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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-nortropine amine and fluoro-substituted aniline exhibited better EC₅₀ values (0.27 μM – 1.2 μM) though they appeared to be partial agonists.

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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].

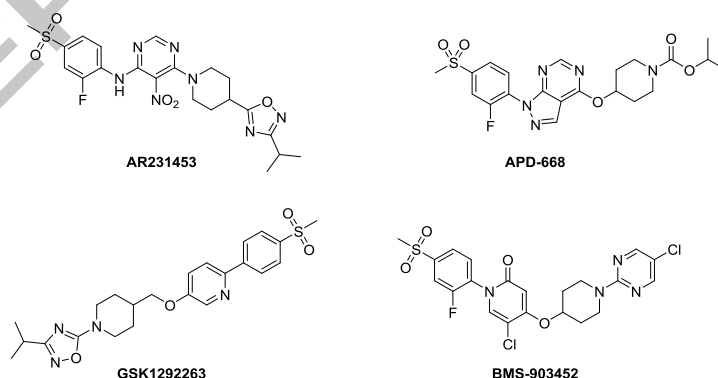


Fig. 1. The structures of GPR119 agonists.

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 pyrimidine moiety to replace 5-nitropyrimidine ring, using the strategy of scaffold hopping. In addition to heterocycle fused pyrimidine, we also studied the effect of

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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.

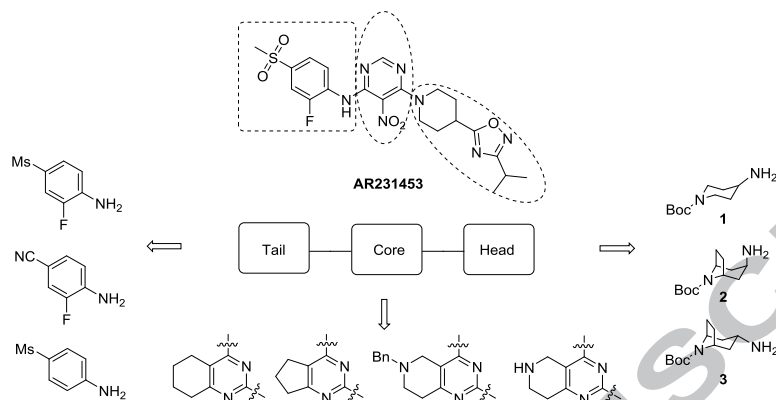
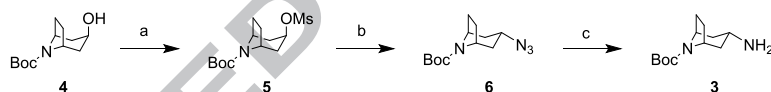


Fig. 2. The design of GPR119 agonists.

2. Results and discussion

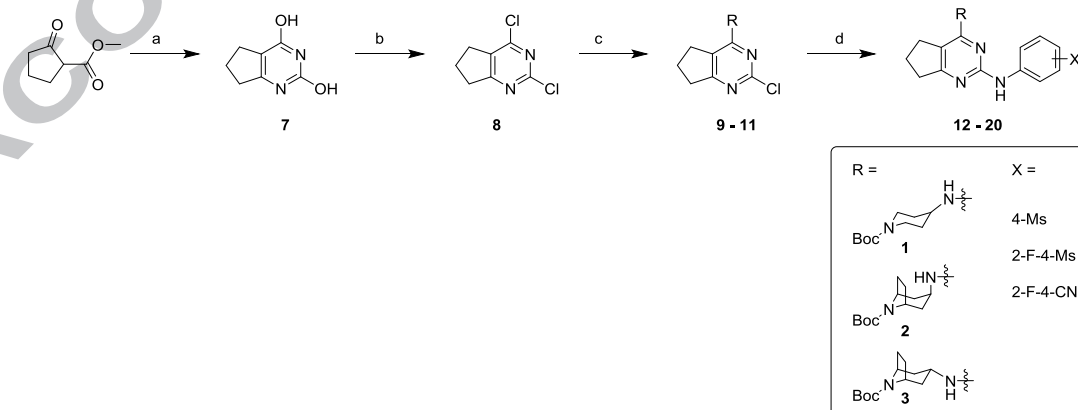
2.1 Chemistry

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 (NaN₃) 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].



