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Discovery of 3-aryl-3-ethoxypropanoic acids as orally active GPR40 agonists

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ABSTRACT

The G protein-coupled receptor 40 (GPR40) mediates enhancement of glucose-stimulated insulin secretion in pancreatic β cells. The GPR40 agonist has been attracting attention as a novel insulin secretagogue with glucose dependency for the treatment of type 2 diabetes. The optimization study of compound **1** led to a potent and bioavailable GPR40 agonist **24**, which showed insulin secretion and glucose lowering effects in rat OGTT. Compound **24** is a potential lead compound for a novel insulin secretagogue with a low risk of hypoglycemia.

In recent years the number of diabetic patients has been increasing all over the world, thus the efficient and suitable treatment for each patient is in high demand.[1] Insulin secretagogues such as sulfonylureas are widely used for patients with a moderate degree of β-cell dysfunction.[2] Sulfonylureas secrete insulin independently of glucose levels, so they may cause hypoglycemia.[3] Their long term therapy also often leads to the gradual diminution of islets activity.[4–6] There are only a few choices of insulin secretagogues with low risk of hypoglycemia such as DPP-4 inhibitors and GLP-1 agonists.[7] Therefore, the novel orally available insulin secretagogues with glucose dependency and strong glucose lowering effects are still in demand.

The G-protein coupled receptor GPR40, highly expressed in human and rodent pancreatic islets,[8–10] is found as an attractive target for new therapy of type 2 diabetes.[8,11] This receptor is activated by medium and long-chain free fatty acids (e.g., palmitic and linolenic acids), and sends signals to downstream pathways resulting in enhancement of insulin secretion by production of inositol triphosphate and release of intracellular Ca^{2+} from endoplasmic reticulum.[12,13] Compared to other mechanisms, there are several advantages of GPR40 as a target for the treatment of type 2 diabetes. The most attractive point is that GPR40 induces insulin secretion depending on the concentration of glucose, indicating that the selective agonist has low risk of hypoglycemia.[14] In addition, the distribution of GPR40 has been limited (mainly in islets), in that side effects associated with GPR40 activation in other tissues rarely occur. Many groups reported a variety of synthetic GPR40 agonists.[15–27] Most of GPR40 agonists have the structure of 3-phenylpropanoic acid mimicking medium or long-chain free fatty acids (Fig. 1). Among these compounds, we chose compound **1** as a starting structure to explore novel GPR40 agonists, because it has very strong in vitro agonistic activity against GPR40 (reported EC_{50} = 8.8 nM; in house data EC_{50} = 6.7 nM) and a simple structure that is easy to modify.[28] On the other hand, it is reported that bioavailability of compound **1** was too poor (F = 0.9% in rats) for oral active agents. In this article, we will show the synthetic efforts to obtain bioavailable compounds starting from compound **1**.

High lipophilicity of **1** seems to cause poor PK profiles. First we replaced the phenyl ring of compound **1** with various hetero aromatic rings to reduce lipophilicity[29,30] (Table 1). The synthesis of pyridine derivative **2** was shown in Scheme 1. (2′,6′-Dimethylbiphenyl-3-yl)methanol (**12**) was reacted with 2,5-dibromopyridine in the presence of NaH. The corresponding bromopyridine **13** and ethyl acrylate were coupled under the microwave-assisted Heck coupling condition.[31] Olefin moiety of compound **14** was reduced with $NaBH_4$ using $NiCl_2$ as a catalyst. Saponification of ethyl ester in compound **15** gave pyridine derivative **2**. Other compounds in Table 1 were synthesized in the similar manner as in Scheme 1.

