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Discovery of 5-aryloxy-2,4-thiazolidinediones as potent GPR40 agonists

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ABSTRACT

Systematic structure–activity relationship (SAR) studies of a screening lead led to the discovery of a series of thiazolidinediones (TZDs) as potent GPR40 agonists. Among them, compound **C** demonstrated an acute mechanism-based glucose-lowering in an intraperitoneal glucose tolerance test (IPGTT) in lean mice, while no effects were observed in GPR40 knock-out mice.

G-Protein-coupled receptor 40, GPR40, which is abundantly expressed in the pancreas, functions as a receptor for long-chain free fatty acids (FFAs). Activation of GPR40 by several naturally occurring medium to long-chain FFAs enhances insulin secretion through the amplification of intracellular calcium signaling (via IP_3 generation) and the exocytosis of insulin secretory granules (via PKC activation). The specificity of these responses is illustrated by lack of glucose-dependent insulin secretion (GDIS) amplification by FFAs following the reduction of GPR40 expression by siRNA.[1] Therefore, there are several potential advantages of GPR40 as a drug target for the treatment of type 2 diabetes. First, since GPR40-mediated insulin secretion is glucose dependent, there is reduced risk of hypoglycemia. Second, the limited tissue distribution of GPR40 in islets suggests that there would be less chance for side effects associated with GPR40 activity in other tissues. Third, the identification of synthetic GPR40 agonists suggests that small molecule drugs can potentially be developed for this target. In addition, GPR40 agonists may potentially be used in combination with other anti-diabetes drugs that have distinct mechanisms of action such as insulin sensitizers or inhibitors of hepatic glucose production. It has been reported by GSK scientists that aryl cyclopropionic acids[2] (compound **A** (Cpd **A**), Fig. 1) and aryl propionic acids[3] (compound **B** (Cpd **B**), Fig. 1) are potent GPR40 agonists. Several examples carboxylic acid GPR40 agonists have also been reported recently.[4] Some thiazolidinedione (TZD) drugs that target peroxisome proliferator-activated receptor (PPARs) are also identified as GPR40 agonists.[5] In our recent efforts to identify novel GPR40 agonists, we screened about 2000 in-house compounds and identified a partial human GPR40 agonist (relative to fatty acids) with a functional EC_{50} of 480 nM.[6] Because of its structural similarity to known TZDs that interact with PPARs, this compound was tested for its binding ability to several PPAR isoforms, and it was found to be inactive in binding assays at concentrations up to 10 μM against human PPAR α-, β-, and γ-isoforms. Herein we report our structure–activity relationship (SAR) studies of this lead which led to the identification of a potent and bioavailable GPR40 agonist (compound **C** (Cpd **C**), Fig. 1).

As shown in Scheme 1, one general method of constructing target compounds starts from key intermediate **3**, which can be prepared by coupling of a phenol or thiophenol (**1**) and a halogen-substituted benzaldehyde (**2**) in the presence of a base. Alternatively, haloarene (**4**) can be coupled to 4-hydroxyl/thiol benzaldehyde (**5**) to obtain intermediate **3**.

The initial route for the preparation of target compounds was developed based on carbanion chemistry (Scheme 2). Dianion (**7**), generated in situ by treatment of heterocycle (**6**) (e.g., 2,4-thiazolidinediones(TZD), 1,2,4-oxadiazolidine-3,5-dinone (OZD), succinimide, and hydantoin) with more than 2 equiv of a strong base such as LDA or NaHMDS in a mixture of THF/HMPA, can be reacted with

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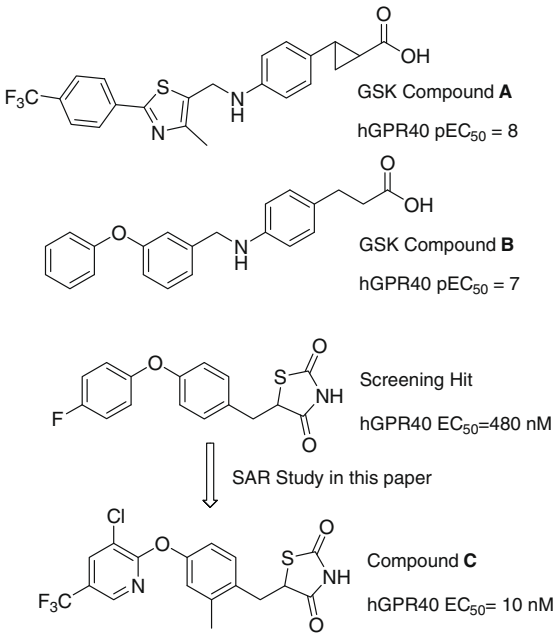
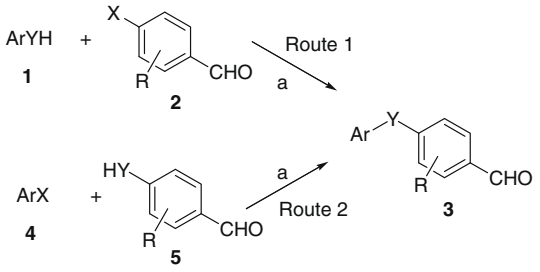
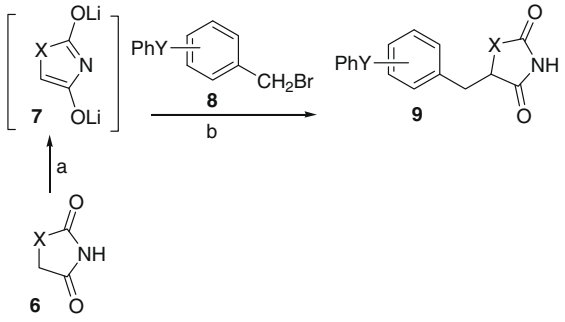


Figure 1. GPR40 agonists.



Scheme 1. Reagents and conditions: (a) Cs_2CO_3 (5 equiv), DMA, 120–150 °C, 1–12 h. X = halide, Y = S or O.



Scheme 2. Reagents and conditions: (a) LDA (2.5 equiv), THF–HMPA (1:1, v/v), −78–0 °C for 30 min, then −78 °C; (b) **8** (1.2 equiv), HMPA, −78 to 0 °C for 2 h. X = S, O, CH_2, CH_2CH_2; Y = O, S.

a commercially available benzylbromide (**8**) to provide the target compound (**9**) as a racemic mixture.

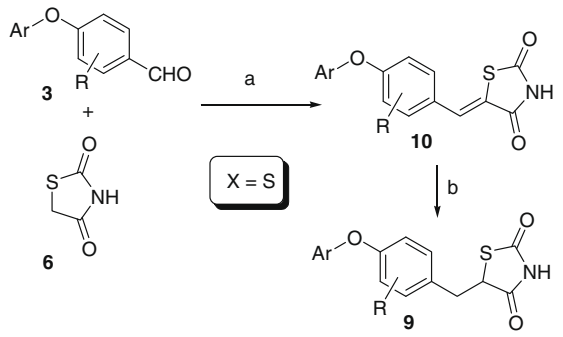
The second route involves direct condensation of the substituted benzaldehyde (**3**), either commercially available or prepared according to Scheme 1, with TZD (X = S) in the presence of sodium acetate at elevated temperature. It was discovered that the condensation proceeds effectively by simply melting all the starting materials in an open flask without use of any solvent. The resulting unsaturated intermediate compound (**10**) was treated with a reducing agent such as lithium borohydride to give the desired product (**9**) as a racemic mixture (Scheme 3).

Most of the target compounds were prepared by a convergent procedure as described for TZD below and shown in Scheme 4. First, unsaturated TZD (**12**) was prepared starting from 4-methoxybenzaldehyde by the same procedure as depicted in the Scheme 3. Further reduction with lithium borohydride gave saturated TZD (**13**) which was deprotected with boron tribromide to yield hydroxyl TZD (**14**). Final convergent coupling with aryl halides was performed using Cs_2CO_3 as a base and with or without copper iodide as the catalyst.

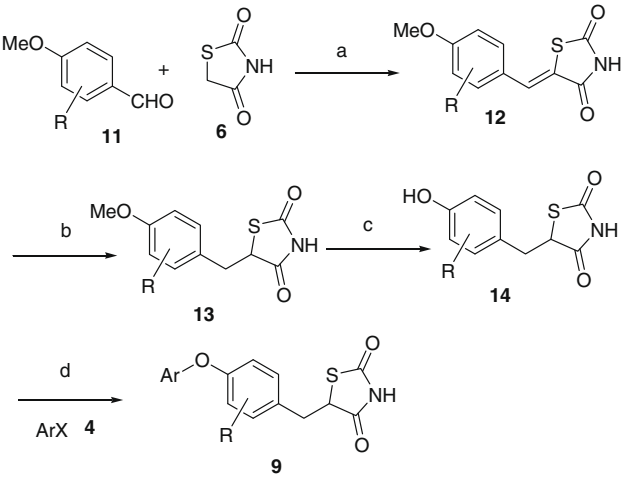
The SAR of lead compound was systematically investigated according to four parts of the molecule which we named as head (TZD), central (phenyl ring), linker, and tail (phenyl ring) as shown in Figure 2.

An initial SAR study on the heterocyclic head part of the lead molecule indicated that the original TZD ring was optimal, and replacement of it with an OZD (X = O), a succinimide (X = CH_2), or other heterocycles significantly reduced the functional GPR40 activity (Table 1).

We then investigated the SAR of the linker region. The results showed that *para*-phenyloxy linker provided the most active agonists (**9**-7). Removal of the *para*-oxygen, switch of the *para*-linker to a *meta*- or *ortho*-linker, or replacement with other linkers resulted in markedly reduced intrinsic potency (Table 2).



Scheme 3. Reagents and conditions: (a) NaOAc (1.5 equiv) neat, 150 °C for 2 h; (b) $LiBH_4$ (1.2 equiv), THF–pyridine (4:1, v/v), reflux, 2 h.



Scheme 4. Reagents and conditions: (a) NaOAc (1.5 equiv) without solvent, 150 °C for 2 h; (b) $LiBH_4$ (1.2 equiv), THF–pyridine (4:1, v/v), reflux, 2 h; (c) BBr_3 (1.2 equiv), CH_2Cl_2, 0 °C–rt, 1 h; (d) Cs_2CO_3 (5 equiv), DMA, 80–120 °C, from 1 to 12 h.

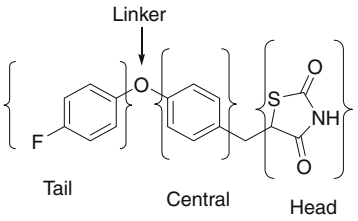
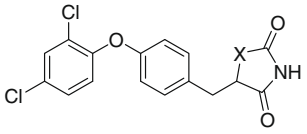


Figure 2. Lead analysis.

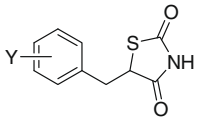
Table 1
SAR on the head part



Entry	X	EC_{50} (nM)*
9-1	S	101
9-2	O	4017
9-3	CH_2	6451
9-4	CH_2CH_2	6% @40 μM
9-5	OCH_2	23% @40 μM
9-6	NMe	2% @40 μM

* FLIPR functional assay (with 1% BSA).[6]

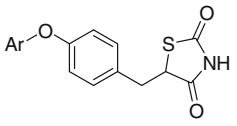
Table 2
SAR on linker part



Entry	–Y–	EC_{50}* (nM)
9-7	*p*-PhO–	307
9-8	*m*-PhO–	4700
9-9	*o*-PhO–	11,270
9-10	*p*-PhS–	5064
9-11	*p*-$PhCH_2$–	9283
9-12	*p*-PhCH=CH–	2094
9-13	*p*-$PhCH_2CH_2$–	1082
9-14	*p*-$PhCH_2O$–	6178
9-15	*p*-PhCO–	5300
9-16	*p*-Ph–	2219

* FLIPR functional assay (with 1% BSA).[6]

