

# Discovery of AM-1638: A Potent and Orally Bioavailable GPR40/FFA1 Full Agonist

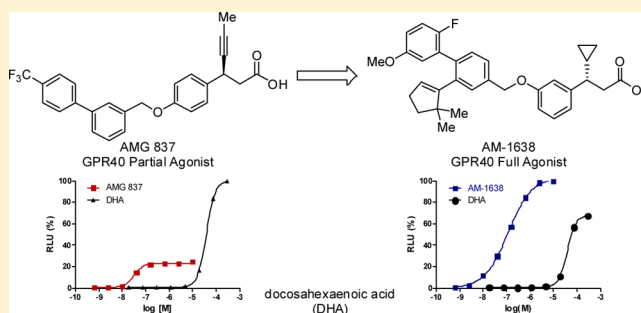
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## S Supporting Information

**ABSTRACT:** GPR40 (FFA1) is a G-protein-coupled receptor, primarily expressed in pancreatic islets, the activation of which elicits increased insulin secretion only in the presence of elevated glucose levels. A potent, orally bioavailable small molecule GPR40 agonist is hypothesized to be an effective antidiabetic posing little or no risk of hypoglycemia. We recently reported the discovery of AMG 837 (**1**), a potent partial agonist of GPR40. Herein, we present the optimization from the GPR40 partial agonist **1** to the structurally and pharmacologically distinct GPR40 full agonist AM-1638 (**21**). Moreover, we demonstrate the improved in vivo efficacy that GPR40 full agonist **21** exhibits in BDF/DIO mice as compared to partial agonist **1**.

**KEYWORDS:** GPR40, full agonist, AM-1638, AMG 837, insulin secretagogue, FFA1



Type II diabetics lose their ability to maintain glucose homeostasis due to defects in both insulin secretion and action.<sup>1</sup> GPR40 (FFA1) is a G-protein-coupled receptor, primarily expressed in pancreatic islets.<sup>2</sup> When activated by medium to long chain fatty acids, GPR40 elicits increased insulin secretion only in the presence of elevated glucose levels.<sup>3</sup>

This alluring mechanism to treat type II diabetes presents the potential of little or no risk of hypoglycemia and has been investigated by multiple groups, leading to the discovery of several clinical candidates.<sup>4–7</sup> We previously described the discovery of AMG 837 (**1**),<sup>8–10</sup> a small molecule partial agonist of GPR40 that displays oral efficacy in a variety of rodent diabetic models without exhibiting hypoglycemia. Because of the robust antidiabetic activity and favorable pharmacokinetic properties, **1** was selected for clinical evaluation. Because the ability of partial agonist **1** to maintain glycemic control was being tested in a clinical setting, we became interested in interrogating GPR40 with full agonists. We hypothesized that a GPR40 full agonist should have a greater ability to induce insulin secretion and thus provide greater glycemic control. In this letter, we describe in detail the structure–activity relationship (SAR) studies that started from the GPR40 partial agonist **1** and culminate with the identification of GPR40 full agonist AM-1638 (**21**) and provide further evidence that GPR40 full agonists demonstrate superior efficacy over partial agonists when evaluated in vivo.

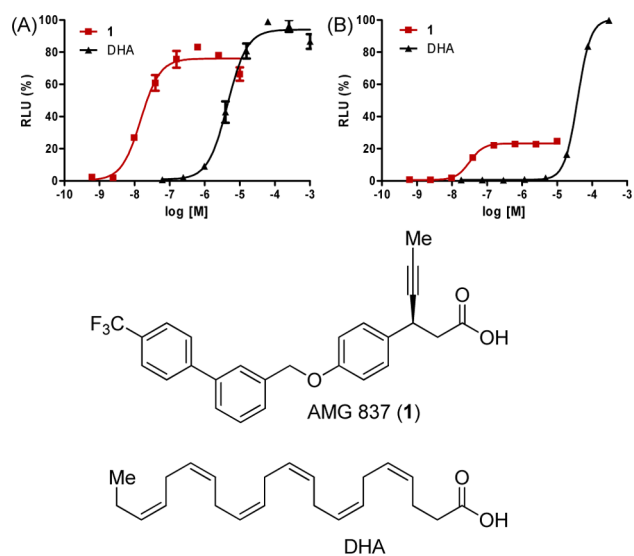
To provide a greater dynamic range with which to assess improvements in intrinsic efficacy, we chose to reevaluate compounds previously synthesized toward discovery of partial agonist **1** in CHO cells transfected with lower levels of GPR40 expression plasmid [from 5.0 (Figure 1A) to 0.05  $\mu\text{g}$  (Figure 1B)].<sup>9</sup> Under the original 5.0  $\mu\text{g}$  plasmid conditions, partial agonist **1** demonstrates 75% of the response ( $E_{\text{max}}$ ) shown by the natural free fatty acid ligand docosahexaenoic acid (DHA) (Figure 1A). In contrast, reducing the expression plasmid to 0.05  $\mu\text{g}$  affords an assay with the appropriate dynamic range to distinguish GPR40 partial and full agonists. As depicted in Figure 1B, partial agonist **1** displays 25%  $E_{\text{max}}$  as compared to DHA. For routine evaluation of compounds, a stable cell line was developed that displays lower GPR40 expression.