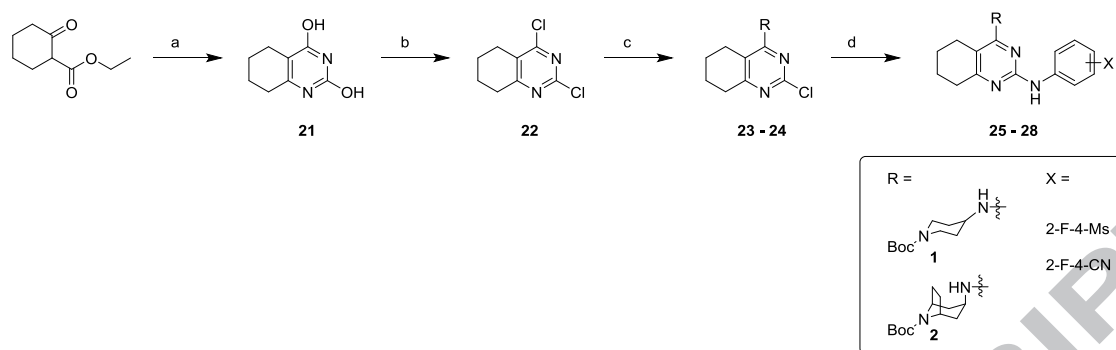
Scheme 1. Reagents and conditions: (a) MsCl, TEA, CH₂Cl₂, 0°C-rt, 5h; (b) NaN₃, MeCN, H₂O, reflux, overnight; (c) 10% Pd(OH)₂/C, H₂, MeOH, 40°C overnight.

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 POCl₃ at reflux conditions to give **8** in 82% yield. 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, Pd₂(dba)₃, X-Phos and Cs₂CO₃ resulted in final compounds **12-20** in 52-86%.



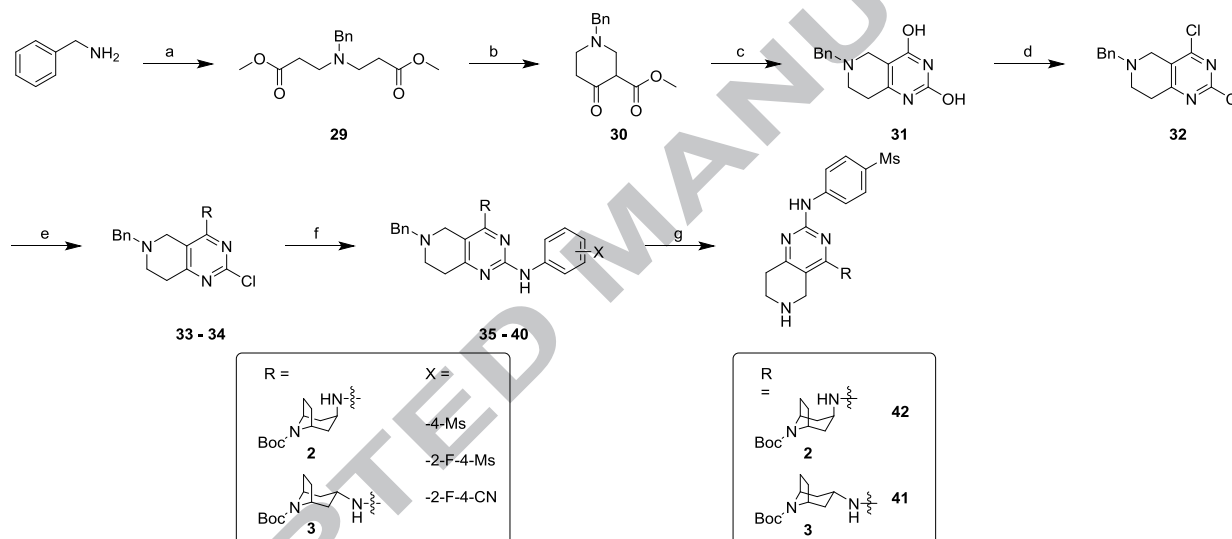
Scheme 2. Reagents and conditions: (a) urea, MeONa, EtOH, 60°C, overnight; (b) POCl₃, reflux, 7 h; (c) **1-3**, DIPEA, r.t., overnight; (d) substituted anilines, Pd₂(dba)₃, X-Phos, Cs₂CO₃, 1,4-Dioxane, reflux, overnight.

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



Scheme 3. Reagents and conditions: (a) urea, MeONa, EtOH, 60°C, overnight; (b) POCl₃, reflux, 7 h; (c) aliphatic amine **1** or **2**, K₂CO₃, DMF, 80°C, 8 h; (d) substituted anilines, Pd₂(dba)₃, X-Phos, Cs₂CO₃, 1,4-Dioxane, reflux, overnight.

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.



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) POCl₃, reflux, 7 h; (e) aliphatic amine **2** or **3**, DIPEA, r.t., overnight; (f) substituted anilines, Pd₂(dba)₃, X-Phos, Cs₂CO₃, 1,4-Dioxane, reflux, overnight; (g) 10% Pd(OH)₂/C, H₂, EtOH, 60°C.

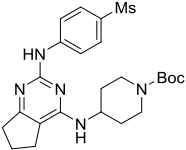
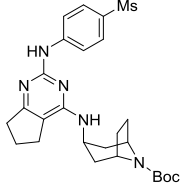
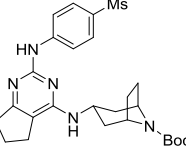
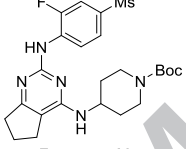
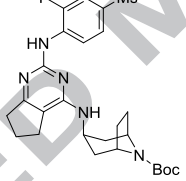
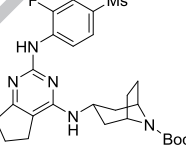
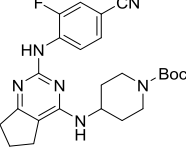
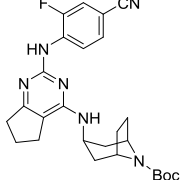
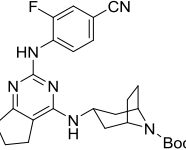
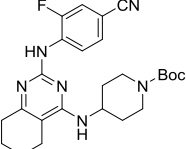
2.2 Biological activity

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 EC₅₀ and %max values. Endogenous GPR119 agonist oleoylethanolamide (OEA) was selected as the positive control. The EC₅₀ 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 (EC₅₀ > 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 (EC₅₀ 0.44 and 0.77 μM, respectively) with rational Clogp 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 EC₅₀ 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 (Clogp: 5.5 and 5.2), often causing pharmacokinetic issues.

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

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	

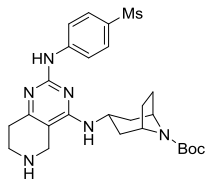
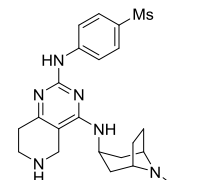
^a% max: cAMP stimulation % compared to maximal effect of OEA

^bClog_P was calculated using ACD software from Discovery Studio 4.5

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 ($EC_{50} = 1.2 \mu\text{M}$) and significantly low lipophilicity (Clog_P: 3.2).

