ACS Medicinal Chemistry Letters

Letter

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ACS Med. Chem. Lett., **Just Accepted Manuscript** • DOI: 10.1021/acsmedchemlett.8b00213 • Publication Date (Web): 06 Jun 2018

Just Accepted

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Discovery of AM-6226. A Potent and Orally Bioavailable GPR40 Full Agonist that Displays Efficacy in Non-Human Primates.

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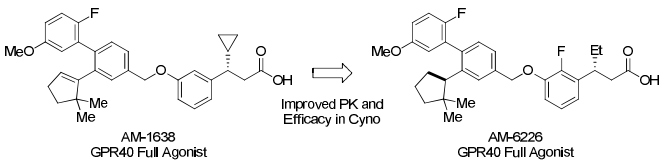
KEYWORDS: GPR40, full agonist, AM-6226, AM-1638, AMG 837, insulin secretagogue, FFA1

Supporting Information Placeholder

ABSTRACT: GPR40 (FFA1) is a G-protein-coupled receptor, primarily expressed in pancreatic islets and enteroendocrine L-cells, when activated elicits increased insulin secretion only in the presence of elevated glucose levels. We recently reported the discovery of AM-1638 (**2**), a full agonist of GPR40. Here in, we present further structure–activity relationships progressing from AM-1638 (**2**) to AM-6226 (**14**) that possesses a profile acceptable for dosing cynomolgus monkeys. The GPR40 full agonist AM-6226 (**14**) is the first molecule to display significant glucose lowering in cynomolgus monkeys providing additional evidence that GPR40 full agonists afford access to a powerful mechanism for maintaining glycemic control.



Type II diabetics lose their ability to maintain glucose homeostasis due to multiple metabolic defects.[1] GPR40 is a G-protein-coupled receptor, primarily expressed in pancreatic islets β-cells and enteroendocrine L-cells[2] that possess the ability to modulate several metabolic defects when activated. Medium- to long-chain fatty acids bind to and elicit GPR40 to increase insulin secretion from β-cells and increased secretion of the gut hormones, glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP).[3,4,5]

Agonists of GPR40 present low risk of hypoglycemia and have been considered by multiple groups leading to the discovery of multiple clinical candidates.[4,5,6,7,8,9] These clinical candidates, such as AMG 837 (**1**), are all partial agonists of GPR40. We previously described the discovery of **1**,[10,11,12] that demonstrations antidiabetic activity in rodent models without exhibiting hypoglycemia. Favorable pharmacokinetic properties and efficacy of **1** led to its selection for clinical evaluation. We became interested in evaluating GPR40 full agonists in a non-human primate model of diabetes, since partial agonist **1**'s ability to maintain glycemic control was being tested in a clinical setting.

We recently described a series of GPR40 full agonists[13,14,15,16,17] that were identified from compounds synthesized in the discovery of **1** by evaluating them in CHO cells transfected with lower levels of GPR40 expression plasmid (0.05 μg). This assay provides greater dynamic range with which to assess improvements in intrinsic efficacy (Figure 1). Optimization of the full agonist series afforded AM-1638 (**2**) that displays greater antidiabetic efficacy in several rodent diabetic models compared to partial agonist **1**,[13] providing compelling evidence that GPR40 full agonists engage a powerful mechanism for maintaining glycemic control.

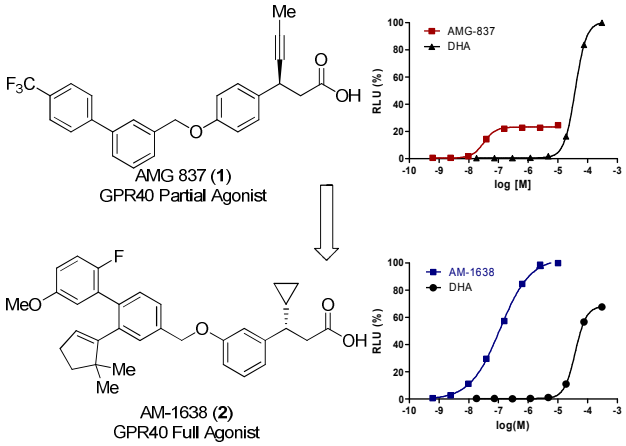
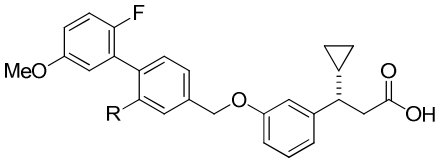
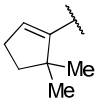
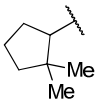
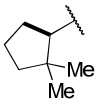
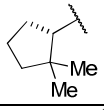


Figure 1. The effect of AMG 837 (**1**), AM-1638 (**2**) and docosahexaenoic acid (DHA) in CHO cells transfected with 0.05 μg of GPR40 expression plasmid.

