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Discovery of the imidazole-derived GPR40 agonist AM-3189

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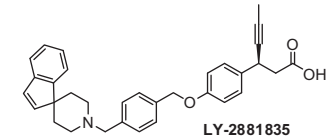
ABSTRACT

As a follow-up to the GPR40 agonist AMG 837, which was evaluated in clinical trials for the treatment of type II diabetes, further optimization led to the discovery of AM-3189 (**13k**). AM-3189 is representative of a new class of compounds with minimal CNS penetration, superior pharmacokinetic properties and in vivo efficacy comparable to AMG 837.

Type 2 diabetes mellitus (T2DM) is a disease characterized by defects in insulin secretion from pancreatic β-cells and/or insulin resistance in target tissues of insulin.[1–3] Insulin secretagogues, such as sulfonylureas and glinides are commonly used to stimulate insulin secretion in diabetic patients.[4] However, these drugs promote insulin secretion independent of blood glucose levels, thereby leading to the risk of hypoglycemia.[4,5] GPR40 (also known as FFAR1) is a novel G protein-coupled receptor (GPCR) that is expressed in pancreatic β-cells and responds to free-fatty acid (FFA) concentrations.[6] Activation of GPR40 potentiates glucose-stimulated insulin secretion and lowers plasma glucose concentrations in multiple animal models of insulin resistance and obesity.[6–8] Because GPR40 mediated insulin secretion is glucose-dependent, it is believed that pharmacologic activation of the receptor should not induce hypoglycemia in either fed or fasted states. Consequently, a GPR40 agonist has potential to be a safe and effective alternative to currently available therapies for T2DM.[9–11] Therapeutic efficacy has been demonstrated in several clinical trials targeting the GPR40 pathway, for example, LY-2881835 (entered Phase I clinical trial),[11] JTT-851 (entered Phase II clinical trial, structure not disclosed)[11] and TAK-875 (entered Phase III clinical trial).[12]



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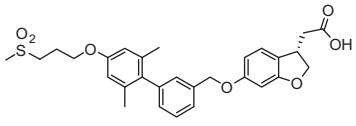

TAK-875

We previously described the development of the GPR40 agonist AMG 837 from a series of beta-substituted propionic acids, which were identified in a high-throughput screen.[13] In multiple animal models, AMG 837 enhances glucose-stimulated insulin secretion and lowers plasma glucose levels.[14] Because GPR40 activity is glucose-dependent, AMG 837 did not induce hypoglycemia in any

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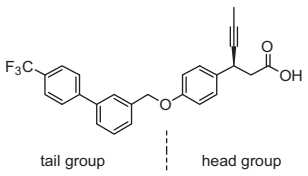
of the models tested.[14] Encouraged by these results, we initiated a Phase I clinical trial of AMG 837.



AMG 837

While the clinical evaluation of AMG 837 was ongoing, we turned our attention to the development of a structurally distinct GPR40 agonist. Although AMG 837 is a carboxylic acid, its physicochemical properties, including low polar surface area (tPSA 47, Table 1), suggest a reasonable possibility of achieving CNS exposure. Additional support comes from a structurally close analog of AMG 837 (with 4′-chloro-2′-ethoxy-(1,1′-biphenyl)-4-yl replacing 4′-(trifluoromethyl)-(1,1′-biphenyl)-3-yl of AMG 837) which showed a brain to plasma ratio of 0.6 3 h after an oral dose of 5 mg/kg in rats. Given that the efficacy of GPR40 agonists is derived peripherally and that they are likely to be dosed chronically, we sought molecules with minimal brain penetration.[15] In general, increasing the polar surface area (PSA) of a molecule tends to decrease its blood–brain barrier permeability.[16] In keeping with this principle, we focused on increasing the PSA of AMG 837, while maintaining its potency and metabolic stability. The approach taken was to introduce polar groups to the tail group and/or the head group of the AMG 837 class of GPR40 agonists.

