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Lead generation and optimization of novel GPR119 agonists with a spirocyclic cyclohexane structure

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ABSTRACT

We describe here the generation of a lead compound and its optimization studies that led to the identification of a novel GPR119 agonist. Based on a spirocyclic cyclohexane structure reported in our previous work, we identified compound **8** as a lead compound, being guided by ligand-lipophilicity efficiency (LLE), which linked potency and lipophilicity. Subsequent optimization studies of **8** for improvement of solubility afforded representative **21**. Compound **21** had no inhibitory activity against six CYP isoforms and showed favorable pharmacokinetic properties and hypoglycemic activity in rats.

GPR119 is a G protein-coupled receptor (GPCR) expressed predominantly in pancreatic beta cells and enteroendocrine cells. Activation of GPR119 promotes glucose-dependent insulin release in the pancreas and secretion of incretins such as glucagon-like peptide-1 (GLP-1) in the gut. GPR119 has the potential to be a suitable target for the development of orally active, small molecule agonists and has received attention from many pharmaceutical companies in recent years.[1]

A number of synthetic GPR119 agonists have been reported to date, several of which have advanced into clinical trials.[2] Reported GPR119 agonists are often composed of three parts as depicted in Fig. 1: a piperidine or a piperazine ring *N*-substituted with a carbamate or a heteroaryl group at the right side, a phenyl group substituted with a methylsulfonyl or a heteroaryl group (X) at the left side, and a linker part connecting the two parts.[2,3] Of the three parts, the *N*-substituted piperidine or piperazine ring as the right side moiety is a characteristic structure.

In our previous work, a novel spirocyclic cyclohexane structure was identified as an alternative to the right side moiety of compound **4** (Fig. 2).[4] The representative **5** having high three-dimensionality showed potent GPR119 agonistic activity with no cytochrome P450 (CYP) inhibitory activity. In the rat intraperitoneal glucose tolerance test (ipGTT), **5** also displayed hypoglycemic activity with insulin secretion dependent on glucose concentration.

However, corneal toxicity was observed in the rat toxicity test of **5**. In general, it is known that compounds with high lipophilicity may show nonspecific off-target action and have high risk of toxicity.[5] We speculated that high lipophilicity of **5** (Clog P = 5.1) was one of reasons for the ocular toxicity.

We therefore undertook the goal to discover a novel GPR119 agonist with reduced lipophilicity, as compared to **5**. We set out to generate a new lead compound having a good balance of agonistic activity and lipophilicity by using ligand-lipophilicity efficiency (LLE)[6] as an index. In seeking a new lead compound, we expected that the spirocyclic cyclohexane structure of **5** would be a versatile right side moiety in GPR119 agonists. Thus, this structure was fixed as the right side moiety and combined with linkers and left side moieties found in known GPR119 agonists. Among them, we were interested in **1** (MBX-2982), **2** (PSN119-2), and **3** (Arena's compound) (Fig. 1) with linkers and left side moieties which could reduce lipophilicity.[2,3] Each of the compounds having the spirocyclic cyclohexane structure as the right side moiety were synthesized and evaluated (Table 1).

All of the spirocyclic cyclohexane intermediates **26**, **28**, **29** and **32** were synthesized from commercially available compounds **22** and **30** as shown in Scheme 1. Lithiation of ester **23** followed by reaction with isobutylene oxide gave the desired lactone **24** with high diastereoselectivity (98:2 d.r.).[4] Reduction of **24** with $LiAlH_4$ and subsequent treatment with *p*-toluenesulfonic acid monohydrate furnished spiro ether **25**. Oxidation of **25** provided spirocyclic carboxylic acid **26**. Curtius rearrangement of **26** in the presence of potassium *tert*-butoxide gave the *tert*-butoxy carbonyl (Boc)-protected amine **27** while maintaining high diastereomeric ratio (98:2 d.r.). Amine **28** was obtained as the corresponding HCl salt by deprotection of the Boc group of **27**. Reduction of **27** with $LiAlH_4$ under reflux and subsequent treatment with HCl in AcOEt gave *N*-methylated amine **29** as the corresponding

