Identification and Synthesis of a Novel Selective Partial PPARδ Agonist with Full Efficacy on Lipid Metabolism In Vitro and In Vivo

Per Sauerberg,* Grith S. Olsen, Lone Jeppesen, John P. Mogensen, Ingrid Pettersson, Claus B. Jeppesen, Jens R. Daugaard, Elisabeth D. Galsgaard, Lars Ynddal, Jan Fleckner, Vladimira Panajotova,[†] Zdenek Polivka,[†] Pavel Pihera,[†] Miroslav Havranek,[†] and Erik M. Wulff

Novo Nordisk A/S, Diabetes Research Unit, Novo Nordisk Park, 2760 Måløv, Denmark, and RE&D VUFB, Podebradska 56/186, 180 66 Praha 9, Czech Republic

Received October 16, 2006

The aim was to identify a novel selective PPARδ agonist with full efficacy on free fatty acid (FFA) oxidation in vitro and plasma lipid correction in vivo. Using the triple PPARα,γ,δ agonist **1** as the structural starting point, we wanted to investigate the possibility of obtaining selective PPARδ agonists by modifying only the acidic part of **1**, while holding the lipophilic half of the molecule constant. The structure−activity relationship was guided by in vitro transactivation data using the human PPAR receptors, FFA oxidation efficacy performed in the rat muscle L6 cell line, and in vivo rat pharmacokinetic properties. Compound **7** ([4-[3,3-bis-(4-bromo-phenyl)-allylthio]-2-chloro-phenoxy]-acetic acid) was identified as a selective, partial agonist with good oral pharmacokinetic properties in rat. Chronic treatment of high fat fed ApoB100/CETP-Tgn mice with **7** corrected the plasma lipid parameters and improved insulin sensitivity. These data suggest that selective PPARδ agonists have the potential to become a novel treatment of dyslipidemia.

Introduction

The peroxisome proliferator-activated receptors (PPARs[a]) are transcription factors known to play a central role in regulating the storage and catabolism of dietary fat. The PPARs were cloned a little more than a decade ago as orphan members of the nuclear receptor gene family that includes the steroid, retinoid, and thyroid hormones receptors.[1,2] There are three PPAR subtypes: PPARα, PPARγ, and PPARδ.[3] The PPAR receptors heterodimerize with the RXR receptor, and the PPAR/RXR heterodimers then bind to DNA sequences known as DR-1 response elements.[4] As with other members of the nuclear receptor gene family, the PPARs are ligand-activated transcription factors. Agonist activation of the receptor results in changes in the expression level of mRNAs encoded by PPAR target genes.

The use of PPARγ activators, for example, rosiglitazone (Table 1) and pioglitazone (glitazones), in the treatment of type 2 diabetes have been established due to their abilities to lower blood glucose and insulin levels and improve insulin sensitivity.[5,6] Similarly, PPARα activators, for example, fenofibrate (Table 1) and clofibrate (fibrates), have been used clinically for more than three decades for their ability to lower plasma triglycerides (TG) and moderately raise high-density lipoprotein (HDL)-cholesterol.[7] There is no drug available targeting the third PPARδ receptor, but there is evidence that PPARδ is involved in lipid homeostasis.

The first proposed pharmacological role for PPARδ was in the regulation of cholesterol homeostasis. Total plasma cholesterol was raised in db/db mice treated with the PPARδ ligand L-165041 ((4-(3-(4-acetyl-3-hydroxy-2-propyl-phenoxy)-propoxy)-acetic acid).[8] Later, an increase in plasma HDL-cholesterol (79%) and a decrease in plasma LDL-c (29%), TG (56%), and insulin (48%) in obese, normo-glycemic rhesus monkeys after treatment with the PPARδ selective agonist GW501516 ({2-methyl-4-[4-methyl-2-(4-trifluoromethyl-phenyl)-thiazol-5-ylmethylsulfanyl]-phenoxy}-acetic acid, Table 1) has increased the therapeutic interest in this third PPAR receptor.[9] An up-regulation of the expression of the reverse cholesterol transporter ATP-binding cassette A1 (ABC-A1) in macrophages, fibroblasts, and intestinal cells and an increase of apolipoprotein A1 (Apo-A1) specific cholesterol efflux was observed as part of the mechanism responsible for the effects of GW501516 on plasma cholesterol parameters. [9] In addition to the effects on cholesterol homeostasis, GW501516 treatment was observed to lower plasma glucose and insulin and improve insulin sensitivity in diabetic ob/ob mice and high fat diet induced insulin resistant mice.[10] Increased oxygen consumption in vivo suggesting a fuel switch from glucose to free fatty acids (FFA), as well as increased FFA oxidation in skeletal muscle, was demonstrated both in vivo and in vitro.[10]

Two different transgenic mouse models overexpressing PPARδ in either adipose tissue[11] or in muscle tissue[12] have shown up-regulation of genes (LPL, FABP, FAT, CD36, CPT1b, and ACS) and proteins (UCP-2) responsible for lipid uptake and metabolism and energy uncoupling. Both types of mice had reduced adipose tissue and were protected against high fat diet induced body weight gain. Supportive for the hypothesis of skeletal muscle being a major target organ were in vitro treatment of C2C12 muscle cells with GW501516 showing up-regulated genes involved with TG hydrolysis and FFA oxidation (LPL, ACS4, CPT1), preferential lipid utilization (PDK4), energy expenditure (UCP1,-2, -3), and lipid efflux (ABCA1/G1).[13,14] Further, GW501516 was found to increase glucose uptake independently of insulin and enhance subsequent insulin stimulation in cultured primary human skeletal muscle cells.[15] As a possible mechanism, the phosphorylation and expression of AMP-activated protein kinase were likewise increased.

* To whom correspondence should be addressed. Telephone: +4544434858. Fax: +4544663939. E-mail: psa@novonordisk.com.

[†] RE&D VUFB.

[a] Abbreviations: FFA, free fatty acid; PPAR, peroxisome proliferator-activated receptor; TG, triglycerides; HDL, high density lipoprotein; ABC-A1, ATP-binding cassette A1; LDL, low density lipoprotein; Apo-A1, apolipoprotein A1; ApoB100/CETP, apo-lipoprotein B/cholesterol ester transfer protein; ADDP, azodicarboxylic dipiperidide; PK, pharmacokinetic; EDL, musculus extensor digitorum longus.

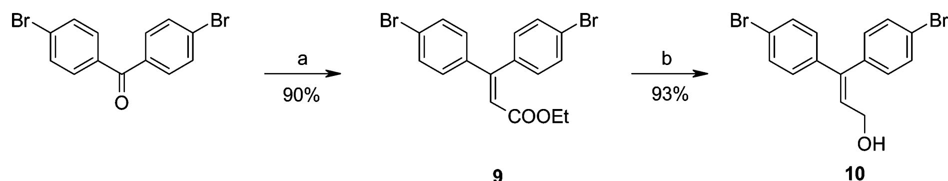
10.1021/jm061202u CCC: $37.00 © 2007 American Chemical Society
Published on Web 03/08/2007

Table 1. In Vitro hPPARα, rPPARα, hPPARγ, and hPPARδ Transactivation of Test and Reference Compounds[a]

Compd no	R	hPPARα(LBD)-GAL4 EC$_{50}$±SEM, μM	hPPARα(LBD)-GAL4 % max ±SEM[b]	hPPARγ(LBD)-GAL4 EC$_{50}$±SEM, μM	hPPARγ(LBD)-GAL4 % max ±SEM[c]	hPPARδ(LBD)-GAL4 EC$_{50}$±SEM, μM	hPPARδ(LBD)-GAL4 % max ±SEM[d] (% of GW)[e]
1		0.39±0.08	93±4	0.59±0.08	57±10	0.54±0.05	149±13 (55)
2		>10	>12	-	<10	0.52±0.02	127±8 (47)
3		-	<10	-	<10	5.9±0.5	123±3 (45)
4		>10	>14	-	<10	1.1±0.1	113±7 (42)
5		>10	>19	>10	>19	0.83±0.10	136±10 (50)
6		-	<10	-	<10	2.3±0.1	155±18 (57)
7		>10	>12	>10	>18	0.053±0.003	200±6 (73)
8		>10	>22	>10	>29	0.017±0.001	179±6 (66)
Rosiglitazone		>10	>24	0.31±0.04	100	-	<10
Fenofibric acid		>10	>94	-	<10	>10	>34
GW501516		3.9±0.3	68±4	>10	>22	0.0079 ±0.0008	272±11 (100)

