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AMG 837: A potent, orally bioavailable GPR40 agonist

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ABSTRACT

The discovery that certain long chain fatty acids potentiate glucose stimulated insulin secretion through the previously orphan receptor GPR40 sparked interest in GPR40 agonists as potential antidiabetic agents. Optimization of a series of β-substituted phenylpropanoic acids led to the identification of (*S*)-3-(4-((4′-(trifluoromethyl)biphenyl-3-yl)methoxy)phenyl)hex-4-ynoic acid (AMG 837) as a potent GPR40 agonist with a superior pharmacokinetic profile and robust glucose-dependent stimulation of insulin secretion in rodents.

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The burden of an epidemic of type 2 diabetes (T2DM) is straining health care systems both in the United States and worldwide. The prevalence of diabetes is expected to grow from 171 million patients worldwide in the year 2000 to 366 million patients (30 million in the United States alone) by 2030.[1] However, a more recent report estimates 23.6 million diabetic patients already in the United States in 2007 with an additional 57 million classified as pre-diabetics.[2] The resulting direct medical costs in the United States for treating diabetes in 2007 were approximately $116 billion with an additional $58 billion in indirect costs.[3]

Type 2 diabetes manifests as multiple metabolic defects.[4] Preeminent among them are an increased resistance of bodily tissues to the effects of insulin along with a marked decrease in the insulin secretory response by the pancreas to glucose loads. Targeting the latter of these two defects, the sulfonylurea insulin secretagogues inhibit the ATP-sensitive potassium channel that is present in the β-cells of the pancreatic islet and elicit a sustained elevation in insulin release.[5] This mechanism of augmenting insulin secretion is insensitive to plasma glucose levels and can lead to overrelease resulting in hypoglycemia with its attendant symptoms of sweating, nervousness, dizziness and confusion. While the sulfonylurea class of antidiabetic agents remain in wide clinical use, an insulin secretagogue whose activity depends on elevated plasma glucose levels would offer a significant benefit to diabetic patients by abating the risk of hypoglycemia.

The ability of free fatty acids to increase insulin secretion has been extensively studied both in vitro and in vivo.[6–8] The mechanism behind this effect was clarified with the identification of GPR40 as an islet expressed receptor for free fatty acids.[9] GPR40 (FFA1) is a $G_{\alpha}q$-coupled Class 1 GPCR and is a member of a small family of fatty acid sensing GPCRs that includes GPR41 (FFA3) and GPR43 (FFA2). GPR40 binds and is activated by medium to long chain fatty acids (>C_6), while the other family members prefer short chain fatty acids.[10] Significantly, the ability of GPR40 to elicit increased insulin secretion manifests only in the presence of elevated glucose levels. Therefore a GPR40 agonist may fulfill the need for an orally bioavailable glucose-dependent insulin secretagogue.

The potential of GPR40 agonists as antidiabetic agents has not gone unrecognized, and several groups have published reports describing potent GPR40 agonists.[11,12] In addition to journal publications, a number of GPR40 agonist series have also been disclosed in the patent literature.[12] Bearing the putative natural ligands of GPR40 in mind, it is not surprising to find that with few exceptions, reported GPR40 agonists are generally lipophilic molecules containing either a carboxylate functionality or a recognizable bioisostere thereof. A common motif in reported GPR40 agonists is an aryl ring situated two atoms away from a carboxylate or equivalent group. Our work began with the identification of a high-throughput

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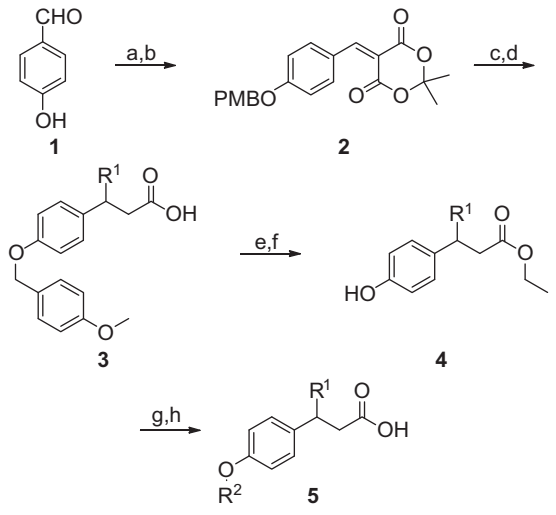
screening (HTS) hit falling into this general class that was quickly expanded into a series of β-substituted phenylpropanoates that displayed potent agonism on the GPR40 receptor (Table 1).[13]

