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Synthesis and Biological Activity of L-Tyrosine-based PPARγ Agonists with Reduced Molecular Weight

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Abstract—A series of PPARγ agonists were synthesized from L-tyrosine that incorporated low molecular weight *N*-substituents. The most potent analogue, pyrrole (**4e**), demonstrated a K_i of 6.9 nM and an EC_{50} of 4.7 nM in PPARγ binding and functional assays, respectively. Pyrrole (**4e**), which is readily synthesized from L-tyrosine methyl ester in four steps, also demonstrated in vivo activity in a rodent model of Type 2 diabetes.

Peroxisome proliferator-activated receptors (PPARs) have been the subject of intense research following the discovery of their physiological roles in the regulation glucose and lipid homeostasis.[1] PPARγ is the molecular target for the thiazolidinedione (TZD) class of insulin sensitizing antihyperglycemic agents. Two of the TZDs, pioglitazone and rosiglitazone (Fig. 1), are currently approved for treatment of Type 2 diabetes.[1]

We recently reported a series of potent tyrosine-based PPARγ agonists, which are exemplified by farglitazar and GW7845 (Fig. 1).[2] Although these compounds contain an asymmetric center, the more potent *S*-enantiomers are conveniently synthesized from naturally-occurring L-tyrosine.[2] Unlike the TZDs,[3] the tyrosine-based PPARγ agonists do not undergo racemization in vivo.[2] Our initial studies[2] suggested that an *N*-benzophenone or *N*-benzoate group was essential for PPARγ activity. As part of an ongoing program to develop new L-tyrosine-based PPARγ agonists, we decided to re-evaluate the structural requirements for the tyrosine *N*-substituents. In addition, a goal of the current study was to employ the X-ray crystal structure of PPARγ[4] in the design of molecules with reduced molecular weight compared to the first generation compounds (Fig. 1), while maintaining robust PPARγ agonist and in vivo antidiabetic activity.

The X-ray crystal structure of farglitazar bound to PPARγ showed that the benzophenone group was inserted into a lipophilic pocket, while the tyrosine–NH and benzophenone C=O formed an intramolecular hydrogen bond.[4] This intramolecular hydrogen bond reduces the basicity and the polarity of the tyrosine amino group.

Through a process of model building into the PPARγ crystal structure,[5] alternative *N*-substituents were selec-

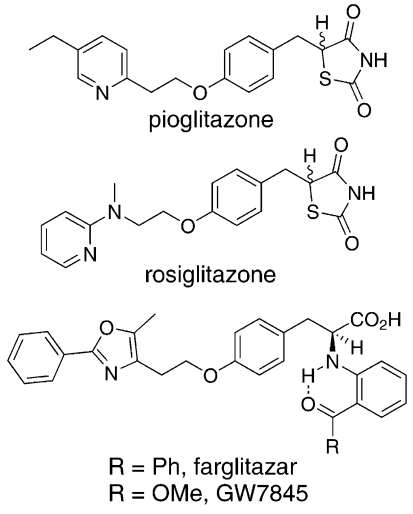

Figure 1. Structures of PPARγ agonists.

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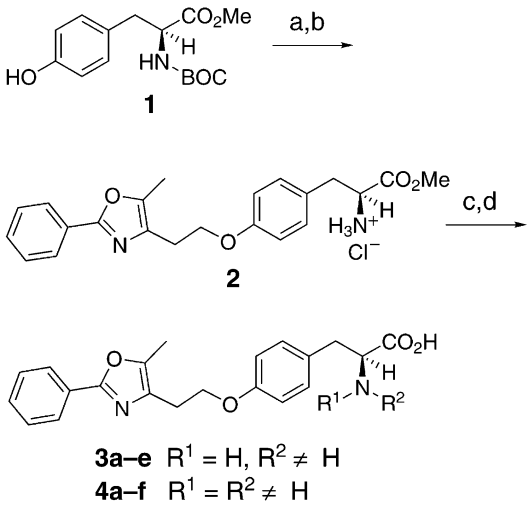
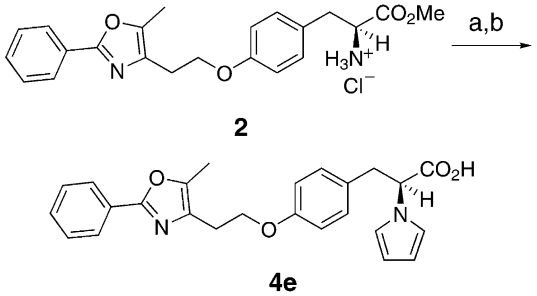
ted that would add a small lipophilic substituent while mimicking some of the effects of the intramolecular hydrogen bond present in the benzophenone analogue. Compounds were built within the PPARγ binding site by removal of the benzophenone group from farglitazar, and growth of alternative substituents onto the tyrosine nitrogen, using the MVP program.[5] The growth process, which generates multiple energy-minimized conformations, was carried out twice, once within the protein binding site and once in aqueous solution. A basicity adjustment was applied to the calculated binding energy to account for the fraction of the molecule in the neutral ionization state at pH 7.[6] The model building predicted that pyrrole, morpholino and *n*-alkyl derivatives would bind to PPARγ effectively at pH 7. Other derivatives, such as amide, piperidine and dimethylpyrrole, were predicted to be less potent due to their polarity, basicity or poor steric fit, respectively.

