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Synthesis and evaluation of novel fused pyrimidine derivatives as GPR119 agonists

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ABSTRACT

A novel series of fused pyrimidine derivatives were designed, synthesized and evaluated as GPR119 agonists. Among them, cyclohexene fused compounds (tetrahydroquinazolines) showed greater GPR119 agonistic activities than did dihydrocyclopentapyrimidine and tetrahydropyridopyrimidine scaffolds. Analogues (**16**, **19**, **26**, **28**, **42**) bearing *endo-N*-Boc-nortropane amine and fluoro-substituted aniline exhibited better EC_{50} values (0.27–1.2 μM) though they appeared to be partial agonists.

1. Introduction

GPR119 is a class A type of G Protein coupled receptor, which is expressed primarily in pancreatic β-cells and the K and L cells of the gastrointestinal tract [1–3]. Activation of GPR119 promotes secretion of incretins such as glucagon-like peptide-1 (GLP-1) in the intestinal tract and glucose dependent release of insulin in pancreatic β-cells [4–8]. The dual mechanism makes GPR119 a promising target for discovery of anti-diabetic agents with low risk of hypoglycemia.

Arena researchers disclosed the first potent and oral small molecule **AR231453** as GPR119 agonist [9]. Following this discovery, numerous synthetic GPR119 agonists have been subsequently reported, some of which have advanced into clinical trials (Fig. 1) [10,11].

We have previously investigated **AR231453** from among the reported agonists, choosing it as the lead compound. A series of 5-nitropyrimidine derivatives bearing azabicyclic alcohols/amines were synthesized and evaluated for their GPR119 agonistic activities [12–14]. Although these 5-nitropyrimidine compounds displayed potent biological activities, nitro-compound always caused hepatotoxicity [15,16]. In our attempts to optimize the core skeleton, we have designed various fused pyridine moiety to replace 5-nitropyrimidine ring, using the strategy of scaffold hopping. In addition to heterocycle fused pyrimidine, we also studied the effect of lipophilic, cycloolefin-fused pyrimidine on the agonistic activity. We herein reported our efforts to expand the SAR study of GPR119 agonists with a series of novel fused pyrimidine derivatives, evaluating them for their human GPR119 activities. We will focus on the various core to see whether or not the pharmacophores are beneficial for bioactivity. The azabicyclic fragments and substituted anilines were retained (Fig. 2). Two conformations (*endo* and *exo*) of azabicyclic amines were explored to study the bioactivity relationship of the new core with *endo/exo* azabicyclic ring.

2. Results and discussion

2.1. Chemistry

4-Amino-3-fluorobenzonitrile, 2-fluoro-4-(methylsulfonyl)aniline and azabicyclic intermediate **2**, were generated according to ~~the~~ reported procedures [17–21]. The *exo* isomer **3** was synthesized in accordance with Scheme 1. Mesylation of *endo-N*-Boc-nortropine **4** with mesyl chloride (MsCl) furnished the mesylate **5** with good yield (95%), which was treated with sodium azide (NaN_3) to afford *exo*-azido compound **6** in 92% yield [22]. Reduction of **6** using 10% palladium(II) hydroxide ($Pd(OH)_2$) on charcoal under hydrogen generated *exo*-azabicyclic amine **3** in 90% yield [23].

The general synthetic procedures of dihydrocyclopentapyrimidine derivatives are outlined in Scheme 2. Commercially available methyl 2-oxocyclopentane-1-carboxylate was reacted with urea and sodium methoxide in ethanol to afford cyclic compound **7** in 88% yield, which was chlorinated by $POCl_3$ at reflux conditions to give **8** in 82% yield.

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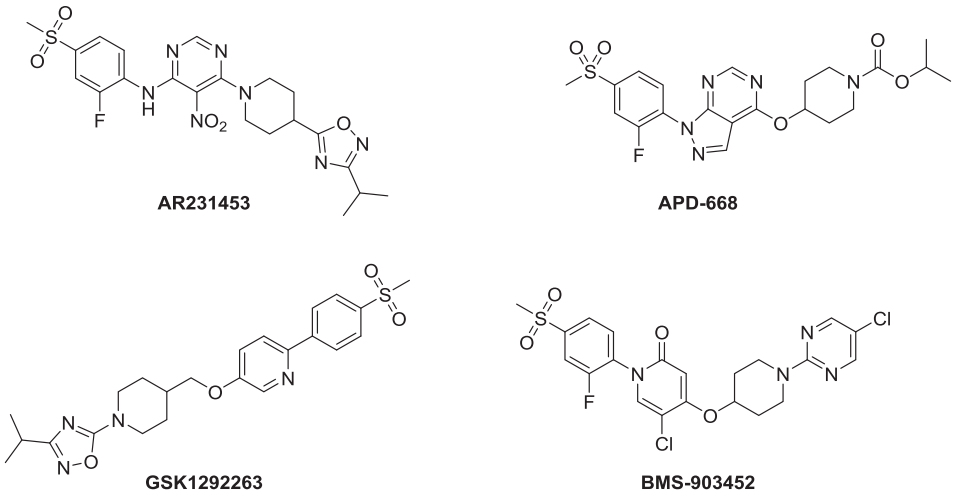


Fig. 1. The structures of GPR119 agonists.

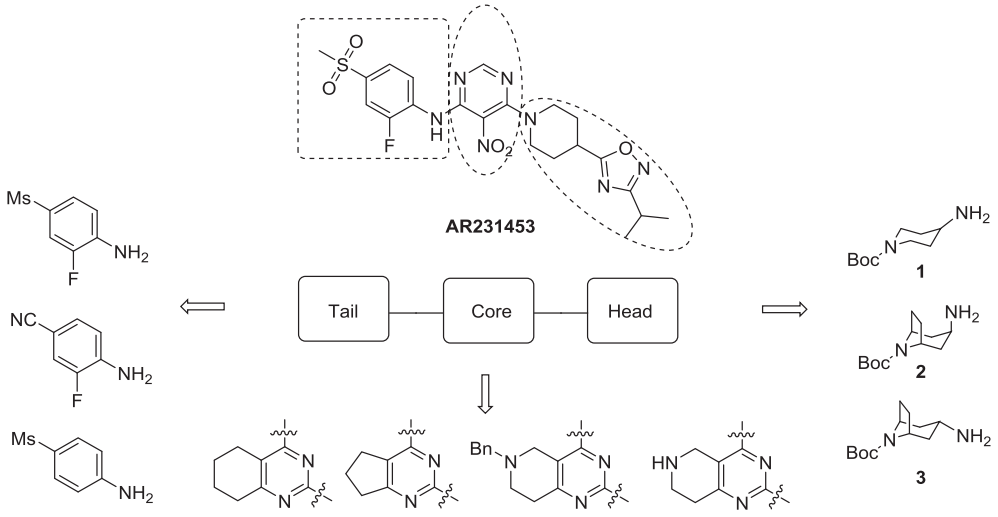
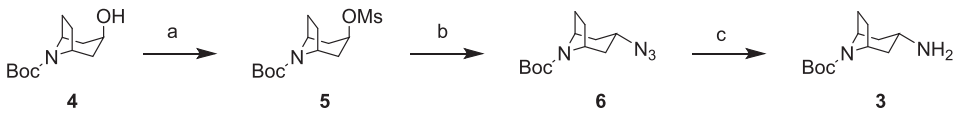
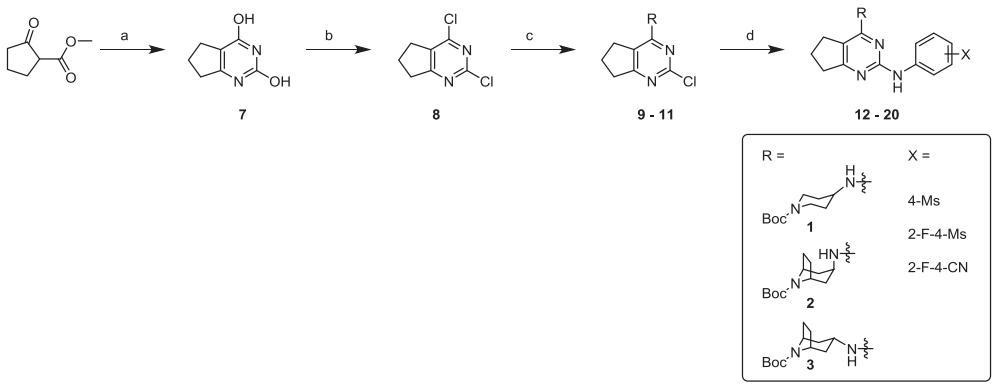


