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Design and synthesis of *1H*-pyrazolo[3,4-c]pyridine derivatives as a novel structural class of potent GPR119 agonists

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Abstract:

Design and synthesis of a novel class of 1H-pyrazolo[3,4- c]pyridine GPR119 receptor agonists are described. Lead compound 4 was identified through the ligand-based drug design approach. Modification of the left-hand aryl group (R1) and right-hand piperidine N-capping group (R2) led to the identification of compound 24 as a single-digit nanomolar GPR119 agonist.

Keywords: Type 2 diabetes mellitus; GPR119 agonist; G-protein coupled receptor; pyrazolopyridine.

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease characterized by hyperglycemia due to impaired insulin secretion and insulin resistance. Long-term hyperglycemia is a major risk factor for diabetic complications such as nephropathy, retinopathy, and neuropathy. The number of people with T2DM worldwide is more than 300 million, and the prevalence is rapidly increasing.[1,2] Despite the availability of multiple antidiabetic agents, a large number of patients fail to achieve the desired glycemic control level.[3] Therefore, there remains a significant need for the development of new medical treatments for T2DM.

GPR119 is a G-protein coupled receptor (GPCR) that is predominantly expressed in the pancreatic β-cells and gastrointestinal L-cells. Activation of the GPR119 receptor increases the cellular cAMP

levels, leading to glucose-dependent insulin secretion from the pancreatic β-cells.[4] In addition, activation of the GPR119 receptor in the gut results in the release of incretins, such as glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), from the enteroendocrine cells.[5] GLP-1 and GIP stimulate insulin secretion from the β-cells in a glucose-dependent manner and protect the β-cells against apoptosis.[6,7] This glucose-dependent dual mechanism of action suggests that GPR119 agonists can improve glycemic control without the risk of inducing hypoglycemia.

Oleoyl-lysophosphatidylcholine and oleoylethanolamide (OEA) have been identified as the endogenous agonists for the GPR119 receptor.[8,9] Many research groups have investigated small-molecule GPR119 agonists,[10,11] which has led to the development of several clinical compounds such as APD668,[12] GSK1292263,[13] MBX-2982[14] (Figure 1).

