Synthesis and Pharmacological Profile of a New Selective G Protein-Coupled Receptor 119 Agonist; 6-((2-Fluoro-3-(1-(3-isopropyl-1,2,4-oxadiazol-5-yl)piperidin-4-yl)propyl)amino)-2,3-dihydro-1*H*-inden-1-one

Masao Sakairi, Masakazu Kogami, Masafumi Torii, Mitsuhiro Makino, Daisuke Kataoka, Ryuji Okamoto, Toshiyuki Miyazawa, Megumi Inoue, Naoki Takahashi, Satoko Harada, and Nobuhide Watanabe*

Drug Discovery Laboratories, Sanwa Kagaku Kenkyusho Co., Ltd.; 363 Shiosaki, Hokusei-cho, Inabe, Mie 511–0406, Japan.
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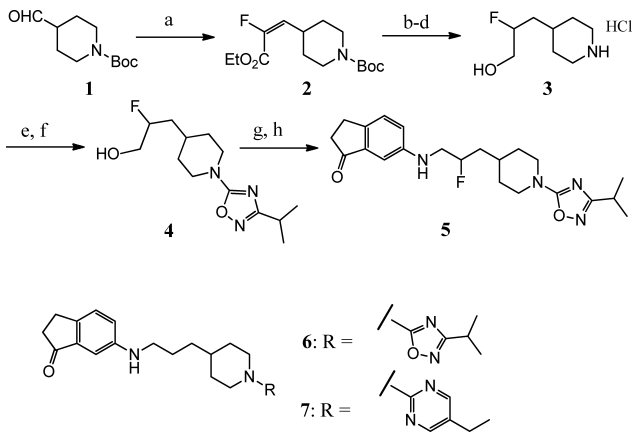
6-((2-Fluoro-3-(1-(3-isopropyl-1,2,4-oxadiazol-5-yl)piperidin-4-yl)propyl)amino)-2,3-dihydro-1*H*-inden-1-one is a potent drug-like G protein-coupled receptor 119 (GPR119) agonist. It is hoped that this compound would be instrumental in probing the pharmacological potential of GPR119 agonists.

Key words glucagon-like peptide-1; G protein-coupled receptor 119 agonist; glucose-dependent insulin secretagogue; gastric emptying

Obesity is strongly associated with insulin resistance and can therefore be problematic in the management of type 2 diabetes mellitus (T2DM).[1,2] Ironically, treatment of T2DM also targets obesity, although oral medications, such as sulfonylureas and thiazolidinediones are known to hardly achieve weight loss. Glucose-dependent insulin secretagogues, such as glucagon-like peptide-1 (GLP-1) analogs and dipeptidyl peptidase-IV (DPP-4) inhibitors have recently emerged as new agents for the treatment of T2DM.[3] Although both GLP-1 analogs and DPP-4 inhibitors improve glycemic control and minimize hypoglycemia, GLP-1 analogs can produce weight loss, while DPP-4 inhibitors merely suppress body weight gain. Very recently, G protein-coupled receptor 119 (GPCR 119, or GPR119) agonists have received considerable attention as a promising therapeutics for the treatment of T2DM.[4,5] GPR119 is a membrane receptor expressed in pancreatic islet β-cells, and its activation enhances insulin secretion in glucose-dependent manner. GPR119 expression has also been detected in murine intestinal L- and K-cell lines. In fact, treatment with AR231453, a GPR119 agonist has been reported to enhance secretion of both GLP-1 and gastric inhibitory polypeptide (GIP) in glucose-challenged mice.[6] It is therefore expected that GPR119 agonists would improve glucose tolerance with minimum hypoglycemia in T2DM patients and produce weight loss by reducing food intake. Based on this hypothesis, a large number of patents and publications regarding GPR119 have been disclosed and several GPR119 agonists such as APD668,[7] MBX-2982 and GSK-1292263A have been under development.[5,8] However, there are some conflicting results from the recent publication, and the high attrition rates of early-stage development also suggest that the potential of GPR119 agonists for treatment of T2DM is still elusive.[9,10] Apparently, drug-like, selective GPR119 agonists provide a useful tool for probing the physiological roles and pharmacological potential of GPR119 agonists. Recently, we reported synthesis and pharmacological profiles of a new series of GPR119 agonists having high potency.[11] This series of agonists (*e.g.* **6**, **7**) feature small species difference in the *in vitro* potency between mouse and human, and good physical properties such as water solubility and molecular weight. Further investigation has been carried out to improve these profiles. In this communication, we are pleased to report the synthesis and pharmacological profile of a new, selective GPR119 agonist, 6-((2-fluoro-3-(1-(3-isopropyl-1,2,4-oxadiazol-5-yl)piperidin-4-yl)propyl)amino)-2,3-dihydro-1*H*-inden-1-one (**5**).[12]