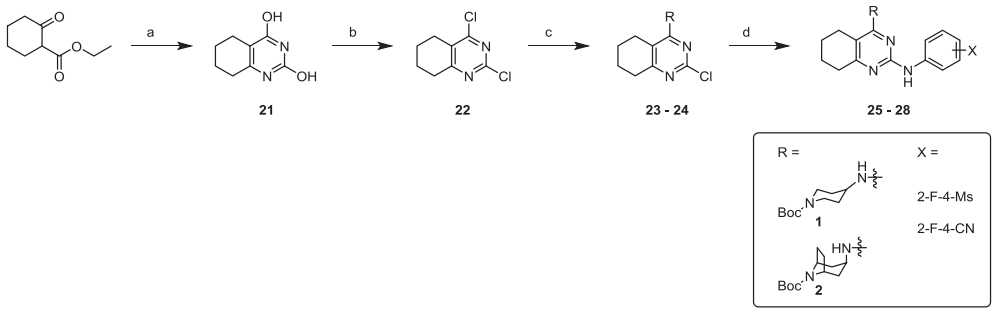
Fig. 2. The design of GPR119 agonists.



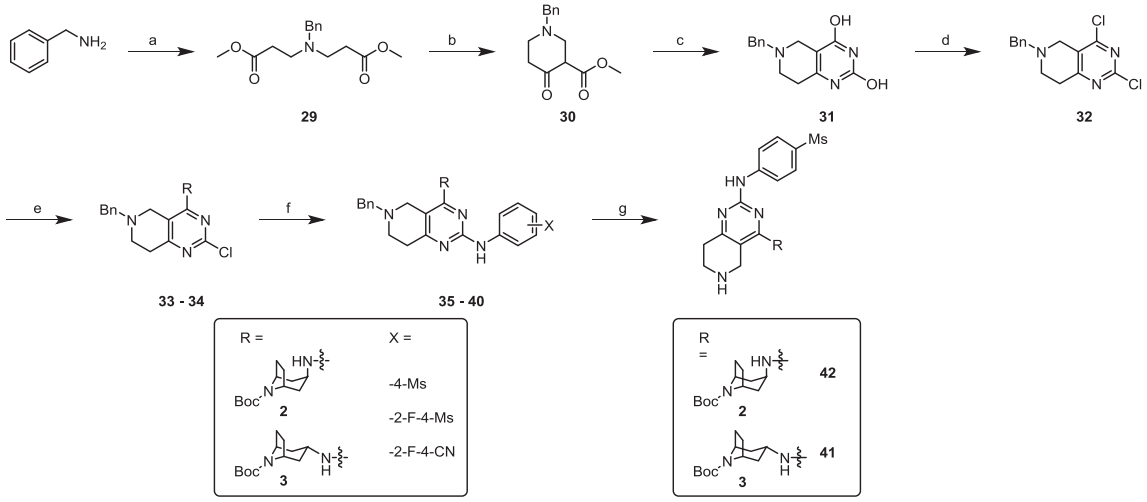
Scheme 1. Reagents and conditions: (a) MsCl, TEA, CH_2Cl_2, 0 °C- rt, 5 h; (b) NaN_3, MeCN, H_2O, reflux, overnight; (c) 10% $Pd(OH)_2/C$, H_2, MeOH, 40 °C, overnight.



Scheme 2. Reagents and conditions: (a) urea, MeONa, EtOH, 60 °C, overnight; (b) $POCl_3$, reflux, 7 h; (c) **1–3**, DIPEA, r.t., overnight; (d) substituted anilines, $Pd_2(dba)_3$, X-Phos, Cs_2CO_3, 1,4-Dioxane, reflux, overnight.



Scheme 3. Reagents and conditions: (a) urea, MeONa, EtOH, 60 °C, overnight; (b) $POCl_3$, reflux, 7 h; (c) aliphatic amine **1** or **2**, K_2CO_3, DMF, 80 °C, 8 h; (d) substituted anilines, $Pd_2(dba)_3$, X-Phos, Cs_2CO_3, 1,4-Dioxane, reflux, overnight.



Scheme 4. Reagents and conditions: (a) methyl acrylate, MeOH, r.t., 6 h. (b) MeONa, toluene, 85 °C, 3.5 h; (c) urea, MeONa, EtOH, 60 °C, overnight; (d) $POCl_3$, reflux, 7 h; (e) aliphatic amine **2** or **3**, DIPEA, r.t., overnight; (f) substituted anilines, $Pd_2(dba)_3$, X-Phos, Cs_2CO_3, 1,4-Dioxane, reflux, overnight; (g) 10% $Pd(OH)_2/C$, H_2, EtOH, 60 °C.

Dichloride **8** was reacted with aliphatic amines under basic conditions to afford intermediates **9–11** in 85%-90% yield. Buchwald-Hartwig coupling of **9–11** with substituted anilines, $Pd_2(dba)_3$, X-Phos and Cs_2CO_3 resulted in final compounds **12–20** in 52–86%.

The general synthetic procedures of tetrahydroquinazoline derivatives **25–28** are outlined in Scheme 3 following the similar synthetic methods as the cyclopentapyrimidine compounds.

The general synthetic procedures of tetrahydropyridopyrimidine derivatives **35–40** were generated as shown in Scheme 4, following similar synthetic methods as for the cyclopentapyrimidine compounds. After synthesizing the benzyl substituted compounds **35–40**, we attempted debenzylation, but only derivatives **41–42**, without fluoro-substituted, were obtained, even though we attempted several debenzylation conditions.

2.2. Biological activity

Target compounds **12–20**, **25–28**, **37–42** were evaluated for their abilities to activate the human GPR119 receptor in a cell-based cAMP assay, which were expressed in EC_{50} and %max values. Endogenous GPR119 agonist oleoylethanolamide (OEA) was selected as the positive control. The EC_{50} values represent the concentration of the tested compounds for 50% cAMP stimulation, while the %max values present the relative response (%) of the tested compounds compared to the maximal effect of OEA.