Table 2
In vitro hGPR119 agonistic activities of **37-42**

Compound	Structure	hGPR119 activity		Clog _P ^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

^bClogP 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_{0-2h}). As outlined in Fig. 3, compound **28** demonstrates a dose-dependent effect but only weakly reduces the area under curve from 0-120 min by 2.6% (23.47±3.20) and 5.3% (22.82±2.36) at the dose of 5mg/kg and 15mg/kg, respectively (Vehicle: 24.09±2.34).

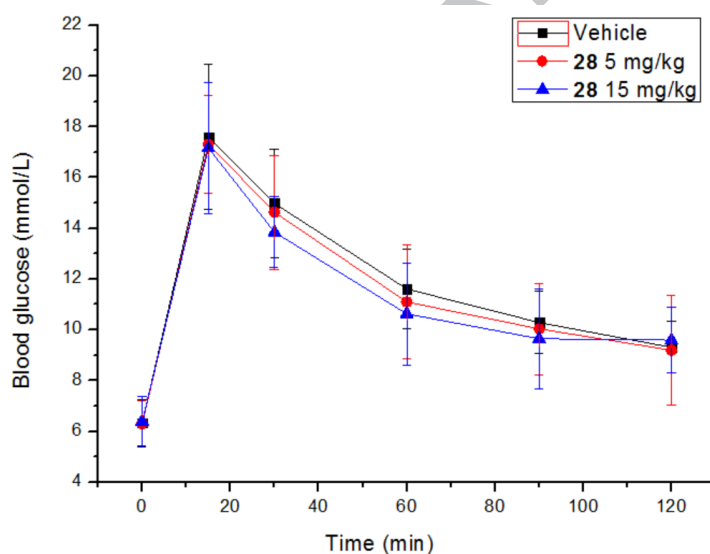


Fig. 3. Single dose of compound **28** on oGTT in C57BL/6N mice (n = 8).

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 EC₅₀ 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 EC₅₀ 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 EC₅₀ 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.

4. Experimental

4.1 Chemistry

All starting materials were obtained from commercial suppliers and used without further purification. ¹H-NMR and ¹³C-NMR spectra were recorded on a Bruker AVANCE III HD 600 (600Hz) 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.).

