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# Discovery of TAK-875: A Potent, Selective, and Orally Bioavailable GPR40 Agonist

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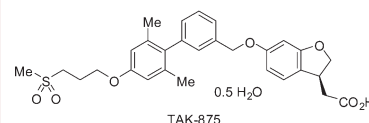
**ABSTRACT** GPR40, one of the G protein-coupled receptors predominantly expressed in pancreatic β-cells, mediates enhancement of glucose-stimulated insulin secretion by free fatty acids. A potent and selective GPR40 agonist is theorized to be a safe and effective antidiabetic drug with little or no risk of hypoglycemia. Cyclization of the phenylpropanoic acid moiety of lead compound **1** produced fused phenylalkanoic acids with favorable in vitro agonist activities and pharmacokinetic profiles. Further optimization led to the discovery of dihydrobenzofuran derivative **9a** ([(3*S*)-6-({2′,6′-dimethyl-4′-[3-(methylsulfonyl)propoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic acid hemi-hydrate, TAK-875) as a potent, selective, and orally bioavailable GPR40 agonist, with a pharmacokinetic profile enabling long-acting drug efficacy. Compound **9a** showed potent plasma glucose-lowering action and insulinotropic action during an oral glucose tolerance test in female Wistar fatty rats with impaired glucose tolerance. Compound **9a** is currently in clinical trials for the treatment of type 2 diabetes mellitus.




**KEYWORDS** GPR40, agonist, GSIS, TAK-875, OGTT, insulin secretagogue

Type 2 diabetes mellitus is characterized by insulin resistance or reduced insulin sensitivity, combined with reduced insulin secretion from pancreatic β-cells. Persistent or uncontrolled hyperglycemia increases the risk of macrovascular and microvascular complications. Accordingly, therapeutic control of blood glucose levels is critically important in the clinical management and treatment of diabetes. Insulin secretagogues such as sulfonylureas and meglitinides are widely used for the treatment of type 2 diabetes.[1] They bind to an ATP-dependent $K^+$ channel in β-cells, causing depolarization of the plasma membrane and opening the voltage-gated $Ca^{2+}$ channels leading to insulin release. Because this action is independent of extracellular blood glucose concentration, use of these agents could cause hypoglycemia,[2] possibly accelerating the loss of function and apoptosis of pancreatic β-cells.[3] Furthermore, the relatively unfavorable cardiovascular risk profiles of sulfonylureas as compared with metformin have been shown in a retrospective analysis encompassing over 90000 diabetics in a British general practice database.[4] Therefore, administration of insulin secretagogues must be carefully controlled. Recently, agents prompting glucose-stimulated insulin secretion (GSIS) have attracted considerable attention, for example, dipeptidyl peptidase-4 (DPP-4) inhibitors or biologically stabilized analogues of glucagon-like peptide-1 (GLP-1).[5]

Free fatty acids (FFAs) provide an important energy source and also act as signaling molecules. FFAs have pleiotropic effects on pancreatic β-cells. Although chronic exposure to high levels of FFAs impairs β-cell function and secretory capacity (lipotoxicity), acute administration of FFAs promotes GSIS.[6,7] In 2003, it was reported that GPR40 contributes to the enhancement of GSIS by FFAs.[8]

GPR40 was identified over 10 years ago as an orphan G protein-coupled receptor (GPCR).[9] It is highly expressed in human and rodent pancreatic β-cells and β-cell lines (MIN6 or INS-1E) and is also found in several areas of the human brain.[8,10,11] GPR40 couples mainly with a G protein α-subunit of the Gq family (Gαq). Several reports suggest that FFAs binding to GPR40 increase the intracellular $Ca^{2+}$ concentration by a Gq-mediated pathway, leading to enhanced insulin secretion in a glucose concentration-dependent manner.[8,12,13] Meanwhile, treatment of MIN6 or INS-1E cells with small interfering RNA (siRNA) specific for GPR40 prevents insulin secretion by stimulation with FFAs.[8,12] This study further supported the hypothesis that GPR40 might serve as an attractive target for a novel insulin secretagogue. Thus, a potent and selective GPR40 agonist could prove to be an

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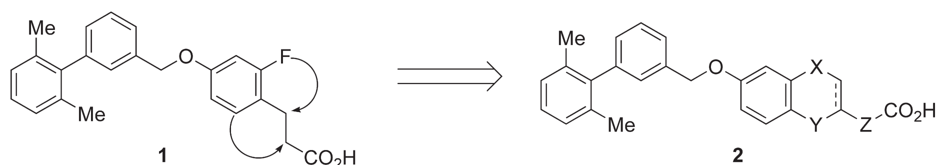
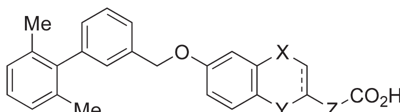



