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Letter

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Potent and Orally Bioavailable GPR142 Agonists as Novel Insulin Secretagogues for the Treatment of Type 2 Diabetes

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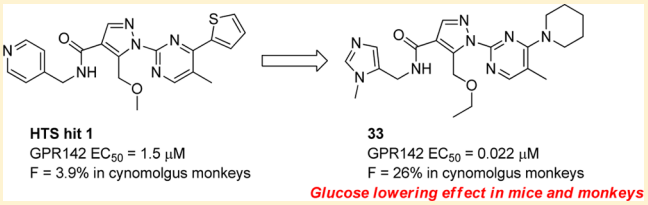
Supporting Information

ABSTRACT: GPR142 is a G protein-coupled receptor that is predominantly expressed in pancreatic β-cells. GPR142 agonists stimulate insulin secretion in the presence of high glucose concentration, so that they could be novel insulin secretagogues with reduced or no risk of hypoglycemia. We report here the optimization of HTS hit compound **1** toward a proof of concept compound **33**, which showed potent glucose lowering effects during an oral glucose tolerance test in mice and monkeys.



KEYWORDS: *GPR142, agonist, insulin secretagogue, diabetes, glucose lowering*

Type 2 diabetes is characterized by high blood glucose resulting from reduced insulin production from pancreatic β-cell or insulin resistance or both of them.[1] Uncontrolled hyperglycemia increases the risk of cardiovascular complications such as coronary heart disease, stroke, nephropathy, neuropathy, and retinopathy in patients with diabetes.[2] Thus, effective glycemic control is important to prevent chronic diabetic complications. Currently, insulin secretagogues such as sulfonylureas and meglitinide are widely used for patients with a moderate degree of β-cell dysfunction.[3] They trigger insulin release independently of blood glucose level. As a result, some of them could increase the risk of hypoglycemia.[4] Therefore, novel glucose dependent insulin secretagogues, such as DPP-IV inhibitors,[5] GLP-1 analogues,[5] GPR40 agonists,[6] and GPR119 agonists,[7] are highly attractive alternatives for the treatment of diabetes.

GPR142 was identified as an orphan G protein-coupled receptor (GPCR). GPR142 is predominantly expressed in pancreatic β-cells and coupled with the Gq signaling pathway, and its endogenous ligands are aromatic amino acids with tryptophan, which is the most potent ligand in inositol phosphate accumulation assay (IP assay).[8] Tryptophan stimulates insulin secretion from isolated mice pancreatic islets only under high glucose conditions and oral administration of tryptophan improves glucose tolerance in mice. These findings indicate that GPR142 agonists could be novel insulin secretagogues with reduced or no risk of hypoglycemia.

High throughput screening (HTS) campaigns identified several hit compounds including compound **1** (Figure 1) as well as phenylalanine derivatives[9,10] already reported. Compound **1** showed modest agonistic activity against human GPR142 (IP assay, EC_{50} = 1.5 μM). Compound **1** potentiated insulin secretion in rat primary islets at high glucose conditions, but not at low glucose conditions (see Supporting Information). This data showed the glucose-dependent insulin secretion of GPR142 agonist. Subcutaneous injection of compound **1** improved glucose tolerance in mice. However, poor pharmacokinetic (PK) profile of compound **1** was inadequate for further proof of concept studies in vivo. High in vivo clearance (1.3 L/h/kg) and very poor oral bioavailability (F = 3.9%) of compound **1** in monkey are presumably due to metabolic instability (Table 4). Moreover, compound **1** inhibited CYP3A4 (IC_{50} = 2.9 μM), which can cause unfavorable drug−drug interaction.

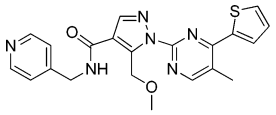


Figure 1. Structure of GPR142 agonist from HTS.

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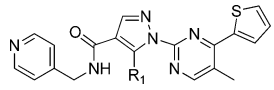
We describe here the optimization of a HTS hit compound **1** to provide a potent orally bioavailable GPR142 agonist and its pharmacological effects in mice and monkeys.

