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Letter

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Design, Synthesis, and Evaluation of Novel and Selective G-protein Coupled Receptor 120 (GPR120) Spirocyclic Agonists

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KEYWORDS: GPR120, FFAR4, insulin sensitization, type 2 diabetes, spirocyclic

ABSTRACT: Type 2 diabetes mellitus (T2DM) is an ever increasing worldwide epidemic, and the identification of safe and effective insulin sensitizers, absent of weight gain, has been a long standing goal of diabetes research. G-protein coupled receptor 120 (GPR120) has recently emerged as a potential therapeutic target for treating T2DM. Natural occurring, and more recently, synthetic agonists have been associated with insulin sensitizing, anti-inflammatory and fat metabolism effects. Herein we describe the design, synthesis, and evaluation of a novel spirocyclic GPR120 agonist series, which culminated in the discovery of potent and selective agonist **14**. Furthermore, compound **14** was evaluated *in vivo* and demonstrated acute glucose lowering in an oral glucose tolerance test (oGTT), as well as improvements in homeostatic measurement assessment of insulin resistance (HOMA-IR; a surrogate marker for insulin sensitization) and an increase in glucose infusion rate (GIR) during a hyperinsulinemic euglycemic clamp in diet-induced obese (DIO) mice.

Type 2 diabetes mellitus (T2DM) is an ever increasing epidemic that affects over 415 million people worldwide.[1] While current therapies such as metformin, sulfonylureas, glitazones, DPP-4 inhibitors, GLP-1 analogs, and SGLT2 inhibitors provide physicians many options for treating T2DM patients,[2,3] eventually patients may become insulin resistant and must be treated with exogenous prandial and/or basal insulin.[4] It has been a long standing goal of diabetes research to identify safe and effective insulin sensitizers[5,6] which do not have the weight gain side effect associated with the glitazones[7] (peroxisome proliferator-activated receptor (PPAR) agonists).[8]

G-protein coupled receptor 120 (GPR120), encoded by the free fatty acid receptor 4 (FFAR4) gene, has gained attention in recent years as a potential target to treat T2DM.[9,10] GPR120 is a member of the rhodopsin family of 7-transmembrane domain G-protein coupled receptors (GPCRs) and has been associated with insulin sensitizing, anti-inflammatory and fat metabolism effects.[11] GPR120 is expressed primarily in the intestine, adipocytes and pro-inflammatory macrophages and can be activated by long chain free fatty acids.[12] In mice, GPR120 is preferentially expressed in pancreatic delta cells and regulates somatostatin secretion from islets.[13]

Agonism of the related GPCR, GPR40 (FFAR1), has also gained attention in recent years as a potential treatment target for T2DM.[14,15] Both GPR120 and GPR40 are activated by long chain fatty acids (LCFA), and in order to better understand the anti-diabetic role of GPR120, identification of selective GPR120 agonists versus GPR40 agonists is essential. Recently, in collaboration with the Olefsky group, we published data regarding the insulin sensitizing and chronic anti-inflammatory effects of selective GPR120 spirocyclic agonist compound A (**14**).[16] Herein we describe the design, synthesis, structure activity relationship (SAR), *in vitro* selectivity, and expanded *in vivo* data for the spirocyclic series.

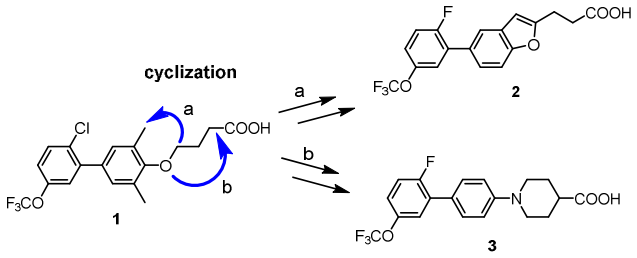


Figure 1. Alternate cyclization modes of early lead **1**.

The inspiration for the spirocyclic series originated from early lead **1** (**Figure 1**). As we recently disclosed, **1** was derived from an ultra high-throughput screening (uHTS) hit, and we utilized the common medicinal chemistry strategy of rigidifying through cyclization of compound **1**, *via* pathway "a" to form a novel benzofuran series represented by compound **2**.[17] Both acyclic analog **1** and cyclic analog **2** had similar human (h) and mouse (m) IP1 and β-Arrestin2 (β-Arr2) GPR120 potency,[18] however cyclic analog **2** lost selectivity versus GPR40 (**Table 1**). We envisioned using a similar rigidifying strategy, but in this case cyclizing *via* route "b" and changing the ether linkage to an amine, to provide piperidine **3** (**Figure 1**). While

this change resulted in loss of potency on h/m IP1 and h/m β-Arrestin2 GPR120 assays for **3** (**Table 1**), it provided an initial baseline point suggesting that the alternate cyclization strategy was a reasonable approach to new analog design.

