Letters

Design and Synthesis of 2-Methyl-2-{4-[2-(5-methyl-2-aryloxazol-4-yl)ethoxy]phenoxy}propionic Acids: A New Class of Dual PPARα/γ Agonists

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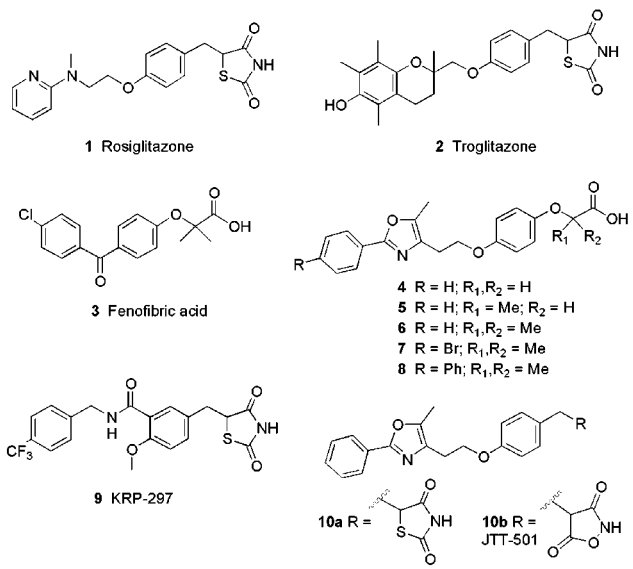
Abstract: Propionic acid derivative **8**, which was designed and synthesized based on putative pharmacophores of known PPARγ- and PPARα-selective compounds, exhibits potent dual PPARα/γ agonist activity as demonstrated by in vitro binding and dose overlap in the newly introduced EOB mouse model for glucose lowering and lipid/cholesterol homeostasis.

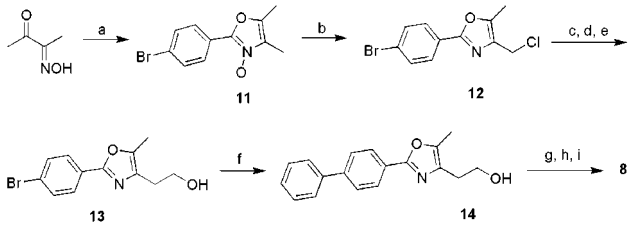
Introduction. Type 2 diabetes is a metabolic disorder that afflicts 120 million worldwide at present and is estimated to rise to over 200 million by the year 2010.[1] In addition to the characteristic combination of insulin resistance and insulin deficiency, the type 2 diabetic often displays cardiovascular risk factors including dyslipidemia (hypertriglyceridemia, low HDL, and small dense LDL), coagulopathy (elevated fibrinogen and plasminogen activator inhibitor-1 (PAI-1)), hypertension, and obesity. The recent publication of the United Kingdom Prospective Diabetes Study (UKPDS)[2] has revealed that in Type 2 diabetes, intensive glucose-lowering therapy is ineffective at reducing cardiovascular complications, despite decreasing microvascular complications such as retinopathy.

The PPARs (peroxisome proliferator activated receptors) were cloned less than a decade ago and are members of the superfamily of nuclear transcription factors that includes the receptors for steroid, retinoid, and thyroid hormones.[3,4] The PPARs form heterodimers with another nuclear receptor, the 9-*cis*-retinoic acid receptor (RXR). This heterodimer complex interacts with critical DNA response elements within promoter regions, and when activated by agonist ligand binding, it leads to gene transcription of proteins involved in control of lipid and carbohydrate metabolism. PPARγ agonists (e.g., rosiglitazone (**1**, Avandia) and troglitazone (**2**, Rezulin)) have displayed clinical utility for increasing insulin sensitivity and improving glycemic control in Type 2 diabetes (Chart 1). In addition, these compounds have been shown to inhibit inflammatory activation of macrophages,[5a] to promote cholesterol efflux in macrophages via the LXR-ABCA1 pathway,[5b] and to inhibit atherosclerosis in the ApoE deficient and LDL receptor deficient mice.[6] Seminal contributions by the Takeda and Pfizer groups have shown that the 5-methyl-2-phenyl-4-oxazolyl thiazolidine-2,4-diones such as **10a** give highly potent in vivo antidiabetic activities in the *ob/ob* mouse.[7] Thiazolidine-2,4-diones (TZDs) are generally selective for PPARγ, although a TZD, KRP-297 (**9**), with activity at PPARα and PPARγ was recently disclosed.[8] The structurally related isoxazolidinedione JTT-501 (**10b**) has also been recently reported with

