



Synthesis and SAR of 1,2,3,4-tetrahydroisoquinolin-1-ones as novel G-protein-coupled receptor 40 (GPR40) antagonists

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ABSTRACT

The development of a series of novel 1,2,3,4-tetrahydroisoquinolin-1-ones as antagonists of G protein-coupled receptor 40 (GPR40) is described. The synthesis, in vitro inhibitory values for GPR40, in vitro microsomal clearance and rat in vivo clearance data are discussed. Initial hits displayed high rat in vivo clearances that were higher than liver blood flow. Optimization of rat in vivo clearance was achieved and led to the identification of **15i**, whose rat oral pharmacokinetic data is reported.

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Type 2 diabetes (T2D), the most commonly occurring form of diabetes, is a condition in which the body resists the insulin that is produced by the pancreas and may fail to make enough insulin to maintain normal glucose levels.¹ The incidence of T2D has increased worldwide in recent years, largely because of growing rates of obesity, and is likely to grow to greater than 366 million by the year 2030.²

One of the recently characterized G-protein-coupled receptor (GPCR) families is the GPR40–43 family,³ comprising GPR40, 41 and 43.⁴ These three family members share ~30–40% sequence identity. Three independent groups have identified GPR40 as a receptor for medium- (C6–C12) and long-chain (C14–C24) fatty acids (FAs).⁵ GPR40 is preferentially expressed in the pancreas with elevated levels reported in the islets and also in the pancreatic β -cell lines.⁶ GPR40-deficient β -cells secrete less insulin in response to FAs, and loss of GPR40 protects mice from obesity-induced hyperinsulinemia, increased hepatic glucose output, hyperglycemia and glucose intolerance.⁷ Conversely, overexpression of GPR40 in β -cells of mice leads to impaired cell function, hypoinsulinemia and diabetes. These results suggest that GPR40 plays a critical role in linking obesity and T2D.

Efforts toward the identification of 1,2,3,4-tetrahydroisoquinolin-1-ones as novel GPR40 antagonists are reported here. A high-throughput screen (HTS) of the Pfizer compound collection identi-

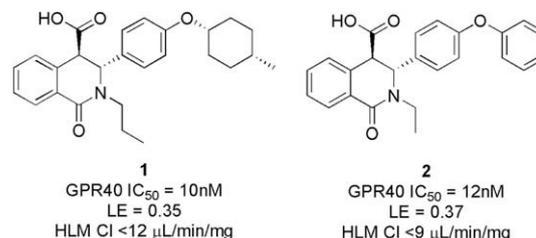


Figure 1. Initial hits from high-throughput screening.

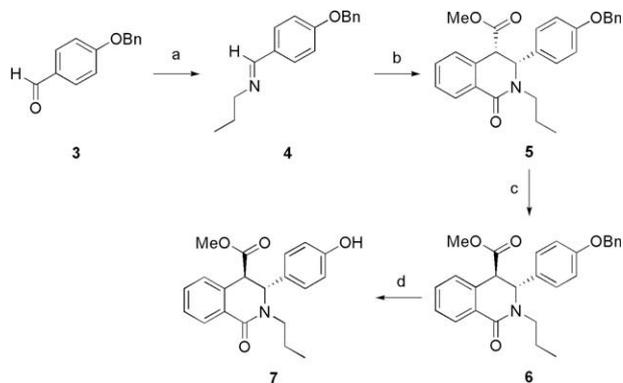
fied compounds **1** and **2** (Fig. 1) as antagonists of GPR40. These hits were extremely attractive based on their high ligand efficiency,⁸ low human liver microsomal (HLM) clearance and promising selectivity in an initial selectivity panel. The above properties motivated an initiation of hit-to-lead chemistry to explore the activity of this class of compounds as GPR40 antagonists.

Initial efforts involved exploration of the SAR of the terminal cyclohexyl and phenyl moieties of **1** and **2**. In order to do this in an efficient manner, a concise synthesis of phenol intermediate **7** was required. Intermediate **7** could then be utilized for late stage diversification and efficient SAR exploration of this region of the molecule.

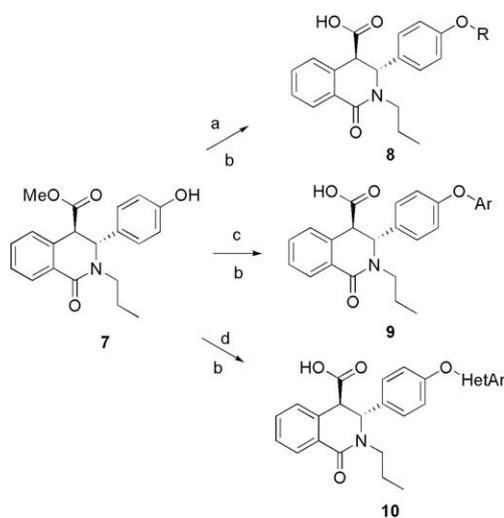
Phenol **7** was accessed in a straightforward fashion following the five step protocol below (Scheme 1). 4-Benzyloxybenzaldehyde **3** was condensed with propylamine to afford imine **4** in excellent yield. Treatment of imine **4** with homophthalic anhydride resulted in the formation of *cis*-lactam **5** in moderate yield along with minor

