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Rational design of a pirinixic acid derivative that acts as subtype-selective PPARγ modulator

Theresa M. Thieme [a], Ramona Steri [a], Ewgenij Proschak [a], Alexander Paulke [a], Gisbert Schneider [b], Manfred Schubert-Zsilavecz [a,*]

[a] Institute of Pharmaceutical Chemistry, ZAFES/LiFF/FIRST, Goethe-University Frankfurt, Max-von-Laue-Str. 9, 60438 Frankfurt, Germany
[b] Institute of Pharmaceutical Sciences, ETH Zurich, Wolfgang-Pauli-Str. 10, 8093 Zurich, Switzerland

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ABSTRACT

Peroxisome proliferator-activated receptor γ (PPARγ) is involved in glucose and lipid homeostasis. PPARγ agonists are in clinical use for the treatment of type 2 diabetes. Lately, a new class of selective PPARγ modulators (SPPARγMs) was developed, which are believed to show less side effects than full PPARγ agonists. We have previously shown that α-substitution of pirinixic acid, a moderate agonist of PPARα and PPARγ, leads to low micromolar active balanced dual agonists of PPARα and PPARγ. Herein we present modifications of pirinixic acid leading to subtype-selective PPARγ agonists and furthermore the development of a selective PPARγ modulator guided by molecular docking studies.

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear hormone receptor superfamily. Three PPAR isotypes have been identified, which are involved in the regulation of glucose and lipid homeostasis.[1–3] PPARα is mainly found in the liver and plays a pivotal role in the regulation of cellular uptake, activation and β-oxidation of fatty acids and in lipoprotein metabolism.[4,5] PPARγ is expressed primarily in adipose tissue and acts as a transcription factor regulating adipocyte differentiation and glucose homeostasis,[6–8] while PPARδ/β is expressed in most cells and plays a role in the regulation of glucose and lipid metabolism.[4,9,10] There are currently no important drugs in clinical use which target PPARδ/β, whereas PPARα and PPARγ agonistic drugs are the most prominent targets for the treatment of metabolic diseases. PPARα agonistic fibrate-type drugs are used in the therapy of several forms of dyslipidemia, PPARγ agonists such as rosiglitazone and pioglitazone are anti-diabetic drugs. Recently dual PPARα/γ agonists were developed, to combine the positive therapeutic effects of both classes and to decrease side effects.[11] However, some of these dual PPARα/γ agonists were dropped from late clinical development due to incidence of edema, heart failure or cardiovascular complications.[12] Thus, it is necessary to develop new drugs with a safer pharmacological profile and less side effects. Selective PPARγ modulators (SPPARγMs), which are believed to bind in a distinct manner to the ligand binding pocket of PPARγ were developed to overcome the side effects of previous therapeutic approaches.[13] This leads to a tissue- and promoter-selective gene expression due to differential cofactor displacement and recruitment to the receptor.[14–16]

Halofenate is known as a hypolipidemic and hypouricemic agent.[17–19] In patients with type 2 diabetes mellitus a significant decrease in fasting plasma glucose was observed in addition to triglyceride and uric acid lowering effects. In vitro and preclinical data had previously demonstrated that halofenate acts as SPPARγM.[20] In vivo halofenate is rapidly and completely modified to its mature circulating free acid form halofenic acid. In vitro studies demonstrated that halofenic acid directly binds to the ligand binding domain of human PPARγ and selectively activates PPARγ with a maximal activation of 10–15% and an EC_{50} of 10–20 μM compared to rosiglitazone. Similar to other known PPARγ modulators, halofenic acid has a weak adipogenic potential and inhibits rosiglitazone-mediated human preadipocyte differentiation.[20]

Pirinixic acid (**1**) is known as moderately active dual agonist of PPARα and PPARγ.[21] We have previously demonstrated that aliphatic substitution in α-position of the carboxylic acid head group of pirinixic acid leads to more potent dual PPARα/γ agonists with a significant preference towards PPARα.[22] Compound **2** with a hexyl residue in α-position is a full agonist of PPARα and γ with an EC_{50} of 1.0 μM for PPARα and 3.6 μM for PPARγ.[22,23]

* Corresponding author. Tel.: +49 69 798 29339; fax: +49 69 798 29332.
E-mail address: schubert-zsilavecz@pharmchem.uni-frankfurt.de (M. Schubert-Zsilavecz).