Using the revised assay conditions in the presence of 0.1% human serum, we discovered that removing the trifluoromethyl moiety of partial agonist **1** and relocating the aryl–aryl linkage of the biphenyl from the meta to the para orientation provides an increase in intrinsic efficacy from 20 to 105%, albeit with a substantial loss of potency [Table 1, ( $\pm$ )-2, 7.7  $\mu\text{M}$ ]. The addition of a 3-methoxy group to the terminal aryl ring of the biphenyl provides a significant increase in potency while maintaining 98% intrinsic efficacy [Table 1, ( $\pm$ )-3, 2.3  $\mu\text{M}$ ].

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**Figure 1.** (A) Effect of **1** and DHA in CHO cells transfected with 5.0  $\mu\text{g}$  of GPR40 expression plasmid. (B) Effect of **1** and DHA in CHO cells transfected with 0.05  $\mu\text{g}$  of GPR40 expression plasmid.

Evaluation of the single enantiomers of full agonist lead **3** revealed that while the majority of the potency resided in (*S*)-**4** (Table 1, 0.65  $\mu\text{M}$ ), compounds in this enantiomeric series are partial agonists (Table 1, **4**, 47%). Thus, we began a chemical optimization of the less active enantiomer (*R*)-**5** due to its demonstration of full agonism (Table 1, 4.0  $\mu\text{M}$ , 99%). Further evaluation of the terminal aryl ring revealed that a 2-fluoro-5-methoxy substitution imparts an increase in potency (Table 1, **6**, 2.3  $\mu\text{M}$ , 97%). The observation that the chirality of  $R_1$  determined whether GPR40 agonists were partial or full prompted us to remove the methylalkyne functionality. To our delight, a simple hydrogen substitution at  $R_1$  maintains full agonism and increased potency (Table 1, **7**, 1.9  $\mu\text{M}$ , 96%). Relocating the 4-phenylbenzyloxy group from A4 on the A ring to A3 affords an additional improvement in potency (Table 1, **8**, 1.2  $\mu\text{M}$ , 104%).

Introduction of a *tert*-butyl substituent on the B ring ortho to the biaryl linkage supplies a further increase in potency (Table 2, **9**, 0.9  $\mu\text{M}$ , 100%). We examined substitutions at  $R_1$  on this more potent scaffold with the knowledge that compounds in the same enantiomeric series as alkynes **5** and **6** maintain full agonism. The general synthesis of agonists **10**–**17** was carried out via a high yielding asymmetric rhodium-catalyzed conjugate addition<sup>11</sup> to construct the requisite phenol (Scheme 1). The biphenyl component was constructed using Suzuki–Miyaura coupling and then reduction of the methyl ester, and the resulting alcohol was exchanged for a chlorine. Facile etherification of the phenol with the biphenyl methyl chloride followed by hydrolysis yields the desired final compounds.<sup>12</sup> A modest increase in potency was observed upon systematically increasing the size of  $R_1$  from hydrogen to methyl and then ethyl (Table 2; **9**, 0.90  $\mu\text{M}$ ; **10**, 0.77  $\mu\text{M}$ ; **11**, 0.68  $\mu\text{M}$ ). A further increase in the size of  $R_1$  to *n*-propyl or *iso*-propyl did not lead to any additional gain in potency (Table 2; **12**, 0.69  $\mu\text{M}$ ; **13**, 2.2  $\mu\text{M}$ ). Realizing that small alkyl substituents were preferred at  $R_1$ , we evaluated a series of alkyl rings. Cyclopropyl and cyclobutyl provided significant increases in potency (Table 2; **14**, 0.37  $\mu\text{M}$ ; **15**, 0.42  $\mu\text{M}$ ), while larger rings such as cyclopentyl and cyclohexyl led to a substantial loss in potency and intrinsic efficacy (Table 2; **16**, 1.31  $\mu\text{M}$ , 59%; **17**, 2.1  $\mu\text{M}$ ,