As shown in Table 1, dihydrocyclopentapyrimidine analogues (**14**, **17** and **20**) possessing *exo*-azabicyclic amine were identified as very weak GPR119 agonists (EC_{50} > 10 μM) and their agonistic activities were much less potent than reference. Replacement of *exo*-azabicyclic amine with *endo*-azabicyclic amine improved the GPR119 activation activities significantly (**13**, **16** and **19**). But introduction of flexible *N*-Boc-4-piperidine-amine group did not affect the biological activities appreciatively. We also examined the structural features of the tail part in compounds **12–20**. The compounds bearing (2-fluoro-4-methylsulfonyl)phenylamino group or (2-fluoro-4-cyano)phenylamino group exhibited greater potency than compounds without a fluorine atom in aromatic fragment. Additionally, analogues (**16** and **19**) containing the *endo*-bicyclic moiety and the fluoro-substituted phenylamino group showed good to moderate potency and moderate agonistic activities (EC_{50} 0.44 and 0.77 μM, respectively) with rational Clog*p* values (4.7 and 5.1 μM).

Next, we turned our attention to tetrahydroquinazoline fragment as core part while keeping the *endo*-azabicyclic amine and fluoro-substituted aniline. As shown in Table 1, superior GPR119 agonistic activity was observed for tetrahydroquinazoline derivatives compared with cyclopentene fused analogues. Among them, compounds **26** and **28** displayed greater EC_{50} values than OEA (0.56 and 0.27 μM, respectively) but moderate level %max values. These results suggest that compound with *endo*-conformation is good for bioactivity. However, analogues **26** and **28** each showed high lipophilicity (Clog*p*: 5.5 and 5.2), often causing pharmacokinetic issues.

To conduct ~~the~~ further SAR studies of the GPR119 agonistic activity, tetrahydropyridopyrimidine derivatives were also evaluated by the human GPR119 receptor activities in a cell-based cAMP assay. As shown in Table 2, the results are similar to the previous work, and this series of compounds with *endo*-moiety showed the moderate potency. Meanwhile, debenzylated **42** exhibited improved middle-level agonistic activity (EC_{50} = 1.2 μM) and significantly low lipophilicity (Clog*p*: 3.2).

Table 1
In vitro hGPR119 agonistic activities of **12–20** and **25–28**.

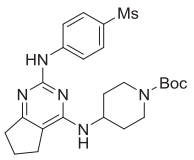
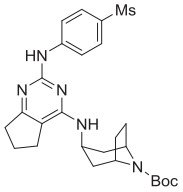
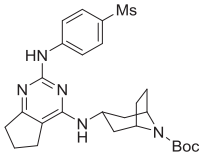
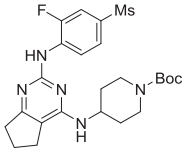
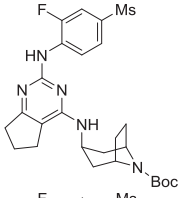
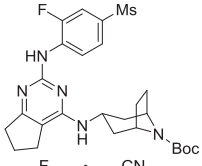
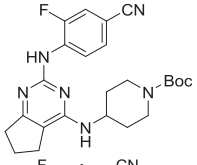
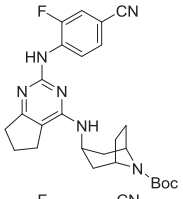
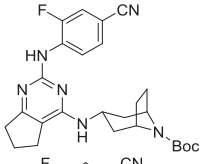
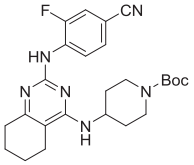
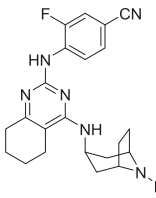
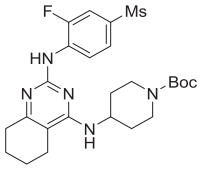
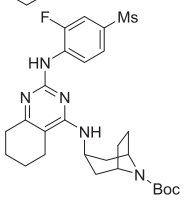
Compound	Structure	hGPR119 activity		ClogP[b]
		EC_{50} (μM)	%max[a]	
12		> 10	35.6	4.0
13		1.8	44.3	4.5
14		> 10	28.7	4.5
15		2.7	42.9	4.2
16		0.44	65.8	4.7
17		> 10	40.2	4.7
18		3.5	46.3	4.6
19		0.77	59.7	5.1
20		> 10	32.1	5.1
25		2.9	50.4	4.7

Table 1 (*continued*)

Compound	Structure	hGPR119 activity		ClogP[b]
		EC_{50} (μM)	%max[a]	
26		0.56	67.2	5.5
27		2.1	54.8	5.0
28		0.27	71.5	5.2
OEA		2.2	100	

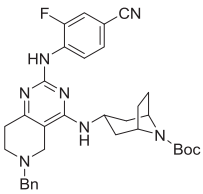
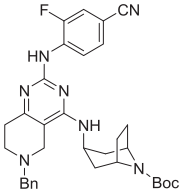
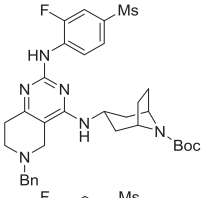
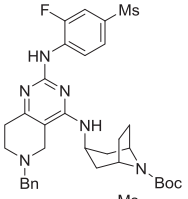
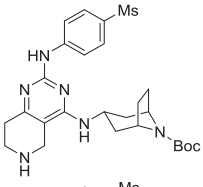
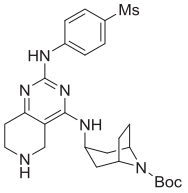
[a] %max: cAMP stimulation % compared to maximal effect of **OEA**.
[b] *ClogP* was calculated using ACD software from Discovery Studio 4.5.

We also evaluate the oral glucose tolerance test (oGTT) of **28** in C57BL/6N mice. For the acute single dose study, vehicle (0.5% carboxymethylcellulose sodium, 10 mL/kg) and **28** (5 and 15 mg/kg) were administered to C57BL/6N mice after 16-hr starvation period, then the oral glucose tolerance test (3 g/kg) was conducted 4 hr after of the single dose; the blood glucose level at 0, 15, 30, 60, 90 and 120 min were recorded by area under curve calculation ($AUC_{0\text{-}2h}$). As outlined in Fig. 3, compound **28** demonstrates a dose-dependent effect but only weakly reduces the area under curve from 0 to 120 min by 2.6% (23.47 ± 3.20) and 5.3% (22.82 ± 2.36) at the dose of 5 mg/kg and 15 mg/kg, respectively (Vehicle: 24.09 ± 2.34).

3. Conclusion

In summary, we designed, synthesized and evaluated a novel series of GPR119 agonists bearing dihydrocyclopentapyrimidine, tetrahydroquinazoline or tetrahydropyridopyrimidine as the core to replace the 5-nitropyrimidine scaffold. As a result, most compounds exhibited stronger EC_{50} values than that of OEA. Compounds containing *endo*-azabicyclic fragment and (2-fluoro-4-methylsulfonyl)phenylamino group or (2-fluoro-4-cyano)phenylamino group exhibited better EC_{50} values even though they appeared to be partial agonists. And tetrahydroquinazoline derivatives showed better GPR119 agonistic activities than did dihydrocyclopentapyrimidine and tetrahydropyridopyrimidine derivatives. But all these compounds displayed weaker potency than 5-nitropyrimidine derivatives. Furthermore, tetrahydroquinazoline **28** with *endo-N*-Boc-nortropane amine and (2-fluoro-4-methylsulfonyl)aniline displayed a good EC_{50} value (0.27 μM) and moderate GPR119 agonistic activity (71.5%). Subsequently, compound **28** displayed the dose-dependent effect in oGTT of C57BL/6N mice but a weak glucose-lowering effect. Further SAR study about various fused pyrimidine compounds is ongoing, and will be reported in due course.

Table 2
In vitro hGPR119 agonistic activities of **37–42**.