Herein, we report the discovery of AM-3189 (**13k**). AM-3189 maintains the in vivo efficacy of AMG 837 while displaying a superior pharmacokinetic profile and minimal CNS exposure.

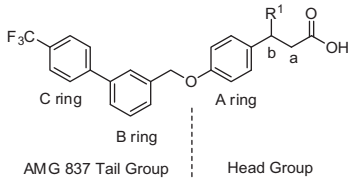
As we reported previously, activity on the GPR40 receptor varies significantly with substitution at the β-carbon relative to the carboxylate.[13] We hoped to modify the beta-substitution of AMG 837 in order to improve potency and desired physiochemical properties. The racemate of AMG 837 (**8a** in the Table 1) displayed the expected twofold decrease in potency on GPR40 compared to the active enantiomer AMG 837 and its activity crossed over to the rat and mouse forms of GPR40.[13] Due to the lack of an efficient method for the asymmetric synthesis of β-substituted 2-phenylpropanoates at the time, racemic compounds were assayed for structure–activity relationship (SAR) studies and early screening purposes. Using the tail group from AMG 837 as a probe, the synthetic route for modification of the beta-substitutions of the carboxylic acids is shown in Scheme 1. Treatment of aldehydes **1** with (4-((tetrahydro-2*H*-pyran-2-yl)oxy)phenyl) magnesium bromide afforded secondary alcohols **2**, which were oxidized by pyridinium chlorochromate (PCC) to yield ketones **3**. The ketones **3** were converted to α,β-unsaturated esters **4** through Peterson-olefination[17] followed by hydrogenation to afford beta-substituted 3-arylpropanoates **5**. After removing the THP protecting group with TFA, the resulting para-substituted phenols **6** were alkylated with 3-(bromomethyl)-4′-(trifluoromethyl)-1,1′-biphenyl in the presence of cesium carbonate to afford the esters **7**, which were hydrolyzed under basic conditions to yield the desired carboxylic acids **8**.

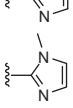
Since GPR40 is a $G_{\alpha q}$-coupled GPCR, compounds were assayed for GPR40 activity in a chemiluminescent system (aequorin) monitoring calcium flux in CHO cells transiently transfected with GPR40 as reported previously (Table 1).[13,14] In the later stage of the project, a CHO cell line stably transfected with human GPR40 was developed. GPR40 aequorin assay was then run in buffer containing 0.01% human serum albumin or in 100% human serum to test activity of compounds in the absence or presence of plasma proteins (Table 2). AMG 837 was included with all assays as a positive control and as a reference compound for benchmarking SAR.

The SAR of the β-substituted 3-phenylpropanoate lead series with AMG 837 tail group is shown in Table 1. Activity on the GPR40 receptor varied significantly with substitution at the β-carbon of the carboxylate. Simple phenyl (**8b**) reduces GPR40 potency 10-fold relative to the methyl acetylene compound (**8a**, racemate of AMG 837). Introducing a nitrogen to the phenyl ring in various positions does not restore the lost potency, thus pyridin-2-yl and pyridin-3-yl compounds (**8c**, **8d**) are equally potent to the simple phenyl analog (**8b**), while the pyridin-4-yl compound (**8e**) is slightly more potent. Five-membered ring heterocycles retain potency slightly more than the phenyl analogs, as evidenced by 2-thiophenyl (**8f**) compared to the simple phenyl (**8b**). Replacing the thiophene ring with a thiazole ring or 2-methylimidazole ring also showed slightly improved potency (**8f** vs. **8g** and **8h**).