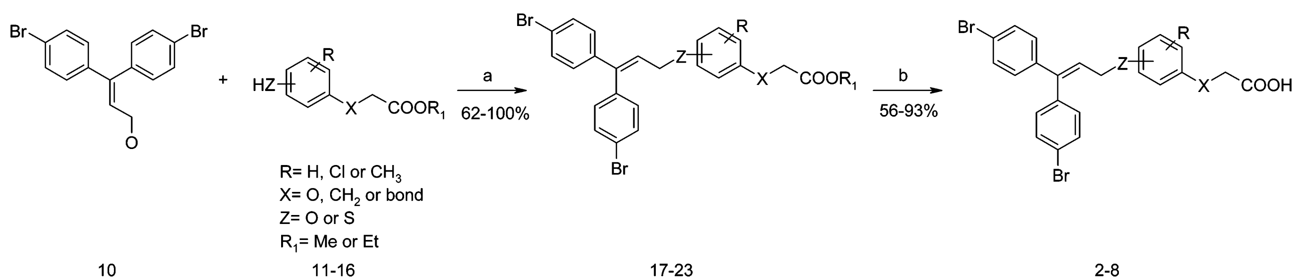
[a] Compounds were tested in at least three separate experiments in at least five concentrations ranging from 0.001 to 30 μM. EC$_{50}$ is the concentration giving 50% of the maximal activity observed for a given compound. [b] For each compound, the efficacy (% max) is given as a relative compared to the maximal activity of NNC 61−4655[32] for PPARα. [c] Rosiglitazone for PPARγ. [d] Carbacyclin for PPARδ. The results are expressed as means ± SEM. If a plateau was not reached at the highest concentration of compound tested (30μM), the effect at this concentration was calculated and the maximal effect was indicated to be greater than this value. In such cases, the EC$_{50}$ was assigned as >10 μM. If the maximal effect of a compound was less than 10%, no EC$_{50}$ could be calculated. [e] Percent relative to GW501516 (272%).

Scheme 1[21 a]



[a] Reagents and conditions: (a) (i) Na, EtOH; (ii) $(EtO)_2POCH_2COOEt$, 70 °C. (b) (i) 1 M DIBAL-H in toluene, toluene, room temperature; (ii) HCl (conc), H_2O, room temperature.

Scheme 2[a]



[a] Reagents and conditions: (a) $P(Bu)_3$, ADDP, THF, 0 °C to room temperature. (b) 1 N NaOH, EtOH.

In addition to the effects on cholesterol homeostasis and glycemic control, PPARδ activation has been suggested to attenuate inflammation and slow the progression of atherosclerosis by direct and indirect mechanisms.[16] Nevertheless, these effects could not be detected in low-density lipoprotein (LDL)-receptor knock-out mice after treatment with the PPARδ agonist GW0742 ({2-methyl-4-[4-methyl-2-(4-fluoro-phenyl)-thiazol-5-ylmethylsulfanyl]-phenoxy}-acetic acid), a close analogue to GW501516.[17] Recently, the same agonist was used to show that PPARδ treatment increased HDL-cholesterol by 50% and lowered cholesterol absorption in wild-type mice by 43%.[18]

Human polymorphism studies further suggested that PPARδ is involved in cholesterol metabolism.[19] Recently, phase 1 clinical trial data from healthy normolipidemic male volunteers treated for 2 weeks with GW501516 showed that HDL-cholesterol increased by 19% and TG decreased by 20%, compared to placebo.[20] GW501516 is reported to be in phase 2 clinical trials.

The aim of the present work was to identify novel selective PPARδ agonists with full efficacy on FFA oxidation in vitro and plasma lipid correction in vivo. The structural starting point was the previously published triple PPARα,γ,δ agonist **1**.[21] The structure−activity relationship (SAR) was guided by in vitro transactivation data using the human PPAR receptors,[22] FFA oxidation efficacy performed in the rat muscle L6 cell line, and in vivo rat pharmacokinetic properties. Compound **7** was identified as a selective, partial agonist with good oral rat pharmacokinetic properties in vivo and full efficacy on plasma lipids in the lipid-metabolism humanized double transgenic apolipoprotein B/cholesterol ester transfer protein (ApoB100/CETP Tgn) mouse model.

Chemistry

We have earlier described the synthesis of 3,3-bis-(4-bromophenyl)-prop-2-en-1-ol, **10**, from 4,4′-dibromo-benzophenone (Scheme 1).[21] Coupling of **10** with the hydroxyl/mercapto-phenylalkylesters **11**−**16** was done under nonoptimized Mitsunobu conditions using ADDP (azodicarboxylic dipiperidide) and tributylphosphine to give the esters **17**−**23** in good to high yields (Scheme 2). Basic hydrolysis of the ester intermediates **17**−**23** gave the desired target molecules **2**−**8**.

Table 2. The Effect of PPAR Agonists on Fatty Acid Oxidation in Rat L6 Muscle Cells[a]

compound	$EC_{50} \pm$ SEM (nM)	% max increase of GW ± SEM[b]
2	212 ± 92	69 ± 5
3	615 ± 265	59 ± 5
4	204 ± 40	62 ± 5
5	224 ± 42	78 ± 11
6	379 ± 113	85 ± 8
7	30 ± 3	109 ± 6
8	3 ± 1	75 ± 14
Rosiglitazone	~5000	58 ± 6
fenofibric acid	~3000	70 ± 1
GW501516	6 ± 1	100

[a] Average of 3−4 experiments made in triplicate. [b] Maximal increase is given as % of maximal increase by GW501516. Maximal increase for GW501516 was 120 ± 21% over basal.

Results and Discussion

We have previously published on our design of the triple PPARα,γ,δ activator **1** (Table 1).[21] The aim of the present work was to investigate if it was possible to obtain selective PPARδ agonists by only modifying the acidic part of **1**, while holding the lipophilic half of the molecule constant.[24] At the same time, we wanted to identify selective PPARδ agonists using transactivation assays to investigate the possibility of getting a full pharmacological response on the desired FFA oxidation/lipid lowering effects with such agonists.

Removal of the ethoxy group α to the carboxylic acid in **1** gave immediately a selective partial PPARδ agonist **2** (Table 1). The PPARδ transactivation potency and efficacy of **2** was retained compared to **1**, but unfortunately, **2** did not give full efficacy in the in vitro FFA oxidation assay (Table 2) compared to GW501516 (which was considered a full PPARδ agonist). Furthermore, the pharmacokinetic (PK) evaluation in rats revealed that **2** had very poor oral bioavailability and a very short half-life (Table 3). This finding is in agreement with a previous observation on a related compound, where extensive beta-oxidation was suggested to be the reason for the poor PK properties.[23] The meta-substituted analogue **3** was a ten times less potent PPARδ agonist than **2**. Compound **3** had slightly better PK properties, but the exposure was still very low. The one carbon shorter analogues of **2** and **3**, **4** and **5**, respectively, were also partial PPARδ agonists. Even though **4** and **5** had

Table 3. Single Dose Rat Pharmacokinetic[a]

	single dose rat pharmacokinetic						
cmpd	dose i.v./p.o. (mg/kg)	C_{max}[b] p.o. (ng/mL)	AUC[c] p.o. (ng × min)/mL	F[d] p.o. (%)	CL[e] (mL/min/kg)	V_{ss}[f] (L/kg)	$T_{1/2}$[g] p.o. (min)
1	1.08/2.29	554	83 534	78	21.4	2.3	85
2	0.20/0.43	bd[h]	bd[h]	bd[h]	bd[h]	bd[h]	bd[h]
3	0.20/0.45	51	67 560	407	27	5.3	953
4	0.20/0.43	252	156 709	210	6.0	1.4	447
5	0.20/0.45	419	119 351	bd[h]	bd[h]	bd[h]	153
6	0.20/0.45	241	84 108	130	7	1.4	181
7	1.06/2.06	508	79 294	46	11.9	0.3	59
8	1.18/2.35	887	179 505	95	10	0.9	151
GW501516	1.09/2.13	454	127 478	136	7	1.0	612

[a] Rats were given either a single dose i.v. ($n = 8$) or a single dose p.o. ($n = 8$) of each of the test compounds. At each of the time points (5, 15, 30, 60, 90, 120, 240, and 360 min), one animal was sacrificed and blood samples were analyzed for compound plasma concentration. [b] Maximum plasma concentration after oral dosing. [c] Estimated area under the plasma−concentration time curve after oral dosing. [d] Oral bioavailability. [e] Clearance. [f] Volume of distribution during steady state. [g] Oral half-life. [h] Below detection limit.