First, we investigated the structure−activity relationship (SAR) of the pyrazole side chain (Table 1). Synthesis of

Table 1. SAR of Pyrazole Side Chains

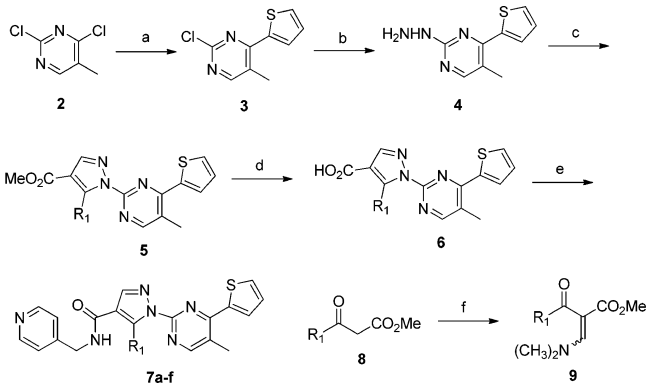


cmpd	R_1	hGPR142 EC_{50} (μM)[a,b]	MLM[c] (% remaining)
1	MOM	1.5	3
7a	Et	1.0	NT
7b	*n*Pr	0.25	1
7c	*n*Bu	0.0070	NT
7d	*n*Pent	1.0	NT
7e	secBu	1.2	NT
7f	CH_2OEt	0.030	NT

[a]Assay protocols are provided in the Supporting Information. [b]Assay results are the average of three replicates; standard deviation was ±20%. [c]% Remaining after incubation for 15 min in mouse liver microsomes. NT = not tested.

compounds **7a−f** started from Suzuki coupling between 2,4-dichloro-5-methylpyrimidine (**2**) and thiopheneboronic acid to give **3** (Scheme 1).[11] Compound **3** was heated with NH_2NH_2

Scheme 1. General Synthesis of Compound[a]

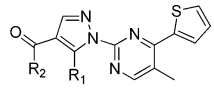


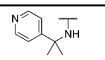
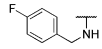
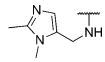
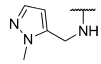
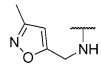
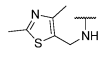
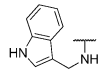
[a]Reagents and conditions: (a) 2-thiopheneboronic acid, $Pd(PPh_3)_4$, K_2CO_3, DME, reflux; (b) NH_2NH_2, pyridine; (c) **9**, HOAc, EtOAc; (d) LiOH, THF/H_2O; (e) 4-(aminomethyl)pyridine, HBTU, iPr_2NEt, DMF; (f) *N*,*N*-dimethylforamide dimethyl acetal, heat.

in pyridine to give **4**. Keto-ester **9** was conveniently prepared by heating **8** with *N*,*N*-dimethylforamide dimethyl acetal. Condensation of **4** and **9** under acidic conditions resulted in methyl ester **5**, which was hydrolyzed to give acid **6**. Finally, coupling of **6** with 4-(aminomethyl)pyridine using HBTU gave compounds **7a−f**. *n*-Propyl (**7b**) and *n*-butyl (**7c**) derivatives were found to improve agonistic activities, but their poor metabolic stability was not improved. The size of alkyl side chains seems to be important for GPR142 agonism.

Next, we explored the 4-pyridyl headgroup in the compound **7b** or **7c** to improve metabolic stability (Table 2). Compounds **10−22** were synthesized with the same method in Scheme 1. Dimethyl substitution (**10**) at benzyl position was tolerated in terms of agonistic activity but did not improve metabolic stability. Substitution on the amide nitrogen (**11**) resulted in

Table 2. Exploration of 4-Pyridyl Head Group



cmpd	R_2	R_1	hGPR142 EC_{50} (μM)[a,b]	MLM/HLM[c] (% remaining)
10		*n*Pr	0.0050	2/NT
11		*n*Pr	3.0	0/NT
12		*n*Bu	inactive	2/NT
13		*n*Bu	0.0010	56/70
14		*n*Pr	15	4.7/NT
15		*n*Bu	0.0010	NT/19
16		*n*Bu	0.030	6/20
17		*n*Bu	0.10	5/22
18		*n*Bu	0.00020	93/NT
19		*n*Bu	0.090	13/NT
20		*n*Pr	>20	11/NT
21		*n*Bu	0.20	10/NT
22		*n*Bu	>20	NT/NT

[a]Assay protocols are provided in the Supporting Information. [b]Assay results are the average of three replicates; standard deviation was ±20%. [c]% Remaining after incubation for 15 min in mouse liver microsomes or human liver microsomes. NT = not tested.

significant loss of GPR142 potency. Replacement of pyridine ring with 4-fluorophenyl ring (**12**) gave an inactive analogue. 5-Substituted imidazole derivative (**13**) showed very potent agonistic activity and modest metabolic stability. In contrast, 4-substitued imidazole derivative (**14**) lost its GPR142 agonistic activity. The orientation of a nitrogen lone pair is likely to be crucial to GPR142 agonism. Methyl substitution at 4-position (**15**) or 2-position (**16**) in the imidazole ring of **13** was tolerated in agonistic activity. However, metabolic stability of both compounds was significantly decreased. A conformationally restricted analogue (**17**), which might resist metabolism, resulted in the loss of agonistic activity and no improvement of metabolic stability. Introduction of carboxylic acid in the headgroup (**18**) was tolerated and improved metabolic stability;