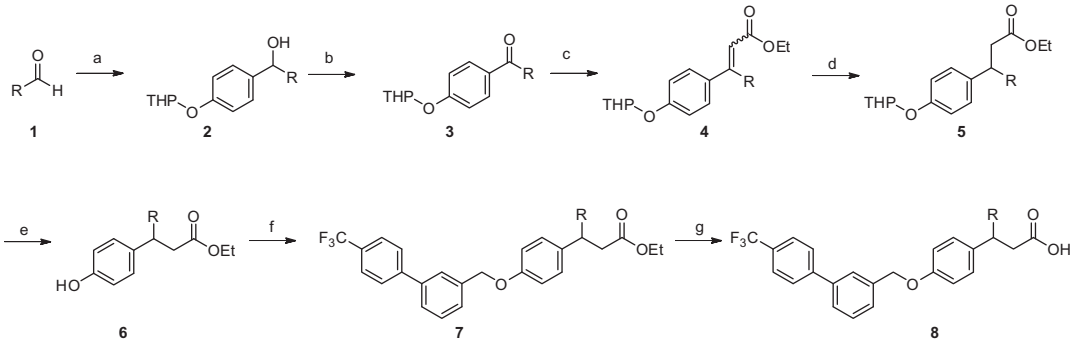
In an attempt to quantify the likely improvement in the balance between physiochemical properties and potency, the lipophilic efficiency (LipE)[18] of this set of compounds was calculated and shown in Table 1. **8h** was revealed to be the best of replacements in terms of lipophilic efficiency among the set shown in Table 1 and it was comparable to that of **8a** (racemate of AMG 837). By incorporating both acidic and basic functional groups, analogs derived from **8h** should be less likely to penetrate the BBB. Therefore, the imidazole arylpropionate was used as the scaffold for the next set of compounds. In designing our analogs, emphasis was placed on increasing polarity and conformational rigidity. We had established in previous work[13] that meta-biaryls yielded the

Table 1
SAR of the β-substituted 2-phenylpropanoate lead series with AMG 837 tail group



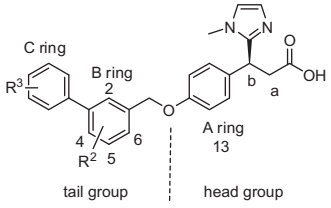
Compound[a]	R[1]	hGPR40[b] EC_{50} (μM)	LogD (pH 7.4)	LipE[c]	tPSA (Å^2)
AMG 837		0.013	3.66	4.23	47
8a		0.025	3.66	3.94	47
8b		0.33	4.84	1.64	47
8c		0.33	3.12	3.36	59
8d		0.40	3.17	3.23	59
8e		0.19	3.13	3.59	59
8f		0.19	4.42	3.30	47
8g		0.12	3.79	3.13	59
8h		0.13	3.01	3.89	62

[a] All compounds reported in this table are racemates except AMG 837.
[b] GPR40 activity was assayed in a chemiluminescent system (aequorin) monitoring calcium flux in CHO cells transiently transfected with GPR40 as reported previously[13,14].
[c] Lipophilic efficiency, LipE = pEC50 – Log *D*.



Scheme 1. General chemistry approach for modification of the beta-substitution of AMG 837. Reagents and conditions: (a) (4-((tetrahydro-2*H*-pyran-2-yl)oxy) phenyl)magnesium bromide, THF, −78 to −20 °C, 70–90%; (b) PCC, CH_2Cl_2, 23 °C, 75–90%; (c) $TMSCH_2CO_2Et$, LiHMDS, THF, −78–23 °C, 60–85%; (d) H_2, Pd/C, EtOAc, 23 °C, 90–100%; (e). TFA, CH_2Cl_2, 23 °C, 80–100%; (f) (1) chiral separation when needed, hexane/*i*-PrOH, OD or AD column, 40–50%; (2) 3-(bromomethyl)-4′-(trifluoromethyl)-1,1′-biphenyl, Cs_2CO_3, DMF, 23 °C; (g) LiOH, THF/water (3/1), 23 °C, 80–99%.