Compound	Structure	hGPR119 activity		ClogP[b]
		EC_{50} (μM)	%max[a]	
37		> 10	24.3	5.9
38		3.1	44.6	5.9
39		> 10	27.5	5.5
40		2.0	48.5	5.5
41		> 10	33.4	3.2
42		1.2	52.1	3.2
OEA		2.2	100	

[a] %max: cAMP stimulation % compared to maximal effect of **OEA**.
[b] *ClogP* was calculated using ACD software from Discovery Studio 4.5.

4. Experimental

4.1. Chemistry

All starting materials were obtained from commercial suppliers and used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE III HD 600 (600 Hz) spectrometer. Chemical shifts are reported in parts per million (ppm) downfield relative to tetramethylsilane as an internal standard. Peak splitting patterns are abbreviated as s (singlet), br s (broad singlet), d (doublet), t (triplet), dd (doublet of doublet), ddd (doublet of doublet of doublet) and m (multiplet). Mass spectra were recorded on a Thermo Fisher (LCQ Fleet). HR-MS spectra were recorded on a AB SCIEX (Triple TOF 5600 +). TLC was performed on silica F254 purchased from Branch of Qingdao Haiyang Chemical Co. and detected by UV light at 254, 365 nm or by charring with sulphuric acid. Column chromatography was performed on silica gel column (200–300 mesh, Branch of Qingdao Haiyang Chemical Co.).

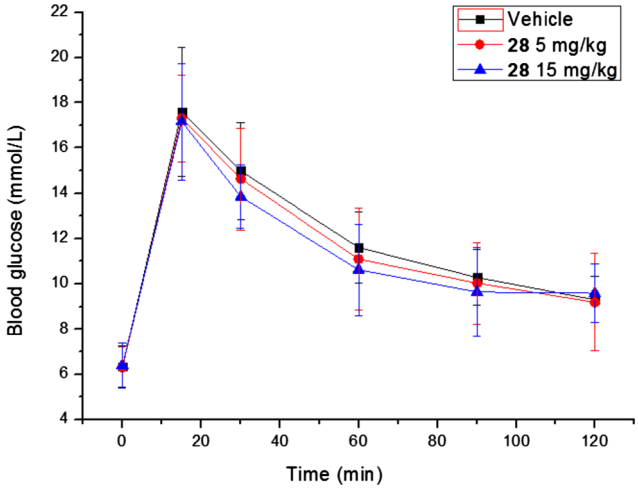


Fig. 3. Single dose of compound **28** on oGTT in C57BL/6N mice (n = 8).

4.1.1. 6, 7-dihydro-5H-cyclopenta[d]pyrimidine-2,4-diol (7)

To the solution of methyl 2-oxocyclopentane-1-carboxylate (1 g, 7.04 mmol) in ethanol (15 mL) was added urea (1.01 g, 8.45 mmol). and sodium methoxide (0.76 g, 14.07 mmol) at 0 °C under nitrogen. The reaction was stirred at 60 °C for overnight. After the reaction completed, the solution was removed under reduced pressure. The residual yellow solid was dissolved in acetic acid (0.8 mL) and H_2O (2 mL). Then the residue was filtered to get yellow solid (0.52 g, 48% yield). ^{1}H NMR (600 MHz, DMSO) δ (ppm): 9.42 (s, 1H), 6.75 (s, 1H), 3.03 (t, J = 7.7 Hz, 2H), 2.42–2.35 (m, 2H), 1.80–1.71 (m, 2H). MS-ESI: $[M-H]^-$: 151.1.

4.1.2. 2, 4-dichloro-6, 7-dihydro-5H-cyclopenta[d]pyrimidine (8)

Compounds **7** (0.5 g, 3.29 mmol) was dissolved in $POCl_3$ (3 mL) at room temperature, then the reaction was heated to reflux for 7 h. The mixture was poured into ice water and adjusted to PH value to 7 with $NaHCO_3$. The mixture was filtered to obtain the yellow product. (0.45 g, 73% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 3.13–3.09 (m, 2H), 3.01 (m, J = 7.6 Hz, 2H), 2.24 (m, J = 15.5, 7.8 Hz, 2H). MS-ESI: $[M-H]^-$:187.1.

4.1.3. General procedure of 2-chloro-6, 7-dihydro-5H-cyclopenta[d] pyrimidin-4-amine (9–11)

To the solution of compound **8** (0.23 g, 1.2 mmol) in THF (5 mL) was added DIPEA (1.8 mmol) and bicyclic amine or piperidin amine (1.44 mmol). The reaction was stirred at r.t. for overnight. Then the mixture was diluted with ethyl acetate, washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by column chromatography to give the product.

4.1.4. tert-butyl 4-((2-chloro-6, 7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)amino)piperidine-1-carboxylate (9)

Yellow solid, 87% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ (pmm): 4.35 (d, J = 7.6 Hz, 1H), 4.27–4.01 (m, 3H), 3.02–2.83 (m, 4H), 2.60 (t, J = 7.4 Hz, 2H), 2.20–2.09 (m, 2H), 2.08–1.98 (m, 2H), 1.46 (s, 9H), 1.33(m, 2H). MS-ESI: $[M-H]^-$:351.2.

4.1.5. tert-butyl (endo)-3-((2-chloro-6, 7-dihydro-5H-cyclopenta[d] pyrimidin-4-yl)amino)-8-azabicyclo[3.2.1]octane-8-carboxylate (10)

Yellow solid, 85% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ (pmm): 4.91

(d, J = 6.1 Hz, 1H), 4.40 (m, 1H), 4.35–4.17 (m, 2H), 2.90 (t, J = 7.8 Hz, 2H), 2.64 (t, J = 7.4 Hz, 2H), 2.30 (m, 1H), 2.21–2.09 (m, 4H), 1.94–1.68 (m, 4H), 1.50 (s, 9H). MS-ESI: $[M-H]^-$:377.2.

4.1.6. tert-butyl (exo)-3-((2-chloro-6, 7-dihydro-5H-cyclopenta[d] pyrimidin-4-yl)amino)-8-azabicyclo[3.2.1]octane-8-carboxylate (11)

Yellow solid, 90% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ (pmm): 4.63–4.49 (m, 1H), 4.31 (m, 2H), 4.25–4.17 (m, 1H), 2.84 (t, J = 7.8 Hz, 2H), 2.58 (t, J = 7.3 Hz, 2H), 2.18–1.93 (m, 6H), 1.93–1.72 (m, 4H), 1.47 (s, 9H). MS-ESI: $[M-H]^-$:377.6.

4.1.7. 6, 7-Dihydro-5H-cyclopenta[d]pyrimidine-2, 4-diamine (12–20)

To the solution of chloro-6, 7-dihydro-5H-cyclopenta[*d*]pyrimidin-4-amine (0.22 mmol) and substituted anilines (0.22 mmol) in 1,4-dioxane (2 mL) was added $Pd_2(dba)_3$ (0.05 mmol), X-Phos (0.05 mmol) and Cs_2CO_3 (0.55 mmol). The reaction was heated to reflux under nitrogen gas for overnight. Then the mixture was diluted with ethyl acetate, washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by column chromatography to give the product.

4.1.8. tert-butyl 4-((2-((4-(methylsulfonyl)phenyl)amino)-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)amino)piperidine-1-carboxylate (12)

Light yellow solid, 60% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.78 (m, 4H), 7.67 (s, 1H), 4.29 (d, J = 7.5 Hz, 1H), 4.17–4.11 (m, 3H), 3.00 (s, 3H), 2.90 (m, 2H), 2.78 (t, J = 7.4 Hz, 2H), 2.57 (t, J = 6.9, 2H), 2.10–2.04 (m, 4H), 1.44 (s, 9H), 1.39 (m, 2H). HRMS-TOF (*m/z*) calcd for $C_{24}H_{33}N_5O_4S$ $[M-H]^-$: 486.2175, found 486.1920. HPLC purity, 97.4%.