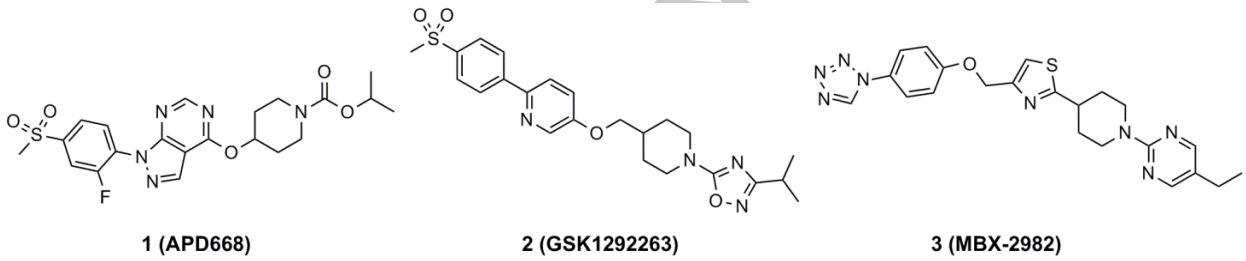


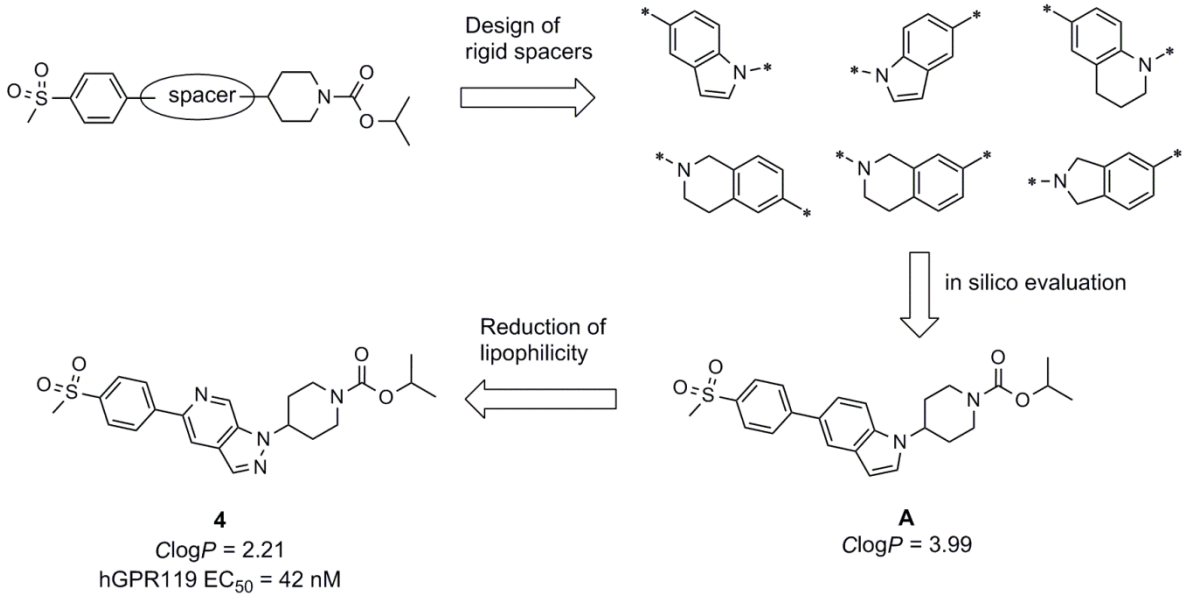
Figure 1. Representative GPR119 agonists.

We envisaged that most GPR119 agonists have a common structural feature: the methylsulfonyl-subsituted-phenyl group linked to a piperidine carbamate group (or its isostere) via a central spacer (Scheme 1).

In our medicinal chemistry program, one of the objectives was to identify an orally active small molecule GPR119 agonist with a novel structure. Therefore, we first pursued a highly potent GPR119 agonist, assuming that the orientation of the methylsulfonylphenyl group and piperidine carbamate group would have an impact on the agonist potency, and we designed compounds focusing on conformationally restricted central spacers as the first step.[15-18]

Evaluation of the designed spacers was conducted based on a ligand-based pharmacophore model. Pharmacophore generation was carried out with the compounds (**1**, **2**, **3**) using the "Common Feature Pharmacophore Generation" protocol in Discovery Studio (BIOVIA).[19] Conformations of the compounds were generated by the "FAST" method, and pharmacophore hypotheses were created with the default parameters of the protocol, with the exception of feature selection; hydrogen-acceptor, hydrophobic, and ring-aromatic features were selected for the generation. As shown in Figure 2, the compounds (**1**, **2**, **3**) overlapped closely and a six-feature hypothesis was created. Various fused rings, such as indole, tetrahydroquinoline, tetrahydroisoquinoline, isoindoline,

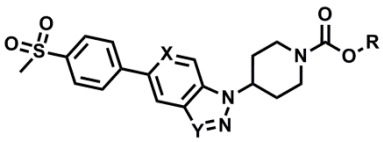
were designed as rigid spacers (Scheme 1). Among them, the indole derivative **A** fit well to the pharmacophore model (Figure 3). Since the lipophilicity of compound **A** itself was relatively high (*C*log*P* = 3.99), we successively designed several more hydrophilic compounds with the same 6-5 fused ring system containing two or more nitrogen atoms based on superposition of the compounds (**1**, **2**, **3** and **A**). The designed compounds as shown in Table 1 were synthesized and evaluated for hGPR119 agonism[20], which led to the identification of the novel 1*H*-pyrazolo[3,4-c]pyridine derivative **4** having an EC_{50} value of 42 nM.