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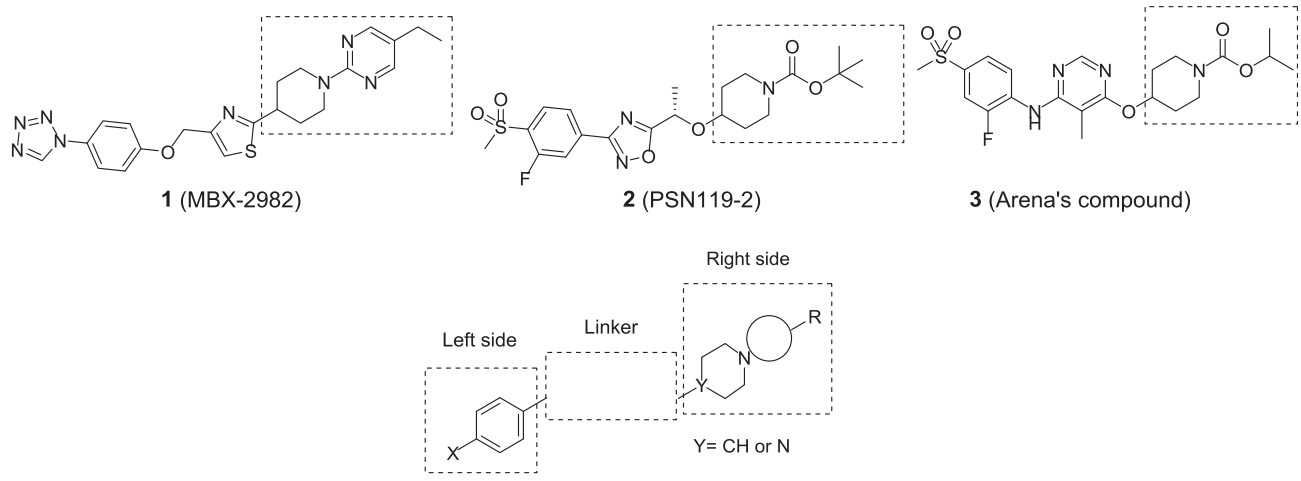
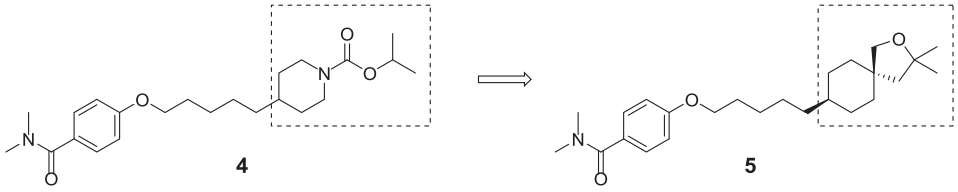


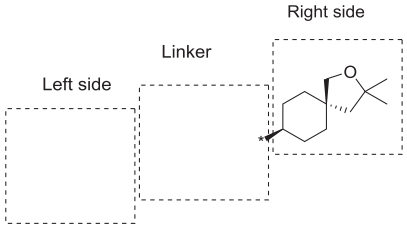
Fig. 1. Representatives and typical structure of GPR119 agonists.

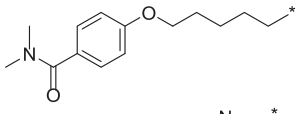
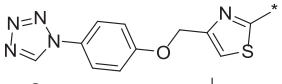
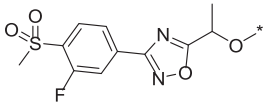
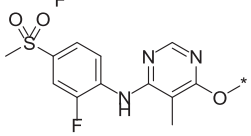


GPR119 EC_{50} (IA)	10 nM (102%)	4 nM (104%)
CYP IC_{50}	< 10 uM	> 10 uM
	CYP 2C8, 2C9, 2C19	CYP 1A2, 3A4, 2C8, 2C9, 2C19, 2D6
CLogP	3.9	5.1