Chart 1. PPAR Agonists



Scheme 1[a]



[a] (a) *p*-Br-benzaldehyde, HOAc, HCl (74%); (b) $POCl_3$, $CHCl_3$ (72%); (c) KCN, KI, DMF (100%); (d) KOH, 2-MeO-ethanol (60%); (e) BH_3−THF, MeOH (72%); (f) $PhB(OH)_2$, $Pd(OAc)_2$, PPh_3 (95%); (g) $(Ts)_2O$, Pyr, DMAP (95%); (h) 2-(4-hydroxyphenoxy)-2-methylpropanoic acid ethyl ester, Cs_2CO_3, DMF (50%), NaOH, EtOH (68%).

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Table 1. Binding IC_{50} and Cotransfection Efficacy Data: Comparison of Compounds **4**−**8** with Reference Compounds[a]

	hPPARγ			hPPARα[b]		
no.	IC_{50} (nM)	CTF eff (%)	EC_{50} (nM)	IC_{50} (nM)	CTF eff (%)	EC_{50} (nM)
4	10591 ± 710	6 ± 1	eff < 20%	10596 ± 2044	28 ± 1	2751 ± 91
5	3120 ± 247	42 ± 4	2751 ± 91	2033 ± 440	65 ± 1	625 ± 125
6	3017 ± 117	55 ± 5	2059 ± 243	1445 ± 346	69 ± 2	717 ± 107
7	4556 ± 308	24 ± 5	1716 ± 398	242 ± 50	58 ± 6	61.9 ± 0.98
8	548 ± 72	123 ± 24	882 ± 89	174 ± 17	92 ± 21	149.5 ± 48.5
1	48 ± 2	100 ± 21	657 ± 345	>10000	9	eff < 20%
2	1285 ± 61	79 ± 1	2235 ± 344	no binding	0	eff < 20%
3	no binding	0	eff < 20%	68000	16	eff < 20%
9	463 ± 88	109 ± 5	1090 ± 305	914 ± 88	54 ± 1	922 ± 185
10a	14 ± 3	61 ± 2	8 ± 1	4086 ± 363	30 ± 0	771 ± 44

[a] Values are the mean of three to five experiments. [b] Gal4-hPPARα was used to eliminate interference by endogenous PPARγ receptors in CV-1 cells.

PPARγ activity similar to troglitazone accompanied by weak activity at PPARα.[9]

PPARα agonists (e.g., gemfibrozil, fenofibric acid (**3**)), on the other hand, produce reductions in serum triglycerides and increases in HDL cholesterol, in some cases accompanied by reductions in serum fibrinogen.[10] Moreover, PPARα receptor activation has also been shown to produce antiinflammatory effects in vascular cells.[11] The combined profile of a dual PPARα/γ agonist thus appears well-suited for treatment of hyperglycemia together with prevention of cardiovascular disease in Type 2 diabetes.[12] Herein we describe our efforts to identify a dual PPARα/γ agonist based on in vivo studies and in vitro binding results. This research has resulted in the identification of **8**, which combines the putative pharmacophores of known PPARγ- and PPARα-selective agents in a single molecule with excellent dual PPARα/γ agonist activity.

Chemistry. The synthesis of **8**, as outlined in Scheme 1, was designed to allow flexibility in the preparation of analogues for SAR studies. Using a modification of the Goto et al. procedure,[13] butanedione monooxime was condensed with 4-bromobenzaldehyde, and the resulting *N*-oxide (**11**) was converted to chloride **12** by treatment with phosphorus oxychloride. The corresponding nitrile was prepared by reaction of chloride **12** with potassium cyanide in the presence of potassium iodide. Following conversion of the nitrile to the corresponding carboxylic acid with potassium hydroxide, the acid was reduced to the desired alcohol (**13**) with borane−THF. Suzuki coupling of alcohol **13** with phenyl boronic acid provides alcohol **14**. Tosylate formation of alcohol **14** was followed by coupling to 2-(4-hydroxyphenoxy)-2-methylpropanoic acid ethyl ester with cesium carbonate in DMF and hydrolysis to afford **8**. Analogues **4**−**6** were prepared via the straightforward coupling of 5-methyl-2-phenyl-4-oxazolyl ethanol with the appropriate (4-hydroxyphenoxy)propanoic (or acetic) acid ethyl ester, followed by hydrolysis to the corresponding acid. Direct coupling of **13** to 2-(4-hydroxyphenoxy)-2-methylpropanoic acid ethyl ester, followed by hydrolysis, affords compound **7**.