In an effort to improve the potency and pharmacokinetic properties of full agonist **2**, we initiated further optimization of the full agonist series of compounds. Saturation of the cyclopentene ring led to an improvement in potency in the (*R*)-enantiomeric series (Table 1, **4**, EC_{50}= 0.08 μM), while loss in potency was observed in the (*S*)-enantiomeric series (Table 1, **5**, EC_{50}= 0.31 μM). While maintaining the 2,2-dimethyl-(*R*)-cyclopentane moiety we next turned our attention to the β-substituent from the carboxylic acid (Table 2). Previous optimization had revealed that small alkyl substituents were preferred at this position. Reducing the size of the β-substituent to a hydrogen (**6**, EC_{50}= 0.45 μM) or methyl (**7**, EC_{50}= 0.17 μM) led to a loss in potency. β-Substituents with similar size to that of the cyclopropane of full agonist **4** displayed similar potency (**8-11**, EC_{50}= 0.08-0.11 μM). The two most potent substituents, the ethyl and cyclopropane, were selected for further optimization, while evaluation of their entipodes displayed a significant decrease in potency and efficacy (**12**, **13**; EC_{50}= 1.96, 1.83 μM; 76%, 89%).

Table 1. The Effect of Biphenyl Modification on GPR40 Activity in the Presence 100% Human Serum



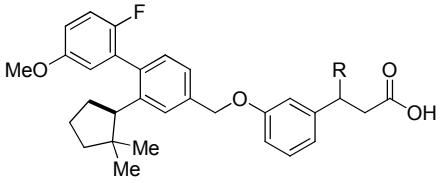
Compound	R	stereo-chemistry	GPR40 EC_{50} (μM)[a]	Efficacy (%)[a,b]
2			0.16	100
3		(*R*,*S*)	0.14	97
4		(*R*)	0.08	101
5		(*S*)	0.31	100

[a] Mean of at least two runs; [b] % Compared to reference agonist 2.

To decrease the electron density of the oxy-aryl ring, we evaluated fluorination of the four possible positions. Installment of a fluorine at the 2 and 5-positions provided full agonists with a minimal loss in potency (Table 3; **14**, 0.12 μM; **16**, 0.09 μM), while fluorination at the 4 and 6-positions led to more significant losses in potency (Table 3; **15**, 0.21 μM; **17**, 0.74 μM). Because they maintained potency the pharmacokinetic properties of the fluorinated full agonist **14** and **16** were evaluated (Table 4). Full agonist **14** displayed low iv clearance and high oral bioavailability. Because of this favorable profile the β-ethyl substituent was exchanged for a cyclopropane that displayed a similar in vitro (Table 3, **18**, EC_{50}= 0.10 μM) and pharmacokinetic profile (Table 4, **18**), albeit a lower oral bioavailibility.

Due to the similar in vitro profiles of the fluorinated compounds **14** and **18**, **14** was selected for further pharmacokinetic evaluation in higher species because of the greater exposure achieved in rats (AUC= 8.22 μM·h). Full agonist **14** displayed both low clearance and good oral bioavailability in dog and cynomologus monkeys (Table 5; Cl=0.21, 0.22 L/h/kg; %F=62, 91), respectively. This profile is a significant improvement over compound **2** with a >4-fold improvement in AUC while fraction unbound in plasma are similar between the two compounds (Table 5). We deemed the profile acceptable to evaluate the antidiabetic activity of full agonist **14**, that we have named AM-6226, in a non-human primate model of diabetes.