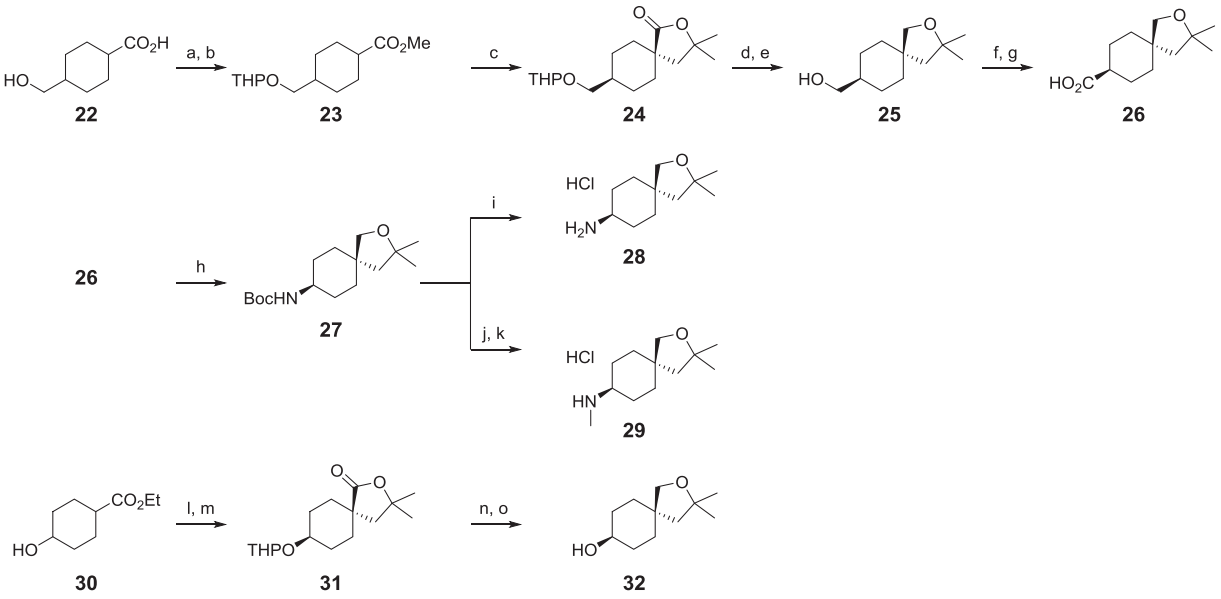
Fig. 2. Our previous work.

Table 1
Lead generation guided by LLE.



Compound	Left side-Linker	GPR119 EC_{50} (nM)	IA (%)	CLogP[a]	LLE[b]
5		4	104	5.1	3.3
6		85	79	3.7	3.3
7		89	109	3.4	3.6
8		13	91	4.0	4.0

[a] The Clog P value was calculated using a software from ChemAxon.
[b] LLE = pEC_{50} − Clog P.



Scheme 1. Synthesis of spirocyclic cyclohexane intermediates **26**, **28**, **29** and **32**. Reagents and conditions: (a) MeI, K_2CO_3, DMF, rt; (b) DHP, (−)-CSA, toluene, rt; (c) isobutylene oxide, LiHMDS, THF, rt, 62% (3 steps); (d) $LiAlH_4$, THF, 0 °C to rt, 79%; (e) *p*-TsOH·H_2O, MeOH, 85 °C, 87%; (f) Dess-Martin reagent, $CHCl_3$, 0 °C; (g) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH, H_2O, rt, 54% (2 steps); (h) DPPA, NEt_3, KO-*t*-Bu, dioxane, 90 °C, 97%; (i) HCl, AcOEt, rt, 66%; (j) $LiAlH_4$, THF, reflux; (k) HCl, AcOEt, rt, 47% (2 steps); (l) DHP, (−)-CSA, toluene, rt; (m) isobutylene oxide, LiHMDS, THF, rt, 69% (2 steps); (n) $LiAlH_4$, THF, 0 °C to rt, 89%; (o) *p*-TsOH·H_2O, MeOH, rt, 91%.

HCl salt. Spirocyclic alcohol **32** was synthesized with high diastereoselectivity (97:3 d.r.) by the same procedure for **25**.

Compounds **6** and **7** were obtained from **26** and **32**, respectively (Scheme 2). Amide **33** was converted from **26**. Reaction of **33** with Lawesson's reagent followed by reaction of ethyl bromopyruvate gave thiazole ester **34**. Reduction of **34** with $LiAlH_4$ and subsequent coupling with the corresponding phenol provided **6**. Reaction of alcohol **32** with 2-bromopropanoic acid followed by reaction with hydroxyl amidine **36** and oxidation of the sulfide group afforded **7**.

Compounds **8–11**, **16** and **17** were synthesized from **32** (Scheme 3). Reaction of **32** with the corresponding dichloropyrimidine and subsequent reaction with the corresponding aniline or phenol afforded the final products.