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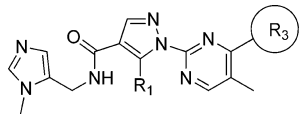
Table 3. Substitution of Thiophene Ring



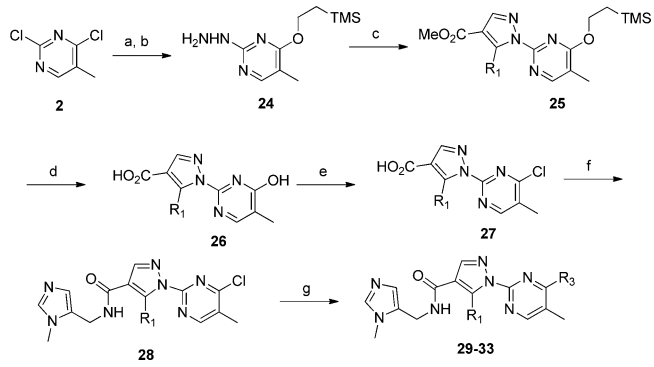
cmpd	R_1	R_3	hGPR142 EC_{50} (μM)[a,b]	HLM/MkLM/RLM[c] (% remaining)	PAMPA pH5/7.4 (x 10^{-6} cm/s)	LogD (PH7.4)
13	*n*Bu		0.0010	74/40/54	3.6/8	3.5[d]
23	CH_2OEt		0.0013	71/34/NT	2.0/4.0	2.2
29	CH_2OEt		0.096	77/66/77	1.2/2.9	NT
30	CH_2OEt		0.025	87/68/NT	0.2/1.6	NT
31	CH_2OEt		0.028	76/78/NT	0.2/1.6	NT
32	CH_2OEt		0.014	69/46/66	0.8/3.1	2.o
33	CH_2OEt		0.022	88/61/53	1.5/3.5	2.0

[a]Assay protocols are provided in the Supporting Information. [b]Assay results are the average of three replicates; standard deviation was ±20%. [c]% Remaining after incubation for 30 min in human, monkey, and rat liver microsomes. [d]Calculated by ACD. NT = not tested.

however, its membrane permeability was too low to achieve oral bioavailability. Pyrazole derivative (**19**) was a modest agonist, which had poor metabolic stability. Isoxazole (**20**), thiazole (**21**), and indole (**22**) derivatives were less potent agonists than imidazole derivatives. By this moment, we selected the imidazole derivative **13** for further optimization.

Introduction of the ethoxymethyl side chain instead of the butyl side chain gave compound **23**, which showed comparable GPR142 agonistic activity and metabolic stability to **13**; moreover, its hydrophobicity (LogD = 2.2) was in an optimal range (Table 3).[12] Thiophene is known to give reactive metabolite in vivo and have potential of idiosyncratic toxicity,[13] in that we investigated substitution of thiophene ring particularly with a nonaromatic saturated group. To facilitate the exploration of replacements for the thiophene, an efficient synthesis was developed to allow the introduction of this moiety at the last step, as shown in Scheme 2. Compound **24** was obtained by treatment of **2** with the sodium salt of 2-(trimethylsilyl)ethanol in THF according to Lluis et al.[14] A two-step sequence to install the pyrazole (as described in Scheme 1) gave compound **25**, which was treated with TBAF in MeCN to liberate the hydroxypyrimidine. The methyl ester was then hydrolyzed to the acid **26**. The acid **26** was heated at reflux with $POCl_3$ to provide 4-chloro pyrimidine acid chloride, which was isolated as an acid **27** after workup in ice water. Coupling with the methyl imidazole amine gave **28**, a key precursor for the preparation of **29**–**33**. Ether and amine derivatives were made by direct nucleophilic aromatic displacement with the appropriate sodium alkoxide or with an excess of the amine in DMF. Compound **29** having ethoxy moiety was found to be a modest GPR142 agonist. A series of alkyl amine analogues (**30**–**33**) showed good GPR142 agonistic activity as well as good metabolic stability. Cyclobutylamine (**30**) and azetidine (**31**) derivatives had poor membrane permeability. Pyrrolidine (**32**) and piperidine (**33**) derivatives had good

Scheme 2. Synthesis of 4-Substituted Analogues to Replace Thiophene[a]



[a]Reagents and conditions: (a) 2-(trimethylsilyl)ethanol, NaH, THF; (b) NH_2NH_2, pyridine; (c) **9**, HOAc, EtOAc; (d) 1. TBAF, THF, MeCN; 2. LiOH, THF/H_2O; (e) $POCl_3$, 110 °C, quench with H_2O; (f) 1-methyl-5-(aminomethyl)-1*H*-imidazole, HBTU, iPr_2NEt, DMF; (g) alkoxide or amine, DMF.

membrane permeability with increasing hydrophobicity. Unfortunately, CYP inhibition of a series of compounds described here was still not appropriate for further development (CYP3A4 IC_{50} = 1.0 μM for **33**). We assessed PK profiles of **33** in monkeys. Compound **33** demonstrated good oral bioavailability (F = 26%), which was consistent with its good metabolic stability (Table 4).

