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Tetrahydroisoquinoline PPARγ agonists: Design of novel, highly selective non-TZD antihyperglycemic agents

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Abstract—Novel tetrahydroisoquinolines have been developed as potent PPAR ligands. Evaluation of these compounds in PPARγ responsive models of type 2 diabetes is described.

Peroxisome proliferator receptors (PPARs) are a subset of the superfamily of ligand-activated transcription factors including receptors for steroid, retinoid, and thyroid hormones.[1] The PPARs form heterodimers with the 9-*cis*-retinoic acid receptor (RXR), initiating a signal transduction cascade leading to gene transcription of proteins involved in the control of lipid and carbohydrate metabolism. There are three known sub-types of PPAR receptors which are designated as PPAR α, γ, and δ. Agonists of PPARα have been reported to produce reductions in serum triglycerides and increases in HDL cholesterol.[2] Further, agonists of PPARα have been shown to reduce weight gain in rodents without affecting food intake.[3] Likewise, PPARδ has been implicated in having a role in dyslipidemia,[4] as well as fertility[5] and cancer.[6]

PPARγ is the best studied of the 3 PPAR sub-types. It is primarily expressed in adipose tissue and in lower levels in skeletal muscle, heart, intestine, liver, smooth muscle, and vascular endothelial cells.[7] PPARγ agonists have been most extensively studied for their ability to enhance the sensitivity of target tissues to the effects of insulin.[8] This increased insulin sensitivity results in reduced plasma glucose, lipid, and insulin levels in animal models of type II diabetes as well as in diabetic humans.[9] There are currently 2 PPARγ agonists being marketed as antidiabetic agents, rosiglitazone (**1**) and pioglitazone (**2**) (Chart 1). Both compounds are members of the thiazolidine-2,4-dione (TZD) class of compounds. Another TZD, troglitazone (**3**) was removed from the market after being associated with idiosyncratic hepatotoxicity. The TZDs were first reported by Takeda to show simultaneous reductions in plasma glucose and insulin concentrations; a clear indication of improved insulin sensitivity.[10,11] In 1995 the target of the TZDs was determined to be PPARγ, by showing direct binding of radiolabeled rosiglitazone (**1**) to PPARγ with high affinity.[12] The TZD class of molecules are selective PPARγ agonists, showing little or no cross reactivity with the other members of the PPAR family.[12] One drawback of this class of compounds is the rapid racemization of the

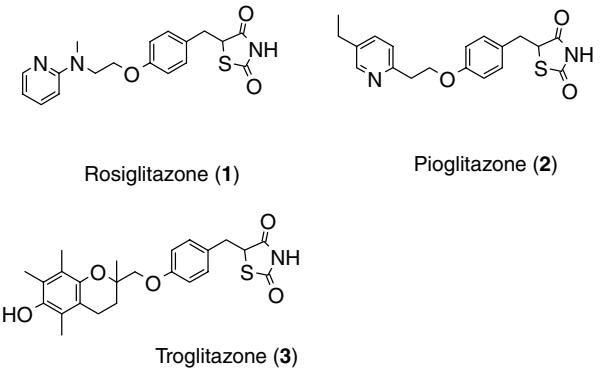

Chart 1. Examples of the TZD class of PPARγ agonists.

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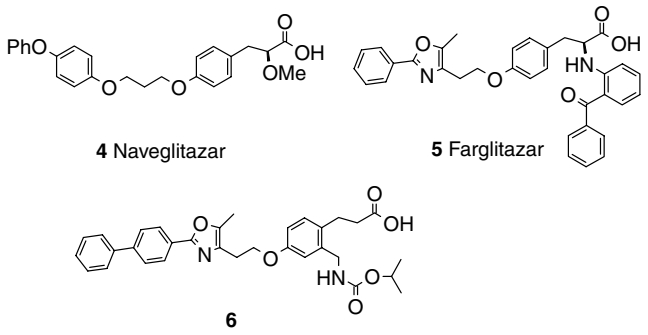
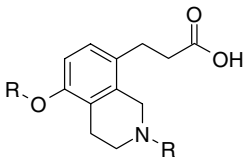