Synthesis of **5** is shown in Chart 1. The piperidine-3-propanol **3** was prepared in 79% yield *via* Horner–Wadsworth–Emmons type reaction of the commercially available aldehyde **1**. 1,2,4-Oxadiazole ring was introduced in 80% yield by a two-step sequence according to a reported protocol.[13] Oxidation of **4** with Dess–Martin reagent followed by reductive amination with the commercially available 6-aminoindan-1-one gave **5** in 46% yield after recrystallization.

Compound **5** *in vitro* potency was determined using GPR119 homogeneous time resolved fluorescence (HTRF) cAMP activation assay, and its GPR119 agonistic activity was determined as percentage of maximum activation (100%) produced by the reference AR231453. As shown in Table 1, compound **5** exhibited good *in vitro* potency (hEC$_{50}$: 33 nM; hE$_{max}$; 100%) across the tested species, although its rat EC$_{50}$ was lower than that in human or mouse. Pharmacokinetic (PK) studies showed that compound **5** has low clearance (0.89 L/h/kg) and good oral bioavailability (58%) in rats, and relatively high clearance (1.71 L/h/kg) and good oral bioavailability (68%) in mice (Table 2). Next, we decided to conduct oral glucose tolerance test (OGTT) with compound **5** and the selected data are shown in Table 3. As shown in Table 3, compound **5** (30, 10 mg/kg, *per os* (*p.o.*)) significantly reduced $AUC_{0-120\,min}$ by 27 and 37% in rats and mice, respectively. On the other hand, administration of **5** to overnight-fasted normal rats produced no reduction in blood glucose level even at a dose



Reagents and conditions: a) $(EtO)_2POCHFCO_2Et$, *n*-BuLi, THF, 0°C; b) H_2, 10% Pd–C, EtOH, rt; c) $LiAlH_4$, THF, 0°C; d) 4 N HCl/dioxane, 0°C; e) BrCN, aq. $NaHCO_3$, CH_2Cl_2; f) $ZnCl_2$, amidine, AcOEt–THF, reflux then HCl–EtOH, reflux; g) Dess–Martin reagent, CH_2Cl_2, rt; h) 6-aminoindan-1-one, $NaBH(OAc)_3$, CH_2Cl_2, rt.

Chart 1. Synthesis of Compound **5** and Related Compounds **6** and **7**

The authors declare no conflict of interest.

* To whom correspondence should be addressed. e-mail: no_watanabe@skk-net.com

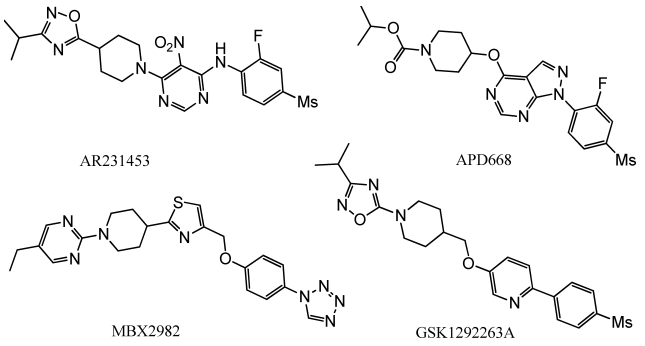


Fig. 1. GPR119 Agonists in Clinical Trials and AR231453

Table 1. Properties of Compound **5**

GPR119 activity (EC_{50}; nM)		Solubility (μM)[c]	
Human	33 (100%)[a]	pH 1.2	80.5
Rat	125	pH 6.5	40.7
Mouse	47 (116%)[a]	Permeability ($\times$10-6 cm/s)[d]	
Protein binding (%)[b]		pH 6.2	50
Human	99.5	CL_{int} (L/h/kg)[e]	
Rat	98.6	RLM	10
Mouse	99.2	HLM	4.6

a) Values in parentheses indicate % maximum (the max. response of AR231453 was defined as 100% activation). *b*) Assayed using Rapid Equilibrium Dialysis (Thermo Fisher Scientific, Waltham, MA, U.S.A.). *c*) Measured using dimethyl sulfoxide (DMSO) solution precipitation method. *d*) Assayed using PAMPA Explorer™ (pION, Woburn, MA, U.S.A.). *e*) Calculated from substrate disappearance rate in liver microsomes as L/h/kg body weight. The assay was carried out by incubation of 5 μM test compound with human (HLM) or rat (RLM) liver microsomes (Xenotech, Lenexa, KS, U.S.A.), NADPH and buffer at 37°C for 30 min and measurement of percent compound remaining by precipitation method followed by LC-MS-MS analysis.