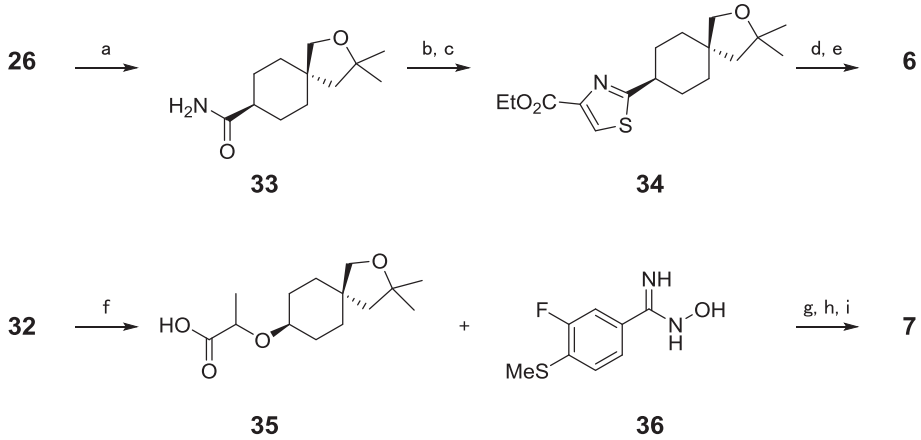
Compounds **12–15** and **20** were synthesized by starting from the corresponding anilines (Scheme 4). The intermediates **45**, **46**, **48** and **49** were coupled with **32** to afford the final products **12–15**. The reaction of **29** with **49** gave the final product **20**.

Compounds **18**, **19** and **21** were converted from **28** (Scheme 5). Reaction of **28** with the corresponding dichloropyrimidine and subsequent methylation of the NH group gave intermediates **50–52**. Reaction of **50** and **51** with aniline **40** afforded the final products **18** and **21**, respectively. Oxidative cleavage of **52**, reductive amination with aniline **40** and treatment under acidic conditions provided the final product **19**.

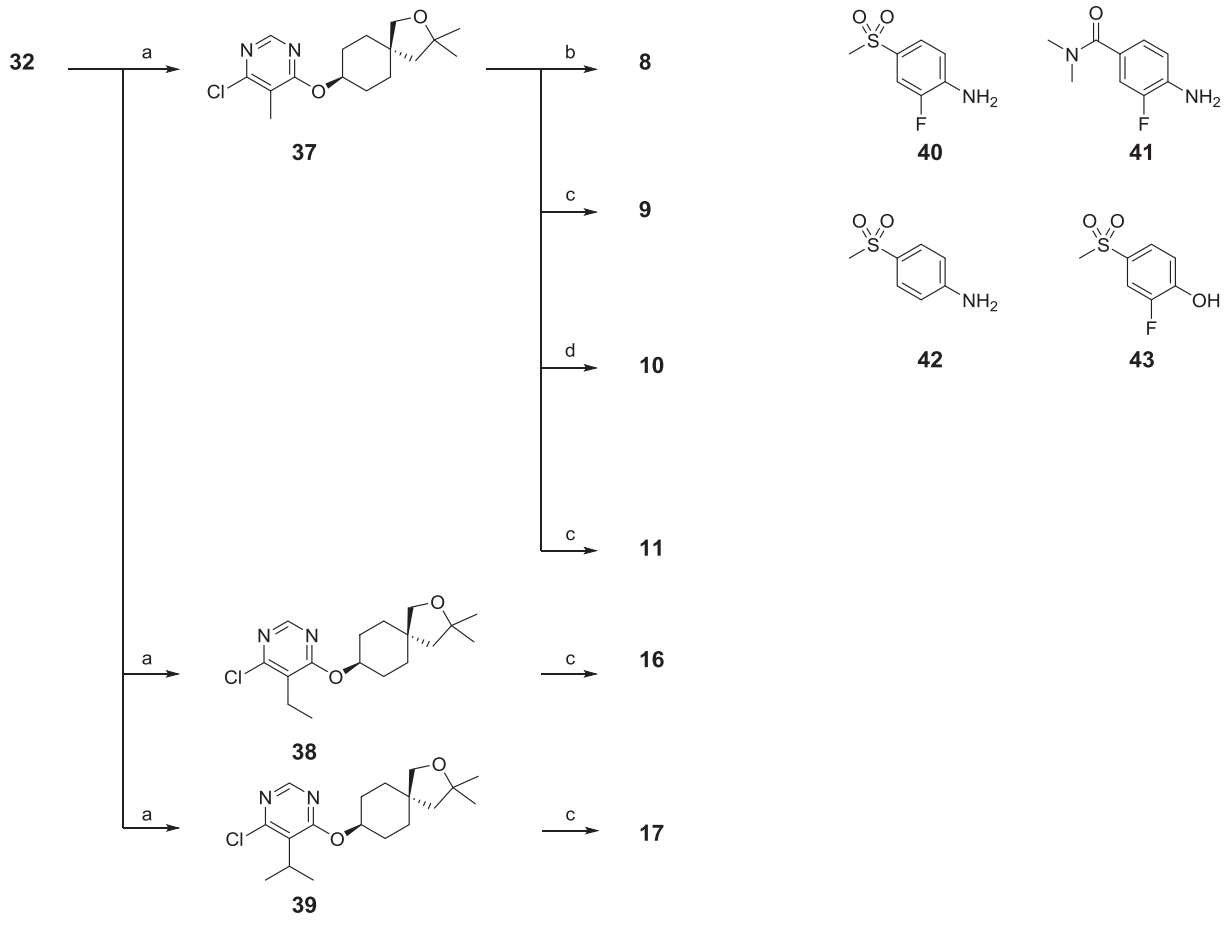
GPR119 agonistic activity was evaluated for the human GPR119 receptor in a cell-based cAMP assay.[7] The results expressed the potency as EC_{50} values and the inherent activity (IA) as percentages which were compared to the test compound with oleoylethanolamide (OEA), an endogenous ligand of GPR119 (defined as 100% activation).