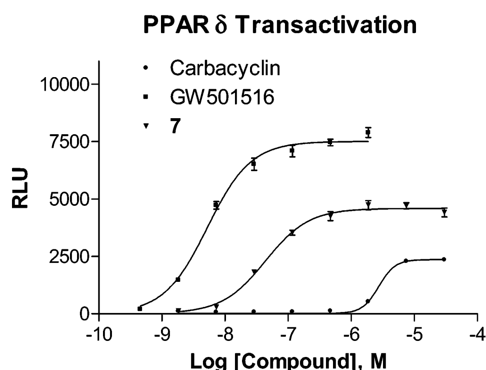


Figure 1. Concentration dependent activation of HEK293 cells transfected with the hPPARδ(LBD)-GAL4 receptor. Comparison of the hPPARδ potency and efficacy between carbacyclin, **7**, and GW501516.

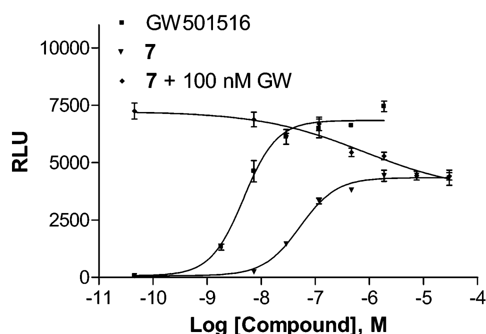


Figure 2. Concentration dependent inhibition of the hPPARδ(LBD)-GAL4 transactivation activity of the full agonist GW501516 by the partial agonist **7**.

better PK properties in the rat (Table 3), none of the compounds induced full FFA oxidation in the muscle cell line in vitro. Examination of the published crystal structure of the PPARδ receptor protein suggested room for a substituent on the central phenyl group (Figure 3).[25] The 3-chlorine-substituted analogue of **4**, compound **6**, had higher PPARδ efficacy but lower potency in the transactivation assay. To improve the potency, the chlorine group was next introduced in the 2-position and oxygen was replaced by sulfur, as in GW501516.[26] In an attempt to block the metabolism, oxygen was also inserted giving compound **7**, which had 73% PPARδ transactivation efficacy (Table 1 and Figure 1) but 113% efficacy in the FFA oxidation assay (Table 2 and Figure 4) compared to GW501516. Further, compound **7** and GW501516 had no effect on glucose oxidation in the rat muscle L6 cell line (data not shown). To verify that **7** really was a partial PPARδ agonist, increasing concentrations of **7**

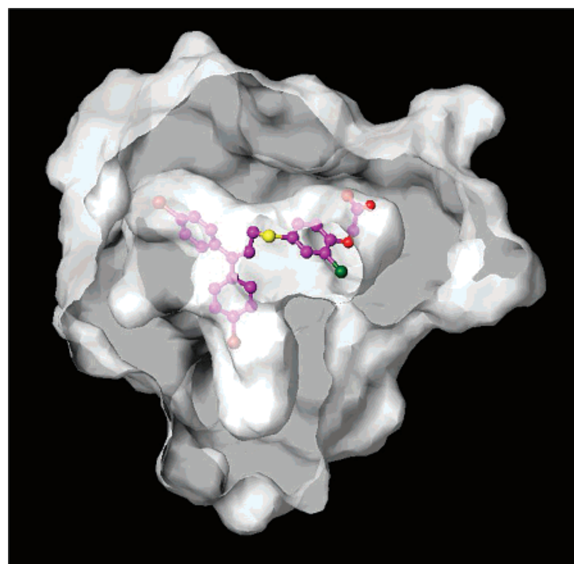


Figure 3. Compound **7** (magenta) docked into the active site of the PPARδ receptor crystallized with GW2433. The Connolly surface was generated around the amino acids within 6 Å from the crystallized ligand (GW2433). The chlorine (green) in compound **7** occupies a pocket not used by compounds **2**−**5** (or GW2433).

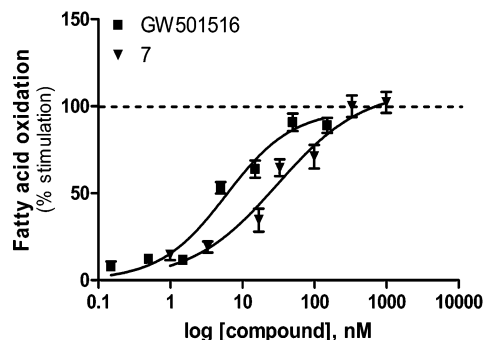


Figure 4. Dose response curves for **7** and GW501516 on fatty acid oxidation in rat L6 muscle cells. Effect of increasing concentration of compound for 48 h on fatty acid oxidation in L6 muscle cells expressed as % increase as compared to untreated cells. The curves represent the average of experiments in triplicate ± SEM.

was added to a fully efficacious concentration (100 nM) of the full agonist GW501516 (Figure 2). The transactivation efficacy of GW501516 was reduced to the max efficacy of **7**, demonstrating the partial agonist/antagonist properties of **7**. Compound **7** was approximately seven times less potent than GW501516, whereas the methyl analogue **8** was nearly equipotent to the

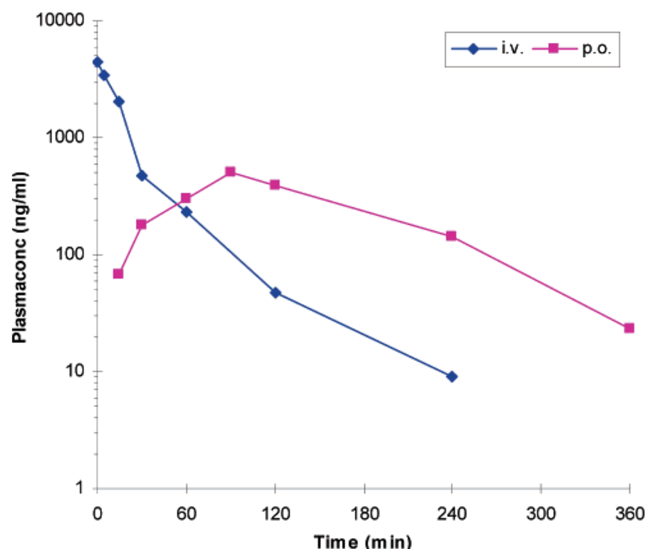


Figure 5. Plasma drug concentration versus time curves obtained from rats dosed with **7** orally by gavage and intravenously. At each time point, one rat was sacrificed and drug plasma concentration was measured.

structurally similar GW501516. The partial agonist **7** further had the desired PK properties with high exposure, good oral bioavailability, low clearance, and sufficiently long half-life (Table 3 and Figure 5). The methyl analogue **8** had slightly higher exposure and a longer half-life, but **8** was not a full agonist in the FFA oxidation assay.

The similar rank order of potency and the actual potencies (EC_{50} values) of the tested compounds in PPARδ transactivation (Table 1) and in FFA oxidation in vitro (Table 2), and the relative lack of FFA oxidation by rosiglitazone and fenofibrate, support previous findings of FFA oxidation in muscles primarily being mediated by PPARδ.[13,14]

Based on the above data, **7** was selected for further in vivo evaluation in mice. Before testing in mice, **7** was evaluated for PPAR activity at the mouse receptors. Transactivation data showed similar mPPARδ activity (**7**: $EC_{50} = 0.30 \pm 0.11$ μM, $167 \pm 15\%$; GW501516: $EC_{50} = 0.054 \pm 0.022$ μM, $269 \pm 39\%$), as seen in the human assay, although potencies were slightly lower. Likewise, **7** had no mPPARα or mPPARγ activity up to 30 μM. The effects on plasma lipids of **7** and GW501516 were evaluated in the double transgenic mouse model (ApoB100/CETP-Tgn). The ApoB100/CETP-Tgn mouse had received the human genes for ApoB100 and CETP. This resulted in a changed plasma lipid profile from a typical rodent profile, with high levels of HDL and low levels of LDL, to a human-like lipid profile, with lower HDL levels and higher LDL levels. In addition to the genetic modifications, the mice were fed a high fat diet to induce dyslipidemia and insulin resistance. At the end of the study, skeletal muscles were removed and tested for FFA oxidation activity ex vivo.