Pyridine derivative **2** showed similar GPR40 agonistic activity to compound **1**. In contrast, pyridine derivative **3** was found to be a weaker agonist than compound **1**. Five-membered hetero aromatic

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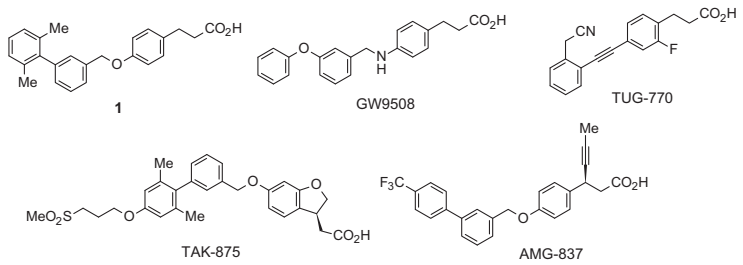
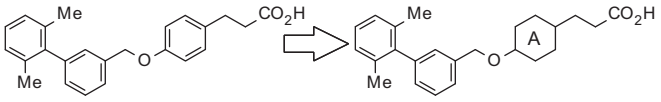


Figure 1. Representative GPR40 agonists.[19–23]

Table 1
GPR40 agonistic activities of heteroaryl derivatives



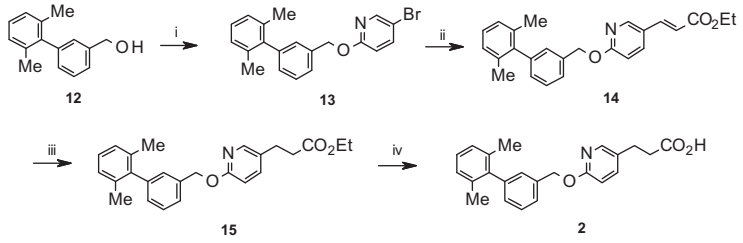
Compound	A	Human EC_{50} (nM)[a]	Rat EC_{50} (nM)[a]	Log*D*[b]
1		6.7	18	>3.3
2		3.5	38	3.0
3		71	210	2.4
4		52	840	>2.4
5		65	540	NT[c]
6		3.8	16	2.5
7		420	310	NT
8		130	1400	2.0
9		9.3	130	NT
10		81	1100	NT
11		2500	9100	NT
GW 9508	—	13	99	NT

[a] Calcium flux assay in GPR40-transfected CHO cells. Means of two or three experiments.
[b] Log*D* values were determined at pH 7.4.
[c] NT = Not tested.

rings were not tolerant (compound **4**, **5**). Next we introduced another nitrogen atom to compound **2** to lower its hydrophobicity (compound **6**–**8**). Only a pyrazine derivative **6** kept strong activity with a lowered log*D* value, while its solubility became worse. Methyl scanning of compound **2** (compound **9**–**11**) resulted in less potent agonists. We assessed PK profiles of compound **2** in rats. Bioavailability of compound **2** ($F = 24\%$) was improved compared to compound **1** ($F = 0.9\%$) probably due to lower lipophilicity of **2** (Table 3).

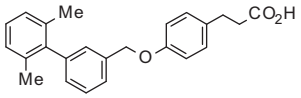
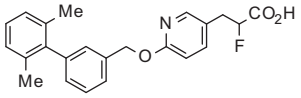
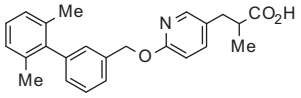
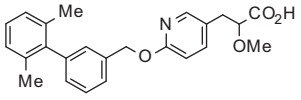
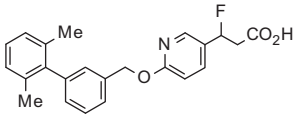
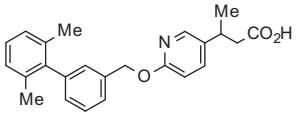
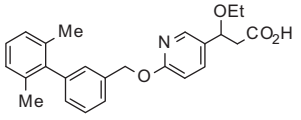
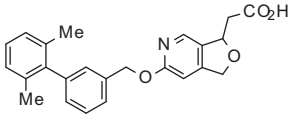
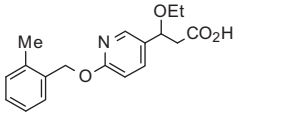
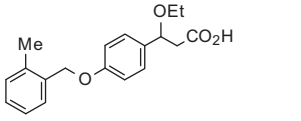
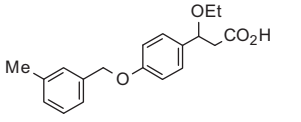
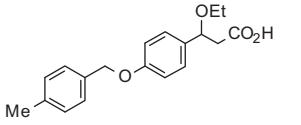
These results encouraged us to take further optimization of compound **2** in order to adjust the PK profiles better. It is well known that fatty acids are metabolized via β-oxidation in the mitochondria,[32] hence compound **2** which contains propanoic acid moiety could be metabolized. Therefore we introduced substituents on the carbon chain of the propanoic acid to block the oxidation (Table 2). The fluoro, methyl and alkoxy substituents were introduced on the α-position (compound **16**–**18**). Only the fluoro group kept activity. The acidity of carboxylic acid has changed by the electronic effect of these substituents. Electron donating substituent (compound **17**, **18**) could decrease the ionic interaction of the carboxylic acid. On the other hand, these substituents on the β-position were tolerant (compound **19**–**21**). Among them, the alkoxy substituent (compound **21**) was promising in terms of strong agonistic activity and preserved lipophilicity. Conformationally restricted derivative **22** did not increase its agonistic activity.

