ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

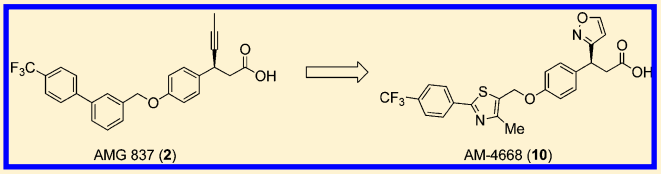
Optimization of GPR40 Agonists for Type 2 Diabetes

Jiwen (Jim) Liu,* Yingcai Wang,* Zhihua Ma, Mike Schmitt, Liusheng Zhu, Sean P. Brown, Paul J. Dransfield, Ying Sun, Rajiv Sharma, Qi Guo, Run Zhuang, Jane Zhang, Jian Luo, George R. Tonn, Simon Wong, Gayathri Swaminath, Julio C. Medina, Daniel C.-H. Lin, and Jonathan B. Houze

Department of Therapeutic Discovery, Metabolic Disorders, Translational Sciences, Amgen Inc., 1120 Veterans Boulevard, South San Francisco, CA 94080, United States

Supporting Information

ABSTRACT: GPR40 (FFA1 and FFAR1) has gained significant interest as a target for the treatment of type 2 diabetes. TAK-875 (**1**), a GPR40 agonist, lowered hemoglobin A1c (HbA1c) and lowered both postprandial and fasting blood glucose levels in type 2 diabetic patients in phase II clinical trials. We optimized phenylpropanoic acid derivatives as GPR40 agonists and identified AMG 837 (**2**) as a clinical candidate. Here we report our efforts in searching for structurally distinct back-ups for AMG 837. These efforts led to the identification of more polar GPR40 agonists, such as AM-4668 (**10**), that have improved potency, excellent pharmacokinetic properties across species, and minimum central nervous system (CNS) penetration.



KEYWORDS: *GPR40, FFAR1, FFA1, GPCR, agonist, type II diabetes, insulin secretagogue*

Type 2 diabetes mellitus (T2DM) is a metabolic dysfunction characterized by increased insulin resistance and impaired insulin secretion, resulting in higher blood glucose levels. Insulin secretagogues, such as sulfonylureas and glinides, are commonly used to increase insulin levels in diabetic patients.[1] However, these drugs promote insulin secretion independent of blood glucose levels, thereby leading to the risk of hypoglycemia.[1,2] Novel insulin secretagogues with a low risk of hypoglycemia are thus desirable.

G-protein coupled receptor 40 (GPR40, IUPHAR recommended name: FFA1) is highly expressed in pancreatic β cells and responds to endogenous fatty acids resulting in amplification of insulin secretion only in the presence of elevated glucose levels.[3−6] Therefore, GPR40 agonists may lower blood glucose levels with decreased risk of hypoglycemia compared to classic insulin secretagogues. On the basis of these findings, GPR40 has drawn considerable attention as a potential therapeutic target for T2DM.[7−13] Robust antiglycemic effects were demonstrated using GPR40 agonists in rodent diabetic models.[7−9,14,15] Positive proof of concept data was obtained in humans using a GPR40 agonist TAK-875 (**1**, Figure 1) in phase II clinical trials: TAK-875 lowered hemoglobin A1c (HbA_{1c}) by an amount similar to that of glimepiride in type 2 diabetic patients at week 12, and hypoglycemic incidents for all doses of TAK-875 were similar to placebo and significantly lower compared to glimepiride treatment groups.[16] TAK-875 is now being evaluated in phase III clinical trials.[17]

We reported the identification of AMG 837 (**2**, Figure 1), which is a potent and selective GPR40 agonist selected for evaluation in human clinical trials.[14,15] AMG 837 shows similar PK properties, potency and efficacy in rats to those of **1**.[7,15] After the discovery of compound **2**, we initiated an effort in searching for structurally distinct GPR40 agonists. We emphasized achieving structural diversity through introducing greater polarity into the molecule. Because compound **2** is a lipophilic acid with low polar surface area (46.5, Table 1), it is likely that **2** has significant central nervous system (CNS) exposure. A close analogue of **2** showed a rat brain to plasma ratio of 0.6 three hours after an oral dose of 5 mg/kg. While GPR40 is expressed in the brain, its function there is unclear. However, its role in modulating insulin secretion is through direct activity on the pancreatic beta cell.[6,13,18] In order to avoid both potential target mediated and off-target effects in the CNS, we aimed to design non-CNS penetrant GPR40 agonists. Since the range of physicochemical properties that allows ready access to the CNS is rather limited,[19] we took the approach of introducing greater polar surface area (PSA) into the molecules to decrease CNS penetration.[20]