GW501516 was tested at 6 mg/kg/day p.o., a dose shown to give full efficacy in mice without liver weight increase.[10] The less-potent compound **7** was tested in three doses: 5, 10, and 20 mg/kg/day p.o. Unlike in previously published studies in high fat fed C57BL/6J mice,[10] PPARδ treatment did not change food intake or body weight in ApoB100/CETP-Tgn mice (data not shown). However, treatment with **7** changed plasma lipid parameters in a dose-dependent manner to a less atherogenic profile, with increased HDL and decreased LDL and TG (Figure 6A,B and Table 4). At the same time, HbA1c levels were lowered, suggesting improved insulin sensitivity (Table 4).

In support of the published literature,[10−15] FFA oxidation in skeletal muscles was increased (Figure 7), indicating that at least part of plasma lipid correcting and insulin resistance improving effects were through this mechanism. The fact that plasma levels of total ketone bodies (Table 4) were decreased rather than increased, as seen after fenofibrate treatment (data not shown), indicated that the beta-oxidation in liver was not part of the lipid lowering mechanism. Interestingly, the partial agonist **7** corrected the plasma lipid and glucose profile as good as or even better than the full agonist GW501516. Compound **7** therefore fulfils the criteria of being a selective partial PPARδ agonist with full efficacy on the desired effects in vivo.

In conclusion, through systematic structural modifications of the PPARpan agonist **1**, we have identified **7**, which is a partial PPARδ agonist in transactivation assay but a full agonist on FFA oxidation in muscle cells both in vitro and in vivo. Further, chronic treatment of high fat fed ApoB100/CETP-Tgn mice with **7** corrected the plasma lipid parameters and improved insulin sensitivity. Although the mechanism behind PPARδ activation is not fully understood and the side effect profile is not explored, the data in this report support the notion that selective PPARδ agonists have the potential to become a novel treatment of dyslipidemia.

Experimental Section

Chemistry. The compounds **17**−**23** and **2**−**8** (Scheme 2) were synthesized without optimization of the reaction conditions. ^{1}H NMR data were recorded on a 300 or 400 MHz spectrometer, with solvent peak as internal reference value (DMSO, 2.50; TMS peak was used in $CDCl_3$).

Ethyl 3-(4-Hydroxy-phenyl)-propionate, 11. To an ice-cooled solution of 3-(4-hydroxy-phenyl)-propionic acid (8.3 g, 50.0 mmol) in ethanol (100 mL) was added dropwise thionyl chloride (3.7 mL, 50.7 mmol). The mixture was stirred at room temperature overnight, concentrated in vacuo, and the residue was purified by kugelrohr distillation to give 9.6 g (99%) of **11** as a colorless oil.

Ethyl 3-{4-[3,3-Bis-(4-bromo-phenyl)-allyloxy]-phenyl}-propionate, 17. Under an atmosphere of nitrogen, azodicarboxylic dipiperidide (504 mg, 2.0 mmol) was added at 0−5 °C to a stirred solution of tributylphosphine (404 mg, 2.0 mmol), **11** (388 mg, 2.0 mmol), and **10**[21](736 mg, 2.0 mmol) in dry THF (50 mL). The reaction mixture was warmed to room temperature and stirred for 48 h. The reaction mixture was concentrated in vacuo, and water and ethyl acetate (75 mL each) were added. The aqueous layer was collected and further extracted with ethyl acetate (2 × 75 mL). The organic layers were combined, washed with water, dried ($MgSO_4$), and evaporated. The crude product was then purified by column chromatography on silica (25% ethyl acetate in heptane as eluent) to give 1.0 g (92%) of the title compound.

3-{4-[3,3-Bis-(4-bromo-phenyl)-allyloxy]-phenyl}-propionic Acid, 2. To a solution of **17** (1.0 g, 2.0 mmol) in toluene (20 mL) and ethanol (50 mL) was added 1 N NaOH (10.0 mL), and the reaction mixture was stirred for 16 h at room temperature. The reaction mixture was concentrated in vacuo, and 1 N HCl (10 mL) was added. The product was extracted with ethyl acetate (3 × 50 mL). The organic layers were combined, washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was recrystallized from warm ethanol (100 mL), which was concentrated to 60 mL and cooled, to give 600 mg (56%) of the title compound. Anal. ($C_{24}H_{20}Br_2O_3$) C, H.

Ethyl 3-(3-Hydroxy-phenyl)-propionate, 12. To an ice-cooled solution of 3-(3-hydroxy-phenyl)-propionic acid (20.0 g, 120 mmol) in ethanol was added dropwise thionyl chloride (8.8 mL, 120 mmol). The mixture was stirred at room temperature overnight, concentrated in vacuo, and submitted to flash chromatography (10% ethyl acetate in toluene eluent) to give 23.3 g (100%) of **12**.

3-{3-[3,3-Bis-(4-bromo-phenyl)-allyloxy]-phenyl}-propionic Acid Ethyl Ester, 18. The compound was synthesized from **12** and **10** as described for compound **17** in a 756 mg (93%) yield.

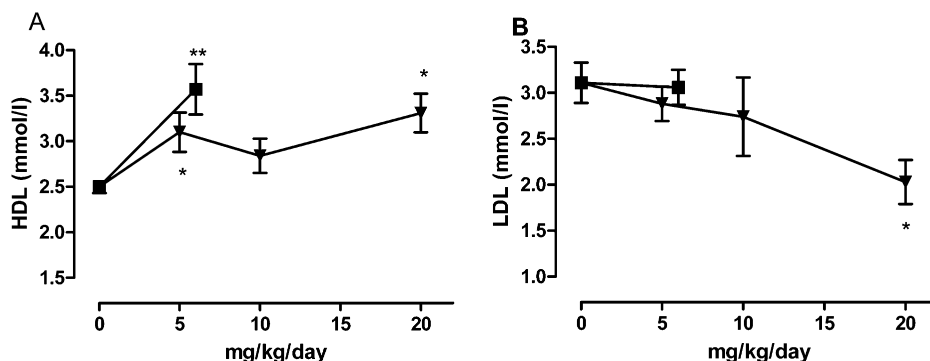


Figure 6. Plasma HDL cholesterol (panel A) and LDL cholesterol (panel B) after 6 weeks treatment of ApoB100/CETP-Tgn mice with GW501516 (■) and **7** (▼).

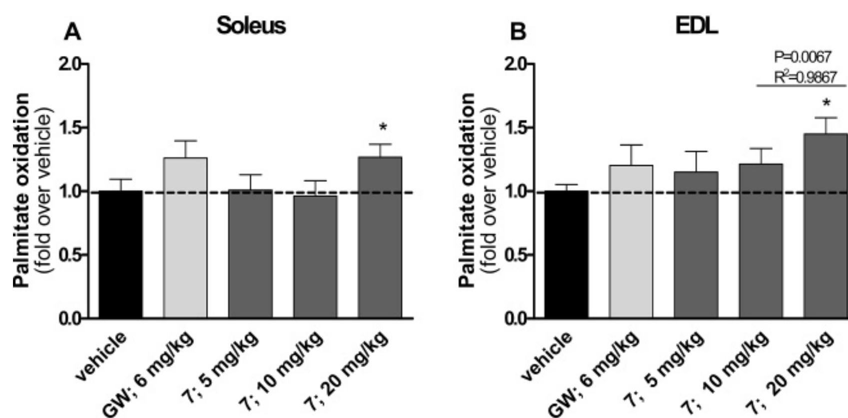


Figure 7. FFA oxidation in skeletal muscle of ApoB100/CETP-Tgn mice after 6 weeks treatment with **7** and GW501516. The mainly oxidative/slow, musculus soleus (type I fibers), is shown in panel A. The mainly glycolytic/fast, musculus extensor digitorum longus (EDL; type II fibers), is shown in panel B. There was a significant correlation between increasing doses of **7** and FFA-oxidation in both soleus and EDL.