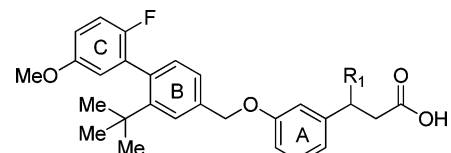
**Table 1. Initial Identification of GPR40 Full Agonists**

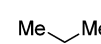





Compound	Position	X	Y	$R_1$	GPR40 $\text{EC}_{50}$ ( $\mu\text{M}$ ) <sup>a</sup>	Efficacy (%) <sup>a,b</sup>
DHA					36	67
<b>1</b>					0.06	20
<b>2</b>	4	H	H	Me	7.7	105
<b>3</b>	4	OMe	H	Me	2.3	98
<b>4</b>	4	OMe	H	Me	0.65	47
<b>5</b>	4	OMe	H	Me	4.0	99
<b>6</b>	4	OMe	F	Me	2.3	97
<b>7</b>	4	OMe	F	H	1.9	96
<b>8</b>	3	OMe	F	H	1.2	104

<sup>a</sup>Mean of at least two runs. <sup>b</sup>Percent compared to reference agonist **21**.

41%). Phenyl and trifluoromethyl substitutions also led to a decrease in potency (Table 2; **18**, 1.2  $\mu\text{M}$ ; **19**, 2.1  $\mu\text{M}$ ).

After identification of the cyclopropane moiety as the favored substituent at  $R_1$ , we returned to optimization of the biphenyl group. We previously observed exchanging a hydrogen at  $R_2$  for a *tert*-butyl substituent leads to a modest increase in potency in an assay run in the presence of 0.1% human serum (Table 3; **20**, 0.48  $\mu\text{M}$ ; **14**, 0.37  $\mu\text{M}$ ). However, when the assay is run in the presence of 100% human serum, this modification affords a 20-fold improvement in potency (Table 3; **20**, 38  $\mu\text{M}$ ; **14**, 1.9  $\mu\text{M}$ ). Further increasing the size of the  $R_2$  substituent to 5,5-dimethylcyclopentenyl moiety provides a GPR40 full agonist that displays the requisite potency in the presence of 100% human serum (Table 3; **21**, 0.71  $\mu\text{M}$ ) to warrant evaluation in vivo. In contrast, the enantiomer of full agonist **21** is both less potent and a partial agonist (Table 3; **22**, 34  $\mu\text{M}$ , 71%).

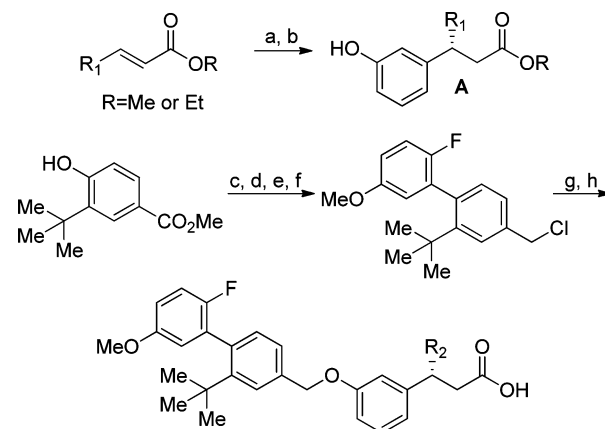
Table 2. Exploration of R<sub>1</sub> SAR


Compound	R <sub>1</sub>	GPR40 EC <sub>50</sub> (μM) <sup>a</sup>	Efficacy (%) <sup>a,b</sup>
9	H	0.90	113
10	Me	0.77	107
11	Et	0.68	110
12	n-Pr	0.69	94
13		2.2	86
14		0.37	103
15		0.42	106
16		1.3	59
17		2.1	41
18		1.2	80
19	CF <sub>3</sub>	2.1	111

<sup>a</sup>Mean of at least two runs. <sup>b</sup>Percent compared to reference agonist **21**.