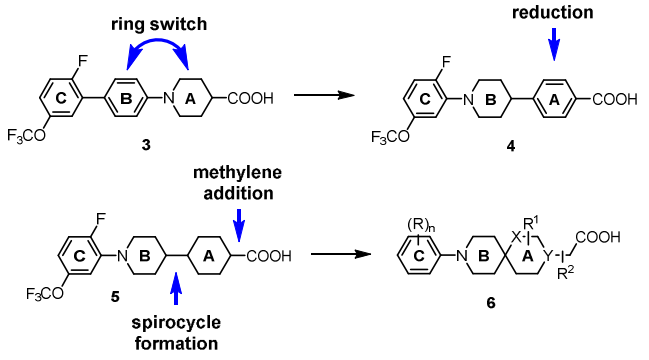


Figure 2. Conceptual formation of the spirocyclic series.

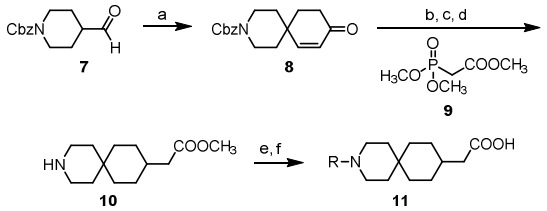
The team was keenly interested in developing a viable novel series with which to build upon, and we felt it was important to diversify our structure class and explore broader chemical space. To this end, we made an initial ring switch of the A- and B-rings on piperidine **3**, to provide the trans-located piperidine **4** (**Figure 2**), which would also facilitate more rapid investigation of the C-ring SAR. Unfortunately, this change resulted in complete loss of GPR120 potency for **4** (**Table 1**).

Table 1. *In Vitro* Potency of Select GPR120 Agonists [a]

Entry	GPR120 EC_{50} (nM) hIP1	mIP1	hβ-Arr2	mβ-Arr2	hGPR40 IP1 EC_{50} (nM)
1	94	16	100	35	>10000
2	63	43	16	21	1829
3	300	89	1600	320	>10000
4	>10000	>10000	>10000	>10000	>10000
5	700	1490	NA	990	>10000

[a]Values are the average of two experiments, each in 10-point titrations. All analogs tested were generally deemed to be full GPR120 agonists under assay conditions.

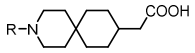
Scheme 1. Synthesis of Spirocyclic Analogs[a]

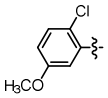
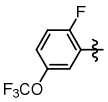
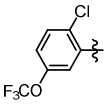
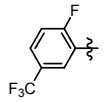
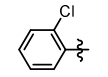
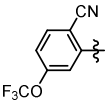
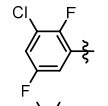
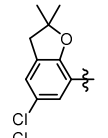
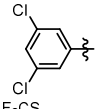
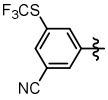
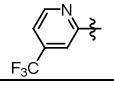


[a]Reagents and conditions: (a) MVK, KOH, EtOH, water, 100 °C, 39% (b) **9**, NaH, THF, 0 °C to 50 °C, 83% (c) Pd/C, H_2 50 psi, Boc_2O, MeOH, 40 °C, 91% (d) TFA, DCM, rt, 91% (e) R-Br, BINAP, $Pd_2(dba)_3$, Cs_2CO_3, dioxane, 100 °C, (f) LiOH, MeOH, THF, water, rt, 15-75% over 2 steps.

We hypothesized that the piperidine B-ring was not the issue since the preceding molecule (**3**) also contained a piperidine-phenyl moiety, but that the phenyl A-ring was not allowing the carboxylic acid to adopt the appropriate conformation for potency. To test this hypothesis, we prepared the cyclohexyl carboxylic acid derivative **5** (**Figure** 2) as a mixture of *cis*- and *trans*-isomers. Gratifyingly, some of the potency lost during the A-/B-ring switch was recaptured by analog **5** (**Table 1**), even as a mixture of isomers. To enhance the ability of the carboxylic acid to adopt the appropriate conformation for potency and inspired by structural minimization overlays with previous potent analogs (see supporting information), we envisioned adding a methylene spacer between the cyclohexyl A-ring and the carboxylic acid, as well as A-/B-ring spirocycle formation to generally maintain similar spacing of the acid moiety and the aromatic C-ring. The aforementioned transformations resulted in the discovery of the novel GPR120 agonist spirocyclic series **6** (**Figure 2**, **Table 2** and **Table 3**).[19]

Table 2. C-Ring SAR[a]



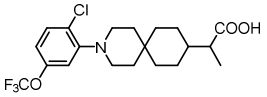
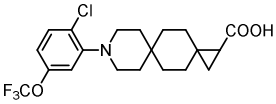
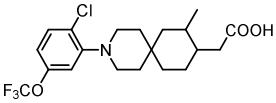
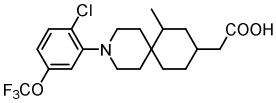
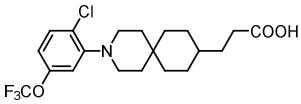
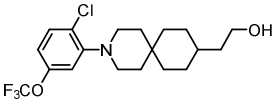
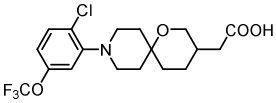
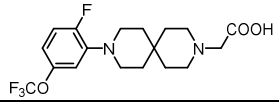
Entry	R	GPR120 EC_{50} (nM) hIP1	mIP1	hβ-Arr2	mβ-Arr2	hGPR40 IP1 EC_{50} (nM)
12		2100	570	2500	5300	>10000
13		130	49	700	470	>10000
14		98	33	66	54	>10000
15		1200	590	2200	3000	>10000
16		>10000	>10000	>10000	>10000	>10000
17		200	66	370	290	2500
18		420	190	1400	1100	8100
19		3300	670	3100	4000	>10000
20		220	51	190	120	6300
21		360	44	480	140	>10000
22		3100	1300	9200	4200	>10000

[a]Values are the average of two experiments, each in 10-point titrations. All analogs tested were generally deemed to be full GPR120 agonists under assay conditions.

The synthesis of the spirocyclic series commenced by ring annulation of aldehyde **7** with methyl vinyl ketone (MVK), in the presence of potassium hydroxide, to afford spirocycle **8** (**Scheme 1**). Key intermediate piperidine spirocycle **10** was prepared by Horner-Wadsworth-Emmons (HWE) reaction of

ketone **8** with phosphonate **9**, followed by concomitant Cbz removal and olefin reduction with hydrogen, in the presence of Boc anhydride (to facilitate purification), and finally Boc removal with TFA. With piperidine spirocycle **10** in hand, we were poised to rapidly evaluate the C-ring SAR of the class by utilizing palladium N-arylation chemistry to provide final analogs **11** after saponification of the methyl ester with lithium hydroxide.

Table 3. Spirocycle Core and Acid Linker SAR[a]

Entry	Structure	GPR120 EC_{50} (nM) hIP1	hβ-Arr2
23		3500	4400
24		2200	3900
25		4100	2700
26		440	580
27		2700	1000
28		4300	330
29		5700	2100
30		>10000	>10000

[a]Values are the average of two experiments, each in 10-point titrations. All analogs tested were generally deemed to be full GPR120 agonists under assay conditions. In cases of possible stereoisomers, the isomers were separated and the data of the most potent is shown but the stereochemistry was not determined.

Due to the nature of our synthesis, we were able to rapidly explore the C-ring SAR, as highlighted by select analogs in **Table 2**. The initial C-ring analogs prepared were based on our previous SAR knowledge from both the acyclic and cyclic analogs **1** and **2** respectively. The first three analogs prepared in the spirocyclic series are listed in order of preparation, with the first (**12**) demonstrating modest GPR120 potency, the second (**13**) demonstrating improved GPR120 potency and the third (**14**) demonstrating sub-100 nM GPR120 potency while maintaining hGPR40 selectivity. The sub-100 nM GPR120 potency for compound **14** was similar to benzofuran lead **2** at h/m IP1 and h/m β-Arrestin2, but proved to have superior GPR40 selectivity.

