Design and Synthesis of *N*-[(4-Methoxyphenoxy)carbonyl]-*N*-[[4-[2-(5-methyl-2-phenyl-4-oxazolyl)ethoxy]phenyl]methyl]glycine [Muraglitazar/BMS-298585], a Novel Peroxisome Proliferator-Activated Receptor α/γ Dual Agonist with Efficacious Glucose and Lipid-Lowering Activities

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Received May 13, 2004

Muraglitazar/BMS-298585 (**2**) has been identified as a non-thiazolidinedione PPAR α/γ dual agonist that shows potent activity *in vitro* at human PPARα (EC_{50} = 320 nM) and PPARγ (EC_{50} = 110 nM). Compound **2** shows excellent efficacy for lowering glucose, insulin, triglycerides, and free fatty acids in genetically obese, severely diabetic *db/db* mice and has a favorable ADME profile. Compound **2** is currently in clinical development for the treatment of type 2 diabetes and dyslipidemia.

Introduction

Type 2 diabetes mellitus is a chronic and devastating disease characterized by hyperglycemia, insulin resistance, and perturbations in fat, protein, and carbohydrate metabolism. According to the World Health Organization, approximately 150 million people are afflicted with the disease worldwide with projections of 300 million by the year 2025.[1] Risk factors for type 2 diabetes include obesity, genetic predisposition, physical inactivity, history of gestational diabetes, impaired glucose tolerance, race, and ethnicity. Patients with type 2 diabetes often also suffer from dyslipidemia in the form of high plasma triglycerides and low HDL-cholesterol levels, both considered risk factors for coronary heart diseases.[2] Current treatments for type 2 diabetes include biguanides, sulfonylureas, insulin formulations, α-glucosidase inhibitors, insulin sensitizers, and insulin secretagogues.[3] Two thiazolidinediones, rosiglitazone[4] and pioglitazone,[5] representing a novel class of insulin-sensitizing peroxisome proliferator-activated receptor γ (PPARγ) agonists have been in clinical use as antidiabetic drugs. Fibrates (e.g., fenofibrate and gemfibrozil), which lower triglycerides and elevate HDL levels, are weak PPARα agonists and have been in clinical use for the treatment of dyslipidemia. A dual PPARα/γ agonist that improves insulin sensitivity, lowers glucose, and corrects lipoprotein abnormalities would be of great interest as a drug for the treatment of type 2 diabetes. A number of PPAR agonists (α- and γ-selective; α/γ dual) have been or currently are in clinical development.[6−9] AZ-242[6] and KRP-297/MK-767[7] are PPARα/γ dual agonists, while GI-262570/farglitazar[9] is a selective PPARγ agonist and GW-9578[10] is a PPARα-selective agonist. In this paper we describe the design, synthesis, and *in vivo* characterization of **2**, a novel, non-thiazolidinedione PPARα/γ dual agonist that shows promise for the treatment of type 2 diabetes and the associated dyslipidemia.

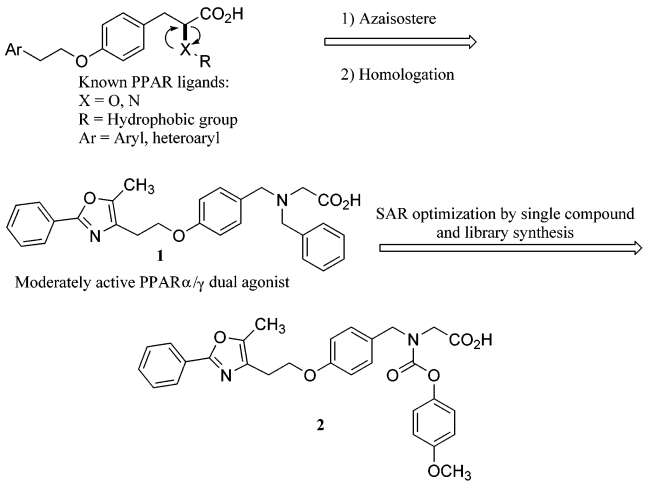

Figure 1. Design of oxybenzylglycines.