Table 4. Treatment Effects on Plasma Parameters in ApoB100/CETP-Tgn Mice

	% change from vehicle treated controls (6 weeks treatment)							
cmpd	total cholesterol	HDL	LDL	triglycerides	total ketone bodies	glucose	insulin	HbA1c
GW501516 (6 mg/kg)	−15	4	−19	−14	−49	−4	−41	−13
7 (5 mg/kg)	−19	24	−7	−15	5	−3	−11	−4
7 (10 mg/kg)	−14	14	−12	−7	−10	−2	−8	−9
7 (20 mg/kg)	2	33	−35	−5	−29	−5	3	−12

3-{3-[3,3-Bis-(4-bromo-phenyl)-allyloxy]-phenyl}-propionic Acid, 3. The ester **18** (755 mg, 1.4 mmol) was hydrolyzed in 1 N NaOH (5.6 mL) and ethanol (15 mL) for 16 h at room temperature. Water (5 mL) was added, and the ethanol was removed by concentration in vacuo. The mixture was neutralized with 6 N HCl. The crude product was extracted with ethyl acetate (3 × 30 mL). The organic layers were combined, dried ($MgSO_4$), and evaporated. The residue was dissolved in toluene, and the product was precipitated with petroleum ether to give 430 mg (60%) of the title compound. Anal. ($C_{24}H_{20}Br_2O_3$) C, H.

Methyl {4-[3,3-Bis-(4-bromo-phenyl)-allyloxy]-phenyl}-acetate, 19. The compound was synthesized from methyl 4-hydroxyphenyl-acetate, **13**,[27]and **10** as described for compound **17** in a 480 mg (62%) yield

{4-[3,3-Bis-(4-bromo-phenyl)-allyloxy]-phenyl}-acetic Acid, 4. To a solution of **19** (473 mg, 0.9 mmol) in THF (5 mL) and ethanol (3 mL) was added 1 N NaOH (3 mL), and the reaction mixture was stirred at 60 °C for 1 h and at room temperature overnight. The title compound was isolated as the sodium salt, by filtration, and washed with ethanol to give 375 mg (81%). Anal. ($C_{23}H_{18}Br_2O_3$, $C_6H_{14}N_4O_2$) C, H, N, arginine salt.

Ethyl 3-(3-Hydroxy-phenyl)-acetate, 14. To an ice-cooled solution of 3-(3-hydroxyphenyl)-acetic acid (21.0 g, 138 mmol) in ethanol was added dropwise thionyl chloride (10.1 mL. 138 mmol). The mixture was stirred at room temperature overnight, concentrated in vacuo, and submitted to flash chromatography (10% ethyl acetate in toluene eluent) to give 23.8 g (77%) of **14**.

Ethyl 3-{3-[3,3-Bis-(4-bromo-phenyl)-allyloxy]-phenyl}-acetate, 20. The compound was synthesized from **14** and **10** as described for compound **17** in a 701 mg (88%) yield.

3-{3-[3,3-Bis-(4-bromo-phenyl)-allyloxy]-phenyl}-acetic Acid, 5. Compound **20** was hydrolyzed as described for compound **3** to give **5** in a 701 mg (76%) yield. Anal. ($C_{23}H_{18}Br_2O_3$) C, H.

Ethyl 3-Chloro-4-hydroxyphenylacetate, 15. To an ice-cooled solution of 3-chloro-4-hydroxyphenylacetic acid (10.0 g, 53.0 mmol) in ethanol was added dropwise thionyl chloride (3.9 mL, 53.5 mmol). The mixture was stirred at room temperature for 48 h, concentrated in vacuo, and submitted to flash chromatography

(graduated from toluene to 5% ethyl acetate in toluene eluent) to give 11.0 g (97%) of **15**.

Ethyl {4-[3,3-Bis-(4-bromo-phenyl)-allyloxy]-3-chloro-phenyl}-acetate, 21. The compound was synthesized from **15** and **10** as described for compound **17** in a 1.0 g (89%) yield.

{4-[3,3-Bis-(4-bromo-phenyl)-allyloxy]-3-chloro-phenyl}-acetic Acid, 6. Compound **21** was hydrolyzed as described for compound **4** to give **6** in a 817 mg (82%) yield, isolated by filtration as the sodium salt. Anal. ($C_{23}H_{19}Br_2ClO_3$, $C_6H_{14}N_4O_2$) C, H, N, arginine salt.

Methyl (2-Chloro-4-mercapto-phenoxy)-acetate, 16. A solution of 2-chlorophenol (25.7 g, 200 mmol), potassium carbonate (41.4 g, 300 mmol), and ethyl bromoacetate (35.1 g, 210 mmol) in 2-butanone (240 mL) was stirred at 100 °C for 24 h. The reaction mixture was filtered and evaporated. The residue was dissolved in toluene (100 mL), washed with water (3 × 25 mL), dried, and evaporated. The residue (37 g, 172 mmol) was dissolved in dichloromethane (50 mL) and chlorosulfonic acid (93 g, 800 mmol) was added slowly at −10 °C. The reaction mixture was stirred at room temperature for 1 h. Ice water (25 mL) was added carefully, and the mixture was extracted with dichloromethane (3 × 100 mL). The combined organic phases were washed with water, dried, and evaporated to give crude ethyl (4-chlorosulfonyl-2-chloro-phenoxy)-acetate as an oil in a 47.5 g (76%) yield.

To a refluxing solution of red phosphorus (17 g, 566 mmol) and I_2 (2.4 g, 9.4 mmol) in glacial acetic acid (100 mL) was added slowly a solution of ethyl (4-chlorosulfonyl-2-chloro-phenoxy)-acetate (47.5 g, 151 mmol) in glacial acetic acid (1000 mL). The reaction mixture was refluxed for 24 h. Water (20 mL) was added carefully, and the mixture was refluxed for an additional 1 h. After cooling, the reaction mixture was filtered and the filtrate was diluted with water (500 mL). The mixture was extracted with dichloromethane (3 × 100 mL), and the organic phases were washed with water. After drying, the dichloromethane phase was evaporated to give crude (2-chloro-4-mercapto-phenoxy)-acetic acid in a 17 g (52%) yield. The crude acid was dissolved in methanol (50 mL), and a solution of acetyl chloride (24 g, 310 mmol) in methanol (200 mL) was added slowly at 10 °C. The reaction mixture was stirred for 2 h. The reaction mixture was evaporated and the residue was purified by column chromatography (eluent: ethyl acetate/heptane (4:1)) to give pure **16** in a 17 g (93%) yield.

Methyl {4-[3,3-Bis-(4-bromo-phenyl)-allylsulfanyl]-2-ethyl-phenoxy}-acetate, 22. Intermediate **10** (2.1 g, 5.5 mmol) and tributylphosphine (1.7 g, 8.6 mmol) were dissolved in dry THF (50 mL) and cooled to 0 °C under an atmosphere of nitrogen. Azodicarbonyl dipiperidide (2.1 g, 8.6 mmol) was added, and the reaction mixture was stirred for 5 min. Compound **16** (1 g, 4.2 mmol) was slowly added (5 min) and the stirring was continued for 12 h at 5 °C. Water (10 mL) was added, and the mixture was extracted with ethyl acetate (2 × 50 mL). The combined organic extracts were dried and evaporated. The residue was purified by HPLC to give the title compound in a 2.4 g (100%) yield.

{4-[3,3-Bis-(4-bromo-phenyl)-allylsulfanyl]-2-chloro-phenoxy}-acetic Acid, 7. The ester **22** (350 mg, 0.6 mmol) was dissolved in ethanol (10 mL). NaOH (1 N, 3 mL, 3 mmol) was added at room temperature, and the reaction mixture was stirred for 18 h at 5 °C, after which it was treated with 1 N HCl (15 mL) and extracted with dichloromethane (2 × 20 mL). The combined organic phases were dried and evaporated to give the title compound in a 230 mg (68%) yield. Anal. ($C_{23}H_{17}Br_2ClO_3S$, $C_6H_{14}N_4O_2$, H_2O) C, H, N, arginine salt.