Changing the *meta*-substitution from -OCF_3 (**13**) to -CF_3 (**15**) resulted in approximately an order of magnitude loss in GPR120 potency and removing the *meta*-substitution as in entry **16** led to complete loss of GPR120 potency. Changing of the *ortho* position from a halogen to -CN (**17**) resulted in a moderate loss of GPR120 potency and reduced the GPR40 selectivity relative to previous analogs. Tri-substituted C-ring variants represented by analogs **18** and **19** led to variable GPR120 potencies, however in all cases the potency was reduced relative to compound **14**. Analogs **20** and **21** are representative analogs with bis-*meta*-substitution and in general demonstrated worse potency when compared to **14**. In an effort to have greater effect on the physicochemical properties of the molecules, we replaced the *ortho*-halide as a pyridine, resulting in only a moderate loss of potency (analog **22** versus analog **15**). Unfortunately, other pyridine analogs resulted in significant loss of GPR120 potency for the series (data not shown). In general, the SAR around the C-ring was challenging and only limited modifications were tolerated, with compound **14** proving to have the optimal C-ring substitutions and substitution pattern to maintain reasonable GPR120 potency and GPR40 selectivity.

With the identification of the *ortho*-Cl, *meta*-OCF_3 substitution pattern for the C-ring, we next turned our attention toward modifying the spirocyclic core (**Table 3**). Substitution on the methylene spacer between the acid and the cyclohexyl ring resulted in significant loss of GPR120 potency, either as a methyl (**23**) or 2,3-cyclopropyl substitution (**24**). Introduction of a methyl group on the cyclohexyl ring also resulted in loss of GPR120 potency (compounds **25** and **26**) relative to **14**. Extending the carboxylic acid by one methylene (**27**) or reducing the acid of **14** to an alcohol (**28**), resulted in significant potency loss. In a similar vein as with the C-ring SAR, introduction of heteroatom replacements for the cyclohexyl (pyran **29** or piperidine **30**) also resulted in substantial loss of GPR120 potency. The SAR observations within the spirocyclic core modifications reaffirmed the challenges we encountered with overall optimization of the series, resulting in no analog having superior GPR120 potency and GPR40 selectivity when compared to spirocyclic lead **14**.

Table 4. PK profile of compound 14[a]

Property	Rat	Mouse	Dog	Rhesus
AUC iv (μM•h)	17.5	14.6	4.6	8.7
Clp (mL/min/kg)	2.3	8.4	9.8	2.7
Vd_{ss} (L/kg)	0.4	8.9	6.7	1.7
$t_{1/2}$ (h)	1.4	18.1	11	9.1
MRT (h)	3.0	26.5	10.5	10.1
F_{oral} (%)	95	100	100	77
PPB (% bound)	99.87	99.79	99.85	99.72
Unbound Cl (Clp/f_{unb}[b])	1769	4000	6533	964

[a]Clp: plasma clearance; $t_{1/2}$: terminal half-life; MRT: mean residence time; F_{oral}: oral bioavailability; AUC: area under the plasma concentration vs time curve following iv dosing; Vd_{ss}: volume of distribution at steady state; PPB: plasma protein binding. [b]f_{unb} = (100-(%PPB))/100.

Often early leads have off target and pharmacokinetic (PK) issues, limiting their utility as tools to better understand biological processes; therefore, while investigating the SAR of the spirocyclic series, we began to profile compound **14** versus common counter screen assays. Ion channel activity for calcium ($Ca_v1.2$ = 21 μM),[20] potassium (IKr > 60 μM),[21] sodium ($Na_v1.5$ = 13 μM)[22] and cytochrome P-450 (CYP3A4, CYP2D6, CYP2C9 > 48 μM)[23] all offered acceptable values. Furthermore, **14** demonstrated minimal binding activity on

PPAR *alpha*, *delta* and *gamma* (>10 μM; see supporting information).

In light of the minimal off-target activity, we continued to profile spirocycle **14** by conducting rat, mouse, dog, and rhesus monkey PK studies (**Table 4**). The clearance and volume of distribution at steady state resulted in a 1.4 h half-life and 3.0 h mean residence time for the rat, while resulting in longer half-life and MRT in the mouse, dog and rhesus. The oral bioavailability across species was high (> 77%), but so too was the unbound clearance (> 964). Unfortunately, all attempts to reduce clearance by introduction of more polar groups or additional substitutions, resulted in significant loss of potency on the GPR120 receptor (*vide supra*). At this time it became clear that **14** was the lead compound in the spirocyclic series, since the overall PK profile allowed for evaluation in more advanced preclinical *in vivo* studies.

