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Discovery of tertiary aminoacids as dual PPARα/γ agonists-I

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Abstract—A novel series of potent dual agonists of PPARα and PPARγ, the alkoxybenzylglycines, was identified and explored using a solution-phase library approach. The synthesis and structure–activity relationships of this series of dual PPARα/γ agonists are described.

Peroxisome proliferator-activated receptors (PPARs)[1] are nuclear hormone receptors which act as transcription factors in the regulation of genes involved in glucose and lipid metabolism, and vessel wall function. They are, therefore, relevant targets in such disease areas as diabetes mellitus, obesity, inflammation, and atherosclerosis. PPARα (expressed highly in the liver and involved in fatty acid oxidation and lipoprotein metabolism) is the target of the fibrate class of hypolipidemic drugs such as fenofibrate[2] and gemfibrozil.[3] PPARγ (predominantly expressed in adipose tissue and implicated in insulin sensitization, glucose and fatty acid utilization as well as adipocyte differentiation) is the target of the thiazolidinedione (TZD)[4] class of antidiabetic drugs such as rosiglitazone[5] and pioglitazone.[6] It has therefore been hypothesized that a dual PPARα/γ agonist that would improve insulin sensitivity, lower glucose and correct lipid abnormalities would be highly beneficial for the treatment of type 2 diabetes and associated dyslipidemia. The clinical utility of dual PPARα/γ agonists has been demonstrated in patients with type 2 diabetes.[7]

In this paper, we report on the initial SAR of the novel alkoxybenzylglycines **1** and **2**, which are shown to be PPAR ligands with potent agonist activities at both PPARα and PPARγ. The concept and design of the alkoxybenzylglycines as PPAR ligands has been discussed in an earlier publication.[8] We decided to initiate our studies on exploring the alkoxybenzylglycine chemotype through the synthesis of tertiary aminoacid analogs with a two-carbon linker, commonly used in the PPAR literature, between the phenyloxazole and the central phenyl ring.

The initial lead compound, benzylamine **2a** (Table 2), displayed modest binding affinity to and promising functional activity at both PPARα and PPARγ. We decided to explore the SAR of this initial lead compound through the preparation of two solution-phase libraries, as described below.

The key intermediate aminoacids **8** and **9** were prepared in good yield, respectively, from 3- and 4-hydroxybenzaldehyde via: (1) reductive amination[9] of **6** and **7** with glycine *tert*-butyl ester/$NaBH_4$ and (2) acidic deprotection of the *tert*-butyl ester (Scheme 1).

Keywords: PPAR; PPAR α/γ dual agonists; Antidiabetic; Oxybenzylglycines.
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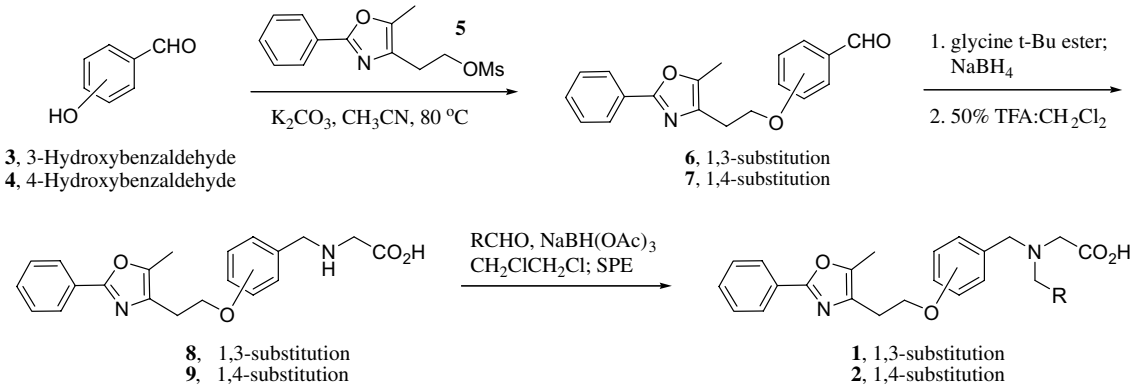


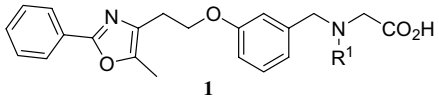
Scheme 1. Synthesis of secondary amines.

