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Structural development studies of PPARs ligands based on tyrosine scaffold

Barbara De Filippis [a], Pasquale Linciano [a], Alessandra Ammazzalorso [a], Carmen Di Giovanni [c], Marialuigia Fantacuzzi [a], Letizia Giampietro [a], Antonio Laghezza [b], Cristina Maccallini [a], Paolo Tortorella [b], Antonio Lavecchia [c, **], Fulvio Loiodice [b, **], Rosa Amoroso [a, *]

[a] Dipartimento di Farmacia, Università "G. d'Annunzio", Via dei Vestini 31, 66100 Chieti, Italy
[b] Dipartimento di Farmacia-Scienze del Farmaco, Università degli Studi di Bari "Aldo Moro", Via Orabona 4, 70126 Bari, Italy
[c] Dipartimento di Farmacia, "Drug Discovery" Laboratory, Università degli Studi di Napoli Federico II, Via Domenico Montesano 49, 80131 Napoli, Italy

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ABSTRACT

PPARs are nuclear receptors with a critical physiological role in lipid and glucose metabolism. As part of our effort to develop new and selective PPAR agonists containing stilbene and its bioisoster phenyldiazene, novel analogs were synthesized starting from tyrosine and evaluated as PPAR agonists. We tested the effects of phenyloxazole replacement of GW409544, a well-known PPARα/γ dual agonist, with stilbene or phenyldiazene moiety, spaced by an ether bridge to tyrosine portion. These structural modifications provided potent and selective PPARγ agonists. Molecular docking studies performed on these new compounds complemented the experimental results and allowed to gain some insights into the nature of binding of the ligands.

1. Introduction

Peroxisome Proliferator-Activated Receptors (PPARs) belong to the II class of nuclear receptors (NRs) superfamily [1]. They are ligand-dependent transcriptional factors involved in the control and expression of several genes implicated in glucidic and lipidic homeostasis and energetic balance [2]. In humans, three different isoforms have been identified: PPARα, PPARβ/δ, and PPARγ. They have different tissue distribution as well as different binding affinity for ligands and recruitment ability of coactivators and corepressors. In their physiological activity, PPARs are activated by long-chain fatty acids, cycloxygenase-derived prostaglandines and their metabolites [3]. PPARα is mostly involved in the control of lipidic catabolism: it induces fatty acid β-oxidation, resulting in decrease of very low density lipoprotein (VLDL), low density lipoprotein (LDL), and increase of high density lipoprotein (HDL) in blood. On vascular cells it exhibits anti-inflammatory and anti-aggregating activity. Fibrates are PPARα agonists currently used as lipid-lowering agents [4]. PPARβ/δ is ubiquitously distributed, with relatively higher levels in brain, adipose tissue and skin. It is involved in lipid metabolism, with a physiological profile similar but non interchangeable with PPARα [5]. No PPARβ/δ agonists approved by health organizations are available. PPARγ is expressed in adipose tissue, where it induces lipogenesis and fat storage, and in skeletal muscle, where it improves insulin sensitivity [6]. It is the target of thiazolidinedione class of insulin-sensitizing drugs, clinically employed in patients with type 2 diabetes [7]. PPARs, therefore, represent valuable therapeutic targets for the treatment of both hyperlipidemia and insulin resistance in metabolic disorders, including metabolic syndrome [8]. For this purpose, in the last years many efforts have been directed toward the combination, in a unique chemical entity, of the insulin-sensitizing effect of PPARγ

Abbreviations: PPARs, peroxisome proliferator-activated receptors; NR, nuclear receptor; PPARα, peroxisome proliferator-activated receptor α; PPARβ/δ, peroxisome proliferator-activated receptor β/δ; PPARγ, peroxisome proliferator-activated receptor γ; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; DCC, dicyclohexylcarbodiimide; HOBt, hydroxybenzotriazole; hPPARα, human PPARα; hPPARγ, human PPARγ; hPPARδ, human PPARδ.

[*] Corresponding author. Dipartimento di Farmacia, Università "G. d'Annunzio", Via dei Vestini 31, 66100 Chieti, Italy

[**] Corresponding authors.

E-mail addresses: antonio.lavecchia@unina.it (A. Lavecchia), fulvio.loiodice@uniba.it (F. Loiodice), ramoroso@unich.it (R. Amoroso).

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activation with the additional lipid-modifying activity of other PPAR subtypes. One of the approaches adopted has been the development of dual PPARα/γ agonists, considered a very attractive option in the treatment of dyslipidemic type 2 diabetes [9]. A basically three-module structure for dual PPARα/γ agonists is always exactly conserved: a carboxylic acid head group, a simple linker group generally bearing a phenylether, and a lipophylic tail [10]. A number of dual agonists belonging to the class of isoxazolidinediones, *N*-substituted tyrosine analogs, α-substituted-3-phenyl propanoic acid derivatives, thiazolidine-2,4-dione and barbituric acid hybrids, and others have been developed and studied (Fig. 1) [11].

An alternative approach for the treatment of metabolic disorders is represented by the development of partial PPARγ agonists or selective PPARγ modulators [12]; in fact, ligands endowed with PPARγ full agonist activity, despite their proven benefits, possess a number of deleterious side effects such as weight gain, peripheral edema, increased risk of congestive heart failure, and higher rate of bone fracture [13]. Some selