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4,4-Dimethyl-1,2,3,4-tetrahydroquinoline-based PPARα/γ agonists. Part. II: Synthesis and pharmacological evaluation of oxime and acidic head group structural variations

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ABSTRACT

Type-2 diabetes (T2D) is a complex metabolic disease characterized by insulin resistance in the liver and peripheral tissues accompanied by a deficiency in pancreatic β-cells. Since their discovery, three subtypes of peroxisome proliferator activated receptors have been identified, namely PPARα, PPARγ and PPARβ/(δ). In this study, we were interested in designing novel PPARγ selective agonists and/or dual PPARα/γ agonists. Based on the typical topology of synthetic PPAR agonists, we focused our design approach on using 4,4-dimethyl-1,2,3,4-tetrahydroquinoline as a novel cyclic scaffold with oxime and acidic head group structural variations.

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The peroxisome proliferator activated receptors are ligand-activated transcription factors belonging to the nuclear hormone superfamily.[1] Since their discovery in 1990 by I. Isseman and S. Green[2], three subtypes of PPARs have been identified, and designated α, γ and δ (β). These receptors have aroused considerable interest in pharmaceutical research due to their extensive involvement in glucose and lipid homeostasis[3–6], and a number of agonists in this class have progressed to the clinical phase, and been marketed as anti-diabetic drugs.[7,8] The success of the hypolipidemic fibrates and glitazones class of insulin sensitizers (full-agonists of PPARα[1] and PPARγ,[9,10] respectively) has incited pharmaceutical companies to concentrate on developing more potent and dual acting agonists belonging to these two subtypes.[6] In the past decade, dual-acting PPARα/γ agonists (e.g., Tesaglitazar, Muraglitazar, Fig. 1) have been viewed as a very attractive option in the treatment of dyslipidemic type-2 diabetes.[6,8,11–16] Moreover such compounds might also avoid or reduce the main side effects (weight gain or edema) induced by the full PPARγ agonists, TZDs.[17]

We have recently reported the design, synthesis and biological data of a novel 4,4-dimethyl-1,2,3,4-tetrahydroquinoline-based series of PPARγ partial agonists and PPARα/γ dual agonists.[18] This study has led to the identification and optimization of lead compound **1,** which has noteworthy dual-activity on both subtypes.

Our lead compound **1** possesses the α-ethoxy-β-phenylpropionic acid head group, which is a potent binding moiety for both PPARα and PPARγ. In order to investigate the impact of variations on the transactivation activity, we first changed the chain length of the oxime of 6-benzoyl-1,2,3,4-tetrahydroquinoline without changing the acidic head, and we subsequently decided to modify this part of the structure. Here, we report the synthesis and in vitro and in vivo biological evaluation of a new series of tetrahydroquinolines.

The synthesis of all the oximes followed on the condensation of the corresponding alkoxylamines on precursor **2,** as previously described[18] (Scheme 1). Saponification of intermediary esters using an aqueous solution of lithium hydroxide quantitatively generated α-hydroxy-acids **3a–c**.

The general strategy of modifying the acidic head is outlined in Scheme 2. In this procedure, appropriate phenols **5a–d** were coupled with the bromo key intermediate **4** under standard substitution conditions. Compounds **6a–d** were further functionalized in the required oxime by treatment with the convenient hydroxyl-