One successful way to introduce greater PSA was by replacing the propynyl group of AMG 837 (**2**) with polar heterocycles. Among the initial heterocycles studied were imidazole, triazole, and tetrazole (Table 1). We initially set the biphenyl moiety to that of compound **3** (Figure 1) because it maximized potency. GPR40 is a $G_{\alpha}q$-coupled GPCR, so activation of GPR40 by the compounds was measured in an aequorin assay using CHO cells stably transfected with human GPR40 and in an inositol phosphate accumulation assay using A9 cells also stably transfected with human GPR40.[14,15] As shown in Table 1, imidazole **4** and triazole **5** showed similar EC_{50} to those of compounds **2** and **3**. Tetrazole **6** was more

Received: December 7, 2013
Accepted: February 6, 2014
Published: February 6, 2014

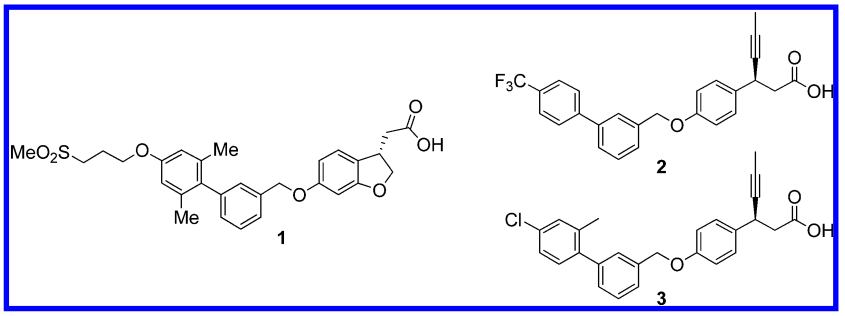
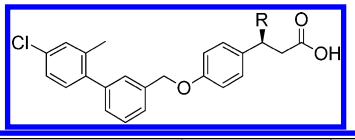


Figure 1. TAK-875 (**1**), AMG 837 (**2**), and a close analogue (**3**).

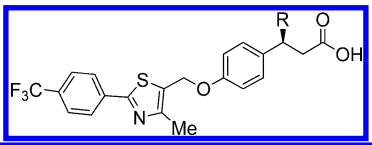
Table 1. GPR40 Activity and Rat Pharmacokinetic Properties of Compounds 4−6



Compd	R	GPR40 Aeq EC_{50} [a] in buffer (nM)	GPR40 IP_3 [b] EC_{50} (nM)	PSA [c] (Å^2)	Clearance (L/h/kg) [d]	MRT (h)	Vdss (L/kg)	F [e] (%)
2	NA	104 [f]	41 [f]	46.5	0.07	10	0.58	84
3	1-propynyl	36 [f]	ND [g]	46.5	ND [g]	ND [g]	ND [g]	ND [g]
4		33 ± 15	7.6 [f]	64.4	0.31	2.4	0.73	48
5		33 ± 10	11 ± 11	77.2	1.5	2.5	3.5	18
6		12 ± 3	1.3 ± 0.66	90.1	2.8	5.2	14.3	53

[a]GPR40 aequorin assay in CHO cells stably transfected with human GPR40. Assay was run in buffer containing 0.01% human serum albumin. Values are reported as the average of at least two determinations. [b]GPR40 IP_3 assay using A9 cells stably transfected with human GPR40 in 0.3% human serum. See refs 14 and 15 for assay protocol. *V* values are reported as the average of at least two determinations. [c]Topological polar surface area was calculated using Daylight Toolkit v4.8.1. [d]IV dose at 0.5 mg/kg. [e]Oral dose at 2 mg/kg. [f]Values are from one experiment. [g]Not determined.