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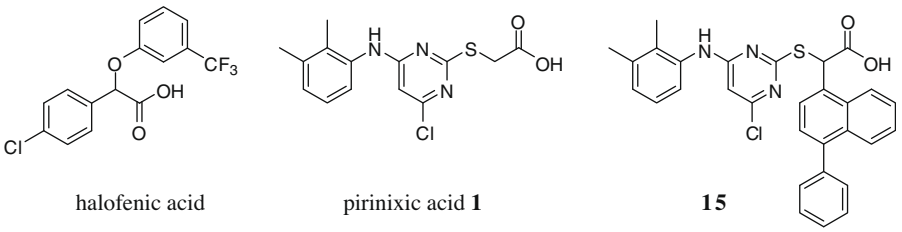


halofenic acid pirinixic acid **1** **15**

Herein, we present a rational approach to turn dual PPARα/γ agonists into selective PPARγ agonists and reduce the maximal activation of PPARγ without loss of potency. We introduced aromatic residues in the α-position guided by molecular docking experiments. Structure–activity-relationship (SAR) studies of PPAR agonists have shown that bulky aromatic residues attached to α-position of the carboxylic group of PPAR modulators turn the selectivity profile towards PPARγ preferential compounds.[22] Molecular docking experiments using the GOLD 4.0 software[24] were performed to predict the binding mode of compound **3** to the binding site of PPARγ-LBD, co-crystallized with the full agonist farglitazar (PDB ID: 1fm9).[25] In order to investigate the selectivity of compound **3** the obtained predicted structure was superposed to PPARα binding domain (PDB ID: 2p54)[26] using PyMOL software (PyMOL Molecular Graphics System v. 0.99, DeLano Scientific, Palo Alto, USA). Two structural differences in the left proximal pocket of PPAR are responsible for the preferential binding of compounds with bulky α-residues to PPARγ. H323 in PPARγ is replaced by Y314 in PPARα, which requires more space in the binding pocket (Fig. 1, left side). Additionally, a phenylalanine residue (F273/PPARα; F282/PPARγ) is displaced towards the binding site lumen in PPARα, leaving less space for the α-substituent. We exploited these structural differences and exchanged the α-hexyl moiety of compound **2** by a α-naphthyl residue (**3**).

Scheme 1 shows the synthesis of compounds **1**–**14** which was published already for compounds **1**–**3**.[22] Compounds **1**–**14** were

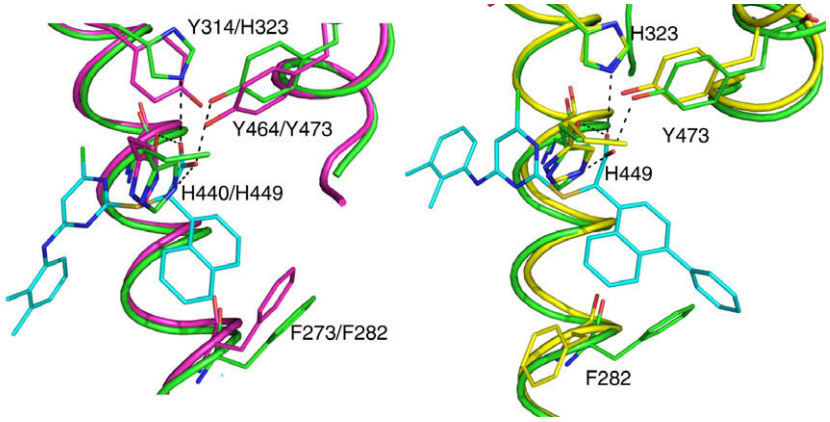
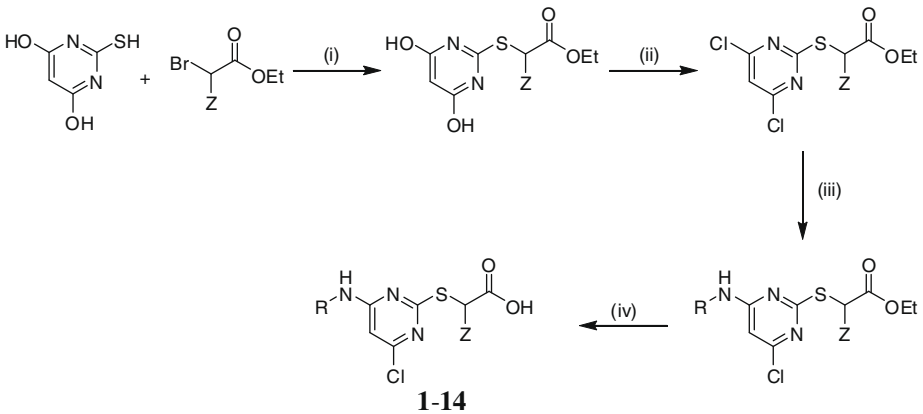
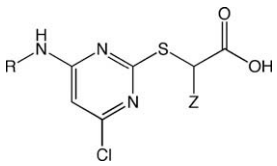


