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Letter

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Identification of an Orally Efficacious GPR40/FFAR1 Receptor Agonist

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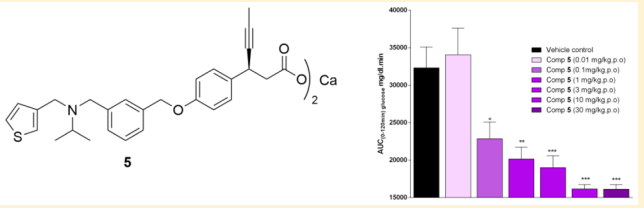
Supporting Information

ABSTRACT: GPR40/FFAR1 is a G protein-coupled receptor predominantly expressed in pancreatic β-cells and activated by long-chain free fatty acids, mediating enhancement of glucose-stimulated insulin secretion. A novel series of substituted 3-(4-aryloxyaryl)propanoic acid derivatives were prepared and evaluated for their activities as GPR40 agonists, leading to the identification of compound **5**, which is highly potent in *in vitro* assays and exhibits robust glucose lowering effects during an oral glucose tolerance test in nSTZ Wistar rat model of diabetes (ED_{50} = 0.8 mg/kg; ED_{90} = 3.1 mg/kg) with excellent pharmacokinetic profile, and devoid of cytochromes P450 isoform inhibitory activity.



KEYWORDS: *GPR40, GPR40 agonist, FFAR1, fatty acids, insulin secretion, type 2 diabetes*

Type 2 diabetes, the most prevalent form of diabetes, is a growing epidemic, which is associated with a high degree of morbidity and mortality.[1−3] Various available oral therapies include insulin secretagogues, such as sulfonylureas; glucose-lowering effectors, for example, metformin; and activators of PPAR-γ, like pioglitazone.[4] However, these available treatments are unable to provide satisfactory glycemic control in many patients.

GPR40 receptor, also known as the long chain free fatty acid 1 receptor (FFAR1), has emerged as one of the potential targets for the effective treatment of type 2 diabetes mellitus (T2DM).[5] GPR40 receptor belongs to the class A family of G-protein coupled receptors. GPR40 is mainly expressed in pancreatic β-cells and activated by long-chain free fatty acids. Thereby, resulting in enhancement of glucose-stimulated insulin secretion (GSIS), which is dependent on elevated glucose levels.[6] Thus, identification of a small molecule GPR40 agonist, with sufficient pharmacokinetic and pharmacodynamics properties, may offer beneficial treatment for type 2 diabetes and associated complications.[7] Additionally, the limited tissue distribution of GPR40 (primarily in pancreatic islets)[8] suggests that there may be less possibilities for adverse events associated with GPR40 inhibition in other tissues.

Due to the promising application of GPR40 agonist in type II diabetes treatment, intensive research has been undertaken in the area, and several structurally diverse small molecule modulators of GPR40 have been reported (Figure 1).[9−16] However, many of them possessed insufficient potency, lack metabolic stability, or toxicity. Two of these molecules, AMG-837[17] and TAK-875,[18] were selected for clinical trials as antihyperglycemic agents. The most advanced compound of the two was fasiglifam (TAK-875) from Takeda in phase III. However, in December 2013 Takeda discontinued the development of fasiglifam due to concerns about liver idiopathic toxicity.[19]

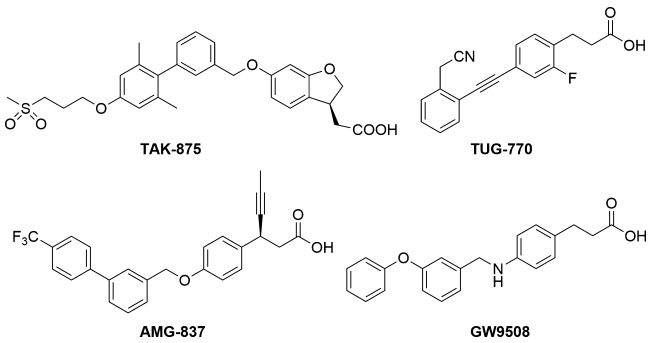


Figure 1. Selected known GPR40/FFAR1 receptor agonist.