As shown in Scheme 1, synthetic access to the lead series was a straightforward extension of a literature procedure[14] involving addition of the desired Grignard reagent to the Meldrum's acid adduct with *para*-methoxybenzyl (PMB) protected *p*-hydroxybenzaldehyde. Hydrolysis and decarboxylation was then carried out in aqueous pyridine to obtain the desired carboxylic acids. In order to vary the benzylic ether portion of the molecule, the head group phenol was generated by esterifying the carboxylic acid **3** with EDC and ethanol followed by selective removal of the PMB ether in refluxing acetic acid. Alkylation of the phenol with a variety of benzylic and non-benzylic halides could be routinely accomplished in DMF with either potassium or cesium carbonate as a base. Hydrolysis of the ester under basic conditions then afforded the desired carboxylic acids **5**.

Since, as noted above, GPR40 is a $G_{\alpha}q$-coupled GPCR, compounds were tested for GPR40 activity in a functional assay monitoring calcium flux in CHO cells transiently transfected with GPR40 (details in Supplementary data). For greater consistency among assay runs, compound **3g** was used as a reference for intrinsic efficacy (defined as 100%) because the most potent endogenous ligand for GPR40, docosahexaenoic acid (DHA), is oxidatively unstable.

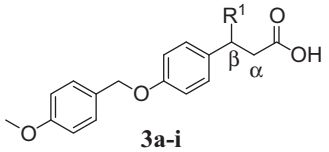
As shown in Table 1, activity on the GPR40 receptor varies significantly with substitution at the β-carbon relative to the carboxylate. While simple alkyl substitution (**3b**) was deleterious to GPR40 activity compared to the unsubstituted parent compound, unsaturated substituents such as alkenes (**3e**), alkynes (**3g**), or an aryl group (**3d**) improved potency to varying degrees. Among the unsaturated substituents, branching was preferred remote from the atom attached to the β-carbon as shown by the greater potency of compound **3e** over **3f**.

The alkyne derivative **3g** was also of interest due to its good potency, particularly in enantiopure form. The separated enantiomers of compound **3g** showed markedly different activity, with the more potent enantiomer **3i** showing the highest potency in the group. Alkyne derivatives also possessed a distinct advantage over alkenes such as compound **3e** in that the GPR40 activity of the alkynes crossed over fully to the rodent receptors whereas non-alkyne substituents showed greatly diminished activity on the rodent receptor (data not shown). Because of the importance of rodent preclinical models for establishing antidiabetic activity in vivo, further optimization was focused on the β-alkynyl series.

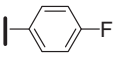


Scheme 1. *Reagents and conditions*: (a) PMB–Cl, K_2CO_3, DMF; (b) Meldrum's acid, piperidine, acetic acid, toluene; (c) R^1MgX, THF; (d) H_2O, pyridine, 100 °C; (e) EtOH, EDC, DMAP; (f) AcOH, Δ; (g) R^2X, Cs_2CO_3, DMF; (h) KOH, EtOH, H_2O.

Table 1
β-Substituted phenylpropanoate lead series



3a-i

Compound	$\mathbf{R^1}$	hGPR40 EC_{50} (±S.D.)[a] (μM)	hGPR40 E_{max} (±S.D.)[b] (%)
3a	–H	1.1 (±0.09)	94 (±5)
3b		3.2 (±0.1)	94 (±5)
3c		0.90 (±0.7)	97 (±9)
3d		0.58 (0.2)	110 (±8)
3e		0.12 (±0.05)	105 (±5)
3f		1.2 (±1)	82 (±19)
3g		0.26 (±0.2)	100
3h		6.7 (±2)	93 (±12)
3i		0.064 (±0.02)	99 (±5)

[a] Mean of at least duplicate runs.
[b] % Compared to reference agonist **3g**.