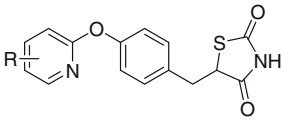
Table 3
SAR on tail part of lead compound



Entry	Ar	EC_{50}* (nM)
9-17	2-Me-Ph	124
9-18	3-Me-Ph	203
9-19	4-Me-Ph	547
9-20	3,5-diMe-Ph	96
9-21	2,3-diMe-Ph	334
9-22	2,4-diMe-Ph	121
9-23	2,5-diMe-Ph	238
9-24	3,4-diMe-Ph	727
9-25	2-Cl-Ph	1189
9-26	3-Cl-Ph	318
9-27	4-Cl-Ph	240
9-1	2,4-diCl-Ph	101
9-28	3,5-diCl-Ph	207
9-29	2-Cl,4-CF_3-Ph	78
9-30	3,5-diMe,4-CN-Ph	120
9-31	1-Naphthyl	135

* FLIPR functional assay (with 1% BSA).[6]

Table 4
SAR on pyridine-2-yl tail



Entry		EC_{50}*(nM)
9-32	3-CF_3-Py	2287
9-33	4-CF_3-Py	237
9-34	5-CF_3-Py	558
9-35	6-CF_3-Py	1635
9-36	3-Me-Py	1087
9-37	4-Me-Py	4282
9-38	5-Me-Py	4474
9-39	6-Me-Py	2620
9-40	5-Cl-Py	1872
9-41	4-CF_3-6-Me-Py	218
9-42	4-Me-6-CF_3-Py	688
9-43	3-Cl-5-CF_3-Py	69
9-44	3,5-diCl-Py	90
9-45	Isoquinolonyl	144

* FLIPR functional assay (with 1% BSA).[6]

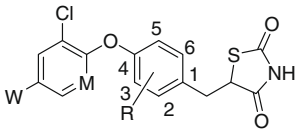
Therefore, we focused our SAR effort on the optimization of the tail region of the lead. Although various aromatic substitutions resulted in reasonable activity, 3,5- or 2,4-di-substitutions (such as **9**-1, **9**-20, and 9-29) are most potent (Table 3). In addition, relatively good potencies were observed when C2 and C3 of the phenyl ring were fused to form a naphthyl ring, as represented by compound **9**-31.

Encouraged by the above results, we went further to investigate various heterocyclic tails. It was discovered that although incorporation of mono-substituted pyridine-2-yl tails did not produce significant improvement compared to the corresponding phenyl analogs, the employment of 3,5-disubstituted pyridine-2-yl tails afforded a series of highly potent GPR40 agonists, especially, 3-chloro-5-trifluoromethylpyridine-2-yl derivative **9**-43, which was the most potent compound in this series (Table 4). Good potency was also observed when R^1, R^2 were connected together to form an isoquinoline (compound **9**-45).

The most exciting results were observed when we moved our SAR study to the central phenyl ring. As shown in Table 5, introduction of substitution(s) on the central phenyl ring led to dramatic changes in potency. 2-Methyl substitution increased potency ~7-fold to give the most potent compound **9**-46 in this series. Slightly bigger groups such as a chloro or a trifluoromethyl somewhat decreased potency (**9**-48, **9**-49, and **9**-50), while a bulkier group such as methoxy suppressed GPR40 activity dramatically (**9**-51, **9**-52). When the central phenyl ring was replaced with a naphthyl moiety, a complete loss of potency was observed (**9**-54 and **9**-55).