Chart 2. Examples of reported non-TZD PPARγ agonists.

resident chiral center under physiological conditions, leading to the development of the TZDs as racemates. It has been shown that only the (*S*)-enantiomer binds to PPARγ, suggesting that only 50% of the drug substance is active in vivo.[13]

Along with their anti-diabetic affects, PPARγ agonists have been implicated in a variety of other disorders including hypertension,[14] dyslipidemia,[15] and inflammation.[16] PPARγ has also been shown to regulate the expression of many genes relevant to carcinogenesis.[17] Highly potent and selective PPARγ agonists would be desirable not only for their well-established value in treating insulin resistance in type 2 diabetes, but also as tools for assessing the role of PPARγ in other disorders.

A variety of non-TZD PPARγ agonists have been reported by our laboratories, and others (Chart 2). A feature of the initially reported non-TZD containing PPARγ agonists was the presence of a heteroatom substituent alpha to a dihydrocinnamic acid, for example, **4**[18] and **5**.[19] In the aminomethyl cinnamate (AMC) series of PPARγ agonists (e.g. **6**), the alpha heteroatom has been moved to the *ortho* position of the aromatic ring, eliminating the epimerizable stereocenter and producing highly PPARγ selective compounds.[20] Further investigation into PPARg agonists led to the discovery of tetrahydroisoquinolines **7** (THQs). Herein, we report our results in this effort.



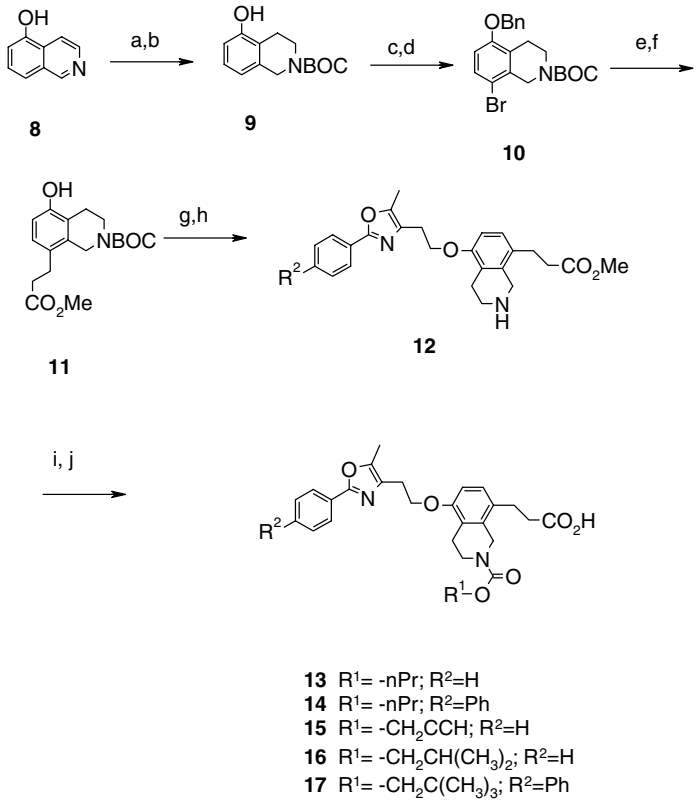
7 Tetrahydroisoquinoline Headpiece

The synthesis of the THQs begins with the catalytic hydrogenation of 5-hydroxy isoquinoline (**8**) to the tetrahydro derivative[21] followed by protection with Boc anhydride to give **9** (Scheme 1). Regioselective bromination, followed by benzylation of the phenol, gives bromide **10**. Heck coupling with methyl acrylate followed by hydrogenolysis of the benzyl group and concomitant reduction of the olefin gave key intermediate **11** in good yield. Coupling with the appropriate tosylate[22] using Cs_2CO_3 in DMF followed by removal of the *N*-Boc-protecting group gives amine **12**. Reaction with an appropriate chloroformate followed by hydrolysis of the methyl ester gives the desired carboxylic acid PPARγ agonists.