The synthesis of tyrosine analogues **3a–e** and **4a–f** is depicted in Scheme 1. Mitsunobu reaction between commercially available *t*-butoxycarbonyl (BOC)-protected L-tyrosine methyl ester **1** and 5-methyl-2-phenyl-4-oxazolylethanol[7] using triphenylphosphine and diisopropylazodicarboxylate in toluene was followed by removal of the BOC protecting group with 4 M HCl in dioxane to afford the amine hydrochloride **2** in 70% overall yield. Intermediate **2** was derivatized on the tyrosine nitrogen to provide the targeted set of analogues following saponification with LiOH (Scheme 1). *N*-Alkyl groups (**3a–b** and **4a**) were introduced by reductive alkylation with the requisite aldehydes. *N*-Cycloalkyl groups (**4b–d**) were introduced through alkylation with dibromoalkylating agents. Compounds **3c** and **3d** were prepared through acylation and sulfonylation, respectively. The pyrimidine compound **3e** was generated by aromatic nucleophilic substitution of 2-chloropyrimidine. Reaction of the free amine from **2** with 2,5-dimethoxytetrahydrofuran or with hexane-2,5-dione in acetic acid provided the pyrrole compounds **4e** and **4f**, respectively (Scheme 1). Presence of sodium acetate in the reaction mixture was found to greatly accelerate pyrrole formation and to be crucial for high yields. Unfortunately, significant racemization (up to 10%) occurred when the pyrroles were formed in acetic acid in the presence of sodium acetate as determined by chiral HPLC. Gratefully, use of a biphasic reaction conditions reported by Jefford[8] led to pyrrole formation without racemization (Scheme 2). Saponification with aqueous LiOH in dioxane or THF provided enantiomerically pure carboxylic acids. The enantiomer of **4e** was also synthesized from D-tyrosine. Use of pyrrole as a primary amine protecting group was first introduced by Bruekelman.[9] Pyrroles have been shown to be stable to strong bases, nucleophiles, and to brief contact with strong acids. Consistent with these reports, compounds **4e** and **4f** are stable to aqueous LiOH, acidic workup, and storage at room temperature. Notably, structurally related racemic pyrroles have been claimed to show in vivo antidiabetic activity.[10]



Scheme 1. Reagents and conditions: (a) 5-methyl-2-phenyl-4-oxazolylethanol, PPh_3, DIAD, $PhCH_3$, 79%; (b) 4 M HCl in dioxane, 100%; (c) **3a**: (i) 2-nitrobenzenesulfonyl chloride, Et_3N, CH_2Cl_2, 98%; (ii) MeI, Cs_2CO_3, DMF, 33%; (iii) thiophenol, K_2CO_3, acetonitrile, 62%; **3b**: CH_3CH_2CHO, $NaBH(OAc)_3$, CH_2Cl_2; **3c**: CH_3COCl, Et_3N, CH_2Cl_2, 98%; **3d**: $MeSO_2Cl$, Et_3N, CH_2Cl_2, 46%; **3e**: 2-chloropyrimidine, Na_2CO_3, EtOH, 15%; **4a**: HCHO, $NaCNBH_3$, HOAc, CH_3CN, 26%; **4b**: 1,5-dibromopentane, Na_2CO_3, EtOH, 42%; **4c**: 2-bromoethyl ether, Na_2CO_3, EtOH, 60%; **4d**: 1,4-dibromobutane, Na_2CO_3, EtOH, 47%; **4e**: 2,5-dimethoxytetrahydrofuran, HOAc, NaOAc, 61%; **4f**: hexane-2,5-dione, HOAc, 36%; (d) LiOH, THF, H_2O, MeOH, 90–100%.

Compounds **3a–e** and **4a–f** were screened against the three human PPAR subtypes in binding and cell-based reporter assays (Table 1). The details of both the binding[2,11] and the functional assays[2] have been reported previously. The compounds (**3–4**) displayed subtype-selectivity for PPARγ over PPARα and PPARδ. However, the selectivity ranged from 3.5-fold with alkyl amine (**3b**) to >700-fold with pyrrole (**4e**) in the cell-based assay. Variation in the polarity of the *N*-substituent was found to have a dramatic effect on the PPARγ activity. As predicted, the acetyl (**3c**) and sulfonyl (**3d**) groups were weak PPARγ agonists with EC_{50} >1 μM, suggesting that polar functionality is poorly tolerated. The *N*-propyl amine (**3b**) and *N,N*-dimethyl amine (**4a**) were more potent than the corresponding mono-methyl analogue **3**, showing that increased lipophilicity was beneficial.