Continuing our research efforts,[20] we explored therapies with distinct mechanism of action for type 2 diabetes. This letter describes the identification of (*S*)-3-(4-((3-((isopropyl-(thiophen-3-ylmethyl)amino) methyl) benzyl)oxy)phenyl)hex-4-ynoic acid calcium salt (**5**) as a novel, potent, and orally efficacious GPR40 agonist. We sought an opportunity in AMG-837 scaffold and focused our efforts in modifying this scaffold. The rational being the introduction of amine substituent in the central phenyl ring of AMG-837, if tolerated, would reduce the lipophilicity and thereby improve the drug-like properties (Figure 2). AMG-837 is very lipophilic (cLog P = 7.6) and likely to have significant CNS exposure.[21] Potential drugs with high lipophilicity and poor physiochemical properties are generally correlated with various side effects and have higher risk of failure in clinical trials.[22]

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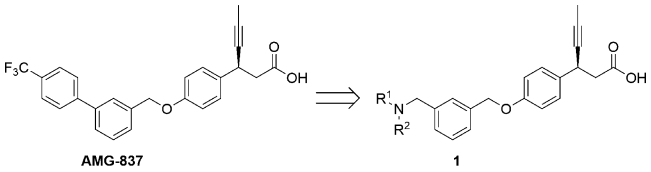
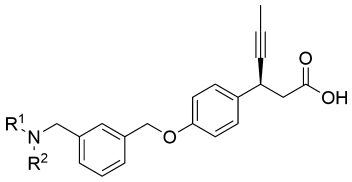
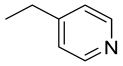
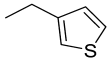
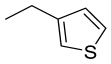


Figure 2. Schematic representation of ligand optimization.

A variety of substituted amines were synthesized and assayed for GPR40 activity using Luciferase transactivation assay. The observation revealed in Table 1 indicates that the pyridyl

Table 1. *In Vitro* Activity of Reference Compounds (TAK-875 & AMG-837) and Compounds 1a−1c



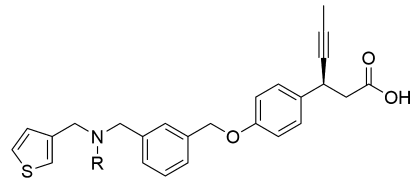
Compound	R^1	R^2	EC_{50} (nM)	cLogP[b]
TAK-875			1.9	5.68
AMG-837			2.2	7.61
1a		H	>1000	3.44
1b		H	118.3	4.32
1c		Me	38	4.67

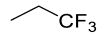
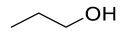
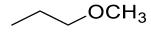
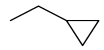
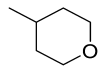
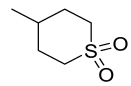
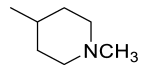
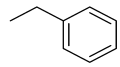
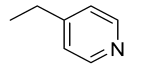
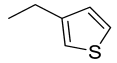
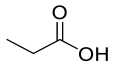
[a]EC_{50} values given are expressed as mean ± SEM of three independent experiments [b]Calculated from Schrödinger 4.4 Software.

compound **1a**, though was significantly less lipophilic when compared to reference AMG-837, however, was also inactive in the *in vitro* assay. The thiophene substituted secondary amine **1b** showed a marked improvement in potency with EC_{50} value of 118 nM, while corresponding *N*-methyl derivative **1c** had EC_{50} of 38 nM. Our optimization of the R1 portion began with a brief examination since relatively steep SAR had been found. The results obtained with analogous **1b** and **1c** suggested that incorporation of the amino-alkyl thiophene substitution may open up new directions for further SAR development.

Accordingly, we focused our investigation to the substituents on the nitrogen atom, and thus, in our optimization campaign, a series of novel 3-methyl amino thiophene derivatives were synthesized and evaluated as GPR40 agonist (Table 2). Compound **2a**, substituted with electron-withdrawing trifluoromethyl group, provided a significant leap in the agonistic activity and found to be extremely potent (EC_{50} = 0.8 nM), while substitution with electron-donating groups OH and OMe (**2b−c**) were relatively less potent. However, the unsubstituted branched alkyls (**2d−e**) were also found to be active. Further, to investigate the effect of cycloalkyls, cyclopropyl was synthesized using our double reductive amination strategy. The smallest, cyclopropyl (**2f**) was very potent (EC_{50} = 1.9 nM), while methyl cyclopropyl (**2g**) was comparatively less

Table 2. *In Vitro* Activity of Compounds 2a−2t



Compound	R	EC_{50} (nM)	cLogP[b]
2a		0.8	5.37
2b		12	3.76
2c		8.6	4.65
2d		8.6	5.13
2e		1.4	5.45
2f		1.9	4.47
2g		16.1	4.82
2h		4.6	5.06
2i		2	5.39
2j		7.7	4.37
2k		7.9	5.36
2l		16.7	3.36
2m		140	4.7
2n		4.4	5.97
2o		1.9	4.85
2p		2.1	5.52
2q		7	6.43
2r		10	5.85
2s		29.6	5.83
2t		weak	4.11