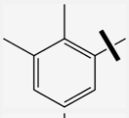
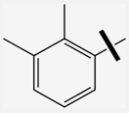
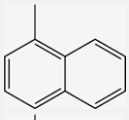
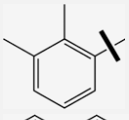
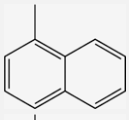
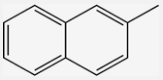
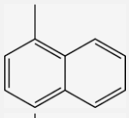
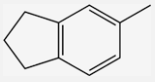
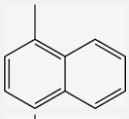
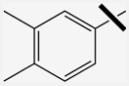
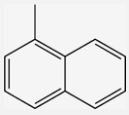
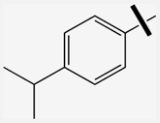
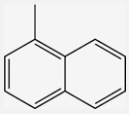
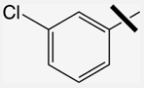
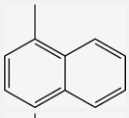
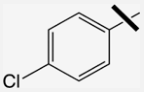
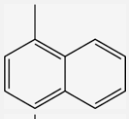
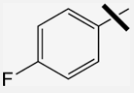
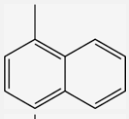
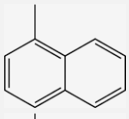
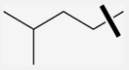
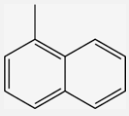
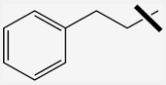
Figure 1. Potential binding modes of compounds **3** and **15** to the PPARγ binding site. Left side: compound **3** (cyan) docked to the ligand binding domain of PPARγ in a conformation induced by a full agonist (PDB ID: 1fm9,[25] green). The structure is superposed with the ligand binding domain of PPARα (PDB ID: 2p54,[26] purple). Right side: compound **15** (cyan) bound to the ligand binding domain of PPARγ in a conformation induced by a partial agonist (PDB ID: 3b3k,[29] yellow). The structure is superposed with the ligand binding domain PPARγ in a conformation induced by a full agonist (PDB ID: 1fm9,[25] green).



Scheme 1. Reagents and conditions: (i) triethylamine, DMF, rt to 80 °C, 1.5 h–1 d; (ii) $POCl_3$, *N*,*N*-diethylaniline, reflux, 2–3.5 h; (iii) R-NH_2, triethylamine, ethanol, reflux, 4–96 h; (iv) KOH, ethanol, rt to 80 °C, 1–24 h.