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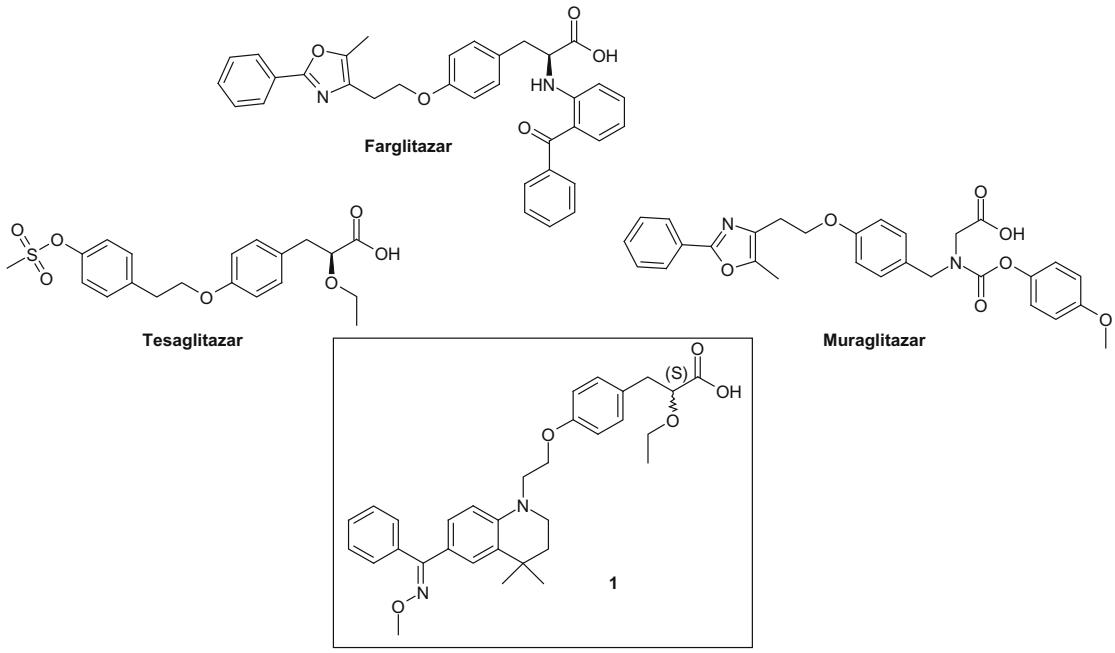
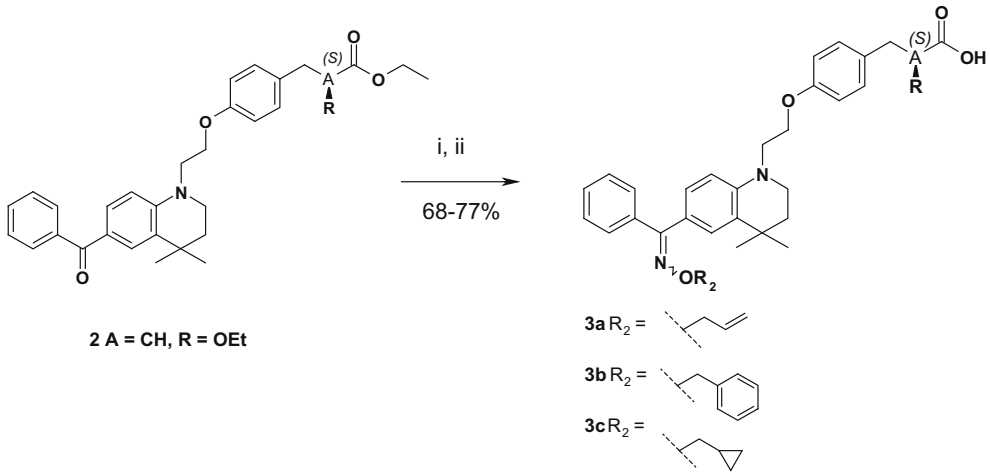


Figure 1. Structures of a PPARγ selective agonist, two PPARα/γ dual agonists (aborted during clinical phase III), and lead compound **1**.



Scheme 1. Reagents and conditions: (i) $R_2O–NH_2$, HCl, pyridine, rt, 5 h; (ii) LiOH, H_2O, THF, 24–36 h.

amine hydrochloride in pyridine, and the desired acids **8a**–**d** were obtained after lithium hydroxide mediated saponification.

The synthesis of synthon **4** was conducted in five steps, and is detailed in our previous paper.[18b]