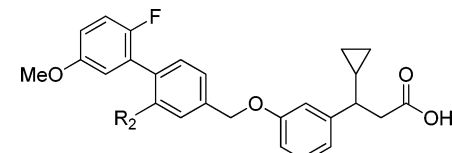
To supply quantities of full agonist **21** sufficient for in vivo studies, a scalable synthesis was developed (Scheme 2). Protection of phenol **23** as a THP ether allowed introduction of the *S,S*-dimethylcyclopentyl group by Suzuki–Miyaura coupling utilizing the Buchwald biarylphosphine 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl (*S*-Phos) as a supporting ligand.<sup>13</sup> Mild removal of the THP group under acidic conditions, followed by activation of the phenol as a triflate and subsequent palladium-catalyzed Suzuki–Miyaura coupling, again employing *S*-Phos, yielded biphenyl **24**. Reduction of the ester and chlorination delivered chloride **26**. Rhodium-catalyzed asymmetric 1,4-addition of 3-hydroxybenzene boronic acid to  $\alpha,\beta$ -unsaturated ester **27** delivered phenol **28** in good yield and enantioselectivity. Alkylation of enantiomerically pure phenol **28** with chloride **26**, followed by ester hydrolysis, affords full agonist **21** in 36% overall yield.




Importantly, full agonist **21** exhibits moderate cross-species plasma clearance and volume of distribution, resulting in plasma half-lives suitable for evaluation of its antidiabetic properties in mouse, rat, and cynomolgus monkey (Table 4).

Scheme 1. <sup>a</sup>

<sup>a</sup>Reagents and conditions: (a) 3-Hydroxybenzene boronic acid, Rh(I)(OH)/(*S,S*)-2,2'-bis(diphenylphosphino)-1,1'-binaphthyl dimer, H<sub>2</sub>O, 1,4-dioxane, 86–96%, 83–96% ee. (b) Chiral resolution to remove minor isomer: Chiralcel OD-H column, 3% *i*PrOH/hexane, 220 nm. (c) (Tf)<sub>2</sub>O, Et<sub>3</sub>N, 4-dimethylaminopyridine, CH<sub>2</sub>Cl<sub>2</sub>, 98%. (d) 2-Fluoro-5-methoxybenzene boronic acid, Pd(PPh<sub>3</sub>)<sub>4</sub>, K<sub>2</sub>CO<sub>3</sub>, DMF, 100 °C, 71%. (e) LiAlH<sub>4</sub>, THF, 0 °C, 72%. (f) SOCl<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>, 74%. (g) A, Cs<sub>2</sub>CO<sub>3</sub>, DMF. (h) LiOH, EtOH, H<sub>2</sub>O.

Table 3. Effect of Biphenyl Modification on GPR40 Activity in the Presence 100% Human Serum

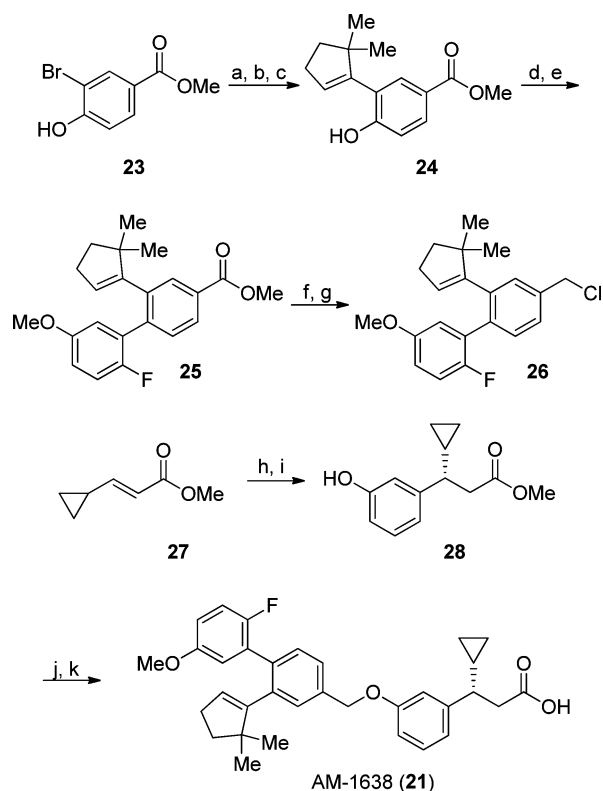


Compound	R <sub>2</sub>	stereo-chemistry	GPR40 EC <sub>50</sub> (μM) <sup>a</sup>	GPR40 EC <sub>50</sub> 100% HS (μM) <sup>a</sup>	Efficacy (%) <sup>a,b</sup>
1	H		0.06		20
20	H	( <i>S</i> )	0.48	38	107
14		( <i>S</i> )	0.37	1.9	103
21		( <i>S</i> )	0.16	0.71	100
22		( <i>R</i> )	1.1	34	76

