen). 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 (Sigma-Aldrich), 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.

$$\%hERGinhibition = 100 - (posthERGcurrent/prehERGcurrent) \times 100$$

5.4. Estimation of LogD at pH 7.4

LogD7.4, 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.[20,21]

5.5. 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.

5.6. Single-crystal X-ray structure analysis

Crystal data for compound **24b**: C27H33N7O3 *MW* = 503.60; crystal size, 0.32 × 0.18 × 0.15 mm; colorless, block; triclinic, space group *P*-1, $a = 5.50960(10)$ Å, $b = 12.9242(2)$ Å, $c = 18.9707(3)$ Å, $\alpha = 89.9541(9)°$, $\beta = 87.6185(9)°$, $\gamma = 79.8701(9)°$, $V = 1328.61(4)$ Å3, $Z = 2$, $Dx = 1.259$ g/cm3, $T = 298$ K, $\mu = 0.688$ mm-1, $\lambda = 1.54184$ Å, $R1 = 0.0902$, $wR2 = 0.2665$, $S = 1.059$.

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.[27,28]

CCDC 2,046,043 for compound **24b** 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.

5.7. 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 KH2PO4–K2HPO4 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 MgCl2, 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.

5.8. 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 KH2PO4, 1.17 mM MgSO4· 7H2O, 25 mM NaHCO3, 2.52 mM CaCl2· 2H2O, 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 declared that there is no conflict of interest.

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