Table 2
Tail group SAR of the imidazole derived analogs



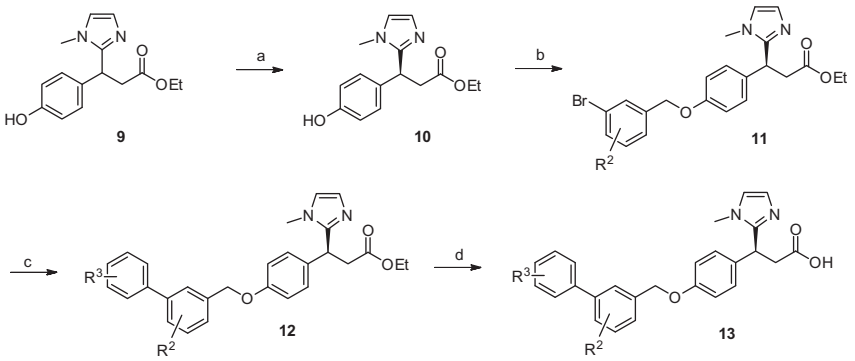
Compound	R^2	R^3	hGPR40 Aeq EC_{50}[a] in buffer (μM)	cLog*D* (pH 7.4)	LipE[b]	tPSA (Å^2)	hGPR40 Aeq EC_{50}[a] in serum (μM)
AMG 837	N/A	N/A	0.080	3.66	3.43	47	8.8
13a	H	2-$MeSO_2$	23	0.32	4.32	96	ND
13b	H	2-CN	0.69	1.3	4.86	86	ND
13c	H	2-MeO	0.41	1.716	4.67	71	ND
13d	H	3-MeO	0.75	1.81	4.31	71	ND
13e	H	4-MeO	0.44	1.86	4.50	71	ND
13f	H	2-CF_3O	0.112	2.74	4.21	71	61
13g	H	2-Me	0.043	2.5	4.87	62	89
13h	H	2-Cl	0.043	2.52	4.85	62	56
13i	H	2-Et	0.048	3.03	4.29	62	65
13j	H	2-Me, 6-Me	0.058	2.96	4.28	62	29
13k (AM-3189)	H	2-Me, 4-Cl	0.033	3.07	4.41	62	10
13l	H	2-Me, 3-Cl	0.061	3.07	4.14	62	22
13m	H	2-Me, 4-F	0.059	2.44	4.79	62	21
13n	H	2-Me, 5-F	0.036	2.52	4.92	62	42
13o	H	2-Cl, 4-Cl	0.069	3.1	4.06	62	30
13p	2-Me	2-Me, 4-Cl	0.24	3.51	3.11	62	23
13q	4-Me	2-Me, 4-Cl	0.097	3.51	3.50	62	9.8

[a] GPR40 aequorin assay in CHO cells stably transfected with human GPR40. Assay was run in buffer containing 0.01% human serum albumin or in 100% human serum.
[b] Lipophilic efficiency, LipE = pEC50 – Log*D*.

most potent molecules so we excluded ortho- and para-biaryls from our designs.

The synthesis of meta-biaryl analogs of **8h** is depicted in Scheme 2. Compound **9** was synthesized as a racemic mixture, using the commercially available 1-methyl-1*H*-imidazole-2-carbaldehyde with the procedure described in Scheme 1. The separated single enantiomer (compound **10**), which yielded more potent compounds, was advanced through the synthesis. The absolute configuration of the compound was later determined by comparison of experimental and calculated vibrational circular dichroism (VCD) spectra[19] and optical rotations[20] to be in the *S*-configuration, which is consistent with the finding from the discovery of AMG 837.[13] The enantiomerically pure material **10** (ee > 98%) was alkylated with substituted 3-bromobenzyl bromides or chlorides in the presence of cesium carbonate to afford ethers **11**, which were converted to compounds **12** using Suzuki cross coupling. The desired carboxylic acids **13** were obtained by hydrolysis of esters **12** under basic conditions. The ee value of compound **10** was found to be preserved during these transformations as all final products **13** had ee% > 98%.