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Scheme 1. Reagents and conditions: (a) $C_3H_7NH_2$, 4 Å MS, CH_2Cl_2 , rt, 16 h, 100%; (b) homophthalic anhydride, CH_3CN , 60 °C, 16 h, 50%; (c) AcOH, 120 °C, 16 h, 95%; (d) MeI, K_2CO_3 , Me_2CO , rt, 16 h, 90%; (e) BBr_3 , CH_2Cl_2 , rt, 16 h, 90%.



Scheme 2. Reagents and conditions: (a) ROH, PPh_3 , DIAD, THF, DMF, 80 °C, 16 h. (b) LiOH, THF, MeOH, H_2O , rt, 16 h; (c) $ArB(OH)_2$, Et_3N , $Cu(OAc)_2$, Py, 1,2-DCE, DMF, 4 Å MS, air, 50 °C, 16 h; (d) 2-Br-HetAr, CuI (cat.), ligand 1 from ref.14 (cat.), K_3PO_4 , MeCN, 100 °C, 16 h.

amounts of *trans*-lactam **6**.⁹ The thermodynamically less stable isomer **5** could be epimerized under acidic conditions, resulting in the exclusive formation of *trans*-lactam **6** in excellent yield.¹⁰ Esterification of acid **6** with iodomethane afforded the intermediate methyl ester, which was subsequently debenzoylated utilizing boron tribromide to yield the required phenol intermediate **7**.¹¹

Phenol **7** underwent a Mitsunobu reaction, followed by saponification, to afford required products **8** in a parallel fashion (Scheme 2).¹² Diaryl ethers **9** were also accessed from phenol **7** utilizing elegant methodology developed by Evans.¹³ S_NAr reaction of phenol **7** with 2-bromoheteroaryls yielded products **10** via a copper-catalyzed Ullman-type coupling.¹⁴

All the compounds were tested in a GPR40 FLIPR calcium mobilization assay.⁵ Simultaneously, compounds were screened in human (HLM) and rat liver microsome (RLM) assays. The effect of different terminal substitution (e.g., replacements for cyclohexyl and phenyl moieties in **1** and **2**) was studied first, and these results are summarized in Table 1. Both compounds **1** and **2** have good GPR40 activity and low human microsomal clearance, but high rat microsomal clearance. Knowing that the desired pre-clinical diabetic model study would be in a rat, improvements in the ro-

Table 1
GPR40 activity, human and rat liver microsome data for antagonists **1**, **2** and **8–10**

Compound	R/Ar/HetAr	GPR40 IC_{50} (nM) ^a	HLM Cl ($\mu L/min/kg$)	RLM Cl ($\mu L/min/kg$)
1	—	10	<12	274
2	—	12	<9	97
8a	Me	2100	<8	<14
8b	CH_2Ph	96	<8	<16
9a	3,5-Di-Me-Ph	2	21	46
9b	3,4-Di-Me-Ph	3	<8	<14
10a		29	<8	<14

^a Values are means of two or more independent experiments.

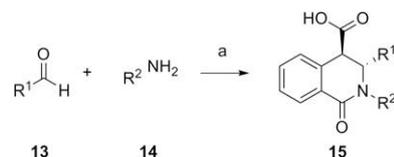
dent in vitro clearance of this series was required, while maintaining our GPR40 potency and excellent human in vitro clearance. Significant reduction in MW (e.g., **8a**) resulted in a concomitant drop-off in potency, but interestingly this compound possessed low rat microsomal clearance. Benzyl substitution (e.g., **8b**) resulted in a compound with moderate GPR40 activity and low human and rat in vitro clearance. Biaryl ethers (e.g., **9b** and **10a**) also provided excellent potency and in vitro clearance.

Compound **8b** was then selected for rat pharmacokinetic (PK) studies.¹⁵ Administration to male Sprague–Dawley (SD) rats surprisingly resulted in iv clearance that was greater than liver blood flow (82.8 mL/min/kg). In order to see whether this high rat iv clearance held true across the series, a number of other compounds were tested and all possessed high rat in vivo clearance.

In an effort to better understand the reason for the higher than predicted in vivo rat clearance, four compounds were cassette

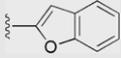
Table 2
Rat in vivo clearance and biliary excretion results

Structure	Rat iv Cl (mL/min/kg)	Percent dose in bile (%)
	111	13.9
	84.5	22.9
	136	69.9
	32.7	64.6



Scheme 3. Reagents and conditions: (a) MeOH, rt, 16 h then DMF, homophthalic anhydride, rt, 16 h then 1 N aq NaOH, rt, 16 h.