Scheme 1. Design of the 1*H*-pyrazolo[3,4-c]pyridine derivative

Table 1

SAR of the 6-5 fused ring spacers



Compound	R	X	Y	hGPR119 EC_{50} (nM)	*C*log*P*[a]
4	*i*Pr	N	CH	42	2.21
5	*t*Bu	N	CH	20	2.60
6	*t*Bu	CH	CH	31	3.43
7	*t*Bu	CH	N	63	2.86

[a] The *Clog*P value was calculated using a software from Daylight Chemical Information Systems, Inc.

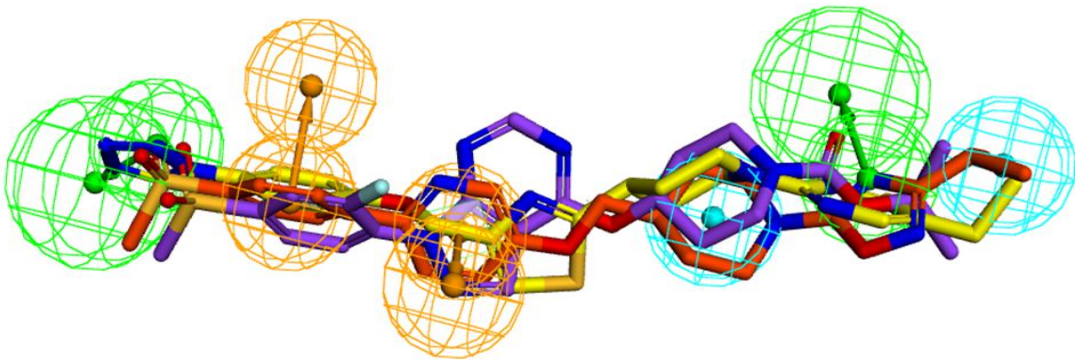


Figure 2. Superposition of compound **1** (carbon atoms are colored purple), **2** (orange), **3** (yellow) and a generated pharmacophore model. The pharmacophore model consists of two hydrogen bond acceptors (green arrows and spheres), two hydrophobic sites (cyan spheres) and two aromatic rings (orange arrows and spheres).

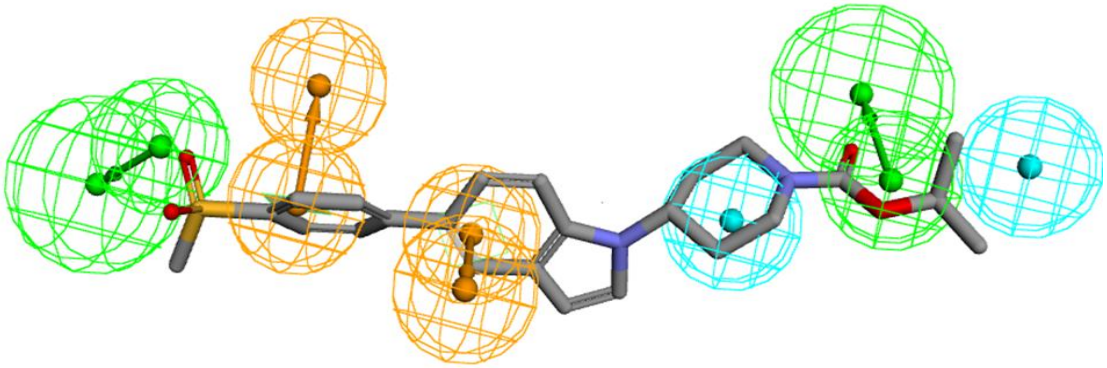
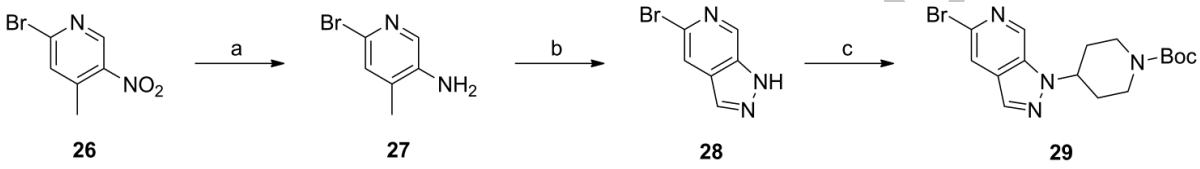


Figure 3. Mapping of compound **A** (gray) onto the pharmacophore model.