Tail group SAR of the imidazole derived analogs is shown in Table 2. The previous SAR study from the discovery of AMG 837 revealed that a simple un-substituted meta-biphenyl tail group was less favorable in terms of potency.[13] Efforts to introduce polarity to the tail group were not successful. When a methylsulfonyl, cyano or methoxy group were added to the different positions of the C ring, all yielded various level of diminished activity (e.g., **13a**–**13e**). This was not entirely unexpected. As endogenous GPR40 ligands are FFAs, the ligand binding site in the receptor is hydrophobic in nature.[21] It was encouraging to find that a



Scheme 2. Chemistry approach for modification of the tail groups of the imidazole head group derived analogs. Reagents and conditions: (a) chiral separation, hexane/i-PrOH, the later-eluting enantiomer on AD or AD-H column, 48%; (b) substituted benzyl halide, Cs_2CO_3, DMF, 23 °C, 90–100%; (c) $Pd(OAc)_2$, S-Phos, K_3PO_4, dioxane, 100 °C, 80–95%; (d) LiOH, THF/water (1/3), 23 °C, 90–99%.

Table 3
Pharmacokinetic properties of compound **13k**

Property	Mouse (n = 2)[a]	Rat (n = 2) [a]	Dog (n = 3)	Cyno (n = 3)
i.v.				
Dose (mg/kg)[b]	1	0.5	0.5	0.5
Cl (L/h/kg)	0.17	0.31	0.15 ± 0.040	0.27 ± 0.030
$T_{1/2}$ (h)	1.5	1.6	5.1 ± 3.8	7.6 ± 0.78
Vd_{ss} (L/kg)	0.78	0.73	0.58 ± 0.079	1.6 ± 0.13
p.o.				
Dose (mg/kg)[c]	5	2	2	5
%F	ca. 100	43	66 ± 0.14	34 ± 0.13
C_{max} (μM)	4.89	0.77	4.40 ± 0.67	1.19 ± 0.33

[a] Plasma concentration data was determined from two mice at each time point.
[b] IV formulations were prepared in 10% *N,N*-dimethylacetamide, 10% ethanol, 30% propylene glycol and 50% sterile water.
[c] Oral formulations were prepared as suspensions using an aqueous vehicle containing 1% Tween 80 in 1% methylcellulose.

Table 4
P-glycoprotein transport properties for compound **13k** determined in MDCK–MDR1 cells

	Efflux ratio	Permeability, apical to basal (10–6 cm/s)	Permeability, basal to apical, (10^{-6} cm/s)
13k (AM-3189)	22	1.4	31

trifluoromethoxy group at the *ortho*-position of the C ring gave an imidazole head group compound with similar potency as AMG 837 (**13f** vs. AMG 837). However, the activity of **13f** is significantly reduced in the presence of 100% human serum. A breakthrough was revealed when a methyl or chlorine was introduced at the *ortho*-position of the C ring (**13g**, **13h**), yielding compounds 2-fold more potent than AMG 837. Adding one more methyl at the other *ortho*-position of the C ring or increasing the size of the substitution relative to the methyl group does not further improve potency (**13i** and **13j** vs. **13g**). With the encouraging results from compounds **13g** and **13h**, the combination of 2-methyl with a methyl, chloro or fluoro were screened (**13k**–**13o**). Compound **13k** (AM-3189) stands out by displaying greater potency both in buffer and in 100% human serum. **13k** (AM-3189) also possesses favorable drug-like properties with a calculated log*D* (pH 7.4) of 3.01, tPSA of 64 $Å^2$ and more favorable lipophilic efficiency (4.4 compared to 3.4 for AMG 837). Adding a methyl group to the *ortho*-positions of the B ring proximal to the C ring of **13k**, targeting restricted rotation of the rotable bond between the B ring and C ring to reduce possible conformers, yielded less potent compounds **13p** and **13q**.