Scheme 2. Reagents and conditions: (a) 2,5-dimethoxytetrahydrofuran, 1,2-dichloroethane, H_2O, 77%; (b) LiOH, dioxane, H_2O, 98%.

Table 1. Activity of tyrosine-based PPAR agonists

Compd	R^1	R^2	Binding affinity, K_i (nM)[a]			Functional activity, EC_{50} (nM)[b]		
			PPARα	PPARγ	PPARδ	PPARα	PPARγ	PPARδ
Pioglitazone			—	630	—	—	580	—
Rosiglitazone			—	120	—	—	43	—
Farglitazar	H	2-(COPh)Ph	490	1.1	1200	450	0.34	—
GW7845	H	2-(CO_2Me)Ph	1100	3.7	1200	3500	0.71	—
3a	H	Me	—	2100	—	—	5000	—
3b	H	*n*-Pr	72	52	—	830	240	—
3c	H	MeCO	—	2600	—	—	3500	—
3d	H	$MeSO_2$	—	620	—	—	2600	—
3e	H	2-pyrimidine	1200	280	—	—	590	—
4a	Me	Me	—	850	—	—	410	—
4b	–$CH_2CH_2CH_2CH_2CH_2$–		—	480	—	—	138	—
4c	–$CH_2CH_2OCH_2CH_2$–		—	158	—	6900	24	—
4d	–$CH_2CH_2CH_2CH_2$–		—	360	—	—	390	—
4e	–CH=CHCH=CH–		560	6.9	2500	3600	4.7	3800
ent-4e	–CH=CHCH=CH–		2600	51	—	1300	20	—
4f	–(Me)CH=CHCH=CH(Me)–		—	980	—	—	710	—

[a]Compounds were assayed for their ability to bind to human PPAR ligand binding domains by scintillation proximity assay.[2,11] The K_i value was calculated according to the equation $K_i = IC_{50}/(1+[L]/K_d)$, where IC_{50} is the concentration of test compound required to inhibit 50% of the specific binding of the radioligand, [L] is the concentration of the radioligand used, and K_d is the dissociation constant for the radioligand at the receptor.
[b]Compounds were assayed for agonist activity on human PPAR-GAL4 chimeric receptors in transiently transfected CV-1 cells as described;[2] EC_{50} = the concentration of test compound that gave 50% of the maximal reporter activity; all data are ±20% for $n=3$; —inactive at 3 μM in the binding assay or 10 μM in the functional assay.

The basicity of the tyrosine nitrogen was a key factor that affected the potency on PPARγ, with compounds designed to reduce the nitrogen basicity demonstrating improved potency in the binding and functional assays. Thus, the pyrimidine **3e** had an EC_{50} of 590 nM, while the morpholinyl derivative **4c** was more potent than the isosteric cycloalkyl analogue **4b**. The pyrrolidinyl compound **4d** showed similar PPARγ potency to its acyclic analogue **4a**, but pyrrole **4e**, which reduces the basicity of the tyrosine nitrogen, was a potent PPARγ agonist with EC_{50} of 4.7 nM in the functional assay and a K_i of 6.9 nM in the binding assay. 2,5-Dimethyl pyrrole **4f** was less potent than its unsubstituted analogue **4e**, most likely due to an unfavorable steric interaction with the receptor. Finally, pyrrole **4e** (synthesized from L-tyrosine) was 10 times more potent at PPARγ and 5 times more potent at PPARα than its enantiomer **ent-4e** (synthesized from D-tyrosine). The higher activity of pyrrole **4e**, with the *S*-configuration, is consistent with the enantioselectivity of the first generation tyrosine-based compounds.[2]

The in vivo antihyperglycemic and antihyperlipidemic efficacy of **4e** were evaluated in male Zucker diabetic fa/fa rats ($n=6$). When dosed orally at 10 mg/kg bid for 14 days, the pyrrole (**4e**) reduced fasting plasma glucose by 40%, and fasting serum triglycerides by 24%, while HDL-cholesterol was raised by 31% compared to vehicle-treated animals.

In summary, the pyrrole (**4e**, MW = 416) was identified as a potent L-tyrosine-based insulin sensitizer with significantly lower molecular weight than the 1st generation compounds farglitazar (MW = 546) and GW7845 (MW = 500). The low basicity of the pyrrole *N*-substituent in **4e** may be key to its potent PPARγ activity.

Acknowledgements

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