We began our SAR studies by synthesizing a number of tertiary amine analogs individually via reductive amination of the corresponding methyl or *tert*-butyl esters of **8** or **9**.[10a] Subsequently, solution-phase libraries of tertiary amines **1** and **2** in both the 1,3- and 1,4-oxybenzylglycine series, respectively, were synthesized by reductive amination of aminoacids **8** and **9** with a diverse set of aromatic, heteroaromatic and aliphatic aldehydes in a parallel format. It is notable that satisfactory purification of the crude reductive amination products was achieved using solid-phase extraction (anion-exchange SAX cartridges).[10b]

A number of tertiary amino acids in the 1,3-substituted alkoxyphenyl series **1** are potent PPARα-selective agonists (**1a**, **1c**, **1e**, **1g**, **1h**, and **1i**). Compounds **1b** and **1d** (Table 1, R^1 = 2-phenylethyl and 4-phenoxybenzyl, respectively) both showed relatively equivalent activity at PPARα and PPARγ (<3-fold difference). Moreover, compound **1d** is 2-fold less potent than rosiglitazone at PPARγ [EC_{50} = 67 nM; EC_{50} (rosiglitazone) = 35 nM] and 2-fold more potent than the reference PPARα/γ dual agonist GW-2331 at PPARα [**1d** EC_{50} = 30 nM; EC_{50} (GW-2331) = 71 nM]. Overall, analogs with an arylalkyl group (e.g., benzyl, heteroarylmethyl) at R^1 showed comparable potencies in terms of PPARα and PPARγ binding affinity as well as functional activity. In the 1,3-alkoxybenzylglycine series **1**, increasing the linker length between the phenyl group and the glycine amine (analogs **1a**–**c**) results in only a modest change in binding or functional activity even though **1c** displays a PPARα EC_{50} which is 19-fold more potent than its PPARγ EC_{50}. Annulation of the *N*-benzyl analog (**1a**) to provide the 1-naphthylmethyl compound **1g** resulted in increased binding and functional activity at both receptors with nearly equivalent activity in their ability to differentiate 3T3L1 preadipocytes. However, the regioisomeric 2-naphthylmethyl analog (**1h**) showed decreased transactivation activity at both receptors.

The 1,4-alkoxyphenyl analog **2a** (Table 2) was considerably less potent (both in terms of binding affinity and EC_{50} values in the PPARα and PPARγ transactivation assays) than its 1,3-alkoxyphenyl counterpart **1a**. Surprisingly, unlike corresponding literature analogs in the α-alkoxy propanoic acid series,[11] tertiary amines

Table 1. In vitro activities against PPARγ and α in the 1,3-substituted series (**11**)[14]

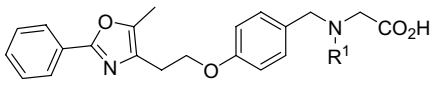


1

Compound	R^1	PPARγ IC_{50} (μM)	HEK (PPARγ) EC_{50} (μM) γK_{act} (% at 1 μM)	K_{act} 3T3L1 (PPARγ) (%)	PPARα IC_{50} (μM)	HEK (PPARα) EC_{50} (μM) αK_{act} (% at 1 μM)
	GW2331[15]	0.670	0.249 ± 0.238 (90%)	105	0.348	0.071 ± 0.035 (79%)
	Rosiglitazone	0.256	0.035 ± 0.0118 (108%)	101	Inactive	Inactive
1a	Benzyl[10a]	0.240	0.295 ± 0.1880 (82%)	104	0.090	0.022 ± 0.0063 (72%)
1b	2-Phenylethyl[10a]	0.134	0.108 ± 0.0718 (102%)	147	0.075	0.044 ± 0.0346 (82%)
1c	3-Phenylpropyl[10a]	0.773	0.901 ± 0.2229 (43%)	118	0.051	0.047 ±0.0080 (62%)
1d	4-Phenoxybenzyl[10a]	0.23	0.067 ± 0.0427 (73%)	116	0.11	0.030 ± 0.0184 (67%)
1e	3-Phenoxybenzyl[10a]	0.11	0.359 ± 0.0515 (90%)	115	0.083	0.062 ± 0.0641 (95%)
1f	4-Benzyloxybenzyl[10b]	0.183	0.412 ±0.0701 (59%)	88	0.387	0.324 ± 0.1679 (56%)
1g	1-Naphthylmethyl[10a]	0.067	0.036 ± 0.0105 (115%)	92	0.034	0.005[a] (85%)
1h	2-Naphthylmethyl[10a]	0.145	1.39 ± 0.4374 (49%)	94	0.149	0.313 ± 0.1080 (65%)
1i	1*H*-indol-2-ylmethyl[10a]	0.540	0.759 ± 0.3733 (58%)	ND[b]	0.246	0.197 ± 0.0966 (62%)
1j	1-[2,2′]Bithiophenyl-5-ylmethyl[10a]	0.101	0.082[a] (32%)	ND[b]	0.080	0.153[a] (27%)