Methyl {4-[3,3-Bis-(4-bromo-phenyl)-allylsulfanyl]-2-methyl-phenoxy}-acetate, 23. The compound was synthesized from methyl (4-mercapto-2-methyl-phenoxy)-acetate[23] (2.06 g, 9.7 mmol) and **10**, as described for compound **22**, in a 4.0 g (88%) yield.

{4-[3,3-Bis-(4-bromo-phenyl)-allylsulfanyl]-2-methyl-phenoxy}-acetic Acid, 8. Compound **23** was hydrolyzed as described for compound **7** to give **8** in a 482 mg (93%) yield. Anal. ($C_{24}H_{20}Br_2O_3S$) C, H.

PPAR In Vitro Transactivation Assay. HEK293 cells were grown in DMEM + 10% FCS. Cells were seeded in T-175 flasks (6×10^6 cells/flask) the day before transfection to give a confluence of ~50% at transfection. A total of 23 μg of DNA containing 17.5 μg pM1(x)LBD(α, γ, or δ), 2.7 μg pRLCMV, 2.2 μg pGL2(Gal4)$_5$, and 0.55 μg pADVANTAGE was transfected per flask using FuGene transfection reagent according to the manufacturers instructions (Roche). The cells were harvested from the flasks 20−24 h after transfection and plated in 96-well plates (110.000 cells/well in media w/o phenol red). The relevant dilutions of test compound were added immediately thereafter. The plates were further incubated for 20 h before measurement of luciferase activity. The luciferase assay was performed using the LucLite kit according to the manufacturers' instructions (Perkin-Elmer). Briefly, media including test compound was aspirated and replaced with PBS. LucLite substrate was added and light emission was quantified on a Packard TopCount.

Human PPAR α, γ, or δ were obtained by PCR amplification using cDNA synthesized by reverse transcription of mRNA from human liver, adipose tissue, and placenta, respectively. Amplified cDNAs were cloned into pCR2.1 and sequenced. The ligand binding domain (LBD) of each PPAR isoform was generated by PCR (PPAR α: aa 167, C-terminus; PPAR γ: aa 165, C-terminus, PPARδ: aa 128, C-terminus) and fused to the DNA binding domain of the yeast transcription factor GAL4 by subcloning fragments in frame into the vector pM1,[28] generating the plasmids pM1αLBD, pM1γLBD, and pM1δLBD. Ensuing fusions were verified by sequencing. The reporter gene was constructed by inserting an oligonucleotide encoding five repeats of the GAL4 recognition sequence (5 × CGGAGTACTGTCCTCCG(AG))[29] into the vector pGL2 promotor plasmid (Promega), generating the plasmid pGL2-(GAL4)$_5$. The plasmid pRLCMV (Promega) encodes a Renilla luciferase used in the validation of the assay. The presence of the pADVANTAGE plasmid (Promega) leads to an enhancement of the luciferase signal.

All compounds were dissolved in DMSO and diluted at least 1:1000 upon addition to the cells.

Raw data is expressed in relative light units. All curve fittings were done by nonlinear regression using GraphPad PRISM (GraphPad Software, San Diego, CA).

In the experiment designed to show **7** was a partial agonist that competes with GW501516 for binding to PPARδ, a dose response curve of **7** was generated in the presence of 100 nM GW501516.

Fatty Acid Oxidation in L6 Line. The rat skeletal muscle cell line L6 was cultured at 37 °C in a humidified atmosphere containing 5% CO_2 in MEM Alpha (Gibco; 32571−028) supplemented with 10% foetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. When confluent, the cells were differentiated by changing the medium to MEM Alpha (Gibco; 32571−028) supplemented with 2% foetal calf serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. Experiments were performed after five days of differentiation.

Differentiated L6 muscle cells were stimulated with PPARδ agonist for 42 h (fresh medium and compound were given after 24 h) in differentiation medium, followed by 6 h of stimulation in differentiation medium supplemented with 0.4 mM palmitate containing 10 μCi/mL [9,10-^{3}H]-palmitic acid (Perkin-Elmer; NET-043) conjugated to 2% fatty acid free BSA (Sigma; A7030) by sonication. The assay was terminated by the addition of 10% TCA. Upon oxidation of the tritiated palmitate, $^3H-H_2O$ is generated and appears in the medium. $^3H-H_2O$ was separated from the medium by equilibration with water in a separate container at 50 °C o/n. The quantity of $^3H-H_2O$ transferred to the water was measured by liquid scintillation counting. Compounds tested were diluted in DMSO as 1000× stocks, giving a final concentration of 0.1% DMSO in the medium upon stimulation. Concentrations ranging from 0 to 30× EC_{50} for the individual compounds (determined in the hPPARδ transactivation assay) were tested.

Glucose oxidation was measured in the same way as fatty acid oxidation, with the exception that the tritiated palmitate was replaced by 10 μCi/mL [5-^{3}H]-D-glucose (Perkin-Elmer; NET-530). As a positive control for the assay, FCCP (mesoxalonitrile 4-trifluoromethoxyphenylhydrazone (Fluka; 21857), a known uncoupler of

oxidative phosphorylation) was included in the glucose oxidation measurements, and in addition, fatty acid oxidation measurement for GW501516 was performed simultaneous with the glucose oxidation assay.

Data were expressed as fold over unstimulated cells, and curve fitting was done by nonlinear regression using GraphPad Prism (GraphPad software, SanDiego, CA). EC_{50} is the concentration giving 50% of maximal response observed for a given compound. To compare the efficacy of each compound, GW501516 was included in every experiment, and maximal response is given as % of maximal response of GW501516. Results are given as mean ± SEM from three to four experiments performed in triplicates.

Pharmacokinetics. The procedure has previously been described in detail.[30] Briefly, the compounds were dosed p.o. and i.v. to male SD rats. The compounds were dissolved in 5% ethanol, 10% HPCD, and phosphate buffer; pH 7.5−8.0. Blood samples were collected in EDTA tubes. Each data point represents one animal.

Plasma samples were analyzed by high turbulence liquid chromatography combined with tandem mass spectrometry.

ApoB100/CETPtgn Mice. ApoB100/CETPtgn double transgenic female mice (B6;SJL-Tgn(CETP)-Tgn(APOB100), Taconic Europe, 8680 Ry, Denmark), 9−11 weeks old, with a mean body weight of 19.2 g ± 1.4, $n = 8$/group, were fed a high fat diet (D01111201, Research Diets Inc., USA) containing 17 kcal % protein, 43 kcal % carbohydrate, and 40 kcal % fat to induce dyslipidemia and insulin resistance. Fresh diet from the freezer was supplied every day (5 g/mouse), which corresponds to ad libitum feeding. The high fat feeding was started 3 weeks prior to administration of compounds and was continued throughout the 6-weeks dosing period. The animals were allowed to adapt to the laboratory conditions during the 3-weeks prefeeding period. Tap water was freely available in the home cages throughout the studies. A normal 12 h/12 h light/dark regime was operative (lights on at 0600 h) in the stables and room temperature was held at 20−21 °C. All animal procedures were conducted according to Novo Nordisk A/S Animal Care approved protocols, and the experiments were done in compliance with internal animal welfare and national guidelines.

Suspensions of compounds were made in the standard vehicle 0.2% CMC (Merch Eurolab 279294T, Lot 1009855) + 0.4% Tween-80 (Merch Eurolab 8.22187.0500, Lot S31764043) in saline. Fresh suspensions were made for seven days of dosing and kept at +4 °C.

The animals were dosed orally by gavage twice daily with either vehicle or test compound. The dose volume was 10 mL/kg.

Body weight and food intake were measured once weekly. Blood was drawn after 23 and 46 days treatment from the orbital plexus (250 μL) in overnight fasted animals under isoflurane anesthesia.

The blood samples were collected in EDTA coated Eppendorf tubes, centrifuged at 4000 × g for 10 min at 4 °C, and plasma was transferred to Hitachi cups for analyses on a Hitachi 912 auto-analyzer (Roche).

The measured parameters were as follows: total cholesterol, HDL, LDL, triglycerides, glucose, HbA1c (all Roche), total ketone bodies, and CETP (Wako) and insulin (Screening and Assay Dept. 244). CETP and insulin were measured by ELISA. The CETP level in plasma was tested after three weeks of treatment with the PPARδ agonists included in this study. The results showed that the CETP level was not reduced. In contrast, CETP levels were found to be up-regulated (data not shown). Why the beneficial effects found (e.g., high HDL) after the treatment cannot be due to lack of CETP.

