Discovery of a Novel Series of Peroxisome Proliferator-Activated Receptor α/γ Dual Agonists for the Treatment of Type 2 Diabetes and Dyslipidemia

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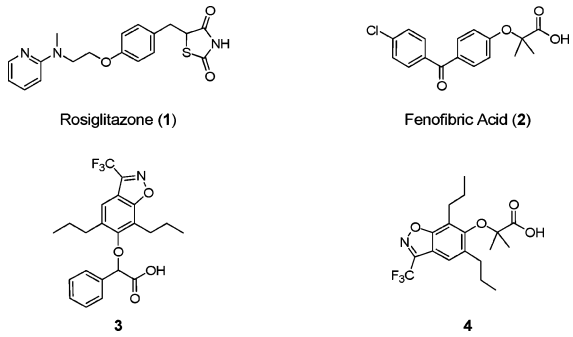
Abstract: A series of 2-aryloxy-2-methyl-propionic acid compounds and related analogues were designed, synthesized, and evaluated for their PPAR agonist activities. 2-[(5,7-Dipropyl-3-trifluoromethyl)-benzisoxazol-6-yloxy]-2-methylpropionic acid (**4**) was identified as a PPARα/γ dual agonist with relative PPARα selectivity and demonstrated potent efficacy in lowering both glucose and lipids in animal models without causing body weight gain. The PPARα activity of **4** appeared to have played a significant role in lowering glucose levels in db/db mice.

Type 2 diabetes is a multifactorial disease characterized by insulin resistance and/or abnormal insulin secretion. This metabolic disorder, which accounts for more than 90% of all diabetes, afflicts an estimated 6% of the adult US population. Its worldwide frequency is expected to grow by 6% annually, reaching a potential total of 200−300 million cases in 2010.[1] Type 2 diabetes and insulin resistance are frequently associated with dyslipidemia and a markedly increased incidence of atherosclerotic cardiovascular disease.[2] Glycemic control is traditionally considered the first priority in the treatment of diabetic patients. However, results from the UK Prospective Diabetes Study have shown that intensive control of hyperglycemia can attenuate microvascular complications, but may not reduce macrovascular disease, the main cause of morbidity and mortality in type 2 diabetes.[3] Accordingly, more aggressive therapeutic approaches that not only lower glucose but also simultaneously reduce cardiovascular risk factors associated with type 2 diabetes are clearly in high demand.

The peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear receptor superfamily.[4] There are three PPAR subtypes encoded by distinct genes: PPARα (NR1C1), PPARδ (NR1C2), and PPARγ (NR1C3).[5] These receptors are important regulators in multiple physiological pathways, such as glucose homeostasis, fatty acid metabolism, inflammation, and cellular differentiation.[6] Glitazones and fibrates are two classes of PPAR drugs currently being marketed for the treatments of insulin resistance and dyslipidemia, respectively. Glitazones (pioglitazone and rosiglitazone (**1**)) are insulin sensitizers functioning through PPARγ activation.[7] Although a direct link between PPARγ and insulin sensitivity has not been fully established, one theory suggests that the hypoglycemic effects of glitazones are, at least in part, derived from their lipid-modulating effects.[8] Activation of PPARγ increases free fatty acid (FFA) uptake in adipose tissue, thus reducing the FFA inhibition of glucose disposal in skeletal muscle. While glitazones lower glucose and improve insulin sensitivity, they suffer from several adverse effects, including weight gain and edema.On the other hand, PPARα-activating fibrates have enjoyed a good safety profile in humans over the past two decades.[9] These hypolipidemics are effective in lowering triglycerides and raising HDL levels and have been demonstrated to significantly reduce the coronary events in type 2 diabetes patients.[10] The lipid modulating effect of fibrates is principally mediated by PPARα-regulated expressions of genes involved in lipid and lipoprotein metabolism in liver, leading to enhanced hepatic fatty acid oxidation, which may also be the basis of the observed independent hypoglycemic effect of fibrates.[11] In addition, fibrates have been shown to reduce body weight in rodents, in sharp contrast to PPARγ activators, which promote weight gain.[11a,12] Current fibrate drugs are low affinity PPARα ligands with only clinically marginal hypoglycemic effects. Further studies will be needed to establish whether more potent PPARα agonists may treat type 2 diabetes without PPARγ-related liabilities.