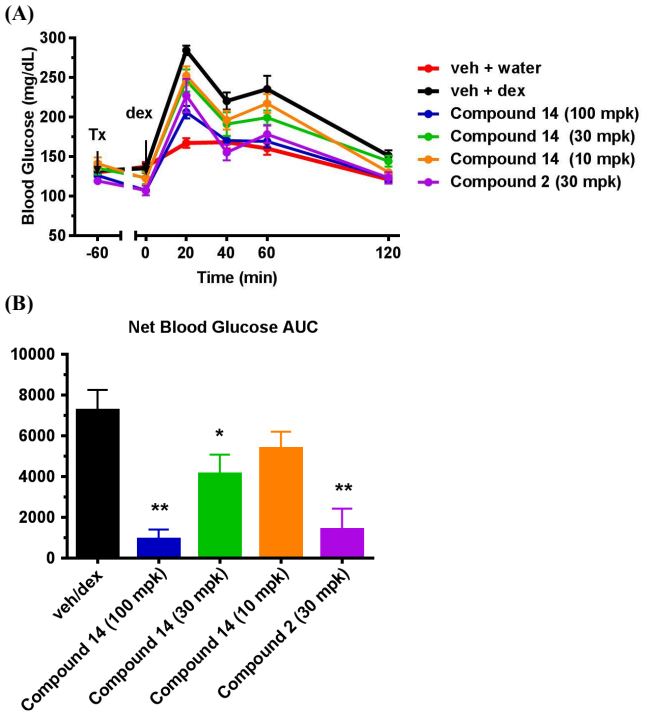


Figure 3. Mouse oral glucose tolerance test (oGTT) dose titration of compound **14** in comparison to GPR120 positive control compound **2**. (A) Blood glucose (mg/dl) over time (minutes). (B) Net blood glucose area under the curve (AUC). Vehicle (veh); dextrose (dex) challenge; Tx = vehicle and compound administration. * $P < 0.05$ compared to vehicle, ** $P < 0.001$ compared to vehicle; two-way ANOVA. Data are represented as mean ± SEM.

Spirocyclic lead **14** was assessed for its ability to improve glucose tolerance in lean mice (**Figure 3**). A dose titration, in an oral glucose tolerance test (oGTT),[24] was conducted with three doses of **14** (10, 30, 100 mpk), utilizing compound **2** (30 mpk) as a positive control and vehicle controls with and without a dextrose challenge. **2** and **14** were orally administered 1 h prior to dextrose challenge and **14** dose dependently reduced blood glucose, with the 100 mpk dose demonstrating comparable reduction to **2** at 30 mpk. As previously disclosed, both **14**[16] and **2**[17] demonstrated on-target effects in wild-type/GPR120 knock out acute mouse oGTT studies.