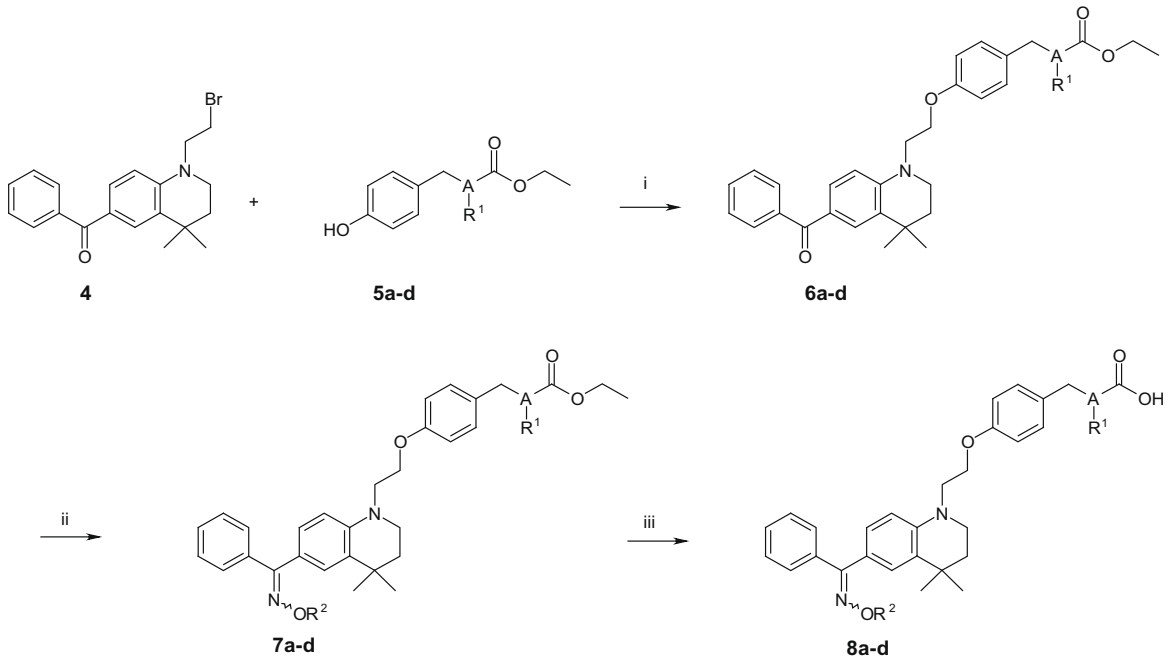
In the first analog we designed, the ethoxy-ether was replaced by the acetate analog. Phenol **5a** was thus prepared in three steps with 85% yield, starting from commercially available *O*-benzyl-L-tyrosine, as shown in Scheme 3. Treatment with isoamylnitrite in a mixture of acetic acid and chloroform yielded **9,** which was subsequently hydrogenated using palladium on charcoal as catalyst.

The trifluoroethoxy homolog of **1** was next envisaged, and required the synthesis of the corresponding phenol **5b**. This was generated from *O*-benzyl-L-tyrosine according to the sequence shown in Scheme 4.

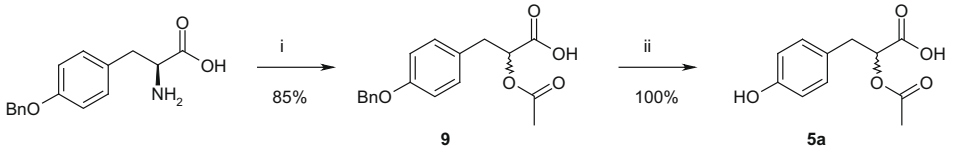
After classical esterification in ethanol, compound **10** was treated with isoamylnitrite in chloroform using a catalytic amount of acetic acid. Under these conditions, diazoester **11** was isolated quantitatively. Insertion of trifluoroethanol catalysed by rhodium acetate (II) generated intermediate **12,** with a modest yield.[19] Finally, catalytic debenzylation under a hydrogen atmosphere in ethanol yielded the desired compound, **5b**.

Supported by the interesting results with Farglitatzar (Fig. 1) we decided to introduce the *N*-(benzoylphenyl)-tyrosine scaffold into our cyclic tail as the selective PPARγ agonist. Thus, DL-tyrosine was first esterified under standard conditions, and the resulting amino-ester **13** was then condensed on 2-benzoylcyclohexanone. Aromatization was carried out in situ, and generated the desired phenol **5c** with a yield of 49%.[20]

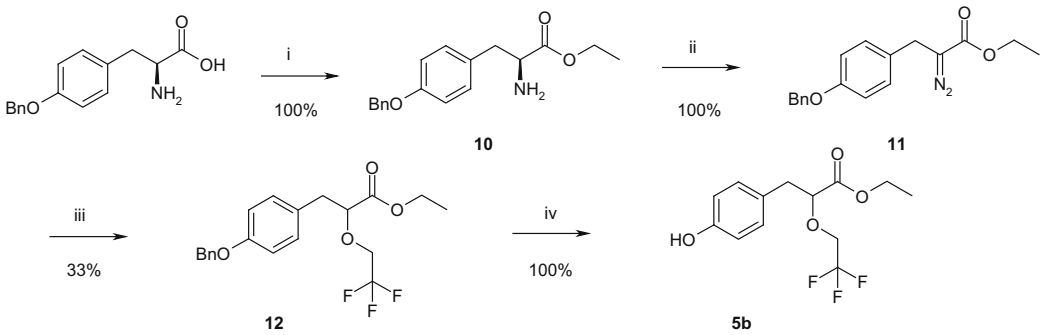
Once Muraglitazar (Fig. 1) had been described in the literature as the most potent PPARα/γ dual agonist, we synthesized one of its homologs for inclusion in our series. As shown in Scheme 6, phenol **4d** was prepared in a four-step sequence, starting from commercially-available 4-benzyloxy-benzaldehyde. First, treatment of the aldehyde with glycine ethyl ester in methanol followed by the reductive action of sodium borohydride gave the amino-es-