Chart 1. PPAR Agonists



We herein report the structural design, synthesis, and preclinical evaluation of a novel series of PPARα/γ dual agonists. Compound **4** demonstrated potent PPARα agonist activity in a cell-based reporter gene expression assay, while only weakly activating PPARγ. It lowered both plasma glucose and lipid in animal models without causing body weight increase, an adverse effect frequently associated with PPARγ agonists.

We recently reported the discovery of a new class of *O*-arylmandelic acid PPAR agonists.[13] Some of them, exemplified by **3**, showed PPARα/γ dual activity in vitro and good in vivo efficacy in lowering both glucose and lipids in rodents. On the other hand, fibrates, such as fenofibrate, are weak PPARα agonists and have enjoyed an excellent tolerability profile. Fenofibrate is the prodrug of the active parent compound, fenofibric acid (**2**). For the structural design of novel PPAR compounds, we combined the putative pharmacophores of these two classes of compounds and generated several hybrid

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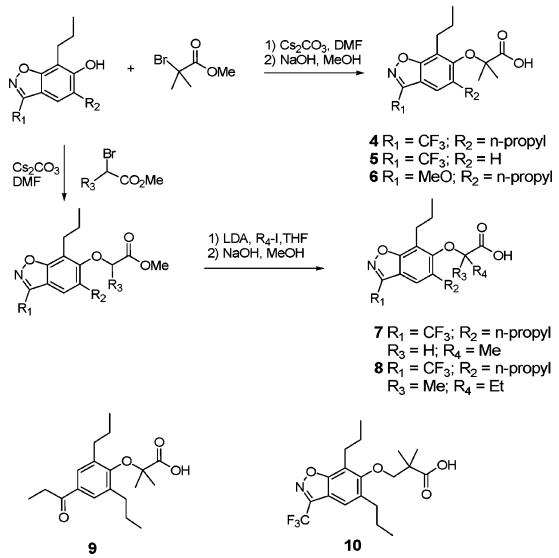
Scheme 1



structures.[14] We hoped that further SAR studies on these hybrid structures would lead us to novel PPARα/γ dual agonists with improved efficacy and safety profiles. The present paper focuses on **4**, obtained by combining the isobutyric acid headgroup of fenofibric acid **2** and the lipophilic aryloxy moiety of the mandelate lead **3**.

A series of 2-aryloxy-2-methyl-propionic acids were prepared via simple condensations followed by hydrolysis as depicted in Scheme 1. Alternatively, the top phenol piece was coupled to the monosubstituted α-bomo ester, followed by alkylation and hydrolysis to produce related analogues with different substitution patterns at the α-position.

The individual compounds **4**−**10** described herein and their affinity for PPAR subtypes[15] and agonist efficacy in a PPAR-GAL4 chimeric transactivation assay in COS-1 cells[16] are summarized in Table 1. Structure **4** was divided into several regions for SAR study and optimization. The effect of substitution at the phenyl ring was first investigated. Compared with the 2,6-dipropyl analogue **4**, which showed 6-fold selectivity for PPARα over PPARγ receptor in the binding assay and potent PPARα agonist activity in the transactivation assay, the monopropyl analogue **5** was essentially inactive, indicating the necessity of bis-substitution. The dimethyl substitution at the α-carbon seemed to be optimal. The corresponding monomethyl analogue **7** exhibited reduced activity. Other α,α-disubstituted analogues, such as **8**, failed to improve potency in the transactivation assay. Extension of the chain length by inserting a methylene group between the aryloxy group and the α-carbon proved to be detrimental, as demonstrated by the inactive analogue **10**. By comparison, the SAR requirement around the isoxazole ring was more flexible. The methoxy analogue **6** showed nonselective PPARα/γ dual binding affinity, and PPARα/γ dual agonist activity in the transactivation assay with enhanced potency. Replacement of the isoxazole ring with a simple propionyl functionality produced **9**, which maintained the potent dual activity. It is important to note that all the compounds in this series demonstrated a high degree of selectivity over PPARδ subtype. No activity was observed for this receptor at the highest concentration tested (10 μM) in the binding assay.