**Figure 1.** Design of fused phenylalkanoic acids.

**Table 1.** In Vitro Activities of Fused Phenylalkanoic Acids




| compd | X | Y | Z | bond type | FLIPR human $EC_{50}$ ($\mu$M)[a] | binding human $K_i$ ($\mu$M)[b] | binding rat $K_i$ ($\mu$M)[b] |
|---|---|---|---|---|---|---|---|
| **3** | $CH_2$ | bond | $CH_2$ | single | 0.028 | 0.21 | 0.52 |
| **4** | $-(CH_2)_2-$ | bond | $CH_2$ | single | 0.023 | 0.059 | 3.7 |
| **5** | $-(CH_2)_3-$ | bond | $CH_2$ | single | 0.54 | 2.4 | >10 |
| **6** | O | bond | $CH_2$ | double | 1.7 | >10 | >10 |
| **7** | O | bond | $CH_2$ | single | 0.022 | 0.17 | 1.1 |
| **8** | $-OCH_2-$ | $CH_2$ | bond | single | 0.049 | 0.27 | >10 |
| **1** | | | | | 0.0057 | 0.032 | 0.054 |

[a] All values are averages of $n = 3$ in the presence of 0.1 % BSA. [b] All values are averages of $n = 2$ or 3 in the presence of 0.2 % BSA.

effective antidiabetic drug with little or no risk of hypoglycemia as observed in sulfonylurea agents. This communication describes the discovery of [(3*S*)-6-({2′,6′-dimethyl-4′-[3-(methylsulfonyl)propoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic acid hemi-hydrate (**9a**, TAK-875), a potent, selective, and orally bioavailable GPR40 agonist that has been selected as a clinical candidate.

Small molecule GPR40 agonists based on scaffolds such as arylalkanoic acids and thiazolidinediones have been reported by several groups.[14] We independently identified a range of synthetic agonists by ligand-based drug design. Potent agonists have also been discovered among the natural ligands, especially polyunsaturated fatty acids such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA).[8,10,11] We therefore speculated that a $\pi-\pi$ interaction might have a great impact on the receptor−ligand interaction. We selected several arylalkanoic acids from our in-house library and screened them for activity using a fluorometric imaging plate reader (FLIPR) assay. This screening identified benzyloxyphenylpropanoic acids as a promising lead series. Optimization of the lipophilic portion of the molecule led to the identification of phenylpropanoic acid **1** that showed in vitro agonist activity and in vivo efficacy in a diabetic animal model.[15] While compound **1** possessed a modest pharmacokinetic (PK) profile, it appeared to be susceptible to $\beta$-oxidation at the phenylpropanoic acid moiety. Therefore, we planned to improve metabolic stability by constructing a fused structure between the *ortho*-position of the phenyl ring and the α- or $\beta$-position of the propanoic acid moiety (Figure 1). In addition, we anticipated that a more constrained structure distinct from the more flexible fatty acids would avoid off-target activities derived from multifunctional fatty acids.

The synthesis of fused phenylalkanoic acids **3**−**8** is detailed in the Supporting Information.

Agonist activities of the prepared compounds were measured by FLIPR assay in the presence of 0.1 % bovine serum albumin (BSA). Binding affinities for human and rat receptors were also measured in the presence of 0.2 % BSA.[16] As depicted in Table 1, fused five- and six-membered ring compounds **3** and **4** exhibited comparable agonist activities (**3**, $EC_{50}$ = 0.028 $\mu$M; **4**, $EC_{50}$ = 0.023 $\mu$M) to the phenylpropanoic acid **1** ($EC_{50}$ = 0.0057 $\mu$M). Expansion of the fused ring to a seven-membered cycle resulted in decreased potency (**5**, $EC_{50}$ = 0.54 $\mu$M). Replacement of the carbon atom of **3** with an oxygen atom retained potency (**7**, $EC_{50}$= 0.022 $\mu$M). However, benzofuran derivative **6** with an unsaturated ring showed weaker activity than **7**, suggesting that a planar template might be unfavorable. Interestingly, the seven-membered analogue **8** fused with the phenyl ring at the α-position instead of the $\beta$-position of the phenylpropanoic acid moiety recovered activity ($EC_{50}$ = 0.049 $\mu$M) against the human receptor. These results suggest that placement of the phenyl ring and the carboxylic group is important in determining activity properly regardless of the size of the fused ring. In our binding assay, affinity for the human receptor showed a trend similar to that observed in the FLIPR assay. Meanwhile, affinity for the rat receptor was decreased in relation to the expansion in ring size (five-membered ring **3** and **7** > six-membered ring **4** > seven-membered ring **8**). Thus, the five-membered fused ring