Of the GPR40 agonists described herein, several compounds were evaluated for their pharmacokinetic (PK) properties in mouse. In general, these compounds possess good PK profiles. For example, Cpd **C** (**9**-46) demonstrated good oral bioavailability in mice at 2 mg/kg dose and excellent overall PK characteristics (F ~100%, AUC_{po} = 18 μM h kg/mg, Cl = 1.9 mL/min/kg, $T_{1/2}$ = 5.1 h, T_{max} = 3 h, C_{max} = 1.4 μM). Thus, Cpd **C** was selected to probe the

Table 5
SAR on central phenyl ring



Entry	R	M	W	EC_{50}* (nM)
9-46	2-Me	N	CF_3	10
9-47	2-Me	CH	CF_3	18
9-48	2-Cl	N	CF_3	26
9-49	2-Cl	N	Cl	63
9-50	2-CF_3	CH	Cl	88
9-51	2-MeO	CH	Cl	1407
9-52	2-MeO	N	CF_3	13,850
9-53	2,6-diMe	N	CF_3	5454
9-54	2,3-CH=CH–CH=CH–	N	CF_3	23,587
9-55	2,3-CH=CH–CH=CH–	CH	CF_3	46%@40 μM

* FLIPR functional assay (with 1% BSA).[6]

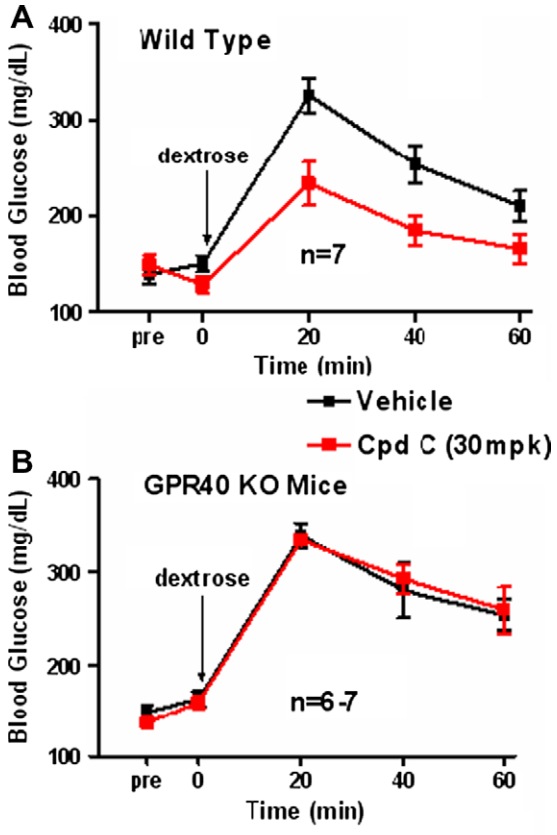


Figure 3. IPGTT results in WT and KO mice.

effects of these small molecule GPR40 agonists on glucose excursion in normal lean mice. To this end, we were excited to find that oral administration of Cpd **C**, one hour prior to dextrose challenge in an intraperitoneal glucose tolerance test (IPGTT), significantly reduced blood glucose excursion in a dose-dependent manner with doses ranging from 3 to 100 mg/kg. The maximum efficacy (73% inhibition of AUC_{GLu}) was achieved at ~30 mg/kg with a corresponding plasma concentration of 37 μM measured 2 h post-dose. In this study, GLP-1 mimetic exendin-4 was included as a positive control which completely inhibited glucose excursion at a concentration of 0.0025 mg/kg. Cpd **C** was also found to significantly enhance glucose-dependent insulin secretion (GDIS) in isolated islets (data no shown).[6]

To demonstrate that the observed Cpd **C**-induced glucose-lowering was GPR40-dependent, the effects of the ligand on blood glucose excursion during an IPGTT were investigated again in a cohort of GPR40-/- mice and littermate WT mice. The administration of 30 mg/kg Cpd **C** again resulted in a significant suppression of glucose AUC during IPGTT in the WT mice (Fig. 3A). In contrast, the same dose of the compound exerted no inhibition of blood glucose excursion in the GPR40-/- mice (Fig. 3B). The above findings demonstrate that robust glucose-lowering in normal WT mice by Cpd **C** is mediated by GPR40.

In summary, a series of thiazolidinediones was discovered as potent GPR40 agonists through systematic SAR studies of a screening lead. Among them, compound **C** possesses excellent pharmacokinetic properties in mouse, and has demonstrated an acute mechanism-based glucose-lowering in intraperitoneal glucose tolerance test in lean mice. Further SAR studies as well as optimization of the overall profiles of these compounds will be reported in due course.

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