[a]EC_{50} values given are expressed as mean ± SEM of three independent experiments [b]Calculated from Schrödinger 4.4 Software.

potent. The larger, cyclopentyl (**2h**) and cyclohexyl (**2k**) also retained the potency. In addition, heterocyclyles (**2j−l**) were

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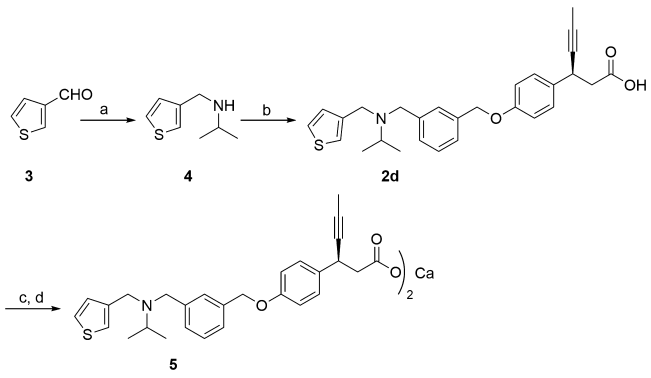
also tolerated, with the notable exception of the *N*-methylpiperidine (**2m**).

In an effort to increase the bulk of *N*-substitution, compound **2n** having benzyl subunit was examined and was also found active as GPR40 agonist, including heteroaryls (**2o**−**p**). Unlike **1a**, the pyridyl compound **2o** showed potent GPR40 activity thereby pointing the importance of methyl thiophene substitution. Next, we introduced the amide groups, which were synthesized via reductive amination of commercially available (2-thiophene)methylamine and aldehyde (**8**), followed by the coupling of resulting amine with corresponding sulfonyl chloride or acids. Notably, sulfonamides **2q** and **2r** showed significant GPR40 agonistic activity, EC_{50} of 7 and 10 nM, respectively. By contrast, amide **2s** was surprisingly weak (EC_{50} = 30 nM), while acid (**2t**) was inactive as GPR40 agonist. In general, a wide variety of substitutions were tolerated, resulting in a series of potent compounds.

With multiple examples of highly potent GPR40 agonists in hand, we next evaluated them against the cytochrome P450 (CYP450) enzymes using recombinant CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4.[23] The number of active compounds tested against CYP450 isoforms were unfortunately found to show strong CYP450 inhibition and unable to pass this hurdle in spite of low nanomolar potency against GPR40 receptor, including most potent compound **2a**. Interestingly, as an exception, compound **2d** (EC_{50} = 8.6 nM) with *N*-isopropyl substituent showed no significant inhibitory activity against CYP450 isoforms (Supporting Information). Compound **2d** was then selected for early stage *in vivo* studies and additional profiling activities.

Synthesis of compound **2d** is depicted in Scheme 1. The reductive amination[24] of commercially available 3-thiophene-

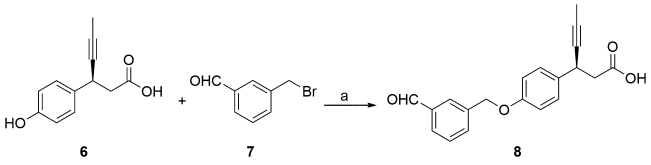
Scheme 1. Synthesis of Compounds 2d and 5



[a]Reagent and conditions: (a) $CH(CH_3)_2NH_2$, $NaB(OAc)_3H$, CH_3COOH, dry THF, 0 °C to r.t., 16 h; (b) **8**, $NaB(OAc)_3H$, CH_3COOH, dry THF, 0 °C to r.t., 16 h; (c) NaOH, $MeCN/H_2O$, r.t., 3 h; (d) $CaCl_2$, $MeOH/H_2O$, r.t., 16 h.

aldehyde (**3**) and isopropyl amine using sodium triacetoxyborohydride resulted in secondary amine intermediate **4**. Compound **4** on further reductive amination under similar conditions with aldehyde intermediate, (*S*)-3-(4-((3-formylbenzyl)oxy)phenyl)hex-4-ynoic acid (**8**), afforded **2d** in high yields. The aldehyde intermediate **8** was obtained from (*S*)-3-(4-hydroxyphenyl)hex-4-ynoic acid (**6**) as shown in Scheme 2. Phenol **6** was synthesized via five-step reported procedure using commercially available 4-hydroxybenzaldehyde and Meldrum's acid.[25] Treatment of **6** with 40% aqueous