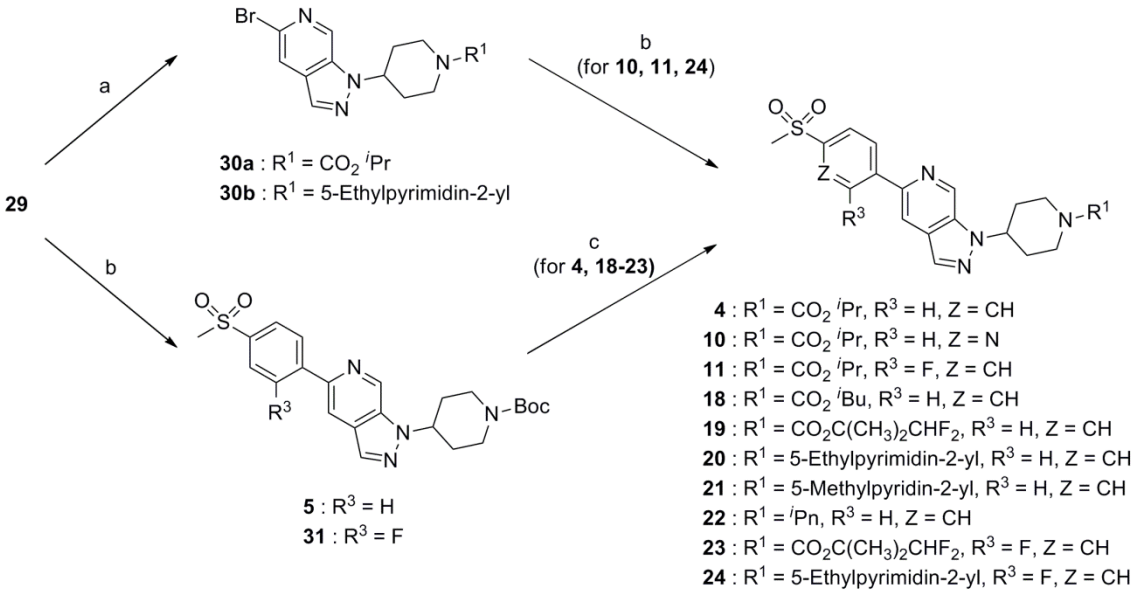
Herein, we describe the synthesis and structure-activity relationships (SARs) of this new series of 1*H*-pyrazolo[3,4-c]pyridine derivatives, focusing on improving the potency without increasing the lipophilicity.

This series of compounds was synthesized from commercially available 2-bromo-4-methyl-5-nitropyridine **26** (Schemes 2-5). Reduction of **26** with hydrogen in the presence of a catalytic amount of Raney-Ni yielded **27** (93% yield). Cyclization of **27** with $NaNO_2$ under acidic conditions led to the formation of **28** (59% yield). Treatment of **28** with *tert*-butyl 4-(methylsulfonyloxy)piperidine-1-carboxylate, followed by separation of the mixture of regioisomers by silica-gel column chromatography provided the key intermediate **29** (36% yield). Removal of the Boc group from **29** followed by treatment with isopropyl chloroformate or