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**Table 2.** Pharmacokinetic Profiles for Fused Phenylalkanoic Acids[a]

| compd | $C_{max}$ (ng/mL) | $AUC_{po}$ (ng h/mL) | *F* (%) |
|---|---|---|---|
| **3** | 285.1 | 1701.3 | 47.8 |
| **4** | 220.7 | 925.7 | 56.9 |
| **7** | 449.7 | 2357.3 | 69.4 |
| **1** | 86.0 | 249.0 | 21.5 |

[a] Rat cassette dosing at 0.1 mg/kg, iv, and 1 mg/kg, po. Average of three rats. *F* means bioavailability.

compounds (**3** and **7**) had the best profiles in terms of potency and species difference.

The PK parameters were evaluated in rats (Table 2). Fused ring compounds (**3**, **4**, and **7**) with favorable activity profiles showed higher oral bioavailability (*F*) (**3**, 47.8%; **4**, 56.9%; **7**, 69.4%) than that of the phenylpropanoic acid **1** (21.5%). Likewise, these compounds (**3**, **4**, and **7**) exhibited >2.6-fold higher maximum plasma concentration ($C_{max}$) and >3.7-fold higher plasma exposure ($AUC_{po}$, area under curve) than that of **1**. These results demonstrate the utility of our strategy to improve PK profiles by cyclization of the phenylpropanoic acid moiety. One of the reasons for the desirable PK profiles of these fused ring compounds is that they might be tolerant to $\beta$-oxidation, as we speculated above.

On the basis of these in vitro activities and PK profiles, we fixed the dihydrobenzofuran as a template for further investigation. Detailed structure−activity relationship (SAR) analyses revealed that the substituents at the 4′-position of the biphenyl scaffold were well-tolerated.[17] These efforts led to the discovery of compound **9a**.

As shown in Table 3, the (*S*)-enantiomer of **9a** exhibited potent agonist activity ($EC_{50}$ = 0.014 $\mu$M) and high binding affinity ($K_i$ = 0.038 $\mu$M) to the human receptor, whereas weaker affinity was found toward the rat receptor as with other fused analogues. A preference for (*S*)-stereochemistry over (*R*) was observed; (*S*)-enantiomer **9a** possesses higher affinity than (*R*)-enantiomer **9b**. This result indicates that the configuration of the acetic acid moiety attached to the dihydrobenzofuran ring makes a significant impact on GPR40 activity, namely, the spatial configuration between the aromatic ring and the carboxylic acid moiety certainly affects the interaction with the GPR40 receptor as expected.

GPR40 belongs to a family of FFAs binding GPCRs, which includes GPR40, GPR41, GPR43, and GPR120.[18,19] While GPR41 and GPR43 are activated by short-chain FFAs, GPR40 and GPR120 are activated by medium- to long-chain FFAs and some eicosanoids. As shown in Table 4, compound **9a** exhibited excellent agonist potency selectivity for GPR40 receptor over other members of the FFA receptor family (for which $EC_{50}$ > 10 $\mu$M). Our medicinal chemistry efforts, moving away from the fatty acid-like structure and reducing lipophilicity, resulted in acquiring specificity for the GPR40 receptor.

Several modeling studies of GPR40 have been published.[20,21] To verify the interaction between the GPR40 and the synthetic agonist **9a**, a modeling study was performed. At the time of this study, bovine rhodopsin structures were the only but attractive templates for homology modeling of family A GPCRs. GPR40 also belongs to the cluster of family A GPCRs, so we chose the X-ray crystallographic structure of bovine rhodopsin as a template. Our method for homology modeling and ligand docking is detailed in the Supporting Information. Figure 2 illustrates a docking model of **9a** inside a GPR40 homology model. In this model, three groups of interactions are observed. The first group involves hydrophilic interactions. The carboxylate of **9a** forms two hydrogen bonds with Arg183 (TM5) and one bond with Arg258 (TM7). In addition, Arg258 (TM7) forms a $\pi-\pi$ interaction with the phenyl ring of the dihydrobenzofuran ring, and the carbamoyl moiety of Asn244 (TM6) locks the arginine residue of Arg258 (TM7) into a fixed conformation. The second group is formed by aromatic and hydrophobic interactions located around the 2′,6′-dimethylbiphenyl moiety, including Tyr12 (TM1), Leu67 (TM2), Trp72 (E-I loop), Phe82 (TM3), and Leu262 (TM7). The third group consists of polar residues at the space between TM1 and TM7, including Ser8 (TM1) and Lys259 (TM7). This modeling study proposed that **9a** exerts its GPR40 activity utilizing a number of different interactions effectively.