All tested compounds in Table 2 have similar or lower E_{max} than that of AMG 837, which indicates that, like AMG 837, these imidazole-derived compounds are partial agonists on the GPR40 receptor.

To confirm the activity difference between a pair of enantiomers that was observed previously,[13] the opposite enantiomer of **13k** (AM-3189) was also synthesized and was found to be >100× less active in the GPR40 aequorin assay.

Similar to AMG 837, while highly potent on GPR40, **13k** (AM-3189) was highly selective over the closely related GPCRs, GPR41 and GPR43. **13k** showed no significant activity in cell-based assays against PPAR-α, -δ, and -γ, despite a possible structural resemblance to some PPAR agonists. An external panel of 64 receptors (MDS Pharma) also revealed no significant activity. Overall, **13k** was both highly potent and selective in vitro.

In addition to its favorable profile in vitro, compound **13k** (AM-3189) distinguished itself by displaying an excellent pharmacokinetic profile in multiple species. As shown in Table 3, **13k** demonstrated low clearance, moderate volume of distribution,

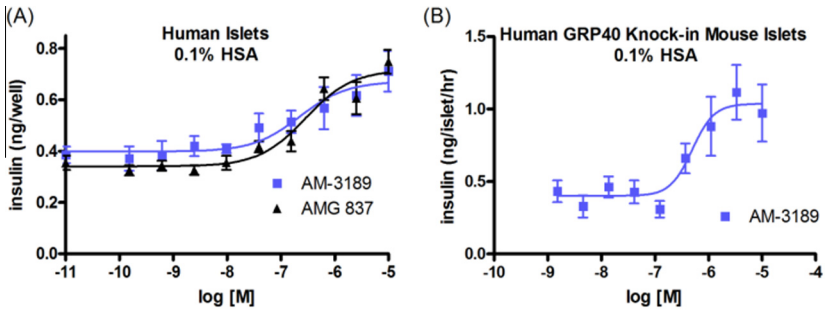


Figure 1. Stimulation of insulin secretion in human islet and human GPR40 knock-in mouse islets by compound **13k** (AM-3189) and AMG 837.

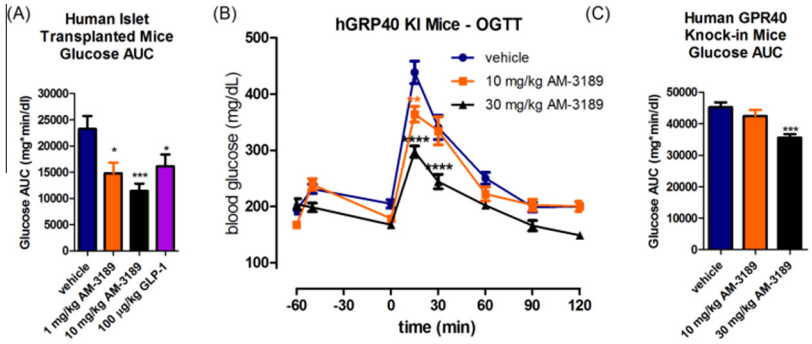


Figure 2. Effect of compound **13k** (AM-3189) during OGTT in human islet transplanted mice and hGPR40 knock-in mice.

and good oral bioavailability. **13k** (AM-3189) does not penetrate the rat CNS as indicated by a rat brain to plasma ratio of 0.04 at 3 h after an oral dose of 5 mg/kg. The reduced CNS penetration may be due to P-glycoprotein mediated efflux as shown in Table 4.

The activity of **13k** (AM-3189) was examined in in vitro insulin secretion assays, first using human islets. As was observed in recombinant cell systems, activity of **13k** and AMG 837 was similar in this assay (Fig. 1A). We also evaluated the activity of **13k** in islets isolated from human GPR40 knock-in mice.[22] In these mice, the mouse GPR40 gene has been replaced with the human GPR40 gene using genetic recombination technology. **13k** increased glucose stimulated insulin secretion from human GPR40 knock-in islets (Fig. 1B).