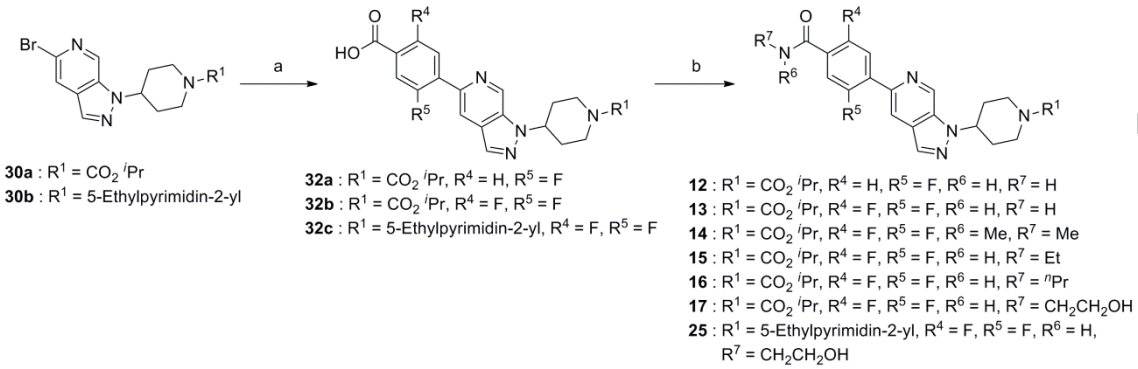
2-chloro-5-ethylpyrimidine yielded **30a** and **30b**, respectively. Suzuki coupling of **30a** or **30b** with an appropriate aryl boronic acid yielded the desired products **10**, **11** and **24** (Scheme 3, a-b). Compounds **4** and **18-23** were prepared from **29** via Suzuki coupling, deprotection and the *N*-capping reaction (Scheme 3, b-c). Amide compounds **12-17** and **25** were prepared by Suzuki coupling of **30a** or **30b**, followed by condensation with an appropriate amine using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI·HCl) and 1,2,3-benzotriazol-1-ol monohydrate (HOBt·H_2O) (Scheme 4).



Scheme 2. (a) H_2, Raney-Ni, THF, rt; (b) $NaNO_2$, AcOH, rt; (c) Cs_2CO_3, *tert*-butyl 4-(methylsulfonyloxy)piperidine-1-carboxylate, DMF, 80 ˚C.

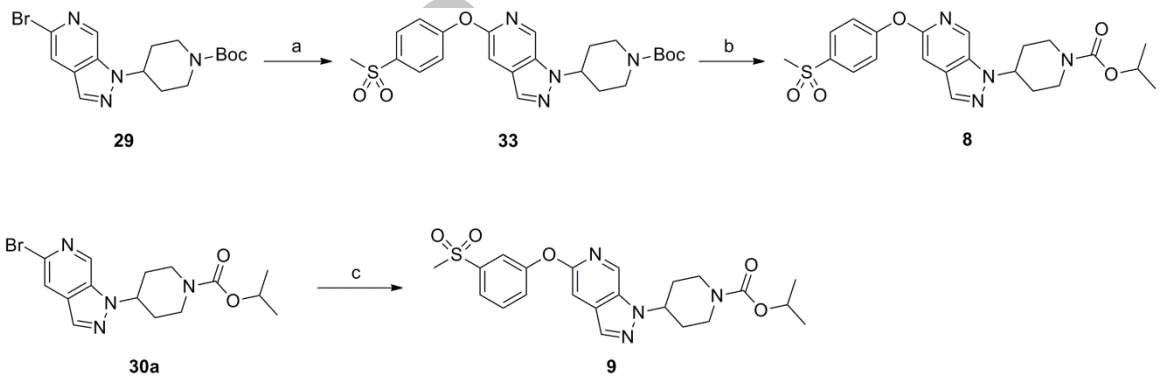


Scheme 3. (a) (i) HCl, MeOH, 1,4-dioxane, rt ; (ii) isopropyl chloroformate, DIPEA, $CHCl_3$, rt or 2-chloro-5-ethylpyrimidine, Cs_2CO_3, DMSO, 180 ˚C; (b) for **5**, **24**, $ArB(OH)_2$, $PdCl_2(dppf)\cdot CH_2Cl_2$, Na_2CO_3, H_2O, DMF, 100 ˚C; for **10**, **11**, **31**, $ArB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, H_2O, EtOH, microwave, 160 ˚C; (c) (1) HCl, MeOH, 1,4-dioxane, rt; (2) for **4**, **18**, alkyl chloroformate, Et_3N, $CHCl_3$, rt; for **19**, **23**, 1-{[(1,1-difluoro-2-methylpropan-2-yl)oxy]carbonyl}-3-methyl-1*H*-imidazol-3-ium iodide, Et_3N, $CHCl_3$, rt; for **22**, *i*PnBr, K_2CO_3, CH_3CN, reflux; for **20**, 2-chloro-5-ethylpyrimidine, Cs_2CO_3, DMSO, microwave, 180 ˚C; for **21**, 2-bromo-5-methylpyridine, Cs_2CO_3, DMSO, 180 ˚C.