Table 1. Binding IC_{50} and TA EC_{50} data

compd	SPA IC_{50} (μM)[a]		TA EC_{50} (μM) or max. % at 3 μM[a]	
	hα	hγ	hα	hγ
4	0.23	1.58	0.30	25%
5	>10	>10	na[b]	na
6	1.51	1.62	0.06	1.10
7	1.03	>10	0.62	21%
8	0.10	>15	0.75	26%
9	0.48	1.22	0.01	1.3
10	>50	>15	na	na
rosiglitazone	>50	0.25	>10	0.02
fenofibric acid	35	>50	15	>100

[a] Mean values are shown ($n = 3$); SD ± 15%; SPA (scintillation proximity assay);[15] TA (chimeric GAL4-hPPAR transactivation assay).[16] [b] Not active at 3 μM.

The in vitro profile of compound **4** was compared with rosiglitazone and fenofibrate, two reference compounds used in the in vivo studies for insulin sensitizing and lipid modulating effects, respectively. In radioligand binding assay, compound **4** was about 150-fold more potent as a PPARα ligand than fenofibric acid, while 6-fold less potent on PPARγ compared with rosiglitazone. In transiently transfected COS-1 cells, compound **4** was a potent agonist of a chimeric hPPARα/GAL4 receptor with an EC_{50} of 0.3 μM. It weakly activated PPARγ with only partial activity starting to show on the titration curve at 3 μM, the highest concentration tested. The PPARγ agonist activity of **4** was further evaluated in a FABP assay.[17] It has been demonstrated that PPARγ is necessary and sufficient for adipocyte differentiation and that PPARγ agonists induce adipogenesis. To examine the effect of **4** on adipocyte differentiation, the levels of mouse adipose fatty acid binding protein (aP2) mRNA expression in differentiating murine 3T3-L1 preadipocytes were determined.[17b] The aP2 mRNA displayed little increase in expression when 3T3-L1 cells were treated with **4** at doses up to 3 μM for 5 days (data not shown). At the highest dose of 10 μM, compound **4** acted as a weak agonist of PPARγ with only 60% maximal expression. In this assay, rosiglitazone increased aP2 expression in a dose-dependent manner with an EC_{50} value of 15 nM.

It has been established that PPARα agonists can exhibit strong species-dependent transactivation profiles. It is desirable to identify a compound with relatively balanced PPARα potency in species used in preclinical animal studies and in man. Similarly constructed transactivation assays were conducted using chimeric PPARα receptors derived from other animal species. The results indicated that compound **4** exhibited comparable agonist activity toward mouse, hamster, and dog PPARα with EC_{50} values of 0.18 μM, 0.46 μM, and 0.55 μM, respectively. Furthermore, compound **4** demonstrated a high degree of selectivity against other members of the nuclear receptor family. In nuclear receptor counterscreens, compound **4** was negative in assays for binding to, and/or activation of, LXRα, LXRβ, RXRα, PXR, GR, ERα, ERβ, TRα, and TRβ at concentrations up to 10 μM.

The results of in vitro experiments demonstrated that compound **4** showed 6-fold selectivity for PPARα over PPARγ receptor in the binding assay and relatively PPARα selective agonist activity in the transactivation assay. Its insulin-sensitizing and lipid-lowering effica-

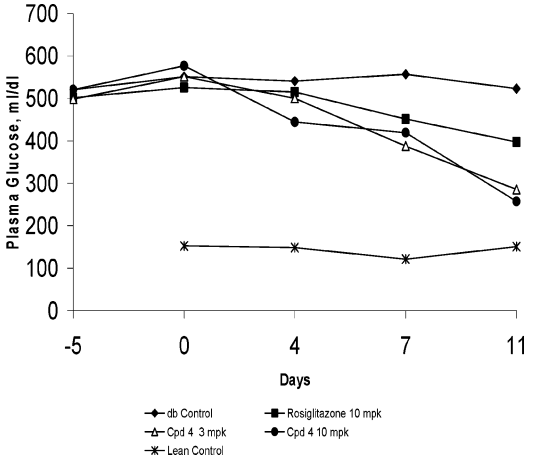


Figure 1. Effect of oral dosing of compound **4** and rosiglitazone on glucose levels in db/db mice ($n = 7$ mice/group). Plasma glucose levels were measured approximately 24 h after dosing on the preceding day.