To determine cytotoxicity of a series of compounds, we evaluated the inhibition of mitochondrial respiratory chain complex 1, which blocks electron transportation and leads to the cell toxicity.[33–35] Unfortunately these derivatives including compound **21** have strong inhibition of complex 1. It was reported that the number of aromatic rings correlated with successful transition of compounds from discovery through clinical trials.[36,37] We hypothesized that the biphenyl moiety which was the common structure of these compounds was crucial for cytotoxicity. Compound **23** was synthesized to remove the biphenyl structure. As we expected, the function of the respiratory chain could keep working normally. However its GPR40 agonistic activity has significantly diminished. According to its log*D* value of 0.7, hydrophobic interaction between the receptor and the ligand might be too weak. In order to restore the binding affinity, the central pyridine ring was



Scheme 1. Synthesis of compound **2**. Reagents and conditions: (i) NaH, 2,5-dibromopyridine, DMF, 70 °C, 93%; (ii) ethyl acrylate, $Pd(OAc)_2$, $P(o\text{-tol})_3$, Et_3N, MeCN, microwave, 80 °C, 88%; (iii) $NaBH_4$, $NiCl_2$, MeOH, 0 °C, 88%; (iv) NaOH aq, EtOH, 50 °C, 84%.

Table 2
GPR40 agonistic activities of substituted propanoic acid derivatives

Compound	R	Human EC_{50}[a] (nM)	Rat EC_{50}[a] (nM)	Log*D*[b]	Complex 1[c] (% inhibition)
1		6.7	18	>3.3	58
16		8.6	250	NT[d]	58
17		65	2100	NT	NT
18		120	>10,000	NT	NT
19		18	96	NT	91
20		10	320	3.3	39
21		14	130	2.8	66
22		49	190	NT	NT
23		230	760	0.7	1.0
24		20	79	1.2	6.0
25		35	78	NT	NT
26		370	290	NT	NT

[a] Calcium flux assay in GPR40-transfected CHO cells. Means of two or three experiments.
[b] Log*D* values were determined at pH 7.4.
[c] %Inhibition of the mitochondrial respiratory chain complex 1 at 100 μM of compound.[39]
[d] NT = Not tested.

changed back to the phenyl ring. As a result, compound **24** recovered strong GPR40 agonistic activity (EC_{50} = 20 nM) and moreover the inhibition of mitochondrial function by **24** was found to be weak. It is reported that small nonpolar substituents at *meta*-position were favored over *ortho*- or *para*-position on the benzyl moiety of propanoic acid series.[38] In contrast, the methyl substituent introduced at *ortho*- or *meta*-position was acceptable in the β-ethoxy-propanoic acid series (compound **24**–**26**).