Table 2. Exploration of β-Substituent SAR



Compound	R	GPR40 EC_{50} (μM)[a]	Efficacy (%)[a,b]
6		0.45	120
7		0.17	106
8		0.08	102
9		0.11	100
4		0.08	101
10		0.09	100
11		0.10	98
12		1.96	76
13		1.83	89

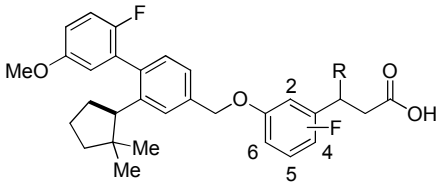
[a] Mean of at least two runs**;** [b] % Compared to reference agonist **2**.

The synthesis of full agonist **14** begins with hydrogenation of the tri-substituted olefin **19** (Scheme 1), followed by ester reduction, which yielded racemic alcohols in excellent yield. Separation of the enantiomers and subsequent chlorination yielded the desired benzylic chloride **20**. Condensation of

aldehyde **21** with Meldrum's acid yielded olefin **22**, and subsequent conjugate addition of ethyl magenesium bromide yielded **23**. Hydroylsis of the diester, removal of methyl ether using dodecylthiol, subsequent esterification, and chiral separation delivered **24** in good overall yield. Alkylation of **24** with **20**, followed by hydrolysis yielded AM-6226.

Sitagliptin and **14** were orally dosed to prediabetic cynomolgus monkeys selected from a high-fat feed colony. The GPR40 full agonist **14** display significant glucose lowering during an oral glucose tolerance test (OGTT) at 1 and 10 mg/kg (Figure 2). Moreover, full agonist 14 and the antidiabetic drug sitagliptin afforded similar reduction in plasma glucose AUC (18% vs. 13%) when 10 mg/kg of either compound was administered orally.

Table 3. Evaluation of Aryl-Fluorine Substitutions



Compound	F Position	R	GPR40 EC_{50} (μM)[a]	Efficacy (%)[a,b]
8	none	Et	0.08	102
14 (AM-6226)	2	Et	0.12	104
15	4	Et	0.21	100
16	5	Et	0.09	105
17[c]	6	Et	0.74	82
18	2		0.10	100

[a] Mean of at least two runs; [b] % Compared to reference agonist 2.
[c] Compound 17 contains a 5,5-dimethyl-1-cyclopenten-1-yl moiety instead of the (1*R*)-2,2-dimethylcyclopentyl moiety.

Table 4. Rat Pharmacokinetic Properties of Full Agonists[a]

Comp.	Fu	oral C_{max} (μM)	oral AUC (μM·h)	Cl (L/h/kg)	Vd_{ss} (L/kg)	iv $t_{½}$ (h)	% F
8	0.006	0.37	1.74	0.67	1.12	2.1	29
4	0.006	0.25	1.83	0.72	1.68	2.0	34
14	0.003	1.18	8.22	0.33	0.81	2.0	72
18	0.003	0.36	2.43	0.49	1.9	3.3	32

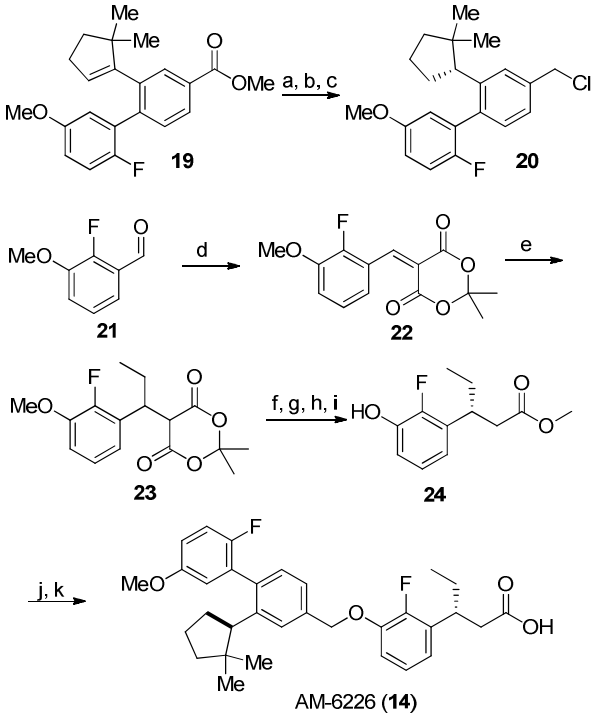
[a] Administered at a dose of 0.5 mg/kg, iv; 2 mg/kg, po, in rats. Data are expressed as mean values (n = 3).