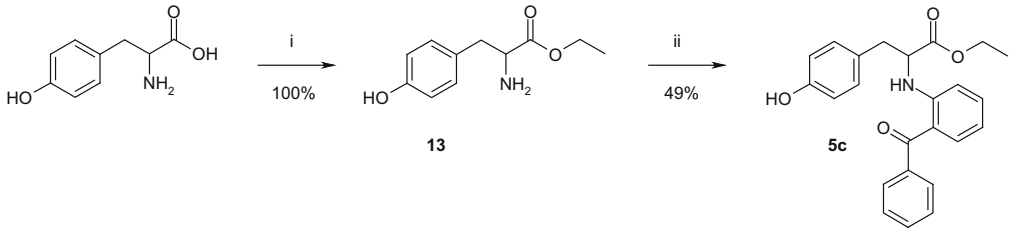
Scheme 2. Reagents and conditions: (i) K_2CO_3, DMF, 60 °C, 24–48 h; (ii) $CH_3O–NH_2.HCl$, pyridine, reflux, 3–5 h; (iii) $LiOH.H_2O$, THF/H_2O, rt, 24–36 h.



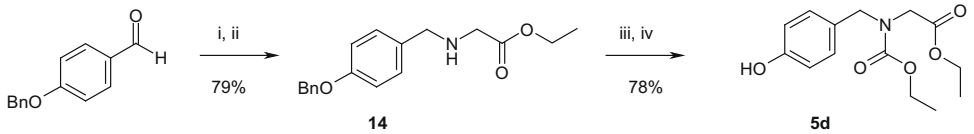
Scheme 3. Reagents and conditions: (i) *i*-$C_5H_{11}ONO$, AcOH/$CHCl_3$, rt, 36 h; (ii) H_2, Pd/C, EtOH, rt, 18 h.



Scheme 4. Reagents and conditions: (i) $SOCl_2$, EtOH, rt, 12 h; (ii) *i*-$C_5H_{11}ONO$, AcOH cat., $CHCl_3$, reflux, 30 min; (iii) $Rh_2(OAc)_4$, CF_3CH_2OH, reflux, 2 h; (iv) H_2, Pd/C, EtOH, rt, 12 h.



Scheme 5. Reagents and conditions: (i) $SOCl_2$, EtOH, rt, 18 h; (ii) 2-benzoylcyclohexanone, Pd/C, anisole, reflux, 3 h.



Scheme 6. Reagents and conditions: (i) glycine-ethyl ester hydrochloride, Et_3N, MeOH, rt, 5 h; (ii) $NaBH_4$, MeOH, rt, 18 h; (iii) ethyl chloroformate, Et_3N, THF, rt, 1 h; (iv) H_2, Pd/C, EtOH, rt, 12 h.

Table 1
Activity of compounds **6a–d** in a cell-based transactivation assay against human PPAR

Compound	A	R^1	R^2	Binding PPARγ	Transactivation EC_{50}[a] nM[b](% activity)	
				K_i (nM)	hPPARα	hPPARγ
1	CH	OCH_2CH_3	CH_3	18	114 (63)	7.85 (95)
3a	CH	OCH_2CH_3	CH_2–CH=CH_2	54.2	73(152)	13(150)
3b	CH	OCH_2CH_3	Bn	18.4	34(137)	7(124)
3c	CH	OCH_2CH_3	CH_2-cyclopropyl	254	9(145)	7.52(165)
8a	CH	OAc	CH_3	>10,000	na	na
8b	CH	OCH_2CF_3	CH_3	33	1000 (50)	125 (100)
8c	CH	NH–Ph–CO–Ph	CH_3	54	10,000 (11)	14 (72)
8d	N–CH2	C(O)OEt	H	>10,000	na	10 000 (69)

[a] Values are means of three experiments (na = not active).
[b] Refers to the maximal activity obtained with each compound expressed as a percentage of the maximum activity of Rosiglitazone at 10^{-6} M for PPARγ and of WY 14,643 at 10^{-5} M for PPARα.

ter product **14** with a 79% yield. Condensation of ethyl chloroformate generated the carbamate, which was finally debenzylated to produce phenol **5d** (Scheme 5).

Compounds were characterized by determining the binding affinity to human PPARγ, using a competitive binding assay with [^{3}H]rosiglitazone, an appropriate radioligand for PPARγ. The binding profiles of compounds were compared to those of the lead derivative **1**. Functional activity was measured in a transient transfection assay using pGAL4hPPARα and pGAL4hPPARγ. The results are given in Table 1.