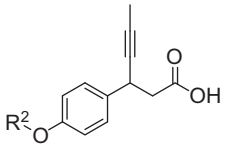
Table 2 details exploration of the tail group. Homologating the benzyl ether (**5a**) to the phenethyl ether (**5b**) resulted in substantial lost potency. Replacement of the methyl ether in **3g** with the strongly electron-withdrawing trifluoromethyl group led to a dramatic loss in both potency and efficacy. In contrast, a significant increase in potency was obtained by attaching a phenyl group in the *meta*-position. The preference for *meta* substitution was less evident with methoxy substituents (**3g** vs **5e**) than the phenyls (**5h** vs **5f**). Even in the *meta*-biphenyl case, however, shortening the benzyl ether to a diaryl ether (**5i**) decreased potency. Further improvement in potency could be obtained by substitution of the terminal arene ring as shown by the trifluoromethylated compound **5j**.

Because of its high potency, compound **5j** was quickly identified as a compound of interest. Knowing that we preferred a single enantiomer to interrogate in vivo activity, a non-racemic synthesis for this compound and others in the series was required. Although the racemates were separable by chiral HPLC, the process was tedious, unpredictable from compound-to-compound, and unsuitable for preparing larger amounts of compound in a timely fashion. A non-racemic synthesis of the key intermediate phenol (**7**) not only accelerated new analog synthesis but also enabled more rapid production of large quantities of compound.

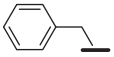
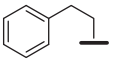
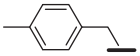
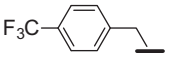
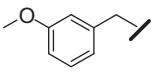
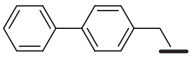
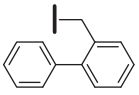
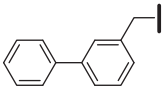
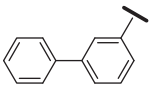
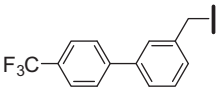
We found it desirable to retain the robust and mild addition of an alkynyl Grignard reagent to a Meldrum's acid adduct in order to form the branched β-center relative to the carboxylic acid. It was decided to employ a chiral resolution in order to quickly develop asymmetric access to the desired building block **7**. The modified synthetic sequence is detailed in Scheme 2.

Condensation of *p*-hydroxybenzaldehyde with Meldrum's acid can be carried out in water to obtain a crystalline adduct.[15] The addition of 1-propynylmagnesium bromide to the highly activated Meldrum's acid adduct occurs smoothly even without protection of the phenol. Hydrolysis and decarboxylation of the Meldrum's acid moiety can be carried out in aqueous 3-pentanone. The racemic hydroxy-acid was then resolved with (1*S*,2*R*) -1-amino-2-indanol by

Table 2
Tail group exploration



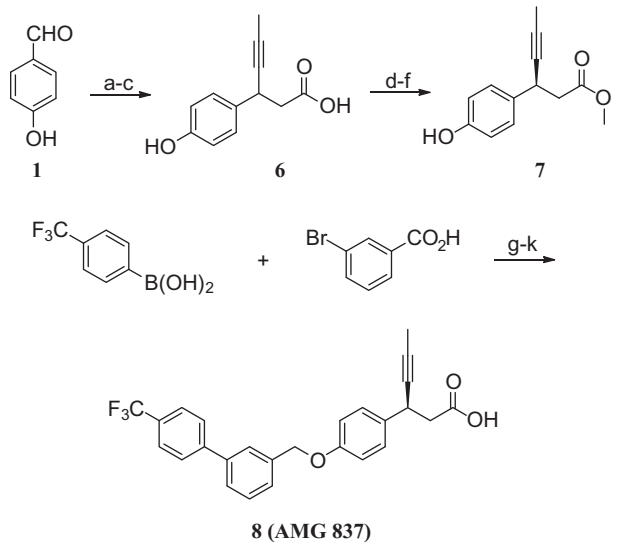
5a-j

Compound	R^1	hGPR40 EC_{50} (±S.D.)[a] (μM)	hGPR40 E_{max} (±S.D.)[b] (%)
5a		0.62 (±0.4)	97 (±2)
5b		6.2 (±4)	126 (±6)
5c		0.18 (±0.1)	97 (±6)
5d		27 (±15)	65 (±54)
5e		0.11 (±0.05)	104 (±3)
5f		1.2 (±0.6)	120 (±5)
5g		0.24 (±0.1)	82 (±8)
5h		0.058 (±0.03)	104 (±4)
5i		0.36 (±0.05)	106 (±1)
5j		0.025 (±0.01)	104 (±4)