Table 3
GPR40 activity and ligand efficiency for antagonists **2** and **15**

Compound	R ¹	R ²	GPR40 IC ₅₀ (nM) ^a	RLM Cl (μL/min/kg)
2	4-PhO~Ph	Et	12	97
15a	4-PhO-Ph	Me	156	27
15b	4-PhO-Ph	CH ₂ (c-C ₃ H ₅)	4	120
15c	3,4-Di-Cl-Ph	C ₃ H ₇	89	<14
15d	3-Cl-Ph	C ₃ H ₇	228	<14
15e		C ₃ H ₇	119	18

^a Values are means of two or more independent experiments.

dosed in a bile duct cannulated (BDC) rat study (Table 2).¹⁵ A wide variation in the percentage of dose found in the bile (even with structurally similar compounds) was observed, which may indicate that (i) biliary excretion is not a series-wide issue, and (ii) this set of compounds suffers from high in vivo clearance for multiple reasons.

In parallel to the above rat BDC study, one representative compound, **12**, was chosen for a dog iv PK study in order to assess whether this was a rodent specific phenomenon.¹⁵ Acid **12** displayed low dog microsomal clearance (DLM Cl = 2.99 μL/min/mg) and low dog in vivo clearance (7.4 mL/min/kg). The fact still remained that we required access to a tool GPR40 antagonist that displayed low rat in vivo clearance and thus needed to break into different chemical space.

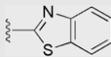
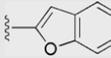
Readily accessible regions of the molecule were varied in parallel to modulate metabolism and reduce clearance, while retaining potency. In order to achieve this, optimization of the chemistry in Scheme 1 was required in order to allow for it to be utilized in a number of parallel arrays (Scheme 3). Two of the most immediate challenges were the solubility of the reagents/products in the existing solvents and the requirement for Dean-Stark conditions on formation of the imine (e.g., **4**). Solutions to these challenges involved utilizing methanol as the solvent of choice for imine formation. The poor solubility of homophthalic anhydride necessitated the use of DMF as the solvent for the formation of the *cis*- and *trans*-lactams (e.g., **5** and **6**). The resulting mixture was converted to all *trans*-lactam (e.g., **6**) by subjection to strong base, rather than the original acidic conditions.

Initially, two libraries were executed utilizing this synthetic sequence. R¹ was fixed as 4-phenoxyphenyl in **13** and the R² moiety was varied in **14**. Following this, R² was fixed as propyl in **14** and the R¹ moiety was varied in **13**. The results from this exercise can be seen in Table 3. Analysis of the amine diversity library revealed that only five compounds had LE > 0.35 (e.g., **15b**), which led us to conclude that the original N-ethyl and N-propyl moieties were already close to optimal. Interestingly, moving to N-methyl (e.g., **15a**) resulted in a significant loss of GPR40 activity, although it displayed low rat in vitro clearance. Analysis of the aldehyde diversity library revealed that 16 compounds had LE > 0.35. Mono-aryl moieties at R¹ (e.g., **15c** and **15d**) resulted in much improved rat in vitro clearance with moderate GPR40 activity.

At this point two low MW acids, **15d** and **15e**, were chosen for rat PK studies, with both compounds displaying low rat in vitro clearance.¹⁵ Administration of **15d** and **15e** to male SD rats resulted in low iv clearance (**15d**—2.96 mL/min/kg, **15e**—3.0 mL/min/kg). This data provided evidence that low rat in vivo clearance could be achieved within this series.

Finally, a full 2D matrix parallel array was executed where the five most ligand efficient amines were crossed with the 20 most ligand efficient aldehydes. This exercise resulted in a number of

Table 4
GPR40 activity and ligand efficiency for antagonists **15**

Compound	R ¹	R ²	GPR40 IC ₅₀ (nM) ^a	RLM Cl (μL/min/kg)
15f	3,4-di-Cl-Ph		13	<14
15g	3,4-di-Cl-Ph	CH ₂ CH(Et) ₂	10	27
15h		CH ₂ CH(Et) ₂	10	<18
15i			20	<14

^a Values are means of two or more independent experiments.

compounds that possessed low rat in vitro clearance and good GPR40 activity (Table 4).

Based on good GPR40 activity, good in vitro permeability (CaCo-2 AB = 12.0 and BA = 18.7 × 10⁻⁶ cm/sec), human in vitro clearance (HLM Cl < 8.0 μL/min/mg) and rat in vitro clearance (RLM Cl < 14.1 μL/min/mg) **15i** was selected for rat PK studies.¹⁵ Administration to male SD rats resulted in satisfactory PK parameters – iv clearance = 4.3 mL/min/kg, V_d = 1.1 L/kg and iv half-life of 5.4 h.

In summary, a novel series of 1,2,3,4-tetrahydroisoquinolin-1-ones have been identified as antagonists of GPR40. Initial hits displayed high rat in vivo clearances that were higher than liver blood flow. Optimization of rat in vivo clearance was achieved and led to the identification of **15i**, which showed satisfactory PK parameters.

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