Scheme 4. (a) $ArB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, H_2O, EtOH, microwave, 160 ˚C; (b) R^6R^7NH, EDCI·HCl, HOBt·H_2O, Et_3N, DMF, rt.

Compounds **8** and **9** were synthesized as shown in Scheme 5. Copper-mediated Ullmann-type coupling of **29** with 4-(methylsulfonyl)phenol using a catalytic amount of CuI and picolinic acid yielded **33** (42% yield). Deprotection of the Boc group followed by treatment with isopropyl chloroformate provided the desired compound **8**. Compound **9** was prepared from **30a** under similar conditions using the copper catalyst described above.



Scheme 5. (a) 4-(methylsulfonyl)phenol, CuI, picolinic acid, K_3PO_4, DMSO, 105 ˚C; (b) (i) HCl, MeOH, 1,4-dioxane, rt; (ii) isopropyl chloroformate, Et_3N, $CHCl_3$, rt; (c) 3-(methylsulfonyl)phenol, CuI, picolinic acid, K_3PO_4, DMSO, 100 ˚C.

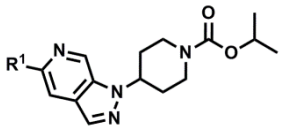
The synthesized compounds were evaluated for their GPR119 agonist potency by using a cAMP assay in a human GPR119 cell line.[20]

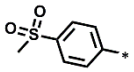
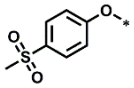
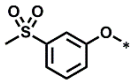
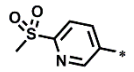
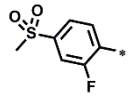
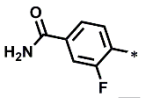
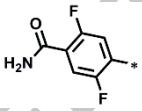
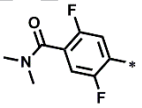
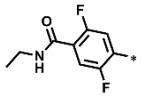
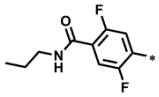
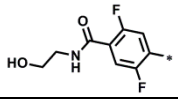
Initially, we conducted an SAR study focusing on the substituent (R^1) at the 5-position of the pyrazolopyridine ring (Table 2) of the representative compound **4**. In this study, we attempted to improve the potency of the compound. However, a high lipophilicity of the compound may cause poor solubility and ADME properties. Therefore, we monitored the LipE value (LipE = pEC_{50} - *C*log*P*)[21,22] to balance the potency and lipophilicity. Insertion of an oxygen atom between the

4-(methylsulfonyl)phenyl moiety and pyrazolopyridine ring of **4** led to a decrease in the potency. This result suggested that an increase in the flexibility of the R^1 substituent lowered the potency as we expected. Replacement of the 4-(methylsulfonyl)phenyl moiety with a 2-(methylsulfonyl)pyridin-5-yl one (**10**) resulted in a decrease of the agonist potency. Meanwhile, installation of a fluorine atom into the 2-position of the 4-(methylsulfonyl)phenyl group yielded compound **11**,[23,24] which showed comparable potency while maintaining the LipE value, as compared to **4**. Subsequently, further modifications of this R^1 substituent were conducted. Replacement of the methylsulfonyl group in **11** with a carbamoyl group (**12**) resulted in a 3-fold decrease of the potency. Installment of an additional fluorine atom (**13**) into the R^1 moiety of **12** was carried out, because the *meta*-fluoro-substituted aryl group is known as a potent left-hand component,[24] which improved the potency by twofold with an improved LipE value (4.89). The carbamoyl moiety of **13** was replaced with dimethylcarbamoyl (**14**), ethylcarbamoyl (**15**), propylcarbamoyl (**16**), and 2-hydroxyethylcarbamoyl (**17**) moieties to evaluate their effect on the agonist potency. The dimethylcarbamoyl derivative (**14**) was equipotent to the carbamoyl derivative (**13**). By contrast, ethylcarbamoyl (**15**), and propylcarbamoyl (**16**) yielded significant improvement in potency, although the LipE value of **16** was lower than that of the compounds **13** and **15**, due to its high lipophilicity. The 2-hydroxyethylcarbamoyl (**17**) showed a 9-fold decrease of the potency, while a high LipE value (4.95) was maintained, due to its low lipophilicity. We selected the 2-fluoro-4-methylsulfonyl-phenyl group (**11**), which conferred high potency with a good LipE value, and the 2,5-difluoro-4-((2-hydroxyethyl)carbamoyl)phenyl group (**17**), which conferred low lipophilicity with a good LipE value, as promising R^1 groups for the combination study described below (Table 4).