The PK profile of **9a** was studied in rats and dogs (Table 5). Compound **9a** showed low plasma clearance (CL) and low volume of distribution ($V_{d(ss)}$), resulting in sustained plasma half-lives (iv $t_{1/2}\lambda$: rat, 4.7 h; dog, 5.9 h) in each species. In addition, oral administration of **9a** exhibited rapid absorption, high $C_{max}$, and high plasma exposure with high bioavailabilities (rat, 76.0%; dog, 92.4%). These results encouraged us to investigate **9a** further as a candidate for clinical development.

**Table 3.** In Vitro Activity Profiles of **9a** and Its Enantiomer **9b**

| compd | stereo | FLIPR | binding | |
|---|---|---|---|---|
| | | human $EC_{50}$ ($\mu$M)[a] | human $K_i$ ($\mu$M)[b] | rat $K_i$ ($\mu$M)[b] |
| **9a** | *S* | 0.014 | 0.038 | 0.14 |
| **9b** | *R* | | 0.37 | 1.6 |

[a] The activity was measured with anhydrous **9a**. The value is an average of $n$ = 3 in the presence of 0.1% BSA. [b] All values are averages of $n$ = 2 or 3 in the presence of 0.2% BSA.

**Table 4.** Selectivity Profile for **9a**[a]

| compd | human GPR40 $EC_{50}$ ($\mu$M)[b] | human GPR41 $EC_{50}$ ($\mu$M)[c] | human GPR43 $EC_{50}$ ($\mu$M)[c] | human GPR120 $EC_{50}$ ($\mu$M)[d] |
|---|---|---|---|---|
| **9a** | 0.014 | >10 | >10 | >10 |

[a] The activities were measured with anhydrous **9a**. [b] The value is an average of $n$ = 3 in the presence of 0.1% BSA. [c] All values are averages of $n$ = 2 in the presence of 0.5% BSA. [d] The value is average of $n$ = 4 in the presence of 0.1% BSA.

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Compound **9a** was assessed for its ability to improve glucose intolerance in female Wistar fatty rats, a model developing obesity and obesity-related features such as impaired glucose tolerance, hyperinsulinemia, and hyperlipidemia.[22] Single oral dosing of **9a** (0.3−3 mg/kg) reduced the blood glucose excursion (Figure 3A) and augmented insulin secretion (Figure 3C) during an oral glucose tolerance test (OGTT), when the compound was administered 1 h before an oral glucose challenge. The area under the curve of plasma glucose ($AUC_{0-120\,min}$) and plasma insulin ($AUC_{pre-30\,min}$) showed that the minimum effective dose was 1 mg/kg (Figure 3B, D, respectively). Because in vitro binding affinity of **9a** for human GPR40 was stronger than that for rat GPR40 and its agonist activity measured on FLIPR was also potent (Table 3), **9a** could be a potent insulinotropic drug in patients with type 2 diabetes.

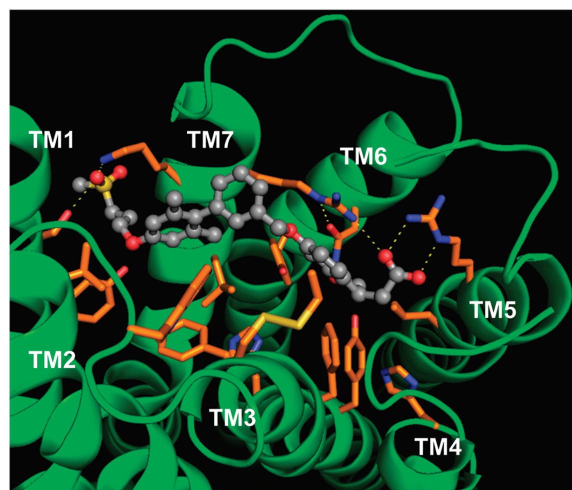


**Figure 2.** Docking model of GPR40 in complex with **9a** (gray).

In conclusion, we discovered compound **9a**, a structurally novel GPR40 agonist showing potent and selective GPR40 agonist activity in vitro, plasma glucose lowering and insulinotropic efficacy in a rat model of diabetes, and an excellent PK profile. Our isosteric conversion of the phenylpropanoic acid moiety developed an efficient strategy to retain potency and improve the PK profile in rats and dogs. Compound **9a** is currently undergoing human clinical trials.