The ability of compounds to modulate PPARγ and PPARα receptors in vitro was determined in both direct binding and cellular cotransfection assays. The DNA-dependent binding assay (ABCD binding) was carried out using scintillation proximity (SPA) technology with PPAR receptors.[23] Tritium-labeled PPARα and PPARγ agonists were used as radioligands for generating displacement curves and IC_{50} values with compounds of interest. Cotransfection assays were carried out in CV-1 cells. For PPARα, interference by endogenous PPARγ in CV-1 cells is an issue, so a GAL4 chimeric system was used in which the DNA-binding domain of the transfected PPARα is replaced by that of GAL4. This system utilizes a reporter gene containing a GAL4 response element, thus eliminating PPARγ interference.[12] Cotransfection efficacy is determined relative to PPARα and PPARγ agonist reference molecules.

As can be seen from the data in Table 1, compounds **13**–**17** are generally highly selective for the PPARγ receptor over PPARα. Some interesting trends are notable in the data. First, increasing the size of the substituent on the oxazole tailpiece from phenyl to biphenyl significantly reduces PPARα activity with little or no effect on PPARγ (**13** vs **14**). The same trend is generally true with the carbamate side chain. Increasing the size of the lipophilic group in the carbamate reduces PPARα activity. Compound **16**, bearing an isobutyl carbamate, is approximately 3-fold less active in the PPARα cotransfection assay than the corresponding propyl carbamate in compound **13**. When comparing our compounds with the marketed TZDs, it can be seen that the PPARγ IC_{50}s are generally twice as potent as **1**, and up to 100 times more potent than **3**. The cellular data reveal even more significant differences in the compounds, with **14** being 200 times more potent in the transfection assay over **1** and 700 times more potent than **3**. The PPARα data show that while the THQ class is not as selective as the TZDs, it shows very little PPARα activity and is significantly more selective against PPARα than the other reported non-TZD PPARγ agonists **5** and **6**. Compound **17** is the exception, showing an in vitro profile equal to the TZDs with respect to selectivity, while maintaining superior PPARγ activity.

After determining the in vitro properties of the compounds, they were subjected to two separate in vivo experiments. In the first, 7-week-old male diabetic (db/db) mice were used to assess test compounds' ability to lower plasma glucose. Mice were dosed daily by oral gavage for 7 days. Treatments were test compounds (30 mg/kg), a positive control agent (**1**, 30 mg/kg) or vehicle [1% carboxymethylcellulose (w/v)/0.25% Tween 80 (w/v); 0.3 ml/mouse]. On day 7, mice were weighed



Scheme 1. Reagents and conditions: (a) PtO_2, AcOH, H_2; (b) Boc_2O, THF (70%, 2 steps); (c) NBS, DMF; (d) BnBr, K_2CO_3, acetone (90%, 2 steps); (e) $Pd(OAc)_2$, methyl acrylate, PrCN; (f) H_2, Pd/C, EtOH (50%, 2 steps); (g) toluene-4-sulfonic acid 2-(5-methyl-2-phenyl-oxazol-4-yl)-ethyl ester (R^2 = H), Cs_2CO_3, DMF; (h) CF_3CO_2H (40%, 2 steps); (i) Et_3N, propyl chloroformate, CH_2Cl_2; (j) NaOH, MeOH yields **13** (70%, 2 steps).