Our initial lead, the oxybenzylglycine **1**, was conceptually derived via homologation of a novel azaisostere structure drawn from known α-alkoxy and α-aminoarylpropanoic acid PPAR ligands (Figure 1).[6,9,11,12] Significant advantages gained from this change include the elimination of a chiral center, simplification of synthesis, and opportunities for rapid generation of diversity from the lead structure. Thus, exploration of the replacement of the *N*-benzyl moiety of **1** was carried out in an effort to optimize the relatively weak *in vitro* potency of **1**. One of the approaches involved the synthesis of a set of carbamate acids, facilitated by iterative solution-phase parallel synthesis. Several of these carbamate acids showed promising oral *in vivo* activity, and **2** was identified from this effort as an optimized candidate for

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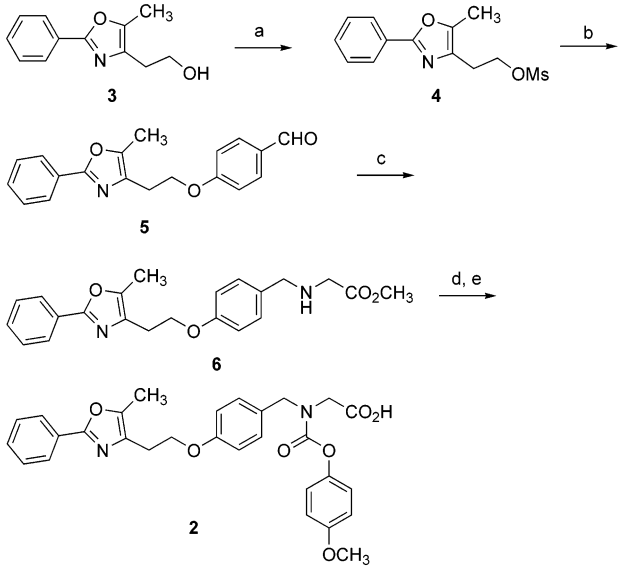
10.1021/jm0496436 CCC: $30.25
Published on Web 11/13/2004

Scheme 1. Synthesis of **2**[a]



[a] (a) $MeSO_2Cl$, Et_3N, CH_2Cl_2, 25 °C, 1 h, >98%; (b) 4-hydroxybenzaldehyde, K_2CO_3, CH_3CN, 95 °C, 24 h, 71%; (c) $H_2NCH_2CO_2Me{\cdot}HCl$, Et_3N, 4 Å molecular sieves, anhydrous $MgSO_4$, MeOH, 25 °C, 12 h; $NaBH_4$, 25 °C, 3.5 h, 80%; (d) 4-methoxyphenyl chloroformate, Et_3N, CH_2Cl_2, 25 °C, 2 h, 98%; (e) $LiOH{\cdot}H_2O$, THF/H_2O (1:1), 25 °C, 2.5 h, 94%.

Table 1. *In Vitro* Receptor Binding and Transactivation Activity Profile of **2**[a]

	hPPARα[b]		hPPARγ[b]	
	IC_{50}	EC_{50}	IC_{50}	EC_{50}
1	1.4	0.02 (103%)	4.8	1.5 (111%)
2	0.25 ± 0.08	0.32 ± 0.1 (70 ± 4%)[b]	0.19 ± 0.07	0.11 ± 0.06 (82 ± 4%)[b]
rosiglitazone	>100 μM	>32 μM	0.25	0.14 (100%)
GW-2331	0.538 ± 0.165	<0.02 (102 ± 3%)	0.316 ± 0.126	0.425 ± 0.07 (64 ± 3%)
fenofibric acid	>10.0	>10.0 (30%)	>25 μM	>100 μM

[a] IC_{50} and EC_{50} are in μM and are defined in the Experimental Section. [b] Intrinsic activity at 1 μM relative to a primary standard (at 1 μM), expressed as a percentage, is represented in parentheses. GW-2331[14] and rosiglitazone[15] were used as primary standards for PPARα and PPARγ activity, respectively.

in vivo efficacy and ADME profiling. Scheme 1 describes a scalable preparation of **2**.

Chemistry

Alkylation of 4-hydroxybenzaldehyde with the phenyloxazole mesylate **4**, prepared readily from commercially available alcohol **3**, yielded aldehyde **5**, which was reacted with glycine methyl ester under standard reductive amination conditions[13] to provide secondary amine **6** in excellent yield. Reaction of amine **6** with 4-methoxyphenyl chloroformate followed by hydrolysis of the methyl ester afforded **2** in 94% yield.