[a] No dose–response or the curve did not top out in 2 other assay runs.
[b] ND, not determined.

Table 2. In vitro activities against PPARγ and α in the 1,4-substituted series (**2**)[14]



2

Compound	R^1	PPARγ IC_{50} (μM)	HEK (PPARγ) EC_{50} (μM) γK_{act} (% at 1 μM)	K_{act} 3T3L1 (PPARγ) (%)	PPARα IC_{50} (μM)	HEK (PPARα) EC_{50} (μM) αK_{act} (% at 1 μM)
	GI 262570 (Farglitazar)	0.217	0.0006 ± 0.0007 (99%)	112	2.87	0.321 ± 0.0783 (59%)
2a	Benzyl[10a]	1.77	3.6[c] (28%)	125	1.4	4.02[c] (24%)
2b	*n*-Heptyl[10b]	2.30	0.136 ± 0.0821 (66%)	87	1.05	0.180 ± 0.0936 (63%)
2c	2-Ethyl-1-butyl[10a]	Inactive	1.8 ± 0.3748 (32%)	ND[a]	ND[a]	8%[c]
2d	2-Benzyloxyethyl[10b]	4.56	1.18 ± 0.3394 (41%)	154	1.8	0.726 ± 0.0697 (56%)
2e	Benzo[1,3]dioxol-5-yl-methyl[10b]	3.29	0.307 ± 0.1358 (72%)	140	1.8	0.128 ± 0.0760 (77%)
2f	3-Phenoxybenzyl[10b]	0.48	0.391 ± 0.0771 (78%)	117	0.088	0.004 ± 0.0003 (107%)
2g	4-Phenoxybenzyl[10b]	0.227	0.052 ± 0.0289 (70%)	152	0.97	0.035 ± 0.0088 (59%)
2h	4-Benzyloxybenzyl[10b]	0.284	0.221 ± 0.0345 (82%)	40	1.05	0.189 ± 0.1535 (106%)
2i	1-Naphthylmethyl[10b]	0.767	0.840 ± 0.7487 (63%)	73	0.14	0.021 ± 0.0111 (90%)
2j	2-Naphthylmethyl[10a]	1.21	1.21 ± 0.05484 (29%)	108	0.68	0.618 ± 0.1654 (39%)
2k	2-Pyridylmethyl[10b]	2.9	0.230 ± 0.0877 (20%)	ND[a]	Inactive at 25 μM	0.692 ± 0.3196 (12%)
2l	5-(2-Chloro-phenyl)-furan-2-yl-methyl[10a]	0.280	0.267 ± 0.1580 (77%)	134	1.8	0.871 ± 0.4624 (55%)
2m	1-[2,2′]Bithiophenyl-5-ylmethyl[10a]	0.102	0.060 ± 0.0369 (44%)	ND[a]	Inactive at 98 μM[b]	0.078 ± 0.0356 (38%)

[a] ND, Not determined.
[b] Poor solubility in test medium.
[c] No dose–response or the curve did not top out in 2 other assay runs.