Table 2. GPR40 Activity and Rat Pharmacokinetic Properties of Compounds 7−9



Compd	R	GPR40 Aeq EC_{50} [a] in buffer (nM)	GPR40 IP_3 [b] EC_{50} (nM)	PSA [c] (Å^2)	Clearance (L/h/kg) [d]	MRT (h)	Vdss (L/kg)	F [e] (%)
7		73 ± 25	16	77.2	0.17	3.7	0.64	ND [f]
8		65 ± 29	ND [f]	90.1	0.46	3.6	1.5	ND [f]
9		41 ± 31	3.8	103.0	4.3	0.74	3.2	6.7

[a]GPR40 aequorin assay in CHO cells stably transfected with human GPR40. Assay was run in buffer containing 0.01% human serum albumin. Values are reported as the average of at least two determinations. [b]GPR40 IP_3 assay using A9 cells stably transfected with human GPR40 in 0.3% human serum. See refs 14 and 15 for assay protocol. Values are from one experiment. [c]Topological polar surface area was calculated using Daylight Toolkit v4.8.1. [d]IV dose at 0.5 mg/kg. [e]Not determined. [f]Oral dose at 2 mg/kg.

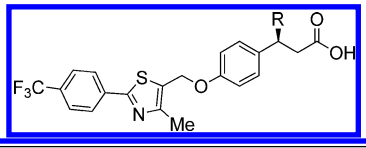
potent, but its rat clearance was high (CL = 2.8 L/h/kg). It was interesting to notice that the clearance values trended higher as the number of nitrogen in the heterocycles increased.

The observations revealed in Table 1 were confirmed in compounds that have different biaryl tail pieces. The phenylthiazole tail (Table 2) has a good degree of structural diversity compared to the biphenyl tails and retains potency on the receptor. With the phenylthiazole tail, tetrazole **9** was also more potent than imidazole **7** and triazole **8** (Table 2). Once again, in vivo rat clearance increased with increasing nitrogen count in the heterocyclic ring β to the carboxylic acid (compounds **7**−**9**).

It was desired to retain the improved potency of the tetrazoles without the increased clearance. The microsomal stability of the tetrazoles was similar to that of the imidazoles and triazoles. It is possible that the tetrazole head piece is

dx.doi.org/10.1021/ml400501x | ACS Med. Chem. Lett. 2014, 5, 517−521

Table 3. GPR40 Activity of Compounds 10−12



Compd	R	GPR40 Aeq EC_{50}[a] in buffer (nM)	GPR40 IP_3 EC_{50}[b] (nM)	PSA[c] ($Å^2$)
2	N/A	104[d]	41	46.5
10 (AM-4668)		36 ± 10	3.6	85.5
11		69 ± 32	ND[e]	85.5
12		57 ± 35	9	85.5

[a]GPR40 aequorin assay in CHO cells stably transfected with human GPR40. Assay was run in buffer containing 0.01% human serum albumin. Values are reported as the average of at least two determinations. [b]GPR40 IP_3 assay using A9 cells stably transfected with human GPR40 in 0.3% human serum. See refs 14 and 15 for assay protocol. Values are from one experiment. [c]Topological polar surface area was calculated using Daylight Toolkit v4.8.1. [d]Values are from one experiment. [e]Not determined.

Table 4. Pharmacokinetic Properties of AM-4668

species	clearance (L/h/kg)[a]	$T_{1/2}$ (h)	Vdss (L/kg)	oral bioavailability[b]
rat	0.09	5.3	0.68	77%
dog	0.15	5.6	0.60	100%
cyno[c]	0.04	14	0.35	65%

[a]IV dose at 0.5 mg/kg. [b]Oral dose at 2 mg/kg. [c]Cynomolgus monkey.

recognized by an efflux transporter, a phase II metabolic enzyme, or both. The recognition seems to be reinforced as the number of heteroatoms in the heterocycles rises. On the basis of this observation, we reduced the number of heteroatoms and evaluated positioning of the remaining ones to maintain the good potency of the tetrazoles. A number of heterocycles were evaluated, and isoxazole **10** (designated AM-4668) was as potent as the corresponding tetrazole (Table 3). Other oxazoles, such as **11** and **12**, were less potent.