13k (AM-3189) was significantly less potent and efficacious on the rodent GPR40 receptor compared to the human receptor. In the presence of 0.01% HSA, the EC_{50} value of **13k** on rat GPR40 was 640 ± 60 nM and the E_{max} (% of AMG 837) value was 41%. Similar weak activity was seen on receptors from other non-primate preclinical species, including mouse, guinea pig, hamster, and pig (data not shown). **13k** (AM-3189) was a potent agonist of dog GPR40 (EC_{50} = 37 nM) but had low efficacy compared to AMG 837 (E_{max} = 37% of AMG 837). **13k** (AM-3189) had good activity against the cynomolgus monkey receptor, with EC_{50} and E_{max} (% of AMG 837) values of 29 nM and 96%, respectively.

Because **13k** (AM-3189) had limited efficacy on rodent GPR40, we used two rodent models expressing human GPR40 to demonstrate efficacy of **13k** (AM-3189). First, we used human islet transplanted nude mice. In this model, endogenous pancreatic β-cells were ablated using streptozotocin, and mice were rescued to euglycemia using human islets transplanted under the kidney capsule.[23] In these mice, **13k** (AM-8139) dosed at 1 and 10 mg/kg po prior to a glucose challenge lowered glucose AUC levels (Fig. 2A). The glucose lowering effect was comparable to that of a 100 µg/kg dose of GLP-1 (Fig. 2A). The efficacy of **13k** was also examined in human GPR40 knock-in mice.[24] Again, **13k** (30 mg/kg po) lowered glucose levels during an OGTT (Fig. 2B andC).

In summary, further optimization of beta-substituted carboxylic acids as GPR40 agonists led to the discovery of **13k** (AM-3189), a potent GPR40 agonist that has good pre-clinical pharmacokinetic properties and low CNS penetration. **13k** (AM-3189) represents a valuable tool in the exploration of the role that the GPR40 receptor may play in type II diabetes.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.11.050.

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22. Insulin secretion was assessed using islets isolated from human GPR40 knock-in mice and was performed as described in Ref. 14. Assays were performed in buffer containing 16.7 mM glucose and 0.1% human serum albumin.
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24. Human GPR40 knock-in animals were generated at Ozgene (Perth, Australia) by replacing the mouse GPR40 gene (a single exon gene) with the human GPR40 gene. Briefly, the targeting vector was created by PCR amplifying two fragments of genomic DNA (from C57Bl/6 mice) corresponding to 5637 bp upstream and 5126 bp downstream of mouse GPR40 and inserting these into the plasmid L-Sniper (Ozgene). Human GPR40 was PCR amplified and fused in between these fragments along with the selectable neomycin resistance marker driven the PGK promoter. The targeting vector was electroporated into

ES cells, selected for by culturing in neomycin, and successful insertion of the human GPR40 was verified by PCR of genomic DNA. Following ES cell injection into blastocysts, generation of chimeric mice and germline transmission of the knockin allele, human GPR40 knock-in mice were created and confirmed by PCR of genomic DNA. mRNA expression of human GPR40, and lack of expression of mouse GPR40, was confirmed by qPCR using RNA from islets isolated from human GPR40 knock-in mice. Oral glucose tolerance tests were performed on female human GPR40 knock-in mice at 28 weeks of age. Mice were dosed via oral gavage at 10 mg/kg of compound **13k** following a 6 h fast. Compound **13k** was formulated in 1% methylcellulose, 1% Tween-80. Glucose was administered by oral gavage at 2 g/kg 1-hour post drug dose. Blood glucose measurements were taken from tail vein samples at various time points using AlphaTRAK (Abbott) blood glucose monitoring system.