Results and Discussion. Compounds **4**−**8** were prepared based on the rational design of a dual PPAR agonist agent using the putative pharmacophores of known PPARγ- and PPARα-selective compounds that were developed and optimized solely by in vivo animal studies. Cloning and expressing both the human PPARα (hPPARα) and PPARγ (hPPARγ) receptors led to an increased mechanistic understanding of their function. This also afforded the opportunity to conduct initial in vitro screens using a displacement study (ABCD binding assays) to determine binding IC_{50}s and cotransfection studies[14] in CV-1 cells to demonstrate functional efficacy. The results using these primary tools to drive the SAR efforts are shown in Table 1. Assessment of compound **4** demonstrates the necessity of methyl substitution α to the carboxylic acid to achieve efficacy. Para substitution of the 2-phenyloxazole in analogue **7** provides a significant boost in binding at the hPPARα receptor (242 nM). This breakthrough led to the synthesis of the *p*-biphenyl-oxazole analogue **8**, which binds to both hPPARα and hPPARγ with IC_{50} values ($\sim K_i$) of 174 and 548 nM, respectively. In addition, compound **8** is shown by cotransfection studies to be an effective agonist at both of these receptors. Compound **8** is also an effective agonist at mouse PPARα (104%, EC_{50} = 2560 nM). This latter finding is important because of substantial interspecies variability in ligand activation of PPARα, in contrast to PPARγ, and the requirement for activation of mouse PPARα in the preclinical animal model.

Binding and cotransfection data for **8** are compared with corresponding data for reference compounds in Table 1. Although not as potent as rosiglitazone (**1**) or Takeda's 5-methyl-2-phenyl-4-oxazolyl thiazolidine-2,4-dione **10a** at binding to hPPARγ, compound **8** shows efficacy comparable to rosiglitazone (**1**) at this receptor. Moreover, **8** is considerably more potent at activating and binding to hPPARα receptors than the reference compounds, including the dual agonist KRP-297 (**9**). Thus, the in vitro profile of **8** as a dual PPARα/γ agonist distinguishes this compound from marketed compounds now known to act on these receptors.

Specificity of **8** was examined in binding and cotransfection studies using a panel of nuclear transcription factors. For the following receptors, **8** either failed to bind or displayed poor affinity (K_i > 10 μM) as a ligand: retinoic acid receptor (RARα, RARβ, RARγ), retinoic acid X receptor (RXRα, RXRβ, RXRγ), glucocorticoid receptor, and thyroid receptor (TRα and TRβ). Furthermore, cotransfection assays for RARα and RXRα demonstrated a lack of agonist activity for **8** at these receptors. Thus, binding and/or activation of nuclear transcription factors by **8** appear to be confined to the PPAR family of receptors.

To determine the ability of **8** to both lower hyperglycemia and positively impact lipid/cholesterol homeostasis in a clinically relevant manner, a new diabetic animal model, the EOB mouse,[15] was created. Briefly,

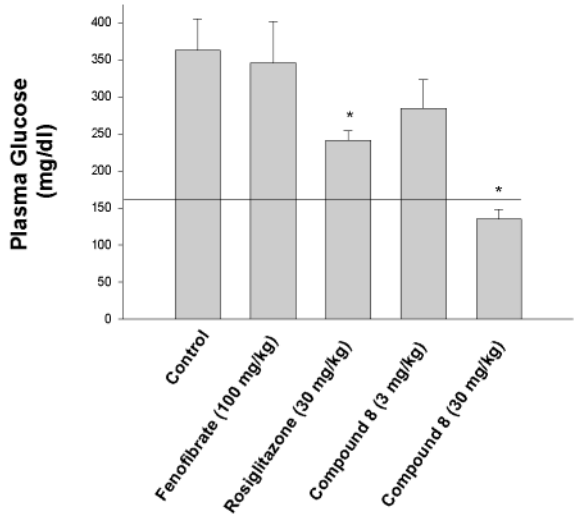

Figure 1. Plasma glucose levels in EOB mice treated via oral gavage for 7 days with fenofibrate, rosiglitazone, or compound **8**. Bars represent means ± SE ($n = 5$ mice per group; $*p <$ 0.05 relative to vehicle control). Horizontal line indicates average plasma glucose levels in untreated chow fed (Purina 5001 diet) human apoA-I transgenic mice (159.4 ± 7.5 mg/dL).