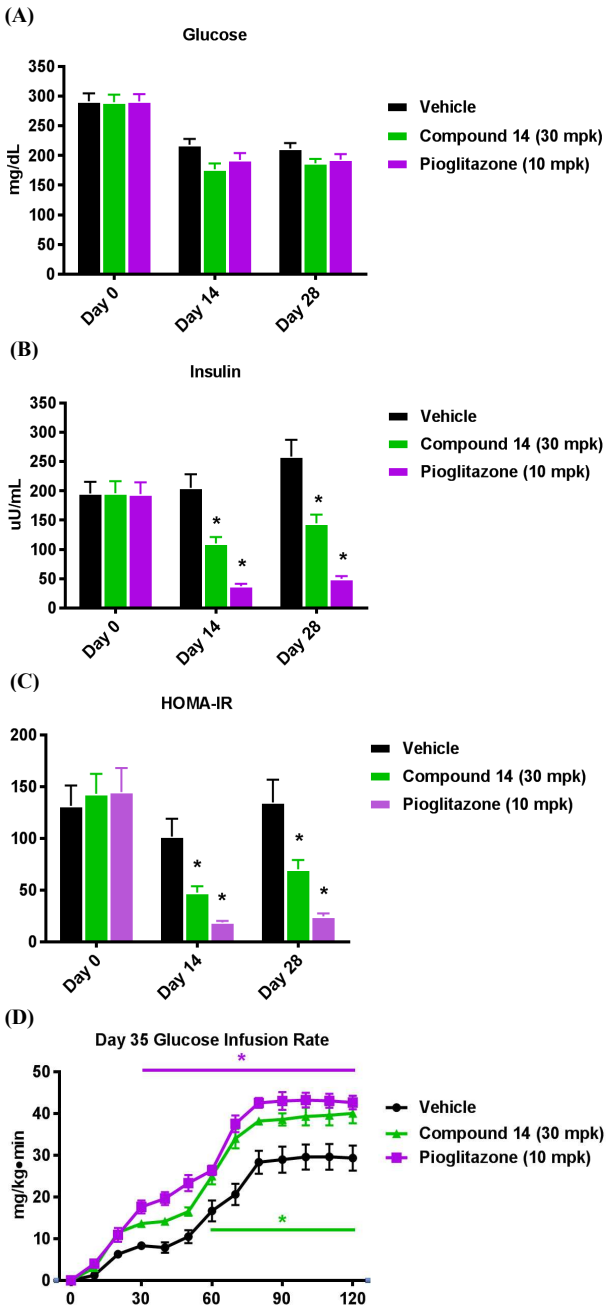


Figure 4. Five week diet induced obese (DIO) mouse study with compound **14** and pioglitazone. (A) Glucose changes over time. (B) Insulin changes over time. (C) Homeostatic model assessment of insulin resistance (HOMA-IR) changes over time; HOMA-IR = glucose (mM) x insulin (μU/mL)/22.5. (D) Glucose infusion rate (GIR) change during hyperinsulinemic euglycemic clamp. For (B-D), * $P < 0.05$ compared to vehicle; one-way ANOVA for (B) and (C); two-way ANOVA for (D). Data are represented as mean ± SEM.

With a superior *in vitro* GPR40 selectivity profile of spirocyclic lead **14** versus **2**, we further evaluated **14** for its ability to improve insulin sensitivity following five week dosing in insulin resistant diet induced obese (DIO) mice using pioglitazone (PPAR *alpha* and *gamma* active) as a positive control (**Figure 4**). While only modest, non-statistically significant reductions in glucose were observed for **14** and pioglitazone (**Figure 4A**), both showed statistically significant reductions in insulin levels on days 14 and 28 of the study (**Figure 4B**).

Furthermore, compound **14** demonstrated statistically significant reduction in homeostatic model assessment of insulin resistance (HOMA-IR),[25] a surrogate marker for insulin resistance, at days 14 and 28 (**Figure 4C**).

As shown in **Figure 4D**, selective GPR120 agonist **14** demonstrated a statistically significant increase in the glucose infusion rate (GIR), which was similar to the known insulin sensitizer pioglitazone, during the hyperinsulinemic euglycemic clamp. These results are indicative of an insulin sensitizing effect of **14**, consistent with previous reports.[16] Notably, after five weeks of treatment, no body weight changes were observed for **14**, which is in contrast to the body weight increase for pioglitazone (see supporting information) and body weight decrease observed with a recently described benzosultam GPR120 agonist.[26]

CONCLUSION

Interest in GPR120 has increased in recent years as a potential type 2 diabetes mellitus target, in particular for its potential role in insulin sensitization. Through systematic modification of an internal hit, we identified a spirocyclic lead series that resulted in the discovery of lead compound **14**. Analog **14** had suitable off-target (including high selectivity versus GPR40 and PPARs) and PK profiles to further evaluate the physiological effects of GPR120 agonism. Lead **14** dose-dependently increased glucose excursion (oGTT) and upon five week administration, reduced insulin levels, decreased HOMA-IR and increased GIR during a hyperinsulinemic euglycemic DIO mouse clamp. The disclosure of further efforts on GPR120 agonism will be forthcoming.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

Experimental procedures and analytical data for the synthesis of all compounds; GPR120 and GPR40 *in vitro* assay procedures; PK protocol; oGTT (including 2.5 h PK) and five week DIO clamp study protocols, including body weight changes (PDF)

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

T2DM, type 2 diabetes mellitus; DPP-4, dipeptidyl peptidase IV; GLP-1, glucagon like peptide 1; SGLT2, sodium glucose co-transporter 2; GPR120, G-protein coupled receptor 120; FFA4, free fatty acid receptor 4; GPCR, G-protein coupled receptor; GPR40, G-protein coupled receptor 40; FFAR1, free fatty acid receptor 1; LCFA, long-chain fatty acid; uHTS, ultra high-throughput screen; IP1, D-myo-inositol-1-phosphate; oGTT, oral glucose tolerance test; DIO, diet induced obese; GIR, glucose infusion rate; HOMA-IR, homeostatic model assessment for insulin resistance.

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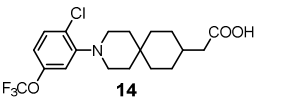

14

h/m GPR120 IP1 EC_{50} = 98/33 nM
h/m GPR120 β-arrestin EC_{50} = 66/54 nM
h GPR40 IP1 EC_{50} = >10000 nM