as high as 100 mg/kg, while other insulin secretagogues, such as nateglinide and glimepiride significantly reduced blood glucose levels below normal, suggesting that **5** induces a glucose-dependent effect on insulin secretion (Fig. 2). In order to see if compound **5** has beneficial effect on gastric emptying, acetaminophen (APAP) absorption test was conducted.[10] Fasted mice were orally treated with 10 mg/kg of **5** followed 0.5 h later by APAP (100 mg/kg, *p.o.*) in a 2 g/kg glucose-containing aqueous solution. Plasma APAP concentrations were determined 10 and 20 min after APAP administration. As expected, compound **5** produced a significant decrease in plasma APAP, indicating delay of gastric emptying (Table 4). Of particular interest, closely related compound **7** showed no effect on gastric emptying in APAP absorption test at a dose as high as 30 mg/kg, *p.o.* (data not shown), although compound **7** produced almost equal GLP-1 secretion compared to that reported for MBX-2982.[11] In addition, another related compound **6** produced no significant reduction in OGTT in mice at a dose of 10 mg/kg, *p.o.* Evidently, these data show superiority of compound **5** over compounds **6** and **7** for oral efficacy.

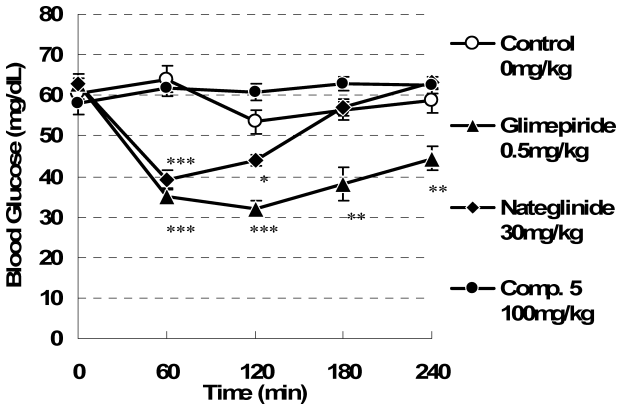


Fig. 2. Effect of 5 on Fasting Normoglycemia in Rats

Male Wistar/ST rats (8 weeks old) were used. Compounds were administered orally at time 0 min to overnight-fasted animals (n=8/group). The plasma glucose levels were measured using a portable glucometer (GLUTEST ProR; Sanwa Kagaku Kenkyusho, Japan). Statistical significance was tested using a one-way ANOVA and the Dunnett multiple comparison test. *p<0.05, **p<0.01, ***p<0.001 *vs.* vehicle control. Data are expressed as the mean±S.E.M.

Table 3. OGTT of Compound **5** in Rats and Mice[a]

Species, dose (mg/kg)	$\Delta AUC_{0-120\,min}$ of cntl (mg/dL·min)	$\Delta AUC_{0-120\,min}$ (mg/dL·min)	% reduction
Rat, 30	9762±477	7156±464**	27
Mouse, 10	12116±843	7668±321***	37

a) Male Wistar/ST rats (8 weeks old) and male C57BL/6J mice (8 weeks old) were used. **5** was administered orally (vehicle; 10% DMSO–5% hydroxypropyl-β-cyclodextrin (HPbCD)/H_2O) to overnight-fasted animals (n=8/group) 30 min before glucose loading (2 g/kg). Plasma glucose levels were measured using a portable glucometer (GLUTEST ProR; Sanwa Kagaku Kenkyusho, Japan). Statistical significance was determined using one-way ANOVA and Dunnett multiple comparison test. **p<0.01, ***p<0.001 *vs.* vehicle control. ΔAUC are expressed as mean±S.E.M.