4.1.9. tert-butyl (endo)-3-((2-((4-(methylsulfonyl)phenyl)amino)-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)amino)-8-azabicyclo[3.2.1] octane-8-carboxylate (13)

Light yellow solid, 74% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.07 (s, 1H), 7.85 (s, 4H), 4.84 (d, J = 6.5 Hz, 1H), 4.38 (m, 1H), 4.34–4.23 (m, 2H), 3.06 (s, 3H), 2.85 (t, J = 7.7 Hz, 2H), 2.64 (t, J = 7.0 Hz, 2H), 2.40–2.28 (m, 1H), 2.17 (q, J = 7.7 Hz, 2H), 2.13 (m, 3H), 1.91–1.89 (m, 4H), 1.52 (s, 9H). HRMS-TOF (*m/z*) calcd for $C_{26}H_{35}N_5O_4S$ $[M-H]^-$: 512.2332, found 512.2180. HPLC purity, 97.9%.

4.1.10. tert-butyl (exo)-3-((2-((4-(methylsulfonyl)phenyl)amino)-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)amino)-8-azabicyclo[3.2.1] octane-8-carboxylate (14)

Light yellow solid, 68% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.69 (s, 1H), 7.84 (m, 2H), 7.76 (m, 2H), 4.61 (m, 1H), 4.24 (m, 3H), 3.00 (s, 3H), 2.76 (t, J = 7.6 Hz, 2H), 2.54 (t, J = 7.0 Hz, 2H), 2.09–2.00 (m, 8H), 1.81 (m, 2H), 1.44 (s, 9H). HRMS-TOF (*m/z*) calcd for $C_{26}H_{35}N_5O_4S$ $[M-H]^-$: 512.2332, found 512.2176. HPLC purity, 98.3%.

4.1.11. tert-butyl 4-((2-((2-fluoro-4-(methylsulfonyl)phenyl)amino)-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)amino)piperidine-1-carboxylate (15)

Yellow solid, 60% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.84 (t, J = 8.2 Hz, 1H), 7.64–7.58 (m, 2H), 7.42 (m, 1H), 4.25 (d, J = 6.8 Hz, 1H), 4.11 (m, 3H), 3.01 (s, 3H), 2.90 (m, 2H), 2.80 (t, J = 7.9 Hz, 2H), 2.58 (t, J = 6.8 Hz, 2H), 2.11–2.00 (m, 4H), 1.44 (s, 9H), 1.37 (m, 2H). HRMS-TOF (*m/z*) calcd for $C_{24}H_{32}FN_5O_4S$ $[M-H]^-$: 504.2081, found 504.1903. HPLC purity, 99.4%.

4.1.12. tert-butyl (endo)-3-((2-((2-fluoro-4-(methylsulfonyl)phenyl) amino)-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)amino)-8-azabicyclo [3.2.1]octane-8-carboxylate (16)

Light yellow solid, 81% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.80 (t, J = 8.0 Hz, 1H), 7.64 (d, J = 8.8 Hz, 1H), 7.57 (m, 1H), 7.42 (m, 1H), 4.74 (d, J = 6.4 Hz, 1H), 4.29 (m, 1H), 4.23–4.15 (m, 2H), 3.00 (s, 3H), 2.79 (t, J = 7.4 Hz, 2H), 2.57 (t, J = 6.9 Hz, 2H), 2.29 (m, 1H), 2.13–2.05 (m, 5H), 1.83 (m, 4H), 1.45 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 167.7, 163.8, 154.4, 149.3, 146.5, 130.6, 127.1, 120.5, 114.8, 109.9, 105.4, 75.7, 64.2, 61.6, 48.9, 48.0, 40.9, 39.5, 32.0, 31.3, 30.2, 24.5 (x3), 22.2, 17.7. HRMS-TOF (*m/z*) calcd for $C_{26}H_{34}FN_5O_4S$ $[M-H]^-$: 530.2238, found 530.2106. HPLC purity, 99.0%.

4.1.13. tert-butyl (exo)-3-((2-((2-fluoro-4-(methylsulfonyl)phenyl) amino)-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)amino)-8-azabicyclo [3.2.1]octane-8-carboxylate (17)

Light yellow solid, 85% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.90 (t, J = 8.2 Hz, 1H), 7.64 (t, J = 9.0 Hz, 2H), 7.39 (s, 1H), 4.67 (m, 1H), 4.41–4.23 (m, 2H), 4.19 (d, J = 8.1 Hz, 1H), 3.05 (s, 3H), 2.82 (t, J = 7.7 Hz, 2H), 2.62 (t, J = 7.2 Hz, 2H), 2.17–2.13 (m, 6H), 2.05 (m, 2H), 1.87 (m, 2H), 1.49 (s, 9H). HRMS-TOF (*m/z*) calcd for $C_{26}H_{34}FN_5O_4S$ $[M-H]^-$: 530.2238, found 530.2084. HPLC purity, 98.5%.

4.1.14. tert-butyl 4-((2-((4-cyano-2-fluorophenyl)amino)-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)amino)piperidine-1-carboxylate (18)

Light yellow solid, 52% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.83 (t, J = 8.4 Hz, 1H), 7.42 (d, J = 8.6 Hz, 1H), 7.36 (dd, J = 11.0, 1.3 Hz, 1H), 7.33 (d, J = 3.3 Hz, 1H), 4.29 (d, J = 7.5 Hz, 1H), 4.17–4.11 (m, 1H), 2.96 (m, 2H), 2.86 (t, J = 7.7 Hz, 2H), 2.64 (t, J = 7.1 Hz, 2H), 2.15 (q, J = 7.7 Hz, 2H), 2.12–2.10 (m, 2H), 1.50 (s, 9H), 1.47–1.42 (m, 4H). HRMS-TOF (*m/z*) calcd for $C_{24}H_{29}FN_6O_2$ $[M-H]^-$: 451.2258, found 451.2108. HPLC purity, 95.6%.

4.1.15. tert-butyl (endo)-3-((2-((4-cyano-2-fluorophenyl)amino)-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)amino)-8-azabicyclo[3.2.1] octane-8-carboxylate (19)

Light yellow solid, 86% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.80 (t, J = 8.5 Hz, 1H), 7.45 (d, J = 8.0 Hz, 1H), 7.36–7.34 (m, 2H), 4.78 (d, J = 6.5 Hz, 1H), 4.35 (m, 1H), 4.32–4.23 (m, 2H), 2.86 (t, J = 7.6 Hz, 2H), 2.64 (t, J = 7.0 Hz, 2H), 2.42–2.28 (m, 1H), 2.17 (q, J = 7.7 Hz, 2H), 2.14–2.12 (m, 3H), 1.89–1.86 (m, 4H), 1.52 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 167.8, 163.8, 154.5, 149.4, 146.5, 130.1, 126.9, 125.4, 115.0, 114.7, 113.9, 105.4, 75.7, 64.2, 48.9, 48.1, 39.4, 34.7, 32.0, 31.3, 30.2, 24.6 (x3), 22.2, 17.7. HRMS-TOF (*m/z*) calcd for $C_{26}H_{31}FN_6O_2$ $[M-H]^-$: 477.2415, found 477.2255. HPLC purity, 97.4%.