Compound **24** was synthesized via Scheme 2. Benzyl moiety was introduced by the reaction of 4-hydroxybenzaldehyde (**27**) and 2-methylbenzyl bromide in the presence of Cs_2CO_3. β-Hydroxyl ester **29** was obtained by aldol reaction of aldehyde **28** and ethyl acetate with the base of LHMDS. The alkylation of hydroxyl substituent at β-position of **29** by iodoethane with excess amount of Ag_2O in toluene reflux gave the ethoxy product **30** in moderate yield. Hydrolysis of ethyl ester **30** in methanol at room temperature gave **24**.

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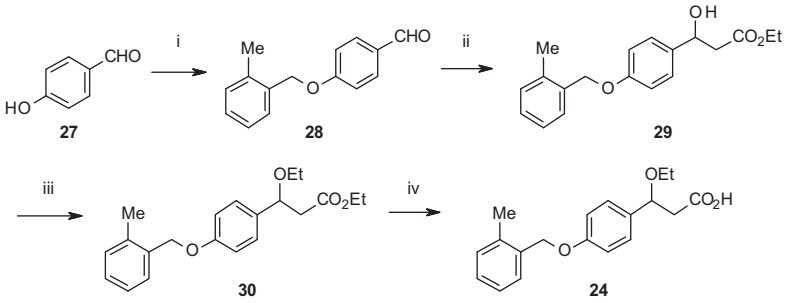
Table 3
PK parameters of compound **2** and **24** in rats[a]

Compound	Cl (L/h/kg)	Vdss (L/kg)	$T_{1/2}$ (h)	C_{max} (μg/ml)	AUC (μg h/ml)	*F* (%)
2	1.2[b]	0.22[b]	0.24[b]	3.5[c]	2.0[c]	24[c]
24	0.37[d]	0.16[d]	0.37[d]	3.3[e]	5.3[e]	64[e]

[a] This experiment was carried out with F344 rats.
[b] The compound was dosed iv at 10 mg/kg.
[c] The compound was dosed po at 10 mg/kg.
[d] The compound was dosed iv at 2 mg/kg.
[e] The compound was dosed po at 3 mg/kg.



Scheme 2. Synthesis of β-ethoxy substituted compound **24**. Reagents and conditions: (i) 2-methylbenzyl bromide, Cs_2CO_3, DMF, rt, quant.; (ii) ethyl acetate, LHMDS, THF, −78 °C, 84%; (iii) Ag_2O, iodoethane, toluene, 100 °C, 38%; (iv) NaOH aq, MeOH, rt, 64%.

PK profiles of **24** were evaluated and found to be improved compared to compound **2** presumably due to interruption of β-oxidation. Low clearance and high plasma exposure were considered to be suitable profiles as an oral agent (Table 3).

We first examined in vitro insulinotropic effects of compound **24** from MIN6 cells.[40,41] The significant increases of insulin were observed as dose dependently from 0.001 to 10 μM compound **24** in the presence of 25 mM glucose (Fig. 2).

Next, the glucose lowering effect of compound **24** was evaluated by an oral glucose tolerant test (OGTT) in diabetic Zucker fatty rats (Fig. 3).[42] Compounds were administrated orally in 9-week-old Zucker fatty rats 30 min prior to the glucose challenge. Compound **24** significantly reduced blood glucose excursion in a dose dependent manner with doses ranging from 1 to 10 mg/kg. Sitagliptin, a marketed DPP-4 inhibitor, was included as a positive control in this study. Compound **24** at a dose of 3 mg/kg showed similar efficacy to sitagliptin at a dose of 10 mg/kg, therefore the potency of compound **24** was seemed to be as 3-fold strong as sitagliptin. As shown in Figure 4, robust increase of insulin secretion was also observed. During this test, exposure of compound **24** was sufficient to exhibit potent in vivo efficacy (Table 4).