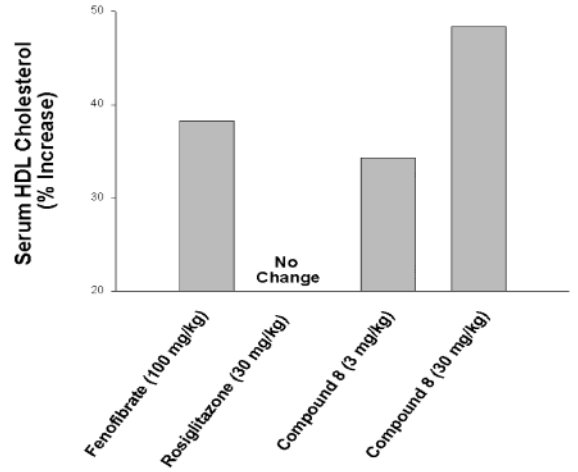

Figure 2. Increase in serum HDL cholesterol levels in EOB mice treated via oral gavage for 7 days with fenofibrate, rosiglitazone, or compound **8**. Bars represent percent HDL cholesterol change from vehicle control for FPLC analyzed serum samples pooled for each group ($n = 5$ mice per group).

EOB mice are commercially available human apoA-I transgenic mice (Jackson Laboratories, Bar Harbor, ME)[16] rendered insulin resistant and hyperglycemic through dietary manipulation and multiple low-dose streptozotocin treatment (for details, see Supporting Information). These mice (Figures 1 and 2) respond to selective PPARγ and PPARα ligands (rosiglitazone and fenofibrate) as demonstrated by plasma glucose lowering and HDL cholesterol elevation, respectively.

When EOB mice were dosed orally by gavage for 7 days, **8** lowered plasma glucose in a dose-dependent fashion with glucose normalization occurring at 30 mg/kg/d (Figure 1). Interestingly, **8** appears to be a more potent glucose lowering agent than rosiglitazone despite the observation that the latter has a higher affinity for PPARγ in vitro. The results in this model, and the recent report of PPARα mediated insulin sensitization,[17] suggest that a dual PPARα/γ agonist may provide better glucose control than currently marketed selective PPARγ

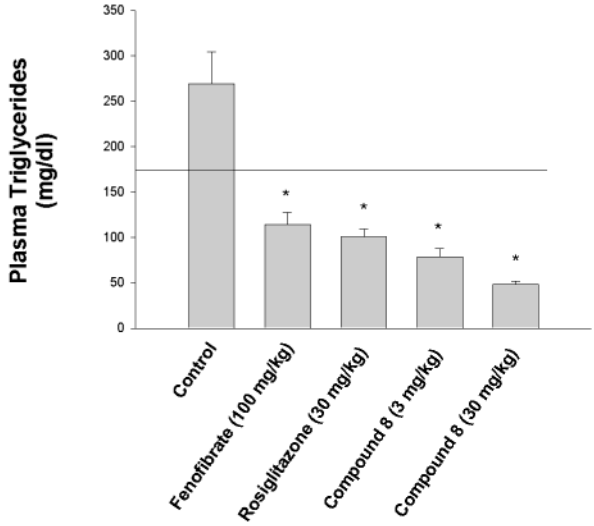

Figure 3. Plasma triglyceride levels in EOB mice treated via oral gavage for 7 days with fenofibrate, rosiglitazone, or compound **8**. Bars represent means ± SE ($n = 5$ mice per group; $*p <$ 0.05 relative to vehicle control). Horizontal line indicates average plasma triglyceride levels in untreated chow fed (Purina 5001 diet) human apoA-I transgenic mice (167.2 ± 10.6 mg/dL).

agents. In addition to normalizing plasma glucose, **8** dose-dependently elevated serum HDL cholesterol levels (Figure 2) and reduced plasma triglycerides (Figure 3). At the glucose normalizing dose of 30 mg/kg/d, HDL cholesterol levels were elevated by 48.4% and plasma triglycerides were reduced by ~5.5-fold, relative to control levels.

In summary, compound **8** was designed and synthesized as a dual PPARα/γ agonist. This profile was demonstrated by in vitro binding to the human and mouse PPAR receptors and in vivo efficacy in a diabetic animal model. Further evaluation of compound **8** is underway.

Supporting Information Available: Experimental details for the binding assays, cotransfection assay, the EOB mouse model, and the intermediate and final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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