4.1.16. tert-butyl (exo)-3-((2-((4-cyano-2-fluorophenyl)amino)-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)amino)-8-azabicyclo[3.2.1] octane-8-carboxylate (20)

Yellow solid, 80% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.78 (t, J = 8.2 Hz, 1H), 7.35–7.29 (m, 3H), 4.57 (m, 1H), 4.26–4.13 (m, 3H), 2.79 (m, 2H), 2.55 (t, J = 7.0 Hz, 2H), 2.09–2.00 (m, 8H), 1.79 (m, 2H), 1.45 (s, 9H). HRMS-TOF (*m/z*) calcd for C26H31FN6O2 $[M-H]^-$: 477.2415, found 477.2259. HPLC purity, 94.2%.

4.1.17. 5, 6, 7, 8-tetrahydroquinazoline-2,4-diol (21)

Follow the similar synthetic procedure of compounds **7**. Yellow solid, 38% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.98 (s, 1H), 7.84 (s, 1H), 2.39 (m, 4H), 1.82 (m, 2H), 1.75 (m, 2H). MS-ESI: $[M-H]^-$:165.3.

4.1.18. 2, 4-dichloro-5, 6, 7, 8-tetrahydroquinazoline (22)

Follow the similar synthetic procedure of compounds **7**. Yellow solid, 50% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 2.78 (m, 4H), 1.9 (m, 2H), 1.79 (m, 2H). MS-ESI: $[M-H]^-$:201.4.

4.1.19. tert-butyl 4-((2-chloro-5,6,7,8-tetrahydroquinazolin-4-yl)amino) piperidine-1-carboxylate(23)

To the solution of compounds **22** (0.3 g, 1.5 mmol) in DMF were added K_2CO_3 (1.95 mmol) and 4-amino-1-Boc-piperidine (2.7 mmol).

The reaction was stirred at 80 °C for 8 h. Then the mixture was extracted with ethyl acetate, washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by column chromatography to give the product (0.5 g, 92% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 4.47 (d, J = 7.7 Hz, 1H), 4.31–4.17 (m, 1H), 4.07 (ds, 2H), 2.95 (ds, 2H), 2.70 (t, J = 6.1 Hz, 2H), 2.26 (t, J = 6.1 Hz, 2H), 2.14–2.00 (m, 2H), 1.94–1.77 (m, 4H), 1.49 (s, 9H), 1.41–1.30 (m, 2H). MS-ESI: [M + H]$^+$: 367.1

4.1.20. tert-butyl 3-((2-chloro-5,6,7,8-tetrahydroquinazolin-4-yl)amino)-8-azabicyclo[3.2.1]octane-8-carboxylate(24)

Follow the similar synthetic procedure of compounds **23**. Yellow solid, 70% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 4.41 (m, 1H), 4.22 (m, 3H), 3.00 (m, 2H), 2.70 (m, 2H), 2.26 (m, 2H), 2.03 (m, 2H), 1.95–1.79 (m, 8H), 1.49 (m, 9H). MS-ESI: [M + H]$^+$: 393.3.

4.1.21. tert-butyl 4-((2-((4-cyano-2-fluorophenyl)amino)-5,6,7,8-tetrahydroquinazolin-4-yl)amino)piperidine-1-carboxylate (25)

Follow the similar synthetic procedure of compounds **12–20**. Yellow solid, 55% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.84 (t, J = 8.4 Hz, 1H), 7.41 (d, J = 8.6 Hz, 1H), 7.34 (dd, J = 11.0, 1.7 Hz, 1H), 7.24 (s, 1H), 4.44 (d, J = 7.2 Hz, 1H), 4.17–4.11 (m, 3H), 2.97 (m, 2H), 2.66 (t, J = 6.1 Hz, 2H), 2.28 (t, J = 6.2 Hz, 2H), 2.09 (m, 2H), 1.88–1.84 (m, 4H), 1.50 (s, 9H), 1.45 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 158.8, 156.3, 152.1, 150.8, 146.5, 130.3, 125.3, 114.9, 114.8, 114.0, 113.8, 101.6, 75.9, 44.4, 39.0, 38.6, 28.3, 27.9, 25.7, 24.5, 18.4, 18.3, 18.0. HRMS-TOF (m/z) calcd for $C_{25}H_{31}FN_6O_2$ [M − H]- : 465.2415, found 465.2250. HPLC purity, 97.0%.

4.1.22. tert-butyl (endo)-3-((2-((4-cyano-2-fluorophenyl)amino)-5,6,7,8-tetrahydroquinazolin-4-yl)amino)-8-azabicyclo[3.2.1]octane-8-carboxylate (26)

Follow the similar synthetic procedure of compounds **12–20**. Light yellow solid, 60% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.80 (t, J = 8.3 Hz, 1H), 7.43 (d, J = 8.7 Hz, 1H), 7.34 (m, 2H), 5.00 (d, J = 5.9 Hz, 1H), 4.36 (m, 2H), 4.26 (s, 1H), 2.66 (t, J = 6.2 Hz, 2H), 2.30 (t, J = 6.3 Hz, 2H), 2.14 (m, 2H), 1.92–1.84 (m, 10*H*), 1.52 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 162.4, 160.2, 156.1, 153.3, 151.3, 134.2, 129.3, 119.0, 118.7, 117.9, 117.8, 105.2, 79.6, 53.4, 52.8, 52.0, 35.7, 35.3, 29.3, 28.6 (x3), 28.2, 28.0, 22.4, 22.2, 21.8. HRMS-TOF (m/z) calcd for $C_{27}H_{33}FN_6O_2$ [M − H]$^-$: 491.2571, found 491.2431. HPLC purity, 95.7%.

4.1.23. tert-butyl 4-((2-((2-fluoro-4-(methylsulfonyl)phenyl)amino)-5,6,7,8-tetrahydroquinazolin-4-yl)amino)piperidine-1-carboxylate (27)

Follow the similar synthetic procedure of compounds **12–20**. Yellow solid, 56% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.92 (t, J = 8.4 Hz, 1H), 7.68 (dd, J = 8.7, 1.7 Hz, 1H), 7.64 (dd, J = 10.6, 2.1 Hz, 1H), 7.35 (s, 1H), 4.45 (d, J = 7.4 Hz, 1H), 4.19–4.12 (m, 3H), 3.07 (s, 3H), 2.97 (m, 2H), 2.67 (t, J = 6.1 Hz, 2H), 2.29 (t, J = 6.2 Hz, 2H), 2.11 (m, 2H), 1.90–1.84 (m, 4H), 1.51 (s, 9H), 1.43 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 158.8, 156.3, 152.1, 150.8, 146.6, 130.9, 126.9, 120.3, 114.5, 110.0, 109.9, 101.5, 75.9, 44.5, 40.9, 39.2, 38.6, 28.4, 27.9, 25.7, 24.5, 18.4, 18.3, 18.0. HRMS-TOF (m/z) calcd for $C_{25}H_{34}FN_5O_4S$ [M − H]$^-$: 519.2316, found 519.2162. HPLC purity, 97.4%.