As expected, compounds **6–8** all showed lower values of lipophilicity than **5**. In addition, they exhibited acceptable GPR119 agonist activity, which indicated that the spirocyclic cyclohexane structure of **5** was a versatile right side moiety in GPR119 agonists. Among **6–8**, pyrimidine derivative **8** expressed the highest LLE value (4.0). Therefore, we selected **8** as a new lead compound having a better balance of agonistic activity and lipophilicity.

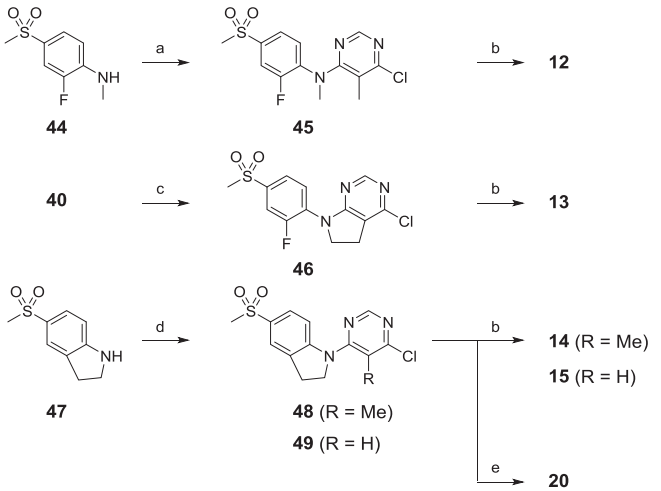
Next, we moved to an optimization process, where we focused on improvement for values of solubility of **8** (Table 2). Compound **8** showed considerably lower values of solubility for Japanese Pharmacopoeia 1st fluid for a dissolution test adjusted to pH 1.2 (JP1) and Fed-state simulated intestinal fluid (FeSSIF). In general, solubility is one factor that governs oral absorption, and compounds with low solubility



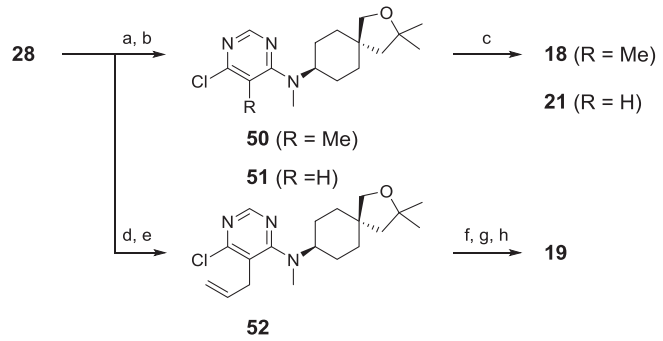
Scheme 2. Reagents and conditions: (a) NH_4Cl, NEt_3, EDC, HOBt, CH_3CN, rt, 96%; (b) Lawesson's reagent, THF, reflux; (c) ethyl bromopyruvate, EtOH, rt, 32% (2 steps); (d) $LiAlH_4$, THF, 0 °C to rt, 94%; (e) 4-(1*H*-tetrazol-1-yl)phenol, PPh_3, DMAD, toluene, rt, 76%; (f) 2-bromopropanoic acid, NaH, dioxane, rt, 62%; (g) EDC, HOBt, *i*-Pr_2NEt, DMF, rt; (h) toluene, reflux; (i) *m*-CPBA, $CHCl_3$, 0 °C, 55% (3 steps).