Results and Discussion

As shown in Table 1, **2** binds with high affinity to PPARα (IC_{50} = 250 nM) and PPARγ (IC_{50} = 190 nM). In transactivation assays **2** shows potent and novel functional activity at the full-length human receptors for PPARα (EC_{50} = 320 nM; intrinsic activity at 1 μM is 70%) and PPARγ (EC_{50} = 110 nM; intrinsic activity at 1 μM is 82%). Thus, **2** shows functional activity comparable to that of rosiglitazone at PPARγ and is considerably more potent than the fibrates (e.g., fenofibric acid) at PPARα. There was no cross-reactivity against other nuclear hormone receptors such as PPARδ, RXRα, RARs, ERα/β, AR, and PR. Furthermore, in a preadipocyte differentiation assay (Table 2), which measures the extent of predominantly PPARγ-mediated differentiation of preadipocytes into triglyceride-loaded adipocytes, **2** induced a 3-fold increase in differentiation, indicating potent activation of PPARγ. This level of activity was comparable to that of rosiglitazone, a known selective PPARγ agonist. WY-14643, a potent and selective PPARα agonist, was used as a negative control in this assay.

Table 2. *In Vitro* Transactivation Activity of **2** in 3T3L-1 Preadipocyte Cells (PPARγ)

compds	triglycerides in cell lysate (mg/dL)[a]
vehicle	15 ± 1
2 (α/γ)	46 ± 2
WY-14643 (α)	13 ± 2
rosiglitazone (γ)	48 ± 3

[a] 3T3L-1 cells were incubated in the presence of 1.0 μM test compound.

Table 3. Glycemic and Lipid Lowering Effects of **2** in Male *db/db* Mice

plasma parameters	vehicle (n = 10 mice/group)	**2** (10 mg kg^{-1} day^{-1}, 14 days)
glucose (mg/dL)	259.0 ± 24.0	120.0 ± 8.0 (−54%[a])
triglycerides (mg/dL)	124.0 ± 5.0	84.0 ± 7.0 (−33%[a])
free fatty acids (mequiv/L)	0.67 ± 0.07	0.25 ± 0.06 (−62%[a])
insulin (ng/mL)	3.9 ± 0.6	2.0 ± 0.2 (−48%[a])

[a] Difference between vehicle and drug treated groups; $p \leq 0.05$.

Table 4. Pharmacokinetic Profile of **2** in Male SD Rats[a]

	oral	intra-arterial
dose (mg kg^{-1})	10.0	5.0
AUC_∞ (μg h mL^{-1})	49.0 ± 7.3	27.7 ± 3.8
C_{max} (μg mL^{-1})	20.8 ± 2.4	
T_{max} (h)	0.4 ± 0.1	
$T_{1/2}$ (h)		7.3 ± 4.0
Cl (mL min^{-1} kg^{-1})		3.0 ± 0.4
V_{ss} (L kg^{-1})		0.6 ± 0.2
F (%)	88	

[a] n = 3.

An *in vivo* study of **2** was carried out in genetically obese male *db/db* mice (at a dose of 10 mpk/day for 14 days). Compound **2** was highly efficacious in this study, reducing levels of glucose (−54%, representing normalization), triglycerides (−33%, representing normalization), nonesterified fatty acids (−62%), and insulin (−48%) (Table 3). Glucose normalization with concomitant reduction in insulin levels suggests insulin sensitizing antidiabetic effects through PPARγ activation. Presumably, the triglyceride lowering effect is predominantly mediated through PPARα activation.

Compound **2** has an excellent ADME profile that is suitable for clinical development (Table 4). It has very good oral bioavailability in rats (88%), with a reasonable plasma $t_{1/2}$ of 7.3 h and low systemic clearance of 3 mL min^{-1} kg^{-1}. In male beagle dogs and cynomolgus monkeys, the oral bioavailability was 18% and 79%, respectively. The oral absorption was rapid, with T_{max} occurring at 0.6 h in both species. The corresponding C_{max} values were 0.12 μg/mL in dogs (1 mg/kg oral dose) and 15.7 μg/mL in monkeys (2 mg/kg oral dose).

In conclusion, **2** is a potent, novel non-thiazolidinedione PPARα/γ dual agonist *in vitro* that demonstrates

highly efficacious glucose and lipid lowering activities *in vivo* along with an excellent ADME profile. Compound **2** is currently in clinical development for the treatment of diabetes and associated dyslipidemia. PPARα/γ dual agonists such as **2** may also be of utility in establishing a therapeutic modality for the treatment of metabolic syndrome (which is characterized by impaired glucose tolerance, hyperinsulinemia, dyslipidemia, and hypertension).