4.1.24. tert-butyl (endo)-3-((2-((2-fluoro-4-(methylsulfonyl)phenyl)amino)-5,6,7,8-tetrahydroquinazolin-4-yl)amino)-8-azabicyclo[3.2.1]octane-8-carboxylate (28)

Follow the similar synthetic procedure of compounds **12–20**. Yellow solid, 58% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.87 (t, J = 8.4 Hz, 1H), 7.69–7.63 (m, 2H), 7.46 (s, 1H), 5.01 (s, 1H), 4.36–4.27 (m, 3H), 3.05 (s, 3H), 2.65 (m, 2H), 2.28 (m, 2H), 2.13 (m, 2H), 1.88 (m, 10*H*), 1.50 (s, 9H). ^{3}C NMR (150 MHz, $CDCl_3$) δ (ppm): 167.1, 160.8, 156.2, 149.3, 147.7, 130.9, 127.9, 120.5, 114.7, 109.8, 101.3, 75.7, 49.0, 47.8, 40.9, 39.7, 33.6, 32.1; 31.3, 30.2, 24.5 (x3), 22.2, 17.8. HRMS-TOF (m/z) calcd for $C_{27}H_{36}FN_5O_4S$ [M − H]$^-$: 544.2394, found 544.2220. HPLC purity, 93.4%.

4.1.25. Dimethyl 3,3′-(benzylazanediyl)dipropionate(29)

To a solution of methyl acrylate (9.4 mL, 98.5 mmol) in methanol (20 mL) was added benzylamine (5 mL, 45.7 mmol). The mixcure was stirred at r.t. for 6 h. After the reaction completed, the solvent was removed under reduced pressure. The residue was extracted with ethyl acetate, washed with brine, dried over $MgSO_4$, and evaporated to give the slightly yellow liquid (10.5 g, 74% yield), which was used directly in next step without purification. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.33– 7.25 (m, 5H), 3.67 (s, 6H), 3.61 (s, 2H), 2.83 (t, J = 7.1 Hz, 4H), 2.50 (t, J = 7.1 Hz, 4H). MS-ESI: [M + H]$^+$: 280.3.

4.1.26. Methyl 1-benzyl-4-oxopiperidine-3-carboxylate(30)

To a solution of sodium methoxide (0.58 g, 10.75 mmol) in toluene (30 mL) was added compound 23 (2 g, 7.17 mmol) dropwise at 0 °C. The reaction was stirred at 85 °C for 3.5 h. Then the reaction was quenched with H_2O (5 mL) and acetic acid (0.6 mL, 10.75 mmol). And the mixture was stirred at r.t. for 1 h. The reaction was extracted with ethyl acetate, dried over $MgSO_4$, evaporated to receive the product (1.2 g, 67% yield) without purification. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.38–7.35 (m, 5H), 3.75 (s, 3H), 3.67 (m, 3H), 3.21 (t, J = 1.5 Hz, 2H), 2.65 (t, J = 5.9 Hz, 2H), 2.43 (ddd, J = 5.9, 4.3, 1.6 Hz, 2H). MS-ESI: [M + H]$^+$: 248.5.

4.1.27. 6-benzyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4-diol(31)

Follow the similar synthetic procedure of compounds **7**. Light yellow solid, 68% yield. ^{1}H NMR (600 MHz, DMSO) δ (ppm): 10.93 (s, 1H), 10.77 (s, 1H), 7.43–7.14 (m, 5H), 3.62 (s, 2H), 3.00 (s, 2H), 2.63 (t, J = 5.7 Hz, 2H), 2.42 (t, J = 5.6 Hz, 2H).

4.1.28. 6-benzyl-2,4-dichloro-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine (32)

Follow the similar synthetic procedure of compound **8**. Yellow solid, 60% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.34–7.17 (m, 5H), 3.72 (s, 2H), 3.52 (s, 2H), 2.74 (t, J = 5.7 Hz, 2H), 2.59 (t, J = 5.6 Hz, 2H).

4.1.29. tert-butyl (exo)-3-((6-benzyl-2-chloro-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidin-4-yl)amino)-8-azabicyclo[3.2.1]octane-8-carboxylate (33)

Follow the similar synthetic procedure of compounds **9–11**. Light yellow solid, 84% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.39–7.28 (m, 5H), 4.63–4.58 (m, 1H), 4.34–4.25 (m, 2H), 4.11 (d, J = 7.8 Hz, 1H), 3.76(s, 2H), 3.22 (s, 2H), 2.82–2.78 (m, 4H), 2.15–2.02 (m, 4H), 1.85 (m, 2H), 1.62 (m, 2H), 1.49 (s, 9H). MS-ESI: [M + H]$^+$: 484.6.

4.1.30. tert-butyl (endo)-3-((6-benzyl-2-chloro-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidin-4-yl)amino)-8-azabicyclo[3.2.1]octane-8-carboxylate (34)

Follow the similar synthetic procedure of compounds **9–11**. Light yellow solid, 82% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.40–7.30 (m, 5H), 4.77 (d, J = 6.8 Hz, 1H), 4.39 (m, 1H), 4.30–4.15 (m, 2H), 3.76 (s, 2H), 3.19 (s, 2H), 2.83 (s, 4H), 2.37–2.23 (m, 2H), 2.07 (m, 2H), 1.80–1.66 (m, 4H), 1.49 (s, 9H). MS-ESI: [M + H]$^+$: 484.4.

4.1.31. tert-butyl (exo)-3-((6-benzyl-2-((4-(methylsulfonyl)phenyl)amino)-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidin-4-yl)amino)-8-azabicyclo[3.2.1]octane-8-carboxylate (35)

Follow the similar synthetic procedure of compounds **12–20**. Light yellow solid, 55% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.83 (s, 4H), 7.38–7.30 (m, 5H), 4.54–4.46 (m, 1H), 4.32–4.22 (m, 2H), 4.05 (d, J = 7.8 Hz, 1H), 3.75(s, 2H), 3.25 (s, 2H), 2.82–2.77 (m, 4H), 2.14–2.05 (m, 4H), 1.82 (m, 2H), 1.65 (m, 2H), 1.51 (s, 9H). MS-ESI: [M + H]$^+$: 619.3.

4.1.32. tert-butyl (endo)-3-((6-benzyl-2-((4-(methylsulfonyl)phenyl) amino)-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidin-4-yl)amino)-8-azabicyclo[3.2.1]octane-8-carboxylate (36)

Follow the similar synthetic procedure of compounds **12–20**. Light yellow solid, 58% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.85 (d, J = 8.8 Hz, 2H), 7.81 (d, J = 8.8 Hz, 2H), 7.39 (m, 4H), 7.32 (m 1H), 4.66 (d, J = 6.3 Hz, 1H), 4.36 (m, 1H), 4.35–4.24 (m, 2H), 3.77 (s, 2H), 3.23 (s, 2H), 3.06 (s, 3H), 2.83 (m, 2H), 2.78 (m, 2H), 2.35–2.24 (m, 2H), 2.06 (m, 2H), 1.83 (m, 2H), 1.73 (m, 2H), 1.52 (s, 9H). MS-ESI: $[M+H]^+$: 619.5.

4.1.33. tert-butyl (exo)-3-((6-benzyl-2-((4-cyano-2-fluorophenyl)amino)-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidin-4-yl)amino)-8-azabicyclo [3.2.1]octane-8-carboxylate (37)

Follow the similar synthetic procedure of compounds **12–20**. Yellow solid, 65% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.82 (t, J = 8.4 Hz, 1H), 7.44–7.35 (m, 6H), 7.33 (m, 1H), 7.27 (s, 1H), 4.64 (m, 1H), 4.33 (m, 2H), 4.04 (d, J = 8.0 Hz, 1H), 3.77 (s, 2H), 3.25 (s, 2H), 2.79 (d, J = 4.6 Hz, 2H), 2.77 (d, J = 4.7 Hz, 2H), 2.12 (m, 4H), 2.06 (m, 2H), 1.86 (m, 2H), 1.51 (s, 9H). HRMS-TOF (m/z) calcd for $C_{33}H_{38}FN_7O_2$ $[M+H]^+$: 584.3149, found 584.3211.