Table 2

SAR of the pyrazolopyridine substituents



Compound	R^1	hGPR119 EC_{50} (nM)	*C*log*P*[a]	LipE[b]
4		42	2.21	5.17
8		177	2.12	4.63
9		266	2.12	4.46
10		197	2.09	4.61
11		27	2.42	5.15
12		97	2.63	4.39
13		52	2.39	4.89
14		46	2.62	4.71
15		10	3.18	4.82
16		14	3.71	4.15
17		92	2.09	4.95

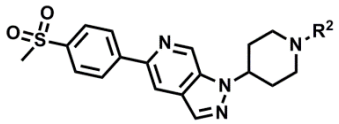
[a] The *C*log*P* value was calculated using a software from Daylight Chemical Information Systems, Inc.

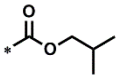
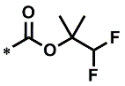
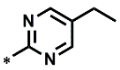
[b] LipE = pEC_{50} - *C*Log*P*

Next, we clarified the effect of the piperidine *N*-substituent (R^2) of **4** on the agonist potency (Table 3). In this study, we simultaneously investigated the relationship between the R^2 group and the human microsomal stability of the compounds. Therefore, we took into account not only the potency, but also the metabolic stability. Replacement of the *iso*-propylcarbamate of **4** with an *iso*-pentyl moiety yielded **22**, which was significantly less potent than **4**. Replacement of the *iso*-propylcarbamate with a *tert*-butylcarbamate (**5**) or an *iso*-butylcarbamate (**18**) improved the potency as compared with that of **4**. However, compound **18** was unstable in the human liver microsomal assay, probably due to the lability of the *iso*-butylcarbamate moiety. To prevent the metabolism without loss of potency, fluorine atoms were introduced into the alkyl group of the carbamate. Fluorinated *tert*-butylcarbamate (**19**) exhibited an increase in potency as compared to **4** and showed great stability in the human liver microsomes as expected. It is likely that replacement of the carbamate moiety in **4** with a carbamate isostere such as pyridine or pyrimidine, would maintain the high agonist potency. In fact, 5-ethylpyrimidine (**20**) resulted in a 3-fold increase in potency as compared to **4**, and 5-methylpyridine (**21**) also conferred good potency. However, improvement of the metabolic stability of these compounds was not obtained. The above rough SAR study on the agonist potency suggested that a hydrogen bond acceptor at the R^2 group was important for obtaining a high potency. We selected a fluorinated *tert*-butylcarbamate group (**19**), which conferred metabolic stability and 5-ethylpyrimidine (**20**), which conferred high potency as a promising R^2 group for the combination study described in Table 4.

Table 3

SAR of the piperidine substituents



Compound	R^2	hGPR119 EC_{50} (nM)	*C*log*P*[a]	LipE[b]	hMS(%)[c]
4		42	2.21	5.17	24.1
5		20	2.60	5.09	NT[d]
18		11	2.82	5.13	79.8
19		23	2.65	4.99	3.9
20		13	2.03	5.85	20.3
21		30	2.27	5.25	44.1
22		220	3.10	3.55	NT[d]

[a] The *C*log*P* value was calculated using a software from Daylight Chemical Information Systems, Inc.