<sup>a</sup>Mean of at least two runs. <sup>b</sup>Percent compared to reference agonist **21**.

Moreover, oral administration of full agonist **21** demonstrates excellent oral bioavailability (mouse, >100%; rat, 72%; and cyno, 71%), affording the GPR40 full agonist **21** and the appropriate pharmacokinetic properties for comparison in vivo to the partial agonist **1**.

Full agonist **21** and partial agonist **1** were compared at a dose of 60 mg/kg for their ability to improve glycemic control in BDF mice with diet-induced obesity (DIO), a model of type II diabetes that develops elevated blood glucose and impaired glucose tolerance.<sup>14</sup> Both compounds reduce blood glucose excursion (Figure 2A; **1** [glucose]<sub>max</sub> 317 mg/dL; **21**,

Scheme 2.<sup>a</sup>

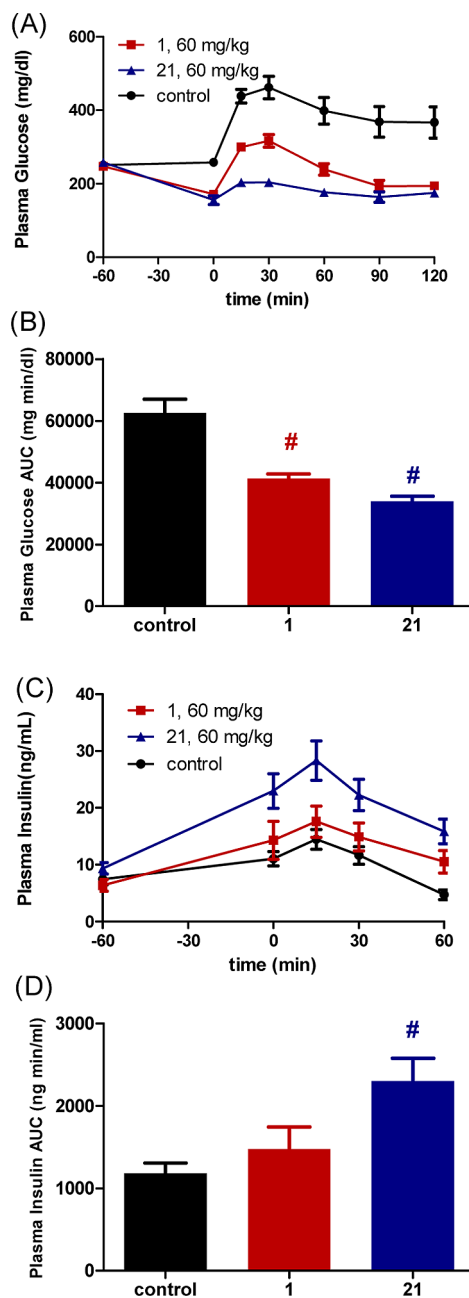
<sup>a</sup>Reagents and conditions: (a) Dihydropyran, pyridinium *p*-toluenesulfonate, 90%. (b) 2-(5,5-Dimethylcyclopent-1-enyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, Pd(OAc)<sub>2</sub>, S-Phos, K<sub>3</sub>PO<sub>4</sub>, DMF/H<sub>2</sub>O, 60 °C, 80%. (c) Pyridinium *p*-toluenesulfonate, MeOH, 90%. (d) PhN(Tf)<sub>2</sub>, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, 88%. (e) 2-Fluoro-5-methoxybenzene boronic acid, Pd(PPh<sub>3</sub>)<sub>4</sub>, K<sub>2</sub>CO<sub>3</sub>, DMF, 90 °C, 91%. (f) LiAlH<sub>4</sub>, THF, 0 °C. (g) SOCl<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>, 86% over two steps. (h) 3-Hydroxybenzene boronic acid, Rh(I)(OH)/(S)-2,2'-bis-(diphenylphosphino)-1,1'-binaphthyl dimer, H<sub>2</sub>O, 1,4-dioxane, 60%, 85% ee. (i) Chiral resolution to remove minor isomer: Chiralcol OD-H column, 3% *i*PrOH/hexane, 220 nm, rt, 40 min. (j) Compound 26, Cs<sub>2</sub>CO<sub>3</sub>, DMF. (k) LiOH, EtOH, H<sub>2</sub>O, 80% over two steps.