Scheme 2. Synthesis of (*S*)-3-(4-((3-Formylbenzyl)oxy)phenyl)hex-4-ynoic Acid (8)



[a]Reagent and conditions: (a) *n*-Bu_4POH, THF/H_2O, 0 °C to r.t., 16 h.

tetrabutylphosphonium hydroxide (*n*-Bu_4POH) in THF, followed by addition of 3-formyl benzyl bromide (**7**), afforded aldehyde intermediate **8**. In order to improve the stability and aqueous solubility, **2d** was converted to its corresponding calcium salt (**5**) via two-step sequence of formation of sodium salt followed by its conversion to calcium salt, with excellent chemical purity for the further pharmacological studies.[26] It should be emphasized that calcium salt was preferred as it offered advantages of crystallinity, API stability, API scale up, and hygroscopicity.

(*S*)-3-(4-((3-((Isopropyl(thiophen-3-ylmethyl)amino)-methyl)benzyl)oxy)phenyl)hex-4-ynoic acid calcium salt (**5**) was next evaluated in cell-based functional IP1 ELISA assay and calcium flux assay. To our delight, **5** was found to be highly potent in both the assays with EC_{50} of 10.5 and 11.6 nM, respectively. Furthermore, **5** was also evaluated in insulin assay in RINm cells and showed dose-dependent insulin secretion with EC_{50} of 20 μM, while TAK-875 showed EC_{50} of 27 μM in the similar assay. All these *in vitro* assays indicate excellent penetration. The compound **5**, with high *in vitro* potency, was next evaluated for physiochemical properties and metabolic stability assays in addition to *in vivo* pharmacokinetic profile. Notably, **5** have good aqueous solubility and acceptable lipophilicity with cLogP in the middle of the ideal range. The compound is stable toward rat liver microsomes and also demonstrated good Caco2 permeability (Supporting Information). These drug-like properties of compound **5** further enabled its potential for *in vivo* validation. The *in vivo* pharmacokinetic investigation in fasted SD rats revealed that **5** exhibited good oral absorption having C_{max} of 2.78 μg/mL with an AUC of 6.6 μg.h/mL and terminal half-life of 2 h, at an oral dose of 3 mg/kg (Table 3). Pharmacokinetic studies in rats showed a fast oral absorption, higher plasma concentration, and an acceptable half-life and demonstrated an excellent oral bioavailability (100%). The desirable pharmacokinetic profile of compound **5** allowed for the progression of the compound into *in vivo* efficacy studies.

In vivo efficacy of the lead compound **5** was evaluated by an oral glucose tolerance test (OGTT) in nSTZ Wistar rat model.[27,28] A single oral administration of **5** at 0.1 mg/kg dose decreases the glucose levels by 30% w.r.t. vehicle control (Figure 3), indicating that even at this low dose, compound **5** markedly reduced the glucose excursion compared to the control. The glucose-lowering potency of **5** at 0.1 mg/kg was found to be similar to that of TAK-875. Single oral doses of **5** robustly lowered the blood glucose excursion during an oral glucose tolerance test in a dose-proportional manner from 0.01 to 30 mg/kg when the compound was administered 1 h before the oral glucose challenge, in nSTZ Wistar rats. It should be noted that compound **5** exhibited a significant blood glucose-lowering effect at a much lower dose with an ED_{50} = 0.8 mg/kg

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Table 3. Pharmacokinetics Properties of 5 in SD Rats

parameters	compd 5
PO dose (mg/kg)	3
T_{max} (h)	1 (0.25−1)
C_{max} (μg/mL)	2.78 ± 0.68
$AUC_{(0-t)}$ (μg·h/mL)	6.61 ± 1.00
$T_{1/2}$, po (h)	2.01 ± 0.19
MRT (h)	2.69 ± 0.55
IV dose (mg/kg)	1
CO (μg/mL)	1.30 ± 0.35
$AUC_{(0-t)}$ (μg·h/mL)	1.10 ± 0.29
Vss (L/kg)	1.05 ± 0.09
CL (mL/min/kg)	13.83 ± 1.56
$T_{1/2}$, iv (h)	1.78 ± 0.16
MRT (h)	1.27 ± 0.05
%F	100

[a]Single dose PK study of compound **5** in fasted female SD rat by oral route. Formulation detail: 5% Tween80 + 5%PEG400 + 90% Na-CMC (0.5%), oral.