With the number of heteroatoms reduced, AM-4668 indeed displayed excellent pharmacokinetic properties, not only in rat but also in dog and cynomolgus monkey (Table 4). It had low clearance, moderate to long half-life, and very good oral bioavailability across species. Unlike some compounds with the propynyl headgroup as **2** and **3**, CNS penetration of AM-4668 was low, as indicated by a rat brain to plasma ratio of 0.02 three hours after an oral dose of 5 mg/kg. In addition, this compound showed no inhibition of the hERG channel and demonstrated low potential to induce drug−drug interaction by various CYP inhibition and induction assays.

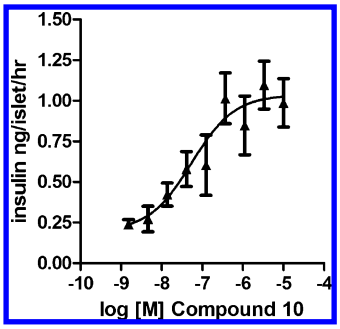


Figure 3. Insulin secretion of AM-4668 (**10**) in islets isolated from hGPR40 knock-in mice.

Since the activity of AM-4668 was weak against rodent GPR40, it was difficult to study its efficacy in standard rodent diabetes models. We therefore evaluated it in mice where the

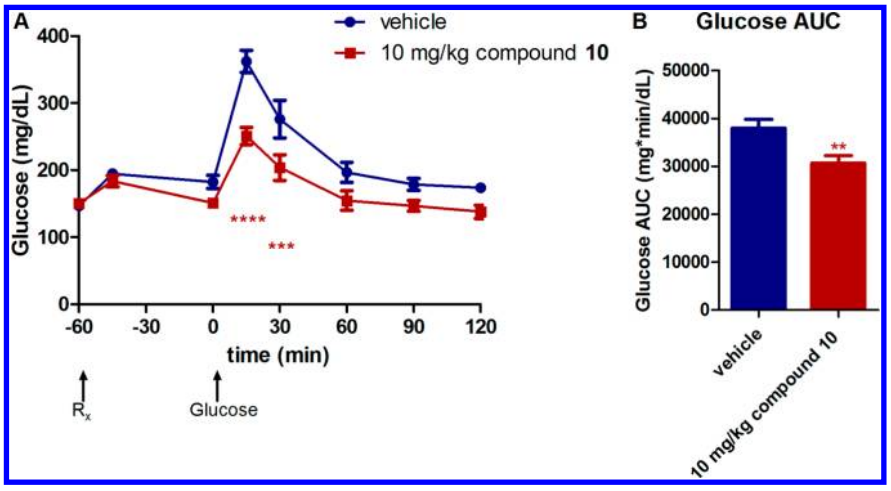
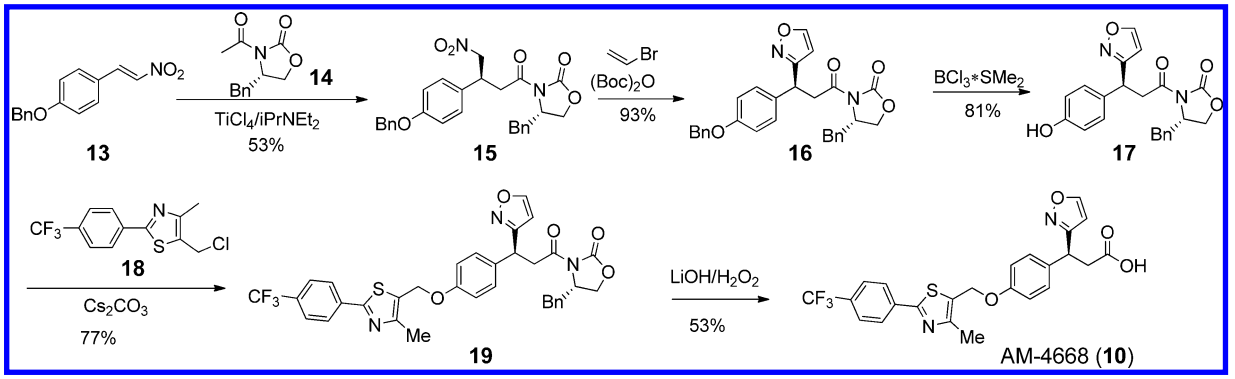


Figure 2. Oral glucose challenge study of AM-4668 (**10**) in hGPR40 knock-in mice.