Table 1. Binding IC_{50} and cotransfection efficacy data[d]

Compound	hPPARγ			hPPARα		
	IC_{50} (nM)[a,b]	EC_{50}[a,b] (nM)	CTF eff[c] (%)	IC_{50}[a,b] (nM)	EC_{50}[a,b] (nM)	CTF eff[c] (%)
Fenofibric acid	>10,000	—	9 ± 4	>10,000	>10,000	75 ± 20
1	48 ± 2	657 ±345	100 ± 21	>10000	Eff <20%	9
3	1285 ± 61	2235 ± 344	79 ± 1	No binding	Eff <20%	0
5	25	5	83	1217	345	66
6	47 ± 16	19 ± 10	92 ± 4	3489 ± 917	845 ± 66	52 ± 3
13	11	12 ± 4*	61 ± 4*	4684	561	47
14	21	3 ± 0.37	55 ± 5	No binding	2393 ± 153*	27 ± 6*
15	24 ± 1.4	77 ± 12	76 ± 4	8399 ± 354*	2780 ± 89	38 ± 2*
16	17 ± 2	8 ± 2	66 ± 3	3415 ± 339	1715 ± 290	40 ± 2
17	33 ± 2*	9 ± 0.41*	56 ± 4*	No binding	Eff <20%	18

[a] Mean values for at least three determinations ± standard error (*n = 2).
[b] Competitive displacement binding assays were performed using scintillation proximity assay (SPA) technology, PPAR receptors, and corresponding radiolabeled ligands.
[c] Maximum efficacy as % of the maximum efficacy of the standard.
[d] PPARδ data are not shown. Only compounds **14** and **15** show significant CTF eff (%) (36% and 65%), but with weak EC_{50}s (2747 and 3021 nM).

and bled (tail snip) at 3 h after dosing. Samples obtained from conscious animals on days 0 and 7 were assayed for glucose. Plasma glucose was measured using a Hitachi 912 metabolic analyzer (Roche, Indianapolis) utilizing the Trinder method.

In a second experiment, compounds were evaluated in a dose–response study using Zucker diabetic fatty (ZDF) rats. Rats (five per group, 8 weeks of age) were dosed via oral gavage for 7 days at doses of 0.01, 0.03, 0.3, and 1 mg/kg. The study was terminated as in the db/db studies. Minimal effective dose (MED) and ED_{50} were determined from the data using a four parameter logistic model (Table 2).[24] The MED is defined as the lowest dose at which the response is statistically significantly better than the response at zero-dose (vehicle).

The results of the db/db mouse study are shown in Figure 1. Percent glucose reduction for each test compound was calculated based on vehicle control glucose levels in each experiment and is summarized in

Table 2. In vivo Efficacy of THQs

Compound	db/db Mouse % glucose reduction at 30 mg/kg (day 7)	ZDF rat MED[b] (mg/kg)
1	29 ± 9	0.28 ± 0.10
13	62 ± 5	0.28 ± 0.19[a]
14	41 ± 8[a]	0.19 ± 0.09[a]
15	37 ± 11	No effect at 1 mg/kg
16	27 ± 9	0.25 ± 0.21
17	49 ± 5[a]	23% at 1 mg/kg[a,c]

[a] Tested as sodium salt of parent acid.
[b] Minimal effective dose (MED).
[c] Percent glucose reduction versus vehicle control.

Table 2. In some cases compounds were evaluated as their sodium salts. The db/db data clearly indicate that all compounds are at least equal to rosiglitazone (**1**) in their ability to lower blood glucose at a single 30 mg/kg dose, with reductions of 62%, 41%, and 49% for **13**, **14**, and **17**, respectively, compared to 29% with compound **1**. In the ZDF dose response study, compounds **13**, **14**, and **16** normalized glucose with MEDs similar to **1**, while **15** and **17** showed poor efficacy. It is unclear why **15** and **17** performed poorly in the ZDF study after promising db/db results, but this may be attributable to uncharacterized species and model differences. The ED_{50} of **14** (sodium salt) was determined to be 0.24 ± 0.08 mg/kg, significantly lower than the ED_{50} of **1**, 0.41 ± 0.12 mg/kg. Further, at a dose of 1 mg/kg **14** reduced plasma triglycerides in treated animals by 56% over the vehicle control group.