[a] Mean of at least duplicate runs.
[b] % Compared to reference agonist **3g**.

repeated crystallizations from hot *iso*-propanol. After acid treatment to break the salt, analysis by chiral HPLC showed 97–98.5% of the desired enantiomer depending on the scale of the resolution. Methyl ester **7** was then obtained through a Fischer esterification. Notably, this sequence requires no chromatography save a quick filtration through silica gel after the esterification step to remove baseline impurities. The absolute configuration of compound **7** was established by derivatization with the Oppolzer camphorsultam auxiliary[16] and obtaining a crystal structure (see Supplementary data). From the chiral building block **7**, the synthesis of the desired *S*-enantiomer of **5j** was carried out in a straightforward fashion. Improvements to this synthetic sequence to allow preparation of multikilogram amounts of compound **8** as well as an enantioselective synthesis have been reported elsewhere.[17,18]

Compound **8** displayed the expected two-fold increase in potency on GPR40 (EC_{50} = 13 [±7] nM) compared to the racemic compound and its activity crossed over to the rat and mouse forms of GPR40 (EC_{50} = 23 and 13 nM, respectively). Because of our interest in the compound, the intrinsic efficacy of compound **8** was determined compared to DHA. Compound **8** was thus found to be a partial agonist on GPR40 with maximal activity 85% of that shown by DHA under our standard assay conditions.[19] In addition to its activity in the Ca^{2+}-flux assay, compound **8** shows functional activity in a



Scheme 2. Non-racemic synthesis of (*S*)-hexynoate **7** and synthesis of AMG 837. *Reagents and conditions*: (a) Meldrum's acid, H_2O, 75 °C, 84%; (b) 1-propynylmagnesium bromide, THF; (c) H_2O, 3-pentanone, 80 °C 85% over two steps; (d) (1*S*,2*R*)-1-amino-2-indanol, *i*-PrOH; (e) HCl, H_2O, EtOAc, 37% over two steps; (f) MeOH, H_2SO_4 (cat.), 95%; (g) Pd/C, *i*-PrOH, H_2O, 90%; (h) $LiAlH_4$, THF, 0 °C, 97%; (i) $SOBr_2$, CH_2Cl_2, 92%; (j) **8**, Cs_2CO_3, acetone, 97%; (k) NaOH, EtOH, H_2O.

mouse β-cell line (MIN6). As shown in Figure 1, compound **8** is a highly potent stimulator of insulin secretion in MIN6 cells with an EC_{50} comparable to that seen in the aequorin Ca^{2+}-flux assay.

While highly potent on GPR40, compound **8** was inactive on the closely related GPCRs GPR41 and GPR43. Despite a possible structural resemblance to some PPAR agonists, compound **8** showed no significant activity in cell-based assays against PPAR-α, -δ, and -γ.

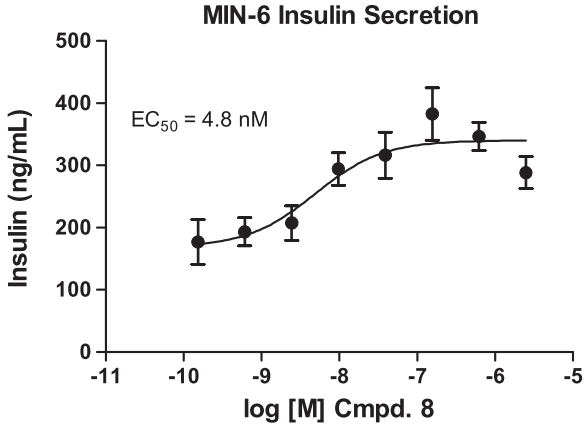


Figure 1. Stimulation of insulin secretion in MIN6 Cells by compound **8.**

Table 3
Pharmacokinetic properties of compound **8**

Property	Mouse	Rat	Beagle Dog	Cynomolgus Monkey
i.v.				
Dose (mg/kg)	0.8	0.5	0.5	0.5
Cl (L/h/kg)	0.07	0.07	0.08	0.06
$T_{½}$ (h)	8	7.2	28	12.4
V_{dss} (L/kg)	0.56	0.58	2.1	0.50
p.o.				
Dose (mg/kg)	5	0.5	2	0.5
%*F*	67	84	100	88
AUC (μg·h/L)	47500	6160	33800	7370
C_{max} (μM)	17	1.4	7.5	2.1