4.1.34. tert-butyl (endo)-3-((6-benzyl-2-((4-cyano-2-fluorophenyl)amino)-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidin-4-yl)amino)-8-azabicyclo[3.2.1] octane-8-carboxylate (38)

Follow the similar synthetic procedure of compounds **12–20**. Yellow solid, 55% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm):8.78 (t, J = 8.5 Hz, 1H), 7.44 (d, J = 8.7 Hz, 1H), 7.39 (m, 4H), 7.37–7.35 (m, 1H), 7.35–7.33 (m, 1H), 7.33–7.30 (m, 1H)., 4.64 (d, J = 6.2 Hz, 1H), 4.34 (m, 1H), 4.29–4.19 (m, 1H), 3.77 (s, 2H), 3.23 (s, 2H), 2.37–2.24 (m, 1H), 2.06 (m, 3H), 1.80 (m, 2H), 1.73 (m, 2H), 1.51 (s, 9H). HRMS-TOF (m/z) calcd for $C_{33}H_{38}FN_7O_2$ $[M+H]^+$: 584.3149, found 584.3295.

4.1.35. tert-butyl (exo)-3-((6-benzyl-2-((2-fluoro-4-(methylsulfonyl) phenyl)amino)-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidin-4-yl)amino)-8-azabicyclo[3.2.1]octane-8-carboxylate (39)

Follow the similar synthetic procedure of compounds **12–20**. Yellow solid, 52% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.89 (t, J = 8.3 Hz, 1H), 7.67 (m, 2H), 7.44–7.35 (m, 5H), 7.33 (m, 1H), 4.64 (m, 1H), 4.33 (m, 2H), 4.06 (d, J = 7.9 Hz, 1H), 3.77 (s, 2H), 3.26 (s, 2H), 3.07 (s, 3H), 2.80 (d, J = 4.3 Hz, 2H), 2.78 (d, J = 4.3 Hz, 2H), 2.12 (s, 3H), 2.04 (s, 1H), 1.85 (s, 3H), 1.50 (s, 9H). HRMS-TOF (m/z) calcd for $C_{33}H_{41}FN_6O_4S$ $[M+H]^+$: 637.2972, found 637.4132.

4.1.36. tert-butyl (endo)-3-((6-benzyl-2-((2-fluoro-4-(methylsulfonyl) phenyl)amino)-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidin-4-yl)amino)-8-azabicyclo[3.2.1]octane-8-carboxylate (40)

Follow the similar synthetic procedure of compounds **12–20**. Yellow solid, 44% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.87 (t, J = 8.1 Hz, 1H), 7.71 (dd, J = 8.8, 1.8 Hz, 1H), 7.64 (dd, J = 10.6, 2.1 Hz, 1H), 7.39 (m, 4H), 7.32 (m, 1H), 7.29 (s, 1H), 4.66 (d, J = 6.5 Hz, 1H), 4.36 (m, 1H), 4.33–4.24 (m, 2H), 3.77 (s, 2H), 3.23 (s, 2H), 3.07 (s, 3H), 2.83 (d, J = 4.8 Hz, 2H), 2.80 (d, J = 5.3 Hz, 2H), 2.36–2.24 (m, 1H), 2.11–2.01 (m, 3H), 1.82 (m, 2H), 1.73 (m, 2H), 1.52 (s, 9H). HRMS-TOF (m/z) calcd for $C_{33}H_{41}FN_6O_4S$ $[M+H]^+$: 637.2972, found 637.4206.

4.1.37. tert-butyl (exo)-3-((2-((4-(methylsulfonyl)phenyl)amino)-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidin-4-yl)amino)-8-azabicyclo[3.2.1]octane-8-carboxylate (41)

To a solution of compound 29 (0.1 mmol) in EtOH (3 mL) was added $Pd(OH)_2/C$ (15 mg). The reaction was stirred at 60 °C for 24 h under hydrogen balloon. Then the mixture was diluted with CH_2Cl_2, and filterer with celite. The filtrate was evaporated to receive the crude product, which was purified by column chromatography to give the yellow solid product.

The yield is 24%. ^{1}H NMR (600 MHz, MeOD) δ (ppm): 7.99 (d, J = 8.7, 2H), 7.83 (d, J = 8.4 Hz, 2H), 4.87–4.74 (m, 1H), 4.31 (m, 2H), 4.01 (s, 2H), 3.52 (t, J = 5.9 Hz, 2H), 3.13 (s, 3H), 2.93 (t, J = 6.0 Hz, 2H), 2.10 (m, 2H), 1.99 (m, 2H), 1.94 (m, 2H), 1.70 (m, 2H), 1.51 (s, 9H). HRMS-TOF (m/z) calcd for $C_{26}H_{36}N_6O_4S$ $[M+H]^+$: 529.2597, found 529. 2717.

4.1.38. tert-butyl (endo)-3-((2-((4-(methylsulfonyl)phenyl)amino)-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidin-4-yl)amino)-8-azabicyclo [3.2.1]octane-8-carboxylate (42)

Follow the similar synthetic procedure of compound **41**. Yellow solid, 18% yield. ^{1}H NMR (600 MHz, MeOD) δ (ppm): 8.02 (d, J = 8.8, 2H), 7.90 (d, J = 8.8 Hz, 2H), 4.58 (m, 1H), 4.34 (m, 2H), 4.03 (s, 2H), 3.72 (m, 2H), 3.14 (s, 3H), 2.32 (m, 2H), 2.21 (m, 2H), 2.02 (m, 2H), 1.90 (m, 2H), 1.77 (m, 2H), 1.51 (s, 9H). HRMS-TOF (m/z) calcd for $C_{26}H_{36}N_6O_4S$ $[M+H]^+$: 529.2597, found 529. 2735.

4.2. Human GPR119 agonistic activity

CHO K1 cells stably transfected with human GPR119 were grown at 37 °C, 95% O_2 and 5% CO_2 in 75 cm flasks containing DMEM/F12 (1:1) media with added 10% FBS (Gibco®), Geneticin (Gibco®) and grown until 90% confluent. Cells were then washed (PBS), lifted with cell dissociation solution (Invtrogen®), counted and used for cAMP accumulation assays and/or passaging (1:10). Following the manufacturer's instructions for the LANCE® Ultra cAMP assay (Perkin Elmer), cell transfected with hGPR119 were centrifuged (1000 rpm, 5 min), re-suspended in cAMP assay buffer (HBSS, 0.1% BSA, 0.5 mM IBMX and 5 mM HEPES) and seeded at 5000 cells/well in optiplate-384 (Perkin Elmer). Cells were treated with compounds over a range of concentrations (10 uM-0.6 uM) and incubated for 1 h. Cell lysis buffers (4X Eu-cAMP tracer solution and 4X ULight™-anti-cAMP solution) were added to each well, and the plates were incubated at room temperature for 1 h before being read on Envision (Perkin Elmer).

4.3. oGTT in C57BL/6N mice

For the acute single dose study, vehicle (0.5% carboxymethylcellulose sodium, 10 mL/kg) and compound **28** (5 and 15 mg/kg) were administered to C57BL/6N mice after 16-hrsstarvation, then the oral glucose tolerance test (3 g/kg) was conducted after 4 h of the single dose, the blood glucose level at 0, 15, 30, 60, 90 and 120 min were recorded for area under curve calculation ($AUC_{0\text{-}2h}$).

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