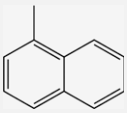
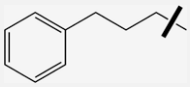
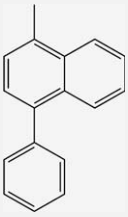
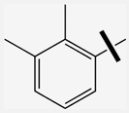
Table 1
In vitro transactivation of human PPARs by compounds **1–15**



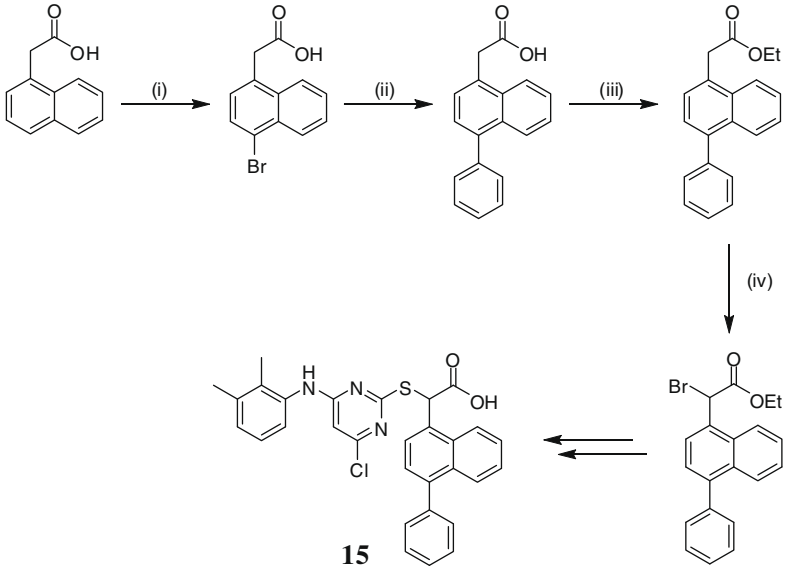
Compound	*Z*	R	Transactivation EC_{50} (μM) ± SD (% activation compared to control)			
			PPARγ		PPARα	
Pioglitazone			0.3 ± 0.05	(100%)	ia	
GW7647			ia		0.23 ± 0.02	(100%)
1	–H		53.7 ± 1	(79%)	36.3 ± 3	(100%)
2	-*n*-Hexyl		3.6 ± 0.2	(139%)	1.0 ± 0.2	(146%)
3			6.2 ± 0.3	(92%)	ia	
4			6.9 ± 0.1	(94%)	ia	
5			6.8 ± 0.3	(92%)	ia	
6			7.8 ± 0.2	(123%)	ia	
7			7.6 ± 0.1	(117%)	ia	
8			7.3 ± 0.3	(70%)	ia	
9			9.0 ± 0.7	(66%)	ia	
10			13.0 ± 0.3	(70%)	ia	
11		-*n*-Butyl	ia		ia	
12			8.9 ± 0.2	(80%)	ia	
13			10.8 ± 0.7	(78%)	ia	

(*continued on next page*)

Table 1 (*continued*)

Compound	Z	R	Transactivation EC_{50} (μM) ± SD (% activation compared to control)		
			PPARγ		PPARα
14			8.9 ± 0.2	(75%)	ia
15			4.5 ± 1.7	(45%)	ia

EC_{50} values were calculated using SigmaPlot2001 (Systat Software GmbH, Germany) based on the mean values of at least three determinations in a Gal4 hybrid transactivation assay for the respective PPAR subtypes. Values in brackets give the relative activation compared to the positive control (pioglitazone for PPARγ and GW7647 for PPARα each at 1 μM); ia: inactive at 10 μM. All substances were inactive at PPARδ at 10 μM (data not shown).



Scheme 2. Reagents and conditions: (i) bromine, glacial acetic acid, 60 °C, 16 h; (ii) phenylboronic acid, tetrakis(triphenylphosphine)-palladium(0), dioxane/water, 100 °C, 16 h; (iii) thionyl chloride, ethanol, 60 °C, 1 h; (iv) NBS, benzoyl peroxide, carbon tetrachloride, 90 °C, 16 h.

synthesized in a four step reaction modified from d'Atri et al.[27] The α-bromosubstituted ethyl esters were commercially available. For the reaction of thiobarbituric acid with the appropriate α-bromo-substituted acid ethyl esters (i) we used triethylamine in DMF instead of NaOH in H_2O/EtOH. The resulting thioether derivatives were treated with $POCl_3$ (ii). The obtained 4,6-dichloro-substituted pyrimidine derivatives were refluxed with the appropriate primary amine in EtOH (iii), using triethylamine. Finally the resulting esters were hydrolyzed with KOH in ethanol (iv) to give the desired carboxylic acids (**1**–**14**).