with nonaromatic R^1 groups (linear, branched alkyl, and cycloalkyl, for example **2b**, R^1 = *n*-hexyl and **2c**, R^1 = 2-ethyl-1-butyl) showed poor PPARα and PPARγ functional activity in this new oxybenzylglycine chemotype. Alkyl substitutions in the 1,3-alkoxybenzylglycine core were, therefore, not explored. Overall, among these two libraries, two of the most promising compounds in vitro were the *N*-4-phenoxybenzyl analogs **1d** (PPARγEC_{50} = 67 nM; PPARαEC_{50} = 30 nM) and **2g** (PPARγEC_{50} = 52 nM; PPARαEC_{50} = 35 nM). Additionally, both compounds showed a superior ability to differentiate 3T3L1 pre-adipocytes into fat-loaded mature adipocytes (a PPARγ-specific activity) to the PPARγ agonist standard rosiglitazone. Due to their excellent in vitro potency, **1d** and **2g** were evaluated in *db*/*db* mice (10 mg/kg dosed po/qd for 2 weeks) for their antidiabetic in vivo activity (with muraglitazar[15b] being used as an internal reference standard). Both these compounds caused an insignificant change in glucose, triglyceride, free fatty acid, and insulin levels in this animal model. Postulating that the inactivity of **2g** (and possibly **1d**) in vivo was probably due to its poor permeability (Caco-2 (apical to basal) < 15 nm/s at pH 6.5 for **2g**) and poor metabolic stability (rate of metabolism = 0.16 nmol/min/mg; 46% remaining after 10 min incubation in mouse liver microsomes), we decided to evaluate **1a** which showed excellent permeability and good metabolic stability in mouse liver microsomes (Caco-2 = 251 nm/s at pH 5.5 and 225 nm/s at pH 7.4; rate of metabolism = 0.048 nmol/min/mg; % remaining:

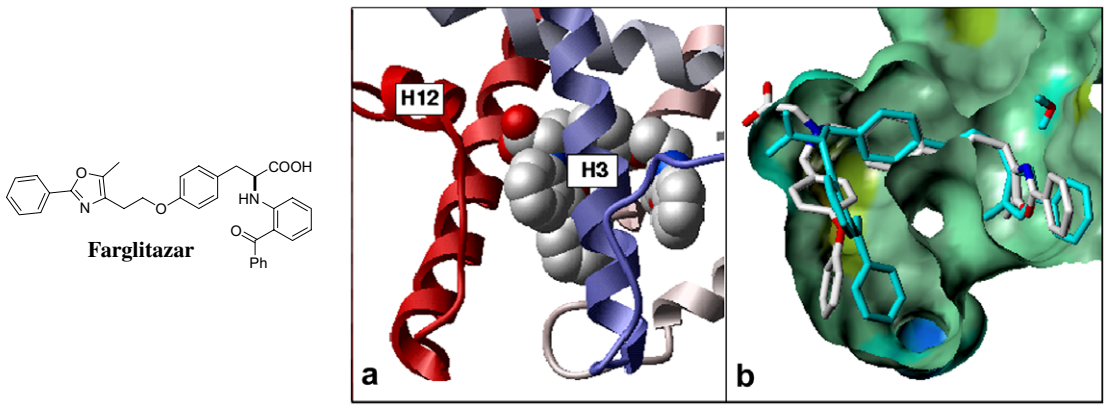


Figure 1. (a) CPK depiction of the published X-ray structure of farglitazar in PPARγ[12] (as part of a heterodimer with RXRα). The carboxylate group is positioned behind helices 3 and 12, while the pendant phenyloxazole group occupies a region behind and to the right of helix 3, forming a U-shaped conformer. (b) The modeled structure of analog **2g** is shown overlaid with farglitazar (in cyan). The carboxylate and phenyloxazole groups of **2g** and farglitazar occupy similar pockets.

Table 3. In vivo data in *db*/*db* mice

Compound		TG	Glucose	NEFA	Insulin
1a	% change	−29	−31	−22	−40
	P value	0.017	0.011	0.256	0.061
Mura	% change	−50	−50	−69	−67
	P value	0.000	0.000	0.000	0.005

84). Administration of **1a,** which is somewhat less active in vitro at PPARγ (EC_{50} = 295 nM), did result in good in vivo efficacy (Table 3).

In the 14-day *db*/*db* mouse model, **1a** (at 10 mg/kg/day) significantly decreased levels of fasted glucose (31%), triglycerides (29%), insulin (40%), and non-esterified fatty acids (22%).