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 H₂O (2 mL). Then the residue was filtered to get yellow solid (0.52 g, 48% yield). ¹H-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 POCl₃ (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 NaHCO₃. The mixture was filtered to obtain the yellow product. (0.45 g, 73% yield). ¹H-NMR (600 MHz, CDCl₃) 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 MgSO₄, 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. ¹H-NMR (600 MHz, CDCl₃) δ (ppm): 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. ¹H-NMR (600 MHz, CDCl₃) δ (ppm): 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. ¹H-NMR (600 MHz, CDCl₃) δ (ppm): 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 Pd₂(dba)₃ (0.05 mmol), X-Phos (0.05 mmol) and Cs₂CO₃ (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 MgSO₄, 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. ¹H-NMR (600 MHz, CDCl₃) 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 C₂₄H₃₃N₅O₄S [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. ¹H-NMR (600 MHz, CDCl₃) 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 C₂₆H₃₅N₅O₄S [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. ¹H-NMR (600 MHz, CDCl₃) 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 C₂₆H₃₅N₅O₄S [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. ¹H-NMR (600 MHz, CDCl₃) 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 C₂₄H₃₂FN₅O₄S [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. ¹H-NMR (600 MHz, CDCl₃) 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). ¹³C-NMR (150 MHz, CDCl₃) 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 C₂₆H₃₄FN₅O₄S [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. ¹H-NMR (600 MHz, CDCl₃) 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 C₂₆H₃₄FN₅O₄S [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. ¹H-NMR (600 MHz, CDCl₃) 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 C₂₄H₂₉FN₆O₂ [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. ¹H-NMR (600 MHz, CDCl₃) 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). ¹³C-NMR (150 MHz, CDCl₃) 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 C₂₆H₃₁FN₆O₂ [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. ¹H-NMR (600 MHz, CDCl₃) 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. ¹H-NMR (600 MHz, CDCl₃) 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. ¹H-NMR (600 MHz, CDCl₃) 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 K₂CO₃ (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 MgSO₄, and evaporated. The residue was purified by column chromatography to give the product (0.5 g, 92% yield). ¹H-NMR (600 MHz, CDCl₃) 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. ¹H-NMR (600 MHz, CDCl₃) 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. ¹H-NMR (600 MHz, CDCl₃) 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). ¹³C-NMR (150MHz, CDCl₃) 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 C₂₅H₃₁FN₆O₂ [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. ¹H-NMR (600 MHz, CDCl₃) 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, 10H), 1.52 (s, 9H). ¹³C-NMR (150 MHz, CDCl₃) 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 C₂₇H₃₃FN₆O₂ [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. ¹H-NMR (600 MHz, CDCl₃) 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, 2.1Hz, 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). ¹³C-NMR (150 MHz, CDCl₃) 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 C₂₅H₃₄FN₅O₄S [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. ¹H-NMR (600 MHz, CDCl₃) 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, 10H), 1.50 (s, 9H). ¹³C-NMR (150 MHz, CDCl₃) 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 C₂₇H₃₆FN₅O₄S [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 mixture 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. $^1\text{H-NMR}$ (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: $[\text{M}+\text{H}]^+$: 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 1h. The reaction was extracted with ethyl acetate, dried over MgSO_4 , evaporated to receive the product (1.2g, 67% yield) without purification. $^1\text{H-NMR}$ (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: $[\text{M}+\text{H}]^+$: 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. $^1\text{H-NMR}$ (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. $^1\text{H-NMR}$ (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. $^1\text{H-NMR}$ (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: $[\text{M}+\text{H}]^+$: 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. $^1\text{H-NMR}$ (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: $[\text{M}+\text{H}]^+$: 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. $^1\text{H-NMR}$ (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: $[\text{M}+\text{H}]^+$: 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. $^1\text{H-NMR}$ (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: $[\text{M}+\text{H}]^+$: 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. $^1\text{H-NMR}$ (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 $\text{C}_{33}\text{H}_{38}\text{FN}_7\text{O}_2$ $[\text{M}+\text{H}]^+$: 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. $^1\text{H-NMR}$ (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 $\text{C}_{33}\text{H}_{38}\text{FN}_7\text{O}_2$ $[\text{M}+\text{H}]^+$: 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. $^1\text{H-NMR}$ (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 $\text{C}_{33}\text{H}_{41}\text{FN}_6\text{O}_4\text{S}$ $[\text{M}+\text{H}]^+$: 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. $^1\text{H-NMR}$ (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 $\text{C}_{33}\text{H}_{41}\text{FN}_6\text{O}_4\text{S}$ $[\text{M}+\text{H}]^+$: 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 CH₂Cl₂, and filtered 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%. ¹H-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 C₂₆H₃₆N₆O₄S [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. ¹H-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 C₂₆H₃₆N₆O₄S [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% O₂ and 5% CO₂ 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 (Invitrogen®), 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 (1000rpm, 5min), re-suspended in cAMP assay buffer (HBSS, 0.1% BSA, 0.5mM IBMX and 5mM HEPES) and seeded at 5000 cells/well in optiplate-384 (Perkin Elmer). Cells were treated with compounds over a range of concentrations (10 μM-0.6 μM) and incubated for 1h. 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 1h 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-hrs starvation, then the oral glucose tolerance test (3 g/kg) was conducted after 4h 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_{0-2h}).

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Highlights

The authors designed novel GPR119 agonists bearing various core moiety from lipophilic cycloolefin fused pyrimidines to polar tetrahydropyridopyrimidines. By synthesis and bio-evaluation of final compounds, we found that the less polar cyclohexene fused compounds displayed the most potent activity. We extended the SAR as a guide for further optimization.

ACCEPTED MANUSCRIPT

Graphical Abstract

Synthesis and evaluation of novel fused pyrimidine derivatives as GPR119 agonists

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