At the final blood collection on day 46, musculus soleus (mainly red muscle, type I oxidative fibers) and musculus extensor digitorum longus (EDL, mainly white muscle, type IIb glycolytic fibers) were gently dissected and immediately transferred to pregassed (95% O_2−5% CO_2) Krebs−Henseleit (KRH) buffer (in mM: 118.5, NaCl; 4.7, KCl; 1.2, KH_2PO_4; 25, $NaHCO_3$; 2.5, $CaCl_2$; 1.2, $MgSO_4$; and 10, HEPES) supplemented with 10 mM glucose and 2% BSA. Fatty acid oxidation capacity was determined by incubating the muscles for 90 min in pregassed KRH buffer supplemented with 10 mM glucose, 60 nM insulin (human from Novo Nordisk) and 0.2 mM palmitate containing 10 μCi/mL [9,10-^{3}H]-palmitic acid (Perkin-Elmer; NET-043) conjugated to 2% fatty acid free BSA (Sigma; A7030, as described in Thompson et al.).[31] Incubations were performed at 30 °C, with gentle agitation (110/min), under continuous gassing with 95% O_2−5% CO_2. The assay was terminated by addition of 10% TCA. Upon oxidation of the tritiated palmitate, $^3H-H_2O$ is generated and appears in the medium. $^3H-H_2O$ was separated from the medium by equilibration with water in a separate container at 50 °C o/n. The quantity of $^3H-H_2O$ transferred to the water was measured by liquid scintillation counting.

Data are presented as mean ± SEM. Statistical analyses were done using one-way ANOVA, followed by Dunnett's multiple comparison test or unpaired t-test with Welch correction. P values less than 0.05 were considered significant, $*p < 0.05$ and $**p < 0.01$.

Glide2.5 Docking. The structure manipulation and surface generation was performed in Maestro version 7.0.[33] Glide calculations were performed with Impact version 3.5.[34,35] Compound **7** was built in Maestro version 7.0, a formal charge of −1 was assigned, and the molecule was minimized with the MMFFs force field. The crystal structure of the PPARδ receptor crystallized with GW2433 was used (1GWX) for docking.[25] Default neutralization zone (10−20 Å) and minimization (0.30 Å) was used in the preparation of the protein. In the generation of the grid, default vdW scaling was used (1.00) and the enclosing box was set 20 Å. Compound **7** was docked with Glide version 2.5 using the SP mode and the van der Waals radii of the ligand atoms were scaled by 0.8. Ten poses were generated in the docking procedure, and the first pose was used.

Acknowledgment. The technical assistance of Bjørn Bentzen, Alice Jørgensen, Otto Larsson, Per S. Klifforth, Nicolai Steffensen, Lene Priskorn, Helle Bach, Annette Zeneca, Dorthe D. Andersen, Trine N. Eriksson, and Helle Fjordvang is highly appreciated.

Supporting Information Available: ^{1}H NMR data on intermediates and final products. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Issemann, I.; Green, S. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature* **1990**, *347*, 645−650.
(2) Mangelsdorf, D. J.; Evans, R. M. The RXR heterodimers and orphan receptors. *Cell* **1995**, *83*, 841−850.
(3) A unified nomenclature system for the nuclear receptor superfamily. *Cell* **1999**, *97*, 161−163.
(4) Kliewer, S. A.; Umesono, K.; Noonan, D. J.; Heyman, R. A.; Evans, R. M. Convergence of 9-*cis* retinoic acid and peroxisome proliferator signaling pathways through heterodimer formation of their receptors. *Nature* **1992**, *358*, 771−774.
(5) Chilcott, J.; Tappenden, P.; Jones, M. L.; Wright, J. P. A systematic review of the clinical effectiveness of Pioglitazone in the treatment of type 2 diabetes mellitus. *Clin. Ther.* **2001**, *23*, 1792−1823.
(6) Boyle, P. J.; King, A. B.; Olansky, L.; Marchetti, A.; Lau, H.; Magar, R.; Martin J. Effects of Pioglitazone and Rosiglitazone on the blood lipid levels and glycemic control in patients with type 2 diabetes melitus: A retrospective review of randomly selected medical records. *Clin. Ther.* **2002**, *24* (3), 378−396.
(7) Staels, B.; Dallongeville, J.; Auwerx, J.; Schoonjans, K.; Leitersdorf, E.; Fruchart, J.-C. Mechanism of action of fibrates on lipid and lipoprotein metabolism. *Circulation* **1998**, *98*, 2088−2093.
(8) Leibowitz, M. D.; Fiévet, C.; Hennuyer, N.; Peinado-Onsurbe, J.; Duez, H.; Berger, J.; Cullinan, C. A.; Sparrow, C. P.; Baffic, J.; Berger, G. D.; Santini, C.; Marquis, R. W.; Tolman, R. L.; Smith, R. G.; Moller, D. E.; Auwerx, J. Activation of PPARδ alters lipid metabolism in db/db mice. *FEBS Lett.* **2000**, *473*, 333−336.
(9) Oliver, W., Jr.; Shenk, J. L.; Snaith, M. R.; Russell, C. S.; Plunket, K. D.; Bodkin, N. L.; Lewis, M. C.; Winegar, D. A.; Sznaidman, M. L.; Lambert, M. H.; Xu, H. E.; Sternbach, D. D.; Kliewer, S. A.; Hansen, B. C.; Willson, T. M. A Selective peroxisome proliferator-activated receptor δ agonist promotes reverse cholesterol transport. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5306−5311.

(10) Tanaka, T.; Yamamoto, J.; Iwasaki, S.; Asaba, H.; Hamura, H.; Ikeda, Y.; Watanabe, M.; Magoori, K.; Ioka, R. X.; Tachibana, K.; Watanabe, Y.; Uchiyama, Y.; Sumi, K.; Iguchi, H.; Ito, S.; Dio, T.; Hamakubo, T.; Naito, M.; Auwerx, J.; Yanagisawa, M.; Kodama, T.; Sakai, J. Activation of peroxisome proliferator-activated receptor δ induces fatty acid β-oxidation in skeletal muscle and attenuates metabolic syndrome. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 15924−15929.

(11) Wang, Y.-X.; Lee, C.-H.; Tiep, S.; Yu, R. T.; Ham, J.; Kang, H.; Evans, R. M. Peroxisome-proliferator-activated receptor δ activates fat metabolism to prevent obesity. *Cell* **2003**, *113*, 159−170.

(12) Luquet, S.; Lopez-Soriano, J.; Holst, D.; Frederich, A.; Melki, J. Rassoulzadegan, M.; Grimaldi, P. Peroxisome proliferator-activated receptor δ controls muscle development and oxidative capacity. *FASEB J.* **2003**, *17*, 209−226.

(13) Holst, D.; Luquet, S.; Nogueira, V.; Kristiansen, K.; Leverve, X.; Grimaldi, P. Nutritional regulation and role of peroxisome proliferator-activated receptor δ in fatty acid catabolism in skeletal muscle. *Biochem. Biophys. Acta* **2003**, *1633*, 43−50.

(14) Dressel, U.; Allen, T. L.; Pippal, J. B.; Rohde, P. R.; Lau, P.; Muscat, G. E. O. The peroxisome proliferator activated receptor γ/δ agonist, GW501516, regulates the expression of genes involved in lipid catabolism and energy uncoupling in skeletal muscle cells. *Mol. Endocrinol.* **2003**, *17*, 2477−2493.

(15) Krämer, D. K.; Al-Khalili, L.; Perrini, S.; Skogsberg, J.; Wretenberg, P.; Kannisto, K.; Wallberg-Henriksson, H.; Ehrenborg, E.; Zierath, J.; Krook, A. Direct activation of glucose transport in primary human myotubes after activation of peroxisome proliferators-activated receptor δ. *Diabetes* **2005**, *54*, 1157−1163.

(16) Lee, C.-H.; Chawla, A.; Urbiztondo, N.; Liao, D.; Boisvert, W. A.; Evans, R. M. Transcriptional repression of atherogenic inflammation: Modulation by PPARδ. *Science* **2003**, *302*, 453−457.