[b] LipE = pEC_{50} - *C*log*P*

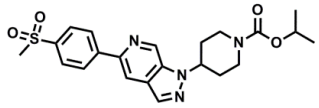
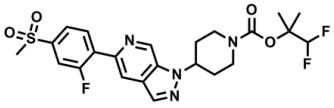
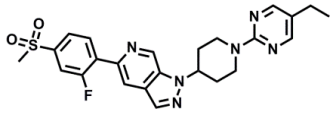
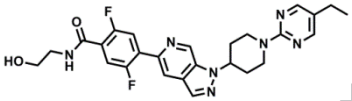
[c] % Metaborized after 15-min incubation with human liver microsomes (1 mg protein/mL).

[d] Not tested.

Finally, we optimized the 1*H*-pyrazolo[3,4-c]pyridine derivatives by a combination of the selected left-hand aryl groups and right-hand piperidine *N*-capping groups (Table 4). The compound **23** showed a higher potency and better metabolic stability than **4**, as expected. However, its aqueous solubility was extremely poor. The compound (**24**), containing a combination of a 4-methylsulfonyl-2-fluorophenyl group (a left-hand substituent) with a 5-ethylpyrimidin-2-yl group (a right-hand substituent), exhibited the highest agonist potency (hGPR119 EC_{50} = 4 nM) among this class of compounds. The 2-hydroxyethylcarbamoyl derivative (**25**) possessed an EC_{50} value of 15 nM, but was metabolically unstable.

Table 4

Combinations of promissing components

Compound	Structure	hGPR119 EC_{50} (nM)	hMS(%)[a]	Solubility (μg/ml)[b]	*C*log*P*[c]	LipE[d]
4		42	24.1	0.67	2.21	5.17
23		10	2.1	<0.06	2.87	5.15
24		4	14.3	0.34	2.25	6.12
25		15	35.8	0.69	1.91	5.92

[a] % Metabolized after 15-min incubation with human liver microsomes (1 mg protein/mL).

[b] Thermodynamic solubility in pH6.8 phosphate buffer.

[c] The *C*log*P* value was calculated using a software from Daylight Chemical Information Systems, Inc.

[d] LipE = pEC_{50} - *C*Log*P*

Given the promising potency along with metabolic stability, we conducted a pharmacokinetic study of compound **24** (TP0456330) in SD rats. The PK parameters are shown in Table 5. Unfortunately, this compound showed poor bioavailability, although it had a low systemic clearance and a low volume of distribution. It exhibited high membrane permeability (112×10^{-6} cm/s at pH6.2) in the PAMPA assay. These results suggested that the low bioavailability of **24** may be due to its low aqueous solubility. To identify compounds for in vivo efficacy studies, further improvement of the aqueous solubility would be required.

Table 5

Pharmacokinetic parameters of **24** in SD rats

Compound	IV (3 mg/kg)			PO (10 mg/kg)	
	CL (mL/h/kg)	Vdss (mL/kg)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng · h/mL)	%F
24	759	537	0.617	1930	12.6

In summary, we identified *1H*-pyrazolo[3,4-c]pyridine derivatives as a novel structural class of GPR 119 agonists using a ligand based pharmacophore model. To identify a highly potent compound in this series, we conducted an exploratory SAR study, which led to the identification of compound **24** (TP0456330) having the most potent agonism of hGPR119 (EC_{50} = 4 nM). However, compound **24** exhibited low bioavailability in rat, probably due to its poor solubility. Further optimization of the *1H*-pyrazolo[3,4-c]pyridine series will be reported in due course.

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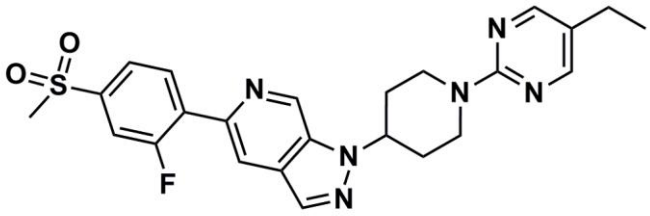
Graphical abstract



24

hGPR119 EC_{50} = 4 nM