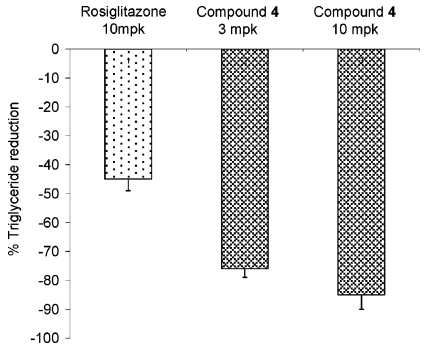


Figure 2. Percentage reduction of elevated triglycerides in db/db mice ($n = 7$ mice/group). Mice were dosed with 10 mpk rosiglitazone or 3 and 10 mpk compound **4** for 11 days.

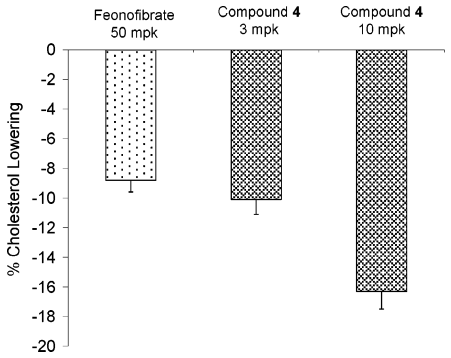


Figure 3. Percentage reduction of serum total cholesterol levels in mature beagle dogs. Dogs were housed individually and dosed via oral gavage daily for 14 days with fenofibrate or compound **4**.

cies in vivo were next examined. The hypoglycemic effect of compound **4** was tested in db/db mice, an obese rodent model of type 2 diabetes characterized by severe insulin resistance, hypertriglyceridemia, and marked hyperglycemia.[16] The mice were treated with compound **4** or rosiglitazone, used as a reference compound in the study, by oral gavage at the indicated dosages for 11 days. Administration of **4** resulted in significant reduction of blood glucose levels: 64% at 3 mpk, and 71% at 10 mpk. By comparison, rosiglitazone produced 34% correction of hyperglycemia at 10 mpk. In the same study, plasma triglyceride levels in db/db mice were also sharply reduced in a dose dependent fashion by the treatment with **4**: 76% at 3 mpk, and 85% at 10 mpk, more than the 45% reduction obtained from rosiglitazone at 10 mpk. As part of this experiment, plasma drug levels after chronic dosing of **4** and rosiglitazone were also measured. Similar exposure levels were obtained for 3 mpk compound **4** and 10 mpk rosiglitazone (237 and 247 μM·h, respectively). It is noteworthy that, despite its weak in vitro activity on PPARγ, compound **4** was more effective in lowering glucose than rosiglitazone, a potent PPARγ selective agonist, suggesting that the PPARα activity of **4** played a significant role in its in vivo hypoglycemic efficacy.[11] This notion is further supported by the potent triglyceride lowering effect exhibited by **4** and the observation that **4** did not induce further increase in body weight versus vehicle control, in sharp contrast to rosiglitazone, which caused significant weight gain (8−11%).

The lipid altering activity of **4** was further assessed in a well-established dog model. Fenofibrate was chosen as a reference compound with well-characterized human and animal efficacy. The daily oral administration of compound **4** to dogs at 3 and 10 mpk for 14 days lowered the average serum cholesterol levels by 10.1% and 16.3%, respectively, in a dose-dependent and time-dependent fashion. Dogs treated with fenofibrate at 50 mpk in parallel showed an average decrease in total cholesterol levels of 8.8%. Non-HDL cholesterol, the sum of LDL- and VLDL-cholesterol, was also determined (data not shown). Both **4** and fenofibrate significantly decreased non-HDL cholesterol levels, and as with total cholesterol, compound **4** exhibited clear superiority over fenofibrate in dose potency.

In summary, compound **4** has been identified as a PPARα/γ dual agonist with relative PPARα selectivity in a cell based transactivation assay. It demonstrated potent efficacy in lowering both glucose and lipids in animal models. More importantly, it did so without causing body weight increase, an adverse effect frequently associated with the glitazones. The PPARα activity of **4** appeared to have played a significant role in glucose lowering in db/db mice. On the basis of its in vitro and in vivo profiles, compound **4** was selected for further evaluation in man.

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Supporting Information Available: Synthetic procedures and characterization data for intermediates and final compounds. This information is available free of charge via the Internet at http://pubs.acs.org.

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