(17) Li, A. C.; Binder, C. J.; Gutierrez, A.; Brown, K. K.; Plotkin, C. R.; Pattison, J. W.; Valledor, A. F.; Davis, R. A.; Willson, T. M.; Witztum, J. L.; Palinski, W.; Glass, C. K. Differential inhibition of macrophage foam-cell formation and atherosclerosis in mice by PPAR α, β/δ, and γ. *J. Clin. Invest.* **2004**, *114*, 1564−1576.

(18) Van der Veen, J. N.; Kruit, J. K.; Havinga, R.; Baller, J. F. W.; Chimini, G.; Lestavel, S.; Staels, B.; Groot, P. H. E.; Groen, A. K.; Kuipers, F. Reduced cholesterol absorption upon PPARδ activation coincides with decreased intestinal expression of NPC1L1. *J. Lipid Res.* **2005**, *46*, 526−534.

(19) Skogberg, J.; Kannisto, K.; Cassel, T. N.; Hamsten, A.; Eriksson, P.; Ehrenborg, E.; Evidence that peroxisome proliferator-activated receptor delta influences cholesterol metabolism in humans. *Arterioscler., Thromb., Vasc. Biol.* **2003**, *23*, 637.

(20) Sprecher, D.; Massien, C.; Patterson, S.; Zalewski, A.; Johnson, A. HDLc effects in healthy subjects administered a peroxisome proliferator activated receptor (PPAR) delta agonist. American Heart Association, 2004; poster 244.

(21) Mogensen, J. P.; Jeppesen, L.; Bury, P. S.; Pettersson, I.; Fleckner, J.; Nehlin, J.; Frederiksen, K. S.; Albrektsen, T.; Din, N.; Mortensen, S. B.; Svensson, L. A.; Wassermann, K.; Wulff, E. M.; Ynddal, L.; Sauerberg, P. Design and synthesis of novel PPAR $\alpha/\gamma/\delta$ triple activators using a known PPARα/γ dual activator as structural template. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 257−260.

(22) The experimental conditions for the in vitro transactivation assays have been changed compared to previously published procedures, giving raise to slightly different results. See Experimental Section.

(23) Sznaiman, M. L.; Haffner, C. D.; Maloney, P. R.; Fivush, A.; Chao, E.; Goreham, D.; Sierra, M. L.; LeGrumelec, C.; Xu, H. E.; Sternbach, D. D. Novel selective small molecule agonists for peroxisome proliferators-activated receptor δ (PPARδ)—Synthesis and biological activity. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1517−1523.

(24) During the preparation of this work, a paper describing a similar type of SAR approach appeared: Adams, A. D.; Yuen, W.; Hu, Z.; Santani, C.; Jones, A. B.; MacNaul, K. L.; Berger, J. P.; Doebber, T. W.; Moller, D. E. Amphipathic 3-phenyl-7-propylbenzisoxazoles; Human PPARγ, δ, and α agonists. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 931−935.

(25) Xu, H. E.; Lambert, M. H.; Montana, V. G.; Parks, D. J.; Blanchard, S. G.; Brown, P. J.; Sternbach, D. D.; Lehmann, J. M.; Wisly, G. B.; Willson, T. M.; Kliever, S. A.; Milburn, M. V. Molecular recognition of fatty acids by peroxisome proliferators-activated receptors. *Mol. Cell* **1999**, *3*, 397−403.

(26) During the preparation of this manuscript, several papers describing a similar improvement in potency appeared: Weigand, S.; Bischoff, H.; Dittrich-Wengenroth, E.; Heckroth, H.; Lang, D.; Vaupel, A.; Woltering, M. Minor structural modifications convert a selective PPARα agonist into a potent, highly selective PPARδ agonist. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4619−4623. Pereira, R.; Gaudon, C.; Iglesias, B.; Germain, P.; Gronemeyer, H.; de Lera, A. R. Synthesis of the β/δ-selective agonist GW501516 and C4-thiazole-substituted analogs. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 49−54. Epple, R.; Azimioara, M.; Russo, R.; Bursulaya, B.; Tian, S.-S.; Gerken, A.; Iskandar, M. 1,3,5-Trisubstituted aryls as highly selective PPARδ agonists. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2969−2973. Epple, R.; Russo, R.; Azimioara, M.; Cow, C.; Xie, Y.; Wang, X.; Wityak, J.; Karanewsky, D.; Gerken, A.; Iskandar, M.; Saez. E.; Seidel, H. M.; Tain, S.-S. 3,4,5-Trisubstituted isoxazoles as novel PPARδ agonists: Part 1. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4376−4380. Epple, R.; Azimioara, M.; Russo, R.; Xie, Y.; Cow, C.; Wang, X.; Wityak, J.; Karanewsky, D.; Bursulaya, B.; Kreusch, A.; Tuntlan, T.; Gerken, A.; Iskandar, M.; Saez. E.; Seidel, H. M.; Tain, S.-S. 3,4,5-Trisubstituted isoxazoles as novel PPARδ agonists: Part 2. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5488−5492. Xu, Y.; Etgen, G. J.; Broderick, C. L.; Canada, E.; Gonzalez, I.; Lamar, J.; Monstrose-Rafizadeh, C.; Oldham, B. A.; Osborn, J. J.; Xie, C.; Shi, Q.; Winneroski, L.; York, J.; Yumibe, N.; Zink, R.; Mantlo, N. Design and synthesis of dual peroxisome proliferator-activated receptor γ and δ agonists as novel euglycemic agents with a reduced weight gain profile. *J. Med. Chem.* **2006**, *49*, 5649−5652.

(27) Mattingly, P. G.; Miller, M. J. Synthesis of 2-azetidinones from serinehydroxamates: approaches to the synthesis of 3-aminonocardicinic acid. *J. Org. Chem.* **1981**, *46*, 1557−1564.

(28) Sadowski, I.; Bell, B.; Broad, P.; Hollis, M. *GAL4* fusion vectors for expression in yeast and mammalian cells. *Gene* **1992**, *118*, 137−141.

(29) Webster, N.; Jin, J. R.; Green, S.; Hollis, M.; Chambon, P. The yeast UASG is a transcriptional enhancer in human HeLa cells in the presence of the GAL4 trans-activator. *Cell* **1988**, *52*, 169−178.

(30) Sauerberg, P.; Pettersson, I.; Jeppesen, L.; Bury, P. S.; Mogensen, J. P.; Wassermann, K.; Brand, C. L.; Sturis, J.; Wöldike, H. F.; Fleckner, J.; Andersen, A.-S. T.; Mortensen, S. B.; Svensson, L. A.; Rasmussen, H. B.; Lehmann, S. V.; Polivka, Z.; Sindelar, K.; Panajotova, V.; Ynddal, L.; Wulff, E. M. Novel tricyclic-α-alkoxyphenylpropionic acids: Dual PPARα/γ agonists with hypolipidemic and antidiabetic activity. *J. Med. Chem.* **2002**, *45*, 789−804.

(31) Thompson, A. L.; Lim-Fraser, M. Y.-C.; Kraegen, E. W.; Cooney, G. J. Effects of individual fatty acids on glucose uptake and glycogen synthesis in soleus muscle in vitro *Am. J. Physiol.* **2000**, *279*, 577−584.

(32) Deussen, H.-J.; Jeppesen, L.; Schärer, N.; Junager, F.; Bentzen, B.; Weber, B.; Weil, V.; Mozer, S. J.; Sauerberg, P. Process development and scale-up of the PPAR agonist NNC 61−4655. *Org. Process Res. Dev.* **2004**, *8*, 363−371.

(33) *Maestro*, version 7.0; Schrödinger, LLC: New York, 1999−2005.

(34) Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shaw, D. E.; Shelley, M.; Perry, J. K.; Francis, P.; Shenkin, P. S. Glide: A new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *J. Med. Chem.* **2004**, *47*, 1739−1749.

(35) Halgren, T. A.; Murphy, R. B.; Friesner, R. A.; Beard, H. S.; Frye, L. L.; Pollard, W. T.; Banks, J. L. Glide: A new approach for rapid, accurate docking and scoring. 2. Enrichment factors in database screening. *J. Med. Chem.* **2004**, *47*, 1750−1759.

JM061202U