With a good affinity for PPARγ (K_i = 54.2 nM), the allyl analog **3a** is a dual agonist PPARα/γ with the same pharmacological profile as compound **1**. Compound **3b** confirms this finding by increasing the activity of PPARα (137% of transactivation at 34 nM).

In this series, compound **3c** seems to be the best analog of lead compound **1**. Indeed, although the binding constant, K_i (254 nM), is slightly lower than for **3a** and **3b**, the transactivation PPARγ tests and K_i values from binding tests (165% at 7.52 nM) are comparable to those found for compound **1**. Instead of PPARα transactivation (145% at 9 nM), as a PPARα/γ dual agonist, compound **3c** could be compared to Tesaglitazar.

On the basis of their in vitro results, these three compounds (**3a–c**) were investigated in absorption and bioavailability tests (Table 2).[21] With a good percentage of absorption (around 95%), these compounds have shown a better metabolic stability than compound **1**; in this series **3c** displays the best results. These values allowed us to confirm that the major degradation site of our compounds is located on the methyl oxime ether (see Table 2, compound **1**).

With a good affinity for the PPARγ receptor (K_i = 33 nM), compound **8b** displays the same dual-agonist pharmacological profile compared to derivative **1**. Nevertheless, the transactivation values, 100% and 50% for PPARγ and PPARα, respectively, associated with EC_{50} values of 125 nM and 1000 nM, indicate low agonist power towards these receptors. Finally, compound **8c** shows good selectivity for PPARγ, but this derivative was not evaluated in vivo with regard to its absorption value with CaCo2 cells (22%). The replacement of an asymmetric carbon (compound **1**) group (compound **6d**) leads to poor performance by this molecule.

The in vitro values of **8b** were comparable to those of compound **1** (Table 1), but the activity observed versus the different parameters tested in vivo was disappointing. The increase in plasma triglycerides suggests that the agonism towards PPARα is not sufficient to regulate the concentration of fatty acids. Moreover the increase in body weight (304%) suggests possible inflammatory effects. The values obtained with compounds **3b** and **3c** are not conclusive.

Table 2
In vivo activity of compounds **1** and **6b** in *ob/ob* model

Compound	Absorption (%)	Bioavailability (%)		*ob/ob* mice 4 days (PO 3 mg/kg)			
	$CaCo_2$	Human hepatocyte microsomes	Rat hepatocyte microsomes	TG	G	Ins	ΔP
Rosiglitazone	100	21	45	−56	−57	−55	0.56
Tesaglitazar	78	100	97	−60	−51	−94	+1.9
1	100	0	0	−5	−12	−20	−33
3a	95	47	20	ND*	ND	ND	
3b	90	59	35	−27	−11	51	0.23
3c	93	84	32	−2	−12	−2	107
8b	100	27	18	9	12	−17	304

TG: triglycerides, G: glycemia, Ins: insulin, ΔP: delta body weight.
* ND not determined

Acknowledgments

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21. The glucose and triglyceride-lowering activities of the compounds prepared were then tested using *ob*/*ob* mice (male C57BL/6 J). The mice were housed in a temperature-controlled room (21.8–24 °C), with a relative humidity of 36–80%, and a 12 h light/12 h dark cycle (light 7:00 am–7:00 pm). Mice had ad libitum access to filtered tap-water (0.22 μm filter) and irradiated pelleted laboratory chow (ref # A03-10, UAR. France) throughout the study. Compounds were prepared as a suspension in HEC 1% (hydroxyl ethyl cellulose) for oral administration (2.5 ml/kg). The mice (n = 8–12 per dose) were dosed by gavage daily between 3.00 pm and 5:00 pm for four days. Randomization was performed based on glycemia values. The body weight gain (ΔP grams) of the animals was determined by measuring the difference between the body weights at the beginning and the end of the study for each mouse. At day five, between 9:00 and 11:00 am, the mice were weighed and blood samples were collected into heparin-containing tubes by retro-orbital puncture (500 μl/mouse) under CO_2 anaesthesia. Plasma sample were prepared by centrifugation (2000*g* for 10 min), and stored at −20 °C. The mice were euthanized by cervical dislocation. For triglycerides and insulin, the percentage change in plasma level as calculated relative to the mean plasma level in the vehicle-treated mice. ANOVA, followed by Dunnett's comparison test (one tailed) was used to estimate the significant difference between the plasma triglycerides, insulin and delta body weight values from the control group and the individual compound-treated groups. The compound is considered active, at the specific dosage administered, if the difference in plasma levels had a $P < 0.05$. All compounds with a $P > 0.05$ were reported as inactive.