Scheme 3. Reagents and conditions: (a) the corresponding 4,6-dichloropyrimidine, NaH, THF, rt, 83–93%; (b) **40**, $Pd(OAc)_2$, (±)-BINAP, NaO-*t*-Bu, dioxane, 100 °C, 12%; (c) the corresponding aniline **40–42**, $Pd(OAc)_2$, 1,1′-bis(di-*t*-butylphosphino)ferrocene, NaO-*t*-Bu, dioxane, 100 °C, 26–57%; (d) **43**, K_2CO_3, TBAI, DMSO, 130 °C, 67%.



Scheme 4. Reagents and conditions: (a) 4,6-dichloro-5-methylpyrimidine, Pd $(OAc)_2$, NaO-*t*-Bu, 1,1′-bis(di-*t*-butylphosphino)ferrocene, dioxane, 120 °C, 16%; (b) **32**, NaH, THF, reflux, 22–69%; (c) 2-(4,6-dichloropyrimidin-5-yl) acetaldehyde, $NaBH(OAc)_3$, THF, rt, 85%; (d) the corresponding 4,6-dichlorolpyrimidine, *n*-PrOH, reflux, 39%; (e) **29**, $Pd(OAc)_2$, DavePhos, K_2CO_3, dioxane, 105 °C, 5%.



Scheme 5. Reagents and conditions: (a) the corresponding 4,6-dichlorolpyrimidine, K_2CO_3, DMSO, rt; (b) MeI, NaH, DMF, rt, 25–70% (2 steps); (c) **40**, $PdCl_2(dppf)$, (±)-BINAP, NaO-*t*-Bu, DMF, 80 °C, 22–38%; (d) 4,6-dichloro-5-allylpyrimidine, *i*-Pr_2NEt, K_2CO_3, DMF, 80 °C; (e) MeI, NaH, DMF, 60 °C, 26% (2 steps); (f) $NaIO_4$, $KOsO_4$, acetone, H_2O, rt, 10%; (g) **40**, $NaBH(OAc)_3$, TFA, $CHCl_3$, rt; (h) conc. HCl, *n*-PrOH, 100 °C, 66% (2 steps).

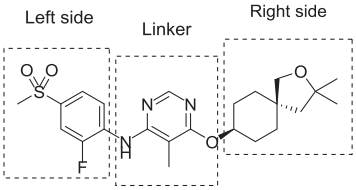
are related to low oral absorption.

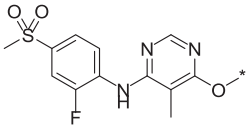
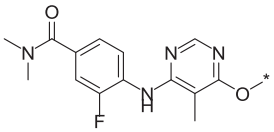
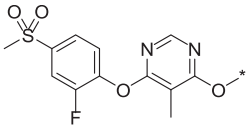
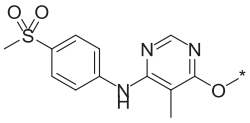
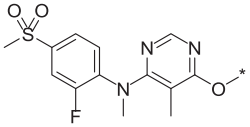
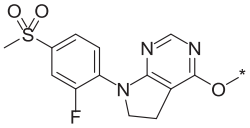
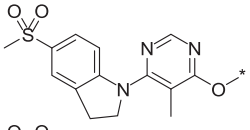
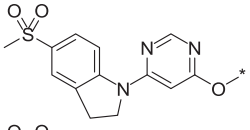
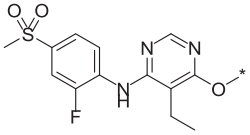
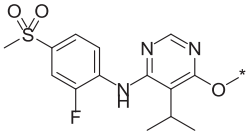
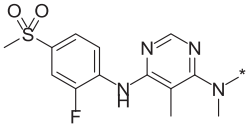
In our previous work[4] and in other reports,[8] conversion of the methylsulfonyl group at the left side to the dimethylcarbamoyl compound exhibited considerable improvement in solubility. Compound **9** transformed by the dimethylcarbamoyl group showed higher values of solubility, particularly against JP1. However, agonistic activity of **9** was decreased about three times compared with **8**.