Table 5. The Pharmacokinetic Properties of Full Agonist **14**

Comp.	Species	Fu	oral dose (mg/kg)	oral AUC (μM·h)	Cl (L/h/kg)	Vd_{ss} (L/kg)	iv $t_{½}$ (h)	% F
2	rat	0.010	2	15.1	0.91	1.1	1.8	72
14	rat	0.003	2	8.22	0.33	0.81	2.0	72
14	dog	0.034	2	11.6	0.21	0.68	4.1	62
2	cyno	0.012	10	18.8	0.81	2.0	2.1	71
14	cyno	0.017	10	80.4	0.22	0.5	3.3	91

[a] Administered at a dose of 0.5 mg/kg, iv. Data are expressed as mean values (n = 3).

Scheme 1. [a]



[a] Reagents and conditions: (a) Pd/C, H_2O, MeOH, 90%; (b) $LiAlH_4$, THF, 0 °C, 96%; Chiral separation of enantiomers; Analytical column (Chiracel-OD (2%IPA in hexane, 45 min run) Peak 1-15.5 mins, Peak 2-38.0 mins) (c) $SOCl_2$, CH_2Cl_2, 87%, (d) $C_6H_8O_4$, H_2O, 90°C, 59%, (e) EtMgBr, THF, 0°C, 93%, (f) DMF/H_2O (10/1), 91%, (g), $C_{12}H_{10}S$, NaOH, NMP, 125°C, 88%, (h) H_2SO_4, MeOH, 80°C, 90%, (i) chiral HPLC (Chiralcel OD column, 3% IPA/hexane, detection at 220 nm) to afford two seperable peaks, 40% for each peak; (j) 20, H_2O, Cs_2CO_3, DMF; (k) LiOH, EtOH, H_2O, 95% over 2 steps.

In conclusion, we have described the further optimization of GPR40 full agonists resulting in the identification of AM-6226 (**14**) that displays an improved pharmacokinetic profile and was suitable for evaluation in cynomolgus monkeys. The anti-diabetic activity that full agonist **14** exhibits in cynomolgus monkeys along with the previously disclosed efficacy of GPR40 full agonists in rodent models of diabetes provides compelling evidence that GPR40 full agonists af-

ford access to a mechanism for maintaining glycemic control and potential for the treatment of type II diabetic patients.

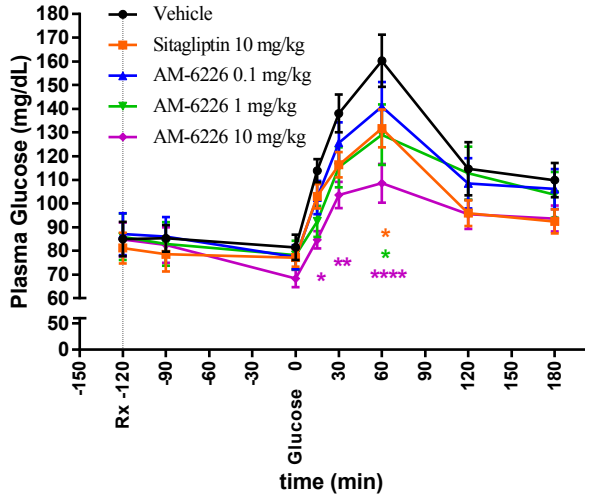


Figure 2. The effect of full agonist **14** (AM-6226) and Sitagliptin during an OGTT in cynomolgus monkeys. Time-dependent changes in plasma glucose after oral administration of either full agonist **14** or Sitagliptin followed by 4 g/kg oral glucose challenge. Values are means ± SE (n = 16). $^{*}p \leq 0.05$, ****$p \leq 0.001$ as compared with control by 2-way ANOVA with Dunnett's test.

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Acknowledgments

In memory of Dr. XianYun Jiao.

Supporting Information

The synthesis and spectroscopic characterization of compound 14, 1NMR and LRMS characterization of final compounds, cell-based and in vivo methods

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