Experimental Section

[(4-Methoxyphenoxycarbonyl)-{4-[2-(5-methyl-2-phenyloxazol-4-yl)ethoxy]benzyl}amino]acetic Acid (2). To a 10 °C solution of amine **6** (16.6 g, 43.7 mmol) in 200 mL of CH_2Cl_2 were successively added Et_3N (9.13 mL, 65.5 mmol) and 4-methoxyphenyl chloroformate (7.65 mL, 50.2 mmol) dropwise while maintaining the reaction temperature at ≤12 °C. The mixture was allowed to warm to 25 °C and stirred for 2 h. Volatiles were removed in vacuo, and the residue was chromatographed (SiO_2; 4:1 to 1:1 hexanes/EtOAc) to afford **2** methyl ester (22.8 g, 98%) as a colorless, viscous oil.

To a solution of the above methyl ester (18.75 g, 35.4 mmol) in THF (275 mL) was added a solution of $LiOH·H_2O$ (4.44 g, 105.9 mmol) in 275 mL of H_2O. The resulting mixture was stirred at 25 °C for 2.5 h, after which the pH was adjusted to ∼4 with 1 N aqueous HCl. The THF was removed in vacuo, the residue was diluted with 800 mL of EtOAc, and the mixture was stirred for 0.5 h at 25 °C. The organic phase was separated, dried ($MgSO_4$), and concentrated in vacuo to afford a solid, which was recrystallized from EtOH to give **2** (8.41 g) as a colorless solid. An additional 8.76 g of product was recovered in two subsequent recrystallizations from the mother liquor to give **2** (17.2 g total) in 94% yield. Mp: 139.1−140.2 °C. ^{1}H NMR (400 MHz, $CDCl_3$): rotamers, δ 2.38/2.39 (s, 3H, oxazole-C*H*$_3$), 3.00/3.01 (s, 2H, oxazole-C*H*$_2$), 3.77/3.78 (s, 3H, ArOC*H*$_3$), 4.02 (s, 2H, −C*H*$_2$COOH), 4.20−4.24 (m, 2H, −CH$_2$C*H*$_2$OAr), 4.55 (s, 1H, ArC*H*$_a$H$_b$N), 4.65 (s, 1H, ArCH$_a$*H*$_b$N), 6.8−7.0 (m, 4H), 7.0−7.1 (m, 2H), 7.2−7.3 (m, 2H), 7.40−7.45 (m, 3H), 7.96−7.98 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): rotamers, δ 10.2 (oxazole-*C*H$_3$), 26.0 (oxazole-*C*H$_2$−), 47.3/47.5 (*C*H$_2$COOH), 51.0/51.2 (Ar*C*H$_2$N), 55.6 (ArO*C*H$_3$), 66.7 (oxazole-CH$_2$*C*H$_2$OAr), 114.3, 114.8, 122.5, 126.0, 127.3, 128.4, 128.7, 129.2, 129.9/130.1, 132.4, 144.8, 145.3, 155.4/155.5, 157.1, 158.5, 159.7, 173.0. Anal. Calcd for $C_{29}H_{28}N_2O_7$: C, 67.43; H, 5.46; N, 5.42. Found: C, 67.45; H, 5.47; N, 5.29. IR (KBr): 2800−3200 (w, br), 1724 (vs), 1511 (s), 1197 (s), 1171 (s) cm^{-1}. UV (MeOH, 13.9 mg/L): λ_{max} 224, 277, 282 (sh), 290 (sh), 302 (sh) nm. LRMS (M + H$^+$): 517.19. HRMS Calcd for $C_{29}H_{29}N_2O_7$: 517.1975. Found: 517.1964.

Acknowledgment. The authors thank Dr. Carl Decicco and Dr. Robert Zahler for proof-reading the manuscript.

Supporting Information Available: Descriptions of PPARα and -γ binding and transactivation assays, preadipocyte differentiation assay, *in vivo* pharmacology; detailed experimental procedures, physical data, elemental analyses, IR, UV, ^{1}H, ^{13}C NMR, LRMS, and HRMS data for **5** and **6**; and synthesis procedure, ^{1}H NMR, and LRMS data for **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

Note Added after ASAP Publication. This manuscript was released ASAP on 11/13/2004 with an incomplete author byline, with errors in the EC_{50} values in the abstract, and with a labeling error in Scheme 1. The correct version was posted on 12/6/2004.

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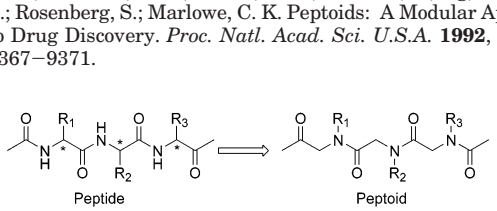

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JM0496436