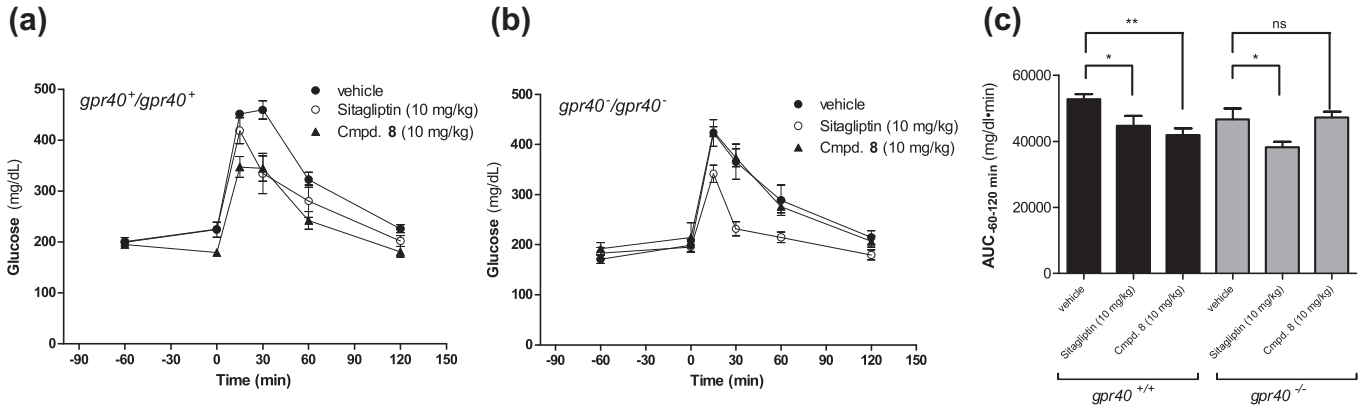


Figure 2. Effect of compound **8** during OGTT in wild type and GPR40 KO mice.

An external panel of 64 receptors also revealed no significant activity with the exception of weak inhibition (IC_{50} = 3 μM) on the α_2-adrenergic receptor. Overall, compound **8** was both highly potent and selective in vitro.

In addition to its favorable profile in vitro, compound **8** distinguished itself by displaying an excellent pharmacokinetic profile in multiple species. As shown in Table 3, compound **8** combines low clearance, long half-life, and high oral bioavailability in four preclinical species.

In order to confirm that the potential antidiabetic activity of compound **8** was mediated by GPR40, an oral glucose tolerance test (OGTT) was carried out in wild-type and GPR40 KO mice. The DPP-4 inhibitor sitagliptin was used at a maximally efficacious dose as a positive control.[20] Compounds were dosed orally 60 min prior to the oral glucose challenge. As shown in Figure 2, compound **8** substantially blunted plasma glucose excursion compared to both vehicle and positive control in wild-type animals consistent with its activity as an insulin secretagogue as shown in MIN6 cells (Fig. 2a). The total glucose AUC was also reduced in a statistically significant manner (Fig. 2c). In contrast, no effect in the OGTT was seen in the GPR40 KO animals after dosing with compound **8** where the positive control retained activity (Fig. 2b). The complete absence of a response in the GPR40 KO animals establishes that the effects of compound **8** are GPR40 mediated. The behavior of compound **8** in this GTT study is consistent with the hypothesis that selective GPR40 agonists could serve as glucose-dependent insulin secretagogues.

In summary, through optimization of a lead series of simple benzyloxy-substituted phenylpropanoic acids, we identified compound **8** as a highly potent agonist of GPR40. Due to its combination of high in vitro potency and selectivity, favorable pharmacokinetic profile, and robust GPR40-mediated in vivo antidiabetic activity, compound **8** (AMG 837) was selected for clinical evaluation.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.10.118.

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