Table 4. Effect of Compound **5** on Gastric Emptying[a]

	Plasma APAP conc. (mM)		$AUC_{0-20\,min}$ (mM·min)
	10 min	20 min	
Control	0.362±0.015	0.389±0.014	5.57±0.21
5	0.312±0.008*	0.336±0.012*	4.80±0.12*

a) Male C57BL/6J mice (8 weeks old) were used. Statistical significance was determined using one-way ANOVA and Dunnett multiple comparison test. *p<0.05 *vs.* vehicle control. Data are expressed as mean±S.E.M.

From a safety point of view, compound **5** was found to have excellent preclinical safety profile with no inhibition of liver metabolic enzymes, such as CYP3A4, CYP2C9, and CYP2D6 (IC_{50} >10 μM), and no induction of CYP3A4 and CYP1A2 at a concentration of 50 μM. Compound **5** also showed no significant inhibition of the human ether-à-go-go related gene channel in patch clamp using human embryonic kidney 293

Table 2. Selected PK Parameter of Compound **5**[a]

Species	Dose, i.v./oral (mg/kg)	i.v. $t_{1/2}$ (h)	CL_p (L/h/kg)	V_{dss} (L/kg)	Oral $t_{1/2}$ (h)	C_{max} (ng/mL)	T_{max} (h)	BA (%)
Rat	0.5/3.0	2.24	0.89	2.31	3.67	315	2.11	58
Mouse	1.0/5.0	1.16	1.71	1.87	1.07	1090	0.17	68

a) Male Wister/ST rats (9 weeks old) and male CD-1 (ICR) mice (7 weeks old) were acclimated to experimental conditions 7–14 d before use, and had free access to food and water throughout the acclimatization period. The animals were fasted overnight, and **5** was administered intravenously *via* the tail vein or orally (by gavage) at the indicated doses (n=3 in rats, n=2 in mice) as a solution in 10% DMSO and 5% HPbC in H_2O. Blood samples were taken periodically and the plasma was analyzed by LC-MS-MS with quantitation against a standard curve.

cells (IC_{50}; 27.4 μM). Finally, compound **5** was negative in Ames mutagenicity test and showed low toxicity in cell toxicity assay using Chinese hamster ovary cells (CC_{50} value of 31 μM). In a panel of 68 receptor binding/ion channel assays, compound **5** (10 μM) showed no specific binding greater than 50% in any of the 68 assays, except for the assay with 5-hydroxytryptamine$_{2B}$ (97% inhibition at 10 μM).[14] In a calcium mobilization assay, compound **5** showed no agonist/antagonist activity towards other human pancreatic islet GPCRs, including GPR40, GPR41, GPR43, and GPR120.[15]

In conclusion, we have demonstrated that compound **5** has drug-like properties and improves glucose tolerance without affecting fasting normoglycemia. It is therefore hoped that compound **5** can play an instrumental role in probing the pharmacological potential of GPR119 agonists.

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12) **5**: Pale yellow needles. mp 88–90°C. IR (KBr) 3402, 2926, 1699, 1598, 1505, 1406, 1263, 837 cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.27 (1H, d, J=8.1 Hz, Ph), 6.93 (1H, dd, J=8.1, 2.4 Hz, Ph), 6.89 (1H, d, J=2.4 Hz, Ph), 4.84 (1H, dddd, J=50.4, 10.5, 6.9, 2.9 Hz, CHF), 4.14 (3H, dd, J=12.6, 2.0 Hz, CH_2N and NH), 3.46–3.21 (2H, m, N(H)CH_2), 3.07 (2H, ddd, J=12.6, 3.9, 2.9 Hz, CH_2N), 3.02 (2H, t, J=5.5 Hz, CH_2Ph), 2.88 (1H, sep, J=6.9 Hz, CH from iPr), 2.68 (2H, t, J=5.5 Hz, $COCH_2$), 1.94–1.68 (4H, m, CH_2 and CH from piperidine and $CFCH_2$), 1.61–1.20 (9H, m, CH_3 from iPr, CH_2 from piperidine and $CFCH_2$). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 207.4, 175.7, 170.8, 147.2, 145.5, 138.1, 127.16, 122.5, 104.2, 90.4 (d, J=169.9 Hz), 48.4 (d, J=21.2 Hz), 46.1, 46.0, 3.92 (d, J=20.1 Hz), 36.9, 31.94, 31.92, 30.94, 27.0, 24.9, 20.4. HR-MS Calcd for $C_{20}H_{29}FN_4O_2$ (M^++1), 401.2347; Found, 401.2352.
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