A possible binding model of the alkoxybenzylglycine analog **2g** in PPARγ, which is based on its similarity to the published structure of the tyrosine-derived PPARγ-selective agonist farglitazar,[12] is illustrated in Figure 1. The modeling was conducted by minimizing structures of the ligand and PPARγ site residues using an Amber force field within the Flo molecular modeling program.[13] By allowing all residues in van der Waals contact with the ligand to co-minimize with the ligand, a molecular binding model was developed which is consistent with observed SAR. According to this model, the residues in electrostatic contact with the carboxylate group of **2g** represent a similar set to that found in the farglitazar structure, namely, S289, H323, Y327, H449, and Y473. In addition, as shown in Panel B, it is likely that a bridging water molecule exists which provides for a H-bond between the phenyloxazole moiety of the ligand and the backbone NH of S342. The lower hydrophobic region is occupied by the pendant diphenyl ether. Transactivation activity is thought to be modulated by the complex and critical conformational changes that helix 12 (H12) adopts to favor its interaction with co-activators and disfavor interactions with co-repressors. Functional activity differences, that is transactivation, may occur due to the manner in which the ligand contacts the bed of Phe residues lining the bottom of the hydrophobic pocket (Phe 282/H3), Phe 360/H7, and Phe 363/H7). These residues appear to make important contacts with other hydrophobic residues on H12, especially Phe 282 which is in close contact with Met 463/H12. Thus, the trajectories of ligand components into this region may be critical for mediating functional activity. For example, compounds **1g** and **1h,** representing regional isomers of a naphthylmethyl moiety in contact with Phe 282, exhibit similar binding affinities with differing degrees of transactivation. In the case of **1a** and **2a**, the extent of H12 contact with the pendant benzylic groups is slightly different between the 1,3 and 1,4 phenyl linkers, resulting in a significant difference in transactivation, while for the 4-phenoxybenzyl pair, **1d** and **2g,** the difference in transactivation disappears, presumably due to sufficient contact with H12 with the larger hydrophobic groups present in both compounds.

In conclusion, we have discovered a novel series of alkoxybenzylglycine dual PPARα/γ agonists as exemplified by the lead compound **2a**. A solution-phase library approach was used to rapidly explore the SAR in this series, which resulted in the discovery of: (1) analogs such as **1d** and **2g** which are potent agonists of PPARα and PPARγ, (2) **1a** as an analog which showed good oral antidiabetic and antidyslipidemic activity in vivo, and (3) several analogs with PPARα/γ profiles that can be used as tool molecules for PPAR research. Further work in the development of the SAR of this lead series based on the novel alkoxybenzylglycine core will be described in a subsequent communication.

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10. Characterized by a) ^{1}H NMR, LC–MS and HPLC; b) LC–MS with HPLC purity >80%. Typical procedure for the solution-phase library: To a solution of aminoacid **8** or **9** (0.074 mmol) in CH_2Cl_2 (2 mL) were added the substituted benzaldehyde (0.37 mmol), $NaBH(OAc)_3$ (0.37 mmol), and HOAc (0.1 mL). The reaction mixture was stirred for 15 h at rt. The product was purified via solid-phase extraction using a Varian SAX cartridge (3 g of sorbent in a 6 mL column, 0.3 mequiv/g) by the procedure outlined below: (a) The column was conditioned with MeOH (10 mL) and CH_2Cl_2 (20 mL), (b) the reaction mixture was loaded onto the SAX column, (c) the column was rinsed with CH_2Cl_2 (10 mL), (d) the column was rinsed with 1% TFA in MeOH (3 mL), and (e) the product was eluted with 1% TFA in MeOH (20 mL). The product solution was concentrated using a Speed Vac for 16 h to afford the desired crude product.
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14. a) IC_{50} and EC_{50} values are represented in micrometer and are averages of 2–6 determinations. IC_{50} values for PPARα and PPARγ were determined by calculating the amount of test compound required to cause half-maximal inhibition of the binding of a fluorescein-labeled PPARα or γ agonist to the PPARα or γ ligand-binding domain, respectively. The fluorescence polarization assay is described elsewhere (Seethala, R. et al. *Anal. Biochem.,* manuscript accepted). The EC_{50} value is defined as the concentration of the test ligand required to elicit 50% of the maximal fold induction of luciferase activity in HEK-293 cells transiently co-transfected with GAL4-PPARα or GAL4-PPARγ and stably co-transfected with the luciferase reporter gene. K_{act} (intrinsic activity) is defined as the activity of a ligand at 1 μM relative to the activity of the primary standards at 1 μM (GW2331[15] for PPARα and rosiglitazone for PPARγ, respectively) expressed as a percentage. None of the compounds tested showed any activity against PPARδ.
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