Next, we focused on molecular planarity, which is known to influence crystal packing. Disruption of molecular planarity would be expected to decrease the efficiency of crystal packing, leading to improved solubility.[9] In the case of **8**, molecular planarity of the two aromatic rings was considered as the cause of low solubility.

Therefore, we investigated how solubility and agonistic activity

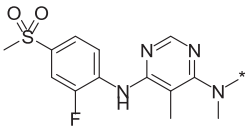
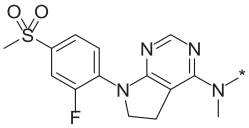
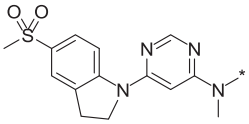
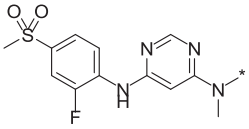
Table 2
SAR and physicochemical properties of pyrimidine derivatives.



Compound	Left side-Linker	GPR119 EC_{50} (nM)	IA (%)	Clog P[a]	LLE[b]	JP1[c] (μM)	FeSSIF (μM)
8		13	91	4.0	4.0	5.3	15.8
9		33	101	4.5	3.7	128.5	65.2
10		59	62	4.1	3.2	< 0.5	33.4
11		139	92	3.9	3.0	4.4	8.6
12		> 1000	17	4.2	–	13.0	42.5
13		5	90	3.7	4.6	18.9	15.2
14		> 1000	38	4.1	–	2.8	25.5
15		15	87	3.6	4.2	7.0	19.8
16		42	82	4.4	3.0	4.6	67.5
17		98	61	4.7	2.3	0.7	4.3
18		45	128	4.3	3.1	> 475	49.8

[a] The Clog P value was calculated using a software from ChemAxon.
[b] LLE = pEC_{50} − Clog P.
[c] Japanese Pharmacopoeia 1st fluid for a dissolution test adjusted to pH 1.2.

Table 3
Fine tuning of pyrimidine derivatives.

Compound	Left side-Linker	GPR119 EC_{50} (nM)	IA (%)	CLogP[a]	LLE[b]	hMs[c] (%)	rMs[d] (%)	JP1[e]/FeSSIF (μM)
18		45	128	4.3	3.1	91	93	> 475 / 49.8
19		39	118	4.0	3.4	99	94	> 475 / 75.3
20		16	108	3.9	3.9	89	80	418.2 / 24.3
21		19	120	3.7	4.0	103	96	473.3 / 31.1

[a] The Clog P value was calculated using a software from ChemAxon.
[b] LLE = pEC_{50} − Clog P.
[c] Percent of compound (5 μM) remaining after 1 h incubation with human liver microsomes (0.2 μg/mL).
[d] Percent of compound (5 μM) remaining after 1 h incubation with rat liver microsomes (0.2 μg/mL).
[e] Japanese Pharmacopoeia 1st fluid for a dissolution test adjusted to pH 1.2.

were affected by disrupting molecular planarity. Compound **10** with the O atom instead of the NH group connecting the two aromatic rings in **8** showed little improvement in solubility and its agonistic activity decreased four times. In addition, the F atom on the benzene ring had little effect in solubility, and the agonistic activity of compound **11** without the F atom decreased about ten times compared with **8**. On the other hand, *N*-methylation of **8** slightly improved solubility and the agonistic activity of *N*-methylated **12** was greatly weakened. These results suggested that the intramolecular hydrogen bond between the F atom and the H atom of the NH group in **8** might be important to achieve highly agonistic activity.[10] This outcome was also indicated that steric repulsion between the *N*-methyl group and the methyl group at the 5-position on the pyrimidine ring in **12** significantly changed the molecular arrangement and led to decreased agonistic activity.