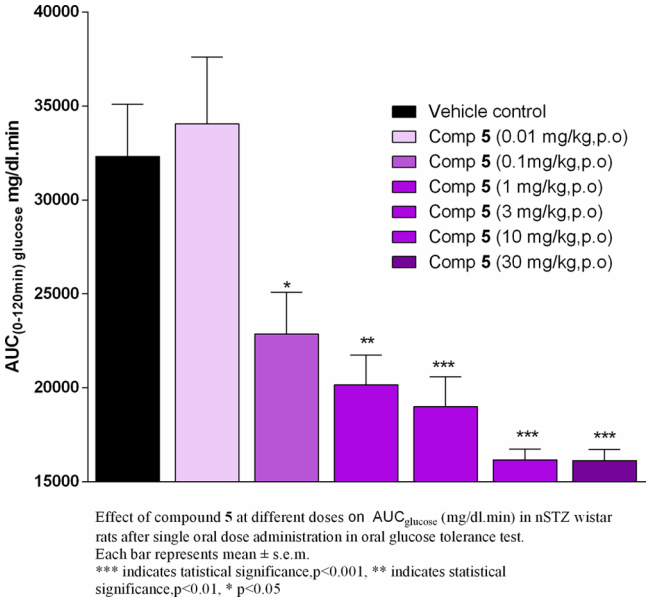

Figure 3. *In vivo* efficacy studies of **5** in nSTZ Wistar rats.

and ED_{90} = 3.1 mg/kg. These results can be at least in part rationalized by the good oral exposure of **5**. Moreover, **5** also showed around 14% AUC-glucose reduction in db/db mice model at 10 mg/kg dose (Supporting Information). It is interesting to note that TAK-875 does not show any measurable decrease in glucose in this mice model.

In conclusion, a series of novel 3-(4-aryloxyaryl)propanoic acid derivatives were prepared and evaluated for their activities as GPR40 agonists. Compound **5** was identified as a structurally distinct GPR40/FFAR1 agonist possessing potent activity *in vitro*, and excellent plasma glucose lowering efficacy in animal models of diabetes with desired pharmacokinetic profile.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.6b00331.

Experimental procedures and analytical data (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

GPCR, G protein-coupled receptor; CMC, carboxymethylcellulose; NMP, *N*-methyl-2-pyrrolidone; T2D, type II diabetes; SAR, structure−activity relationship; GSIS, glucose-stimulated insulin secretion

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(26) Spectral data of calcium (*S*)-3-(4-((3-((isopropyl(thiophen-3-ylmethyl)amino)methyl) benzyl)oxy) phenyl) hex-4-ynoate (**5**): White powder; mp: 124.5 °C. IR (KBr): ν = 3435, 2960, 2918, 2868, 2818, 1608, 1550, 1508, 1440, 1383, 1359, 1240 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ = 7.43−7.42 (m, 2H), 7.28−7.24 (m, 6H), 7.04 (d, J = 4.4 Hz, 1H), 6.89 (d, J = 8.4 Hz, 2H), 5.02 (s, 2H), 4.02 (s, 1H), 3.50 (d, J = 7.2 Hz, 4H), 2.84−2.77 (sept, J = 6.4 Hz, 1H), 2.43 (dd, J_1 = 6.8 Hz, J_2 = 7.2 Hz, 1H), 2.28 (dd, J_1 = 6.8 Hz, J_2 = 7.2 Hz, 1H), 1.73 (s, 3H), 0.99 (d, J = 6.4 Hz, 6H). ^{13}C NMR and DEPT (100 MHz, DMSO-d_6): δ = 177.78 (C), 157.23 (C), 142.11 (C), 141.4 (C), 137.46 (C), 135.81 (C), 128.83 (CH), 128.62 (CH), 128.40 (CH), 127.94 (CH), 127.69 (CH), 126.26 (CH), 122.18 (CH), 114.77 (CH), 83.18 (C), 77.32 (C), 69.66 (CH_2), 52.89 (CH_2), 48.59 (CH), 48.48 (CH_2), 46.86 (CH_2), 33.52 (CH), 17.88 (CH_3), 3.78 (CH_3). MS (EI): m/z (%) = 462.05 (100) $(M+H)^+$. HPLC (% Purity) = 99.38%; Calcium Content ($C_{56}H_{60}CaN_2O_6S_2$) Calcd.: 4.17%. Found: 3.99%.
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