**Table 5.** PK Parameters for **9a** (Hemi-hydrate) in Fasted Rats and Dogs[a]

| | iv | | | po | | | | |
|---|---|---|---|---|---|---|---|---|
| species | CL (mL/h/kg) | $V_{d(ss)}$ (mL/kg) | $t_{1/2}\lambda$ (h) | $t_{1/2}\lambda$ (h) | $C_{max}$ ($\mu$g/mL) | $T_{max}$ (h) | $AUC_{po,\,0-24\,h}$ ($\mu$g h/mL) | $F$ (%) |
| rat | 34.16 | 208.49 | 4.7 | 4.1 | 5.77 | 1.0 | 65.00 | 76.0 |
| dog | 29.79 | 224.67 | 5.9 | 7.5 | 3.29 | 2.0 | 29.45 | 92.4 |

[a] Administered at a dose of 1 mg/kg, iv; 3 mg/kg, po, in rats. Administered at a dose of 0.5 mg/kg, iv; 1 mg/kg, po, in dogs. The values for $C_{max}$ and AUC were expressed as equivalent of anhydrous **9a**. Data are expressed as mean values (rats, $n$ = 3; dogs, $n$ = 4).

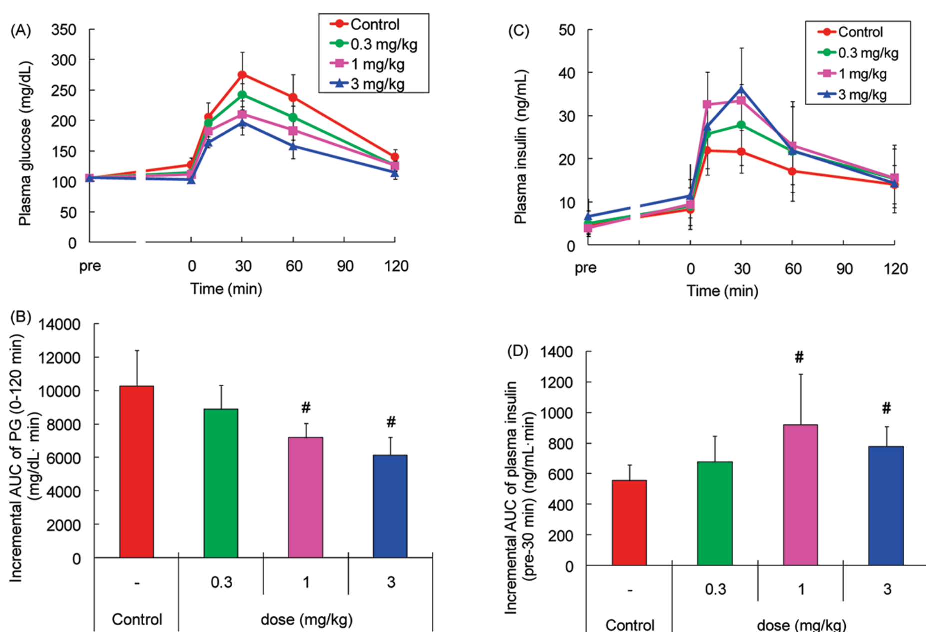


**Figure 3.** Effects of **9a** during an OGTT in female Wistar fatty rats. Panels A and C show time-dependent changes of plasma glucose (PG) and plasma insulin after oral administration of **9a** followed by 1 g/kg oral glucose challenge, respectively. Data in panels B and D represent incremental $AUC_{0-120\,min}$ of PG levels and incremental $AUC_{pre-30\,min}$ of plasma insulin levels shown in panels A and C, respectively. Values are means ± SDs ($n$ = 6). $^{\#}p \leq 0.025$ as compared with control by one-tailed Williams' test.

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Detailed studies of SAR and pharmacological evaluation will be reported elsewhere.

**SUPPORTING INFORMATION AVAILABLE** Experimental procedures and analytical data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

## AUTHOR INFORMATION

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