dx.doi.org/10.1021/ml400501x | ACS Med. Chem. Lett. 2014, 5, 517−521

Scheme 1. Synthesis of Isoxazole AM-4668 (10)



human GPR40 gene has been replaced with the mouse GPR40 gene but is under the control of the endogenous mouse GPR40 promoter.[21] Oral administration of AM-4668 at 10 mg/kg dose 1 h before oral glucose challenge significantly reduced the blood glucose levels (Figure 2A). The glucose AUC in the compound-treated animals is 19% lower than that in the vehicle-treated animals (Figure 2B). In a separate mouse oral PK study, the plasma concentration of AM-4668 at 1 mg/kg dose was about 2 μM for the first 4 h, which corresponded to an 8 nM unbound concentration. In addition, AM-4668 induced insulin secretion in pancreatic islets isolated from human GPR40 knock-in mice with an EC_{50} of 55 nM (Figure 3).[22] Because AM-4668 does not effectively enter the CNS, these results demonstrate that agonism of GPR40 by this compound in the CNS is likely not necessary for its glucose lowering effects in this model.

The synthesis of AM-4668 is shown in Scheme 1. Michael addition of (*S*)-3-acetyl-4-benzyl-2-oxazolidinone (**14**) to *trans*-β-nitrostyrene **13** in the presence of titanium chloride and Hunig's base produced compound **15**.[23] Generation of the nitrile oxide[24] from the nitro group in **15** and reaction with vinyl bromide generated the isoxazole compound **16**. Deprotection of the benzyl group of **16** using boron trichloride methyl sulfide complex afforded phenol **17**, which was alkylated using commercially available choloromethylthiazole **18** to yield compound **19**. Finally the oxazolidinone in compound **19** was removed in the presence of lithium hydroxide and hydrogen peroxide to afford AM-4668 (**10**).

In summary, we replaced the propynyl group of AMG 837 (**2**) with heterocycles and introduced greater PSA into the molecules. It is likely that the polar heterocycles in the head piece significantly reduced CNS penetration of the molecules. After optimization for potency and PK, we identified isoxazole AM-4668 (**10**), which is more potent than AMG 837 (**2**), possesses excellent pharmacokinetic properties across species, and reduces the plasma glucose levels in an OGTT study in human GPR40 knock-in mice.

■ ASSOCIATED CONTENT

Supporting Information

Detailed synthetic experimental procedures and characterization for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*(J.L.) E-mail: jiwenl@amgen.com.
*(Y.W.) E-mail: yingcai02@gmail.com.