In order to eliminate the steric repulsion, cyclization of the two methyl groups in **12** was investigated.[11] As anticipated, this cyclization boosted the agonistic activity significantly, and the thus-obtained **13** showed an EC_{50} value of 4 nM and LLE value of 4.6. The similar improvement in agonistic activity was observed in the indoline derivative[10,12] **15** which maintained coplanarity compared with compound **14**. These results suggested that maintaining of coplanarity in the two aromatic rings was important to demonstrate potent agonistic activity. Unfortunately, cyclized compounds **13** and **15** exhibited little improvement in solubility against only acidic medium (JP1) as compared with **8**.

Another approach to decrease the efficiency of crystal packing while maintaining the coplanar arrangement was investigated. The methyl group at the 5-position of the pyrimidine ring was converted to either an ethyl group or an isopropyl group. Although ethyl derivative **16** showed slight improvement in solubility against FeSSIF, the agonistic activity diminished about three times compared with **8**. In isopropyl derivative **17**, no improvement in solubility was observed and further agonistic activity attenuation occurred.

Finally, we investigated increasing basicity as a way to improve solubility. We expected that conversion of the ether linkage of **8** to an *N*-methyl group could improve solubility. More than expected, compound **18** demonstrated significant improvement of values of solubility for the two media, especially for acidic medium (JP1). The agonistic activity of **18** was only about three times less than **8**.

Optimization of **8** led to compounds **9** and **18** which exhibited good solubility and acceptable agonistic activity. Metabolic stability of **9** was moderate (percentage remaining at 1 h = 77% in human liver microsomes, 44% in rat liver microsomes), whereas **18** exhibited high metabolic stability as shown in Table 3.

Further optimization of **18** was carried out with the aim of enhancing agonistic activity, while maintaining good solubility and metabolic stability (Table 3). Cyclized products **19** and **20** with favorable profiles in solubility and metabolic stability, especially the indoline derivative **20**, showed about three times improvement in agonistic activity compared with **18**. This result suggested that steric repulsion between the substituent at the 5-position of the pyrimidine and the *N*-methyl group attenuated the agonistic activity of **18** and **19**, when compared with the ether forms **8** and **13**, respectively. Therefore, compound **21** without the methyl group at the 5-position of pyrimidine in **18** was synthesized and evaluated. As a result, improvement of agonistic activity (EC_{50} = 19 nM) was observed while maintaining good solubility and metabolic stability. The lipophilicity of **21** (Clog P = 3.6) was considerably lower than that of **5** (Clog P = 5.1), resulting in a better LLE value (4.0).

We finally succeeded in identifying a novel GPR119 agonist **21** which exhibited reduced lipophilicity compared with **5** and additionally demonstrated good solubility and metabolic stability.

The pharmacokinetic profiles of compound **21** were evaluated in Sprague-Dawley rats (Table 4). Compound **21** exhibited long-lasting and good oral availability. In addition, **21** showed no inhibitory activity (IC_{50} > 50 μM) against all six CYP isoforms (CYP 3A4, 2D6, 1A2, 2C9,

Table 4
Phamacokinetic profiles of **21** in Sprague-Dawley rats.

Rat IV (1 mg/kg)				Rat PO (3 mg/kg)
t1/2β (h)	CL (L/h/kg)	Vdss (L/kg)	MRT (h)	BA (%)
3.7	0.8	4.0	5.1	99

2C8, 2C19).

The effect of compound **21** on plasma glucose levels in the intraperitoneal glucose tolerance test (ipGTT) in Sprague-Dawley rats was evaluated.[13] Glucose solution was intraperitoneally administered at 16 h after oral administration of **21** (10 mg/kg) or vehicle. The plasma glucose levels were measured at 0, 30 and 60 min after glucose loading. Plasma glucose levels just before glucose loading were slightly different between the vehicle and **21** groups. The plasma glucose levels at 30 and 60 min after glucose loading in **21** group were 14% and 11% lower than that in the vehicle group, respectively. Compound **21** suppressed the increase in peak plasma glucose levels after glucose loading.

In summary, lead generation studies guided by LLE led to the identification of compound **8** having a spirocyclic cyclohexane structure, which was a versatile right side moiety in GPR119 agonists. Further efforts to improve solubility succeeded in discovery of a novel GPR119 agonist **21**. Compound **21** exhibited orally active, long-lasting pharmacokinetic profiles and showed a hypoglycemic effect after 16 h of administration in rats. Further identification of novel GPR119 agonists with a spirocyclic cyclohexane structure will be reported in due course.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2018.12.041.

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