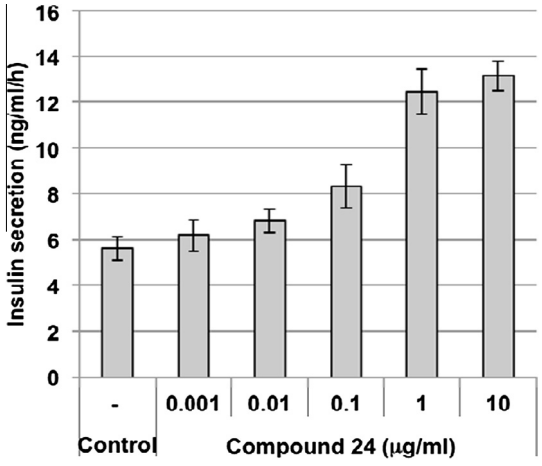


Figure 2. Effects of compound **24** on insulin secretions from MIN6 cells.[40,41] Values are expressed as the mean ± S.E. (4 wells).

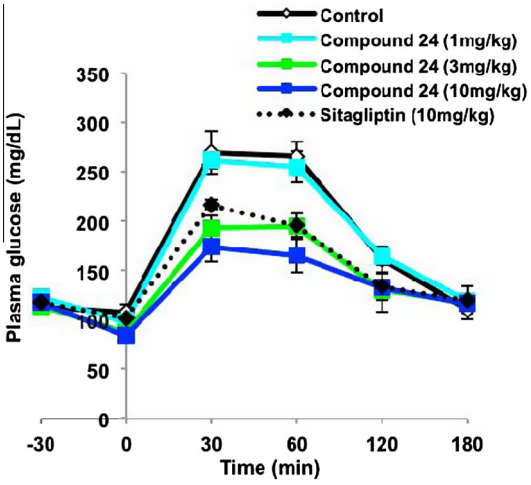


Figure 3. Effects of compound **24** and Sitagliptin on plasma glucose levels during OGTT.[42] Values are expressed as the mean ± S.E. ($n = 6$). The compound was administrated before 30 min of glucose administration (2 g/kg).

Finally, We confirmed that compound **24** was inactive up to 10 μM on GPR120 and PPAR α,γ,δ receptors,[10,43] which have also the fatty acids as their ligands.

In conclusion, we discovered a series of propanoic acid derivatives as a lead structure for potent and orally bioavailable GPR40 agonists. Starting from compound **1**, introduction of substituents on propanoic acid moiety improved the PK profiles, and removal of the biphenyl structure lowered the toxicity risks. Among them, compound **24** was found to be a promising lead compound. Compound **24** increased insulin secretion from MIN6 cells and lowered plasma glucose level in rat OGTT. Further optimization on this series is ongoing and will be reported in due course.

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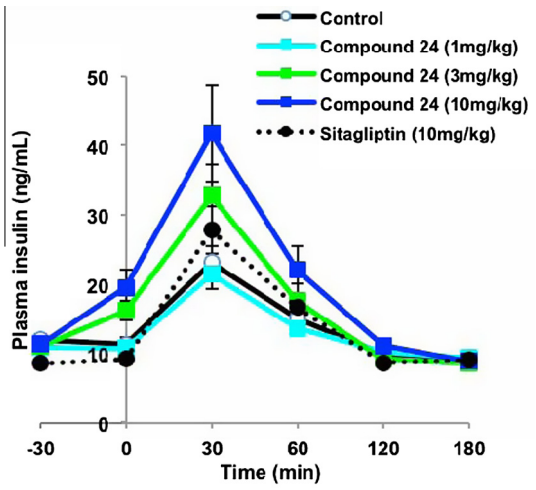

Figure 4. Effects of compound **24** and sitagliptin on plasma insulin levels during OGTT.[42] Values are expressed as the mean ± S.E. ($n = 6$). The compound was administrated before 30 min of glucose administration (2 g/kg).

Table 4
PK parameters of compound **24** during OGTT

Compound **24**	C_{max} (μg/ml)	AUC (μg h/ml)
1 mg/kg	4.1	4.8
3 mg/kg	14	19
10 mg/kg	51	78

Acknowledgments

MIN6 cells were licensed in from Prof. Jun-ichi Miyazaki at Osaka University. We thank Prof. Miyazaki and the staffs at his laboratory for their assistance in the use of MIN6 cells and useful advice.

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