Table 4. Pharmacokinetic Properties of Full Agonist 21<sup>a</sup>

species	oral C <sub>max</sub> (μM)	t <sub>max</sub> (h)	Cl (L/h/kg)	Vd <sub>ss</sub> (μM)	iv t <sub>1/2</sub> (h)	% F
mouse	14	1	0.18	0.45	2.0	>100
rat	3.6	3.5	0.91	1.1	1.8	72
cyno	5.1	2	0.81	2.0	2.1	71

<sup>a</sup>Administered at a dose of 1 mg/kg, iv; 5 mg/kg, po, in mice. Administered at a dose of 0.5 mg/kg, iv; 2 mg/kg, po, in rats. Administered at a dose of 0.5 mg/kg, iv; 2 mg/kg, po, in cyno. Data are expressed as mean values (*n* = 3).

[glucose]<sub>max</sub> 204 mg/dL) and area under the curve of blood glucose (AUC 0–60 min) (Figure 2B) upon administration 1 h before an oral glucose tolerance test (OGTT) as compared to the control (Figure 2A; control [glucose]<sub>max</sub> 462 mg/dL). However, full agonist 21 shows greater efficacy in blunting glucose excursion than partial agonist 1. Moreover, full agonist 21 imparts greater 46% improvement in AUC<sub>glucose</sub>, while partial agonist 1 provides 34%. Partial agonist 1 does not display a statistically significant increase in plasma insulin as compared to the control. However, full agonist 21 elicits a statistically significant increase in both plasma insulin at all time



**Figure 2.** The effect of full agonist 21 and partial agonist 1 during an OGTT in BDF/DIO mice. Time-dependent changes in (A) plasma glucose and (C) plasma insulin after oral administration of either full agonist 21 or partial agonist 1 followed by 1 g/kg oral glucose challenge. (B) Incremental AUC<sub>0–120 min</sub> of plasma glucose levels. (D) Incremental AUC<sub>–60–60 min</sub> of plasma insulin levels. Values are means ± SDs (*n* = 6). #*p* ≤ 0.025 as compared with control by one-tailed Williams' test.

points and in the AUC<sub>insulin</sub> (Figure 2C,D). This is in accord with our original hypothesis and further confirms that the greater intrinsic efficacy observed *in vitro* for full agonist 21 affords advantages *in vivo* for maintaining plasma glucose homeostasis as compared to partial agonist 1.

In conclusion, we have described the SAR leading from the GPR40 partial agonist 1 to a structurally and pharmacologically distinct series of GPR40 agonists, exemplified by the orally bioavailable GPR40 full agonist 21. The antidiabetic activity that full agonist 21 exhibits in BDF/DIO mice provides

compelling evidence that GPR40 full agonists afford access to a powerful mechanism for maintaining glycemic control and great potential for the treatment of type II diabetic patients.

## ■ 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.

## ■ REFERENCES

(1) DeFronzo, R. A. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes. *Diabetes* **2009**, *58*, 773–795.

(2) Itoh, Y.; Kawamata, Y.; Harada, M.; Kobayashi, M.; Fujii, R.; Fukusumi, S.; Ogi, K.; Hosoya, M.; Tanaka, Y.; Uejima, H.; Tanaka, H.; Maruyama, M.; Satoh, R.; Okubo, S.; Kizawa, H.; Komatsu, H.; Matsumura, F.; Noguchi, Y.; Shinohara, T.; Hinuma, S.; Fujisawa, Y.; Fujino, M. Free fatty acids regulate insulin secretion from pancreatic  $\beta$  cells through GPR40. *Nature* **2003**, *422*, 173–176.

(3) Stoddart, L. A.; Smith, N. J.; Milligan, G. International union of pharmacology. LXXI. Free fatty acid receptors FFA1, -2, and -3: Pharmacology and pathophysiological functions. *Pharmacol. Rev.* **2008**, *60*, 405–417.