Therefore, we selected **33** as a proof of concept compound for in vivo studies in mice (Figure 2) and monkeys (Figure 3). C57BL/6J mice were dosed orally with compound **33**. After that, mice were challenged with an oral glucose, and blood samples were collected for the assay of blood glucose levels. Compound **33** (1–30 mg/kg) decreased blood glucose concentration in C57BL/6J mice in a dose dependent manner. The maximum glucose lowering effect of **33** is similar to that of

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Table 4. Monkey PK Parameters of 33

cmpd	Cl (L/h/kg)[a]	V_{dss} (L/kg)[a]	$t_{1/2}$ (h)[b]	F (%)[b]
1	1.3	1.0	NA[c]	3.9
33	1.0	0.77	1.2	26

[a]Compounds were dosed iv at 0.5 mg/kg. [b]Compounds were dosed po at 1 mg/kg. [c]Not applicable.

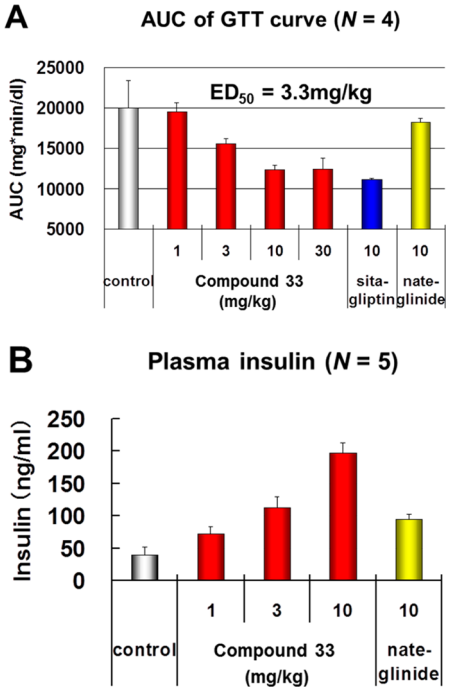

Figure 2. In vivo efficacy of **33** in mice. (A) Oral glucose tolerance test (C57BL/6J mouse). (B) Plasma insulin level at 15 min after bolus dosing (*ob/ob* mouse).

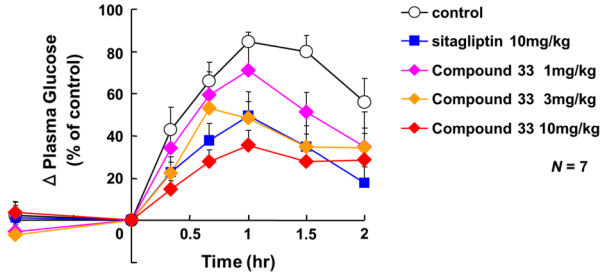

Figure 3. In vivo efficacy of **33** in monkeys with impaired glucose tolerance.

sitagliptin,[15] a marketed DPP-IV inhibitor. Because plasma insulin level of C57BL/6J mice was so low that it was difficult to detect the increase, we confirmed the plasma insulin increase based on GPR142 activation by using *ob/ob* mice, the diabetic animal model that strongly secreted insulin. Obese *ob/ob* mice were dosed orally with compound **33**, and blood samples were collected 20 min after dosing for insulin measurement. Compound **33** (1−10 mg/kg) increased plasma insulin concentration in a dose dependent manner.

Compound **33** was evaluated for its ability to improve glycemic control in cynomolgus monkeys (Figure 3). Single oral dosing of **33** (1−10 mg/kg) reduced the blood glucose level in a dose dependent manner during an oral glucose tolerance test. Furthermore, at a dose of 10 mg/kg, compound **33** tended to show greater efficacy than sitagliptin.[15]

In conclusion, we described the optimization leading from HTS hit compound **1** to the orally bioavailable compound **33**, a structurally novel potent GPR142 agonist. The antidiabetic effect that compound **33** demonstrated in mice and monkeys strongly suggests that GPR142 agonists have great potential for the treatment of type II diabetes. Detailed pharmacological evaluation and further optimization of this series will be reported elsewhere.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures and analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

HTS, high throughput screening; CYP, cytochrome P450; HBTU, *o*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexa-fluoro-phosphate; MLM, mouse liver microsome; HLM, human liver microsome; MkLM, monkey liver microsome; RLM, rat liver microsome

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