PPARγ activity of all synthesized compounds was screened by a cell-based reporter gene assay using the Dual-Glo Luciferase Assay System (Promega) as described previously[28] (Table 1). In brief, Cos7 cells were transfected with pFR-Luc (Stratagene), pRL-SV40 (Promega) and the Gal4 fusion receptor plasmids of the respective subtype (pFA-CMV-Gal4-DBD-hPPAR-LBD). Incubation with test compounds for 14 h leads to luminescence which could be read out with a GENios Pro luminometer (Tecan). Pioglitazone was used as positive control for PPARγ, GW7647 for PPARα, each at 1 μM. The activities of the screened compounds are given as percentages compared to the data obtained from 1 μM of the positive controls, with the resulting activity designated as 100%. Introducing a hexyl residue in α-position of the carboxylic acid head group (**2**) enhances PPARα and PPARγ potency to an EC_{50} of 1.0 μM and 3.6 μM, respectively with full activation of both isotypes. Insertion of a naphthalene residue in α-position of the carboxylic acid head group (**3**) led to a complete loss of PPARα activity, as expected from the molecular docking studies. Replacement of the 2,3-dimethyl-aniline residue of the naphthalene substituted pirinixic acid by different substituted aromatics led to subtype-selective PPARγ agonists which show no PPARα agonistic activity at 30 μM.

Implementation of bulkier aromatic residues (**4**, **5**), variation of the methyl groups (**6**) or insertion of isopropyl in *para*-position of the aniline (**7**) has no or only marginal consequence on PPARγ activity. While an insertion of halogen-substituted aniline residues led to a decrease of PPARγ activation between 60 and 70% with no effect on potency (**8**–**10**). An unsubstituted alkyl chain (**11**) resulted in a complete loss of activity while substitution with isopen-

tylamine (**12**), 2-phenethylamine (**13**) or 3-phenylpropylamine (**14**) had marginal impact on potency and decreased activation down to 75–80%.

Molecular docking studies suggest that substitution by a larger substituent in α-position of the carboxylic acid head group might cause a distinct binding manner of the ligand (Fig. 1). Comparison of the PPARγ ligand binding domain co-crystallized with farglitazar (PDB ID: 1fm9)[25] and a partial agonist (PDB ID: 3b3k)[29] reveals a conformational change of F282 (Fig. 1, right side).[30,29] We therefore expected that an elongation of the naphthyl group might reduce the maximal activation of PPARγ. The *para*-position of the naphthalene residue seemed to be suitable for the introduction of a bulky substituent that would possibly cause a conformational change of F282. Introducing a phenyl residue in *para*-position of the naphthalene actually led to a subtype-selective PPARγ modulator (**15**) with a transactivation of 45% and an EC_{50} of 4.5 μM (Table 1).

Scheme 2 shows the synthesis of compound **15**, which was done by bromination of 1-naphthylacetic acid in *para*-position of the naphthalene using bromine in glacial acetic acid (i). The next step (ii) was a Suzuki-coupling with phenylboronic acid and tetrakis(triphenylphosphine)-palladium(0) in a mixture of dioxane and water. It was followed by an esterification (iii) using thionyl chloride and ethanol. The bromination in α-position of the carboxylic acid head group was done with NBS (*N*-bromosuccinimide) and benzoyl peroxide in carbon tetrachloride (iv). The subsequent reactions were done as in case of the other compounds.

In this study we have demonstrated that the variation of the α-position of the carboxylic acid head group of pirinixic acid has an impact not only on PPAR subtype selectivity, but also on maximally achievable activity. Through this effect we obtained several subtype-selective PPARγ agonists with EC_{50} values in low micromolar range. By insertion of a phenyl residue in *para*-position of the naphthalene moiety we obtained a subtype-selective PPARγ modulator, as suggested by automated ligand–receptor docking. These studies may be considered as a example to turn lead structures established as PPARα or PPARγ selective or dual agonists into exclusive PPARγ modulators.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.03.008.

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