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Wajchenberg, B. L. β-Cell failure in diabetes and preservation by clinical treatment. *Endocr. Rev.* **2007**, *28*, 187−218.
(2) Maedler, K.; Carr, R. D.; Bosco, D.; Zuellig, R. A.; Berney, T.; Donath, M. Y. Sulfonylurea induced beta-cell apoptosis in cultured human islets. *J. Clin. Endocrinol. Metab.* **2005**, *90*, 501−506.
(3) Edfalk, S.; Steneberg, P.; Edlund, H. Gpr40 is expressed in enteroendocrine cells and mediates free fatty acid stimulation of incretin secretion. *Diabetes* **2008**, *57*, 2280−2287.
(4) Morgan, N. G.; Dhayal, S. G-protein coupled receptors mediating long chain fatty acid signalling in the pancreatic beta-cell. *Biochem. Pharmacol.* **2009**, *78*, 1419−1427.
(5) Steneberg, P.; Rubins, N.; Bartoov-Shifman, R.; Walker, M. D.; Edlund, H. The FFA receptor GPR40 links hyperinsulinemia, hepatic steatosis, and impaired glucose homeostasis in mouse. *Cell Metab.* **2005**, *1*, 245−258.
(6) Briscoe, C. P.; Tadayyon, M.; Andrews, J. L.; Benson, W. G.; Chambers, J. K.; Eilert, M. M.; Eillis, C.; Elshourbagy, N. A.; Goetz, A. S.; Minnick, D. T.; Murdock, P. R.; Sauls, H. R., Jr; Shabon, U.; Spinage, L. D.; Strum, J. C.; Szekeres, P. G.; Tan, K. B.; Way, J. M.; Ignar, D. M.; Wilson, S.; Muir, A. I. The orphan G protein-coupled receptor GPR40 is activated by medium and long chain fatty acids. *J. Biol. Chem.* **2003**, *278*, 11303−11311.
(7) 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.
(8) Sasaki, S.; Kitamura, S.; Negoro, N.; Suzuki, M.; Tsujihata, Y.; Suzuki, N.; Santou, T.; Kanzaki, N.; Harada, M.; Tanaka, Y.; Kobayashi, M.; Tada, N.; Funami, M.; Tanaka, T.; Yamamoto, Y.; Fukatsu, K.; Yasuma, T.; Momose, Y. Design, synthesis, and biological activity of potent and orally available G protein-coupled receptor 40 agonists. *J. Med. Chem.* **2011**, *54*, 1365−1378.
(9) Naik, H.; Vakilynejad, M.; Wu, J.; Viswanathan, P.; Dote, N.; Higuchi, T.; Leifke, E. Safety, tolerability, pharmacokinetics, and pharmacodynamic properties of the GPR40 agonist TAK-875: results from a double-blind, placebo-controlled single oral dose rising study in healthy volunteers. *J. Clin. Pharmacol.* **2012**, *52*, 1007−1016.
(10) Walsh, S. P.; Severino, A.; Zhou, C.; He, J.; Liang, G.-B.; Tan, C. P.; Cao, J.; Eiermann, G. J.; Xu, L.; Salituro, G.; Howard, A. D.; Mills, S. G.; Yang, L. 3-Substituted 3-(4-aryloxyaryl)-propanoic acids as GPR40 agonists. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3390−3394.
(11) Alquier, T.; Poitout, V. GPR40: good cop, bad cop? *Diabetes* **2009**, *58*, 1035−1036.
(12) 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.
(13) 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 β cells through GPR40. *Nature* **2003**, *422*, 173−176.
(14) 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.
(15) 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.
(16) Burant, C. F.; Viswanathan, P.; Marcinak, J.; Cao, C.; Vakilynejad, M.; Xie, B.; Leifke, E. TAK-875 versus placebo or glimepiride in type 2 diabetes mellitus: a phase 2, randomised, double-blind, placebo-controlled trial. *Lancet* **2012**, *379*, 1403−1411.
(17) Takeda news release. http://www.takeda.com/news/2013/20130516_5780.html.
(18) Kotarsky, K.; Nilsson, N. E.; Flodgren, E.; Owman, C.; Olde, B. A human cell surface receptor activated by free fatty acids and thiazolidinedione drugs. *Biochem. Biophys. Res. Commun.* **2003**, *301*, 406−410.
(19) Wager, T. T.; Chandrasekaran, R. Y.; Hou, X.; Troutman, M. D.; Verhoest, P. R.; Villalobos, A.; Will, Y. Defining desirable central nervous system drug space through alignment of molecular properties, in vitro ADME, and safety attributes. *ACS Chem. Neurosci.* **2010**, *1*, 420−434.
(20) Kelder, J.; Grootenhuis, P. D. J.; Bayada, D. M.; Delbressine, L. P. C.; Ploemen, J.-P. Polar molecular surface as a dominating determinant for oral absorption and brain penetration of drugs. *Pharm. Res.* **1999**, *16*, 1514−1519.
(21) 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 by 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 were 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 **10** following a 6 h fast. Compound **10** was formulated in 1% methylcellulose and 1% Tween-80. Glucose was administered by oral gavage at 2 g/kg 1 h post drug dose. Blood glucose measurements were taken from tail vein samples at various time points using AlphaTRAK (Abbott) blood glucose monitoring system.
(22) Insulin secretion was assessed using islets isolated from human GPR40 knock-in mice and was performed as described in ref 15. Assays were performed in buffer containing 16.7 mM glucose and 0.1% human serum albumin.
(23) Mulzer, J.; Zuhse, R.; Schmiechen, R. Enantioselective synthesis of the antidepressant Rolipram by Michael addition to a nitroolefin. *Angew. Chem., Int. Ed.* **1992**, *31*, 870−872.
(24) Mukaiyama, T.; Hoshino, T. The reactions of primary nitroparaffins with isocyanates. *J. Am. Chem. Soc.* **1960**, *82*, 5339−5342.