Finally, the pharmacokinetic parameters of the sodium salt of acid **14** were evaluated in two species and are summarized in Table 3. Similar results were obtained in both rats and dogs with oral bioavailabilities of 35% and 37%, respectively.

In summary we have developed a novel class of non-TZD containing PPARγ agonists. These compounds exhibit potent PPARγ binding with the isolated receptor and in cellular assays, while showing extraordinary selectivity against PPARα. Further, compounds have shown efficacy in two separate in vivo models of diabetes. Compound **14** has shown an excellent in vivo profile in the db/db and ZDF models

Table 3. Pharmacokinetic parameters for the sodium salt of **14**[a]

Parameter	F344 male rat	Male beagle dog
Half life (iv)	5 h	3.58 ± 0.52 h
Volume of distribution (iv)	0.59 L/kg	0.38 ± 0.08 L/kg
Clearance (iv)	1.37 mL/min/kg	1.24 ± 0.30 mL/min/kg
Tmax (oral)	1 h	4 ± 0 h
Oral bioavailability	35%	37 ± 7%

[a] iv 1 mg/kg, oral 10 mg/kg.

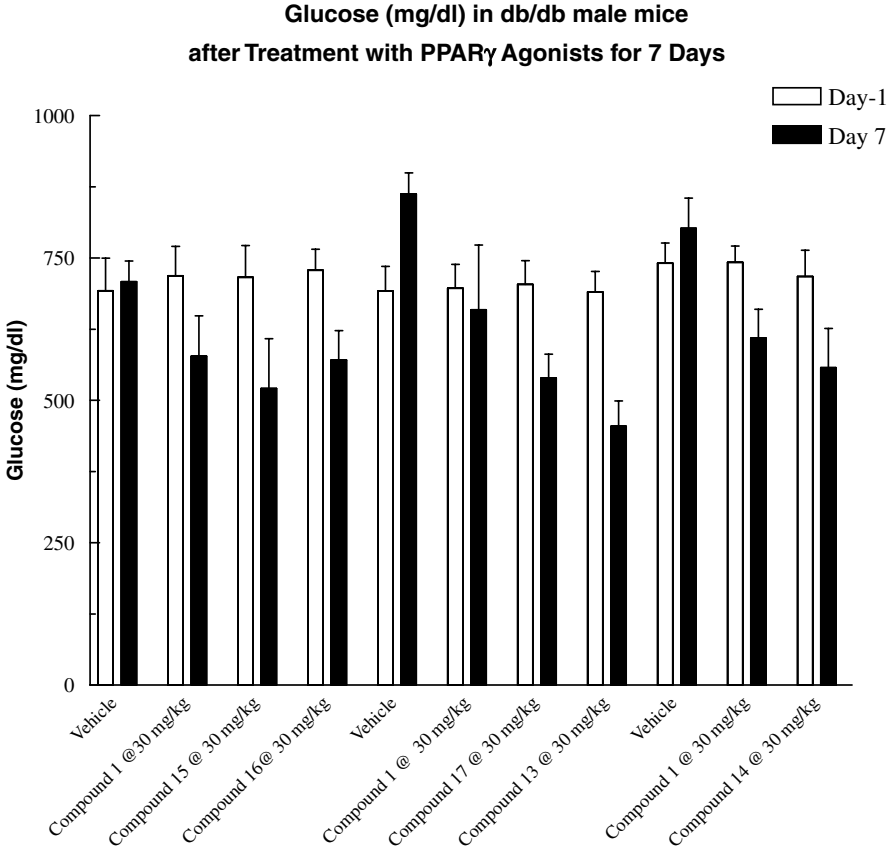


Figure 1. Plasma glucose levels on days 1 and 7 in db/db mice treated via oral gavage for 7 days with rosiglitazone (**1**), or compound. The figure contains data from three separate experiments and shows compound data relative to control and rosiglitazone data for each experiment.

of type II diabetes, and also shows desirable pharmacokinetic properties. Further evaluation of compound **14** is underway.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.09.028.

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