(4) For a review, see Medina, J. C.; Houze, J. B. GPR40 (FFAR1) modulators. *Annu. Rep. Med. Chem.* **2008**, *43*, 75–85.

(5) For a review, see Bharate, S. B.; Nemmani, K. V.S.; Vishwakarma, R. A. Progress in the discovery and development of small-molecule modulators of G-protein-coupled receptor 40 (GPR40/FFA1/FFAR1): An emerging target for type 2 diabetes. *Expert Opin. Ther. Pat.* **2009**, *19*, 237–264.

(6) Negoro, N.; Sasaki, S.; Mikami, S.; Ito, M.; Suzuki, M.; Tsujihata, Y.; Ito, R.; Harada, A.; Takeuchi, K.; Suzuki, N.; Miyazaki, J.; Santou, T.; Odani, T.; Kanzaki, N.; Funami, M.; Tanaka, T.; Kogame, A.; Matsunaga, S.; Yasuma, T.; Momose, Y. Discovery of TAK-875: A potent, selective, and orally bioavailable GPR40 agonist. *ACS Med. Chem. Lett.* **2010**, *1*, 290–294.

(7) Christiansen, E.; Due-Hansen, M. E.; Urban, C.; Merten, N.; Pfeleiderer, M.; Karlsen, K. K.; Rasmussen, S. S.; Steensgaard, M.; Hamacher, A.; Schmidt, J.; Drewke, C.; Petersen, R. K.; Kristiansen, K.; Ullrich, S.; Kostenis, E.; Kassack, M. U.; Ulven, T. Structure-activity study of dihydrocinnamic acids and discovery of the potent FFA1 (GPR40) agonist TUG-469. *ACS Med. Chem. Lett.* **2010**, *1*, 345–349.

(8) Woo, J. C. S.; Cui, S.; Walker, S. D.; Faul, M. M. Asymmetric syntheses of a GPR40 receptor agonist via diastereoselective and enantioselective conjugate alkylation. *Tetrahedron* **2010**, *66*, 4730–4737.

(9) Lin, D. C-H.; Zhang, J.; Zhuang, R.; Li, F.; Nguyen, K.; Chen, M.; Tran, T.; Lopez, E.; Lu, J. Y. L.; Li, X. N.; Tang, L.; Tonn, G. R.; Swaminath, G.; Reagan, J. D.; Chen, J.-L.; Tian, H.; Lin, Y.-J.; Houze, J. B.; Luo, J. AMG 837: A novel GPR40/FFA1 agonist that enhances insulin secretion and lowers glucose levels in rodents. *PLoS One* **2011**, *6*, e27270.

(10) Houze, J. B.; Zhu, L.; Sun, Y.; Akerman, M.; Qiu, W.; Zhang, A. J.; Sharma, R.; Schmitt, M.; Wang, Y.; Liu, J.; Liu, J.; Medina, J. C.; Reagan, J. D.; Luo, J.; Tonn, G.; Zhang, J.; Lu, J. Y.-L.; Chen, M.; Lopez, E.; Nguyen, K.; Yang, L.; Tang, L.; Tian, H.; Shuttleworth, S. J.; Lin, D. C-H. AMG 837: A potent, orally bioavailable GPR40 agonist. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1267–1270.

(11) Hayashi, T.; Yamasaki, K. Rhodium-catalyzed asymmetric 1,4-addition and its related asymmetric reactions. *Chem. Rev.* **2003**, *103*, 2829–2844.

(12) Walker, S. D.; Borths, C. J.; DiVirgilio, E.; Huang, L.; Liu, P.; Morrison, H.; Sugi, K.; Tanaka, M.; Woo, J. C. S.; Faul, M. M. Development of a scalable synthesis of a GPR40 receptor agonist. *Org. Process Res. Dev.* **2011**, *15*, 570–580.

(13) Barder, T. E.; Walker, S. D.; Martinelli, J. R.; Buchwald, S. L. Catalysts for Suzuki–Miyaura coupling processes: Scope and studies of the effect of ligand structure. *J. Am. Chem. Soc.* **2005**, *127*, 4685–4696.

(14) Karasawa, H.; Nagata-Goto, S.; Takaishi, K.; Kumagai, Y. A novel model of type 2 diabetes mellitus based on obesity induced by high-fat diet in BDF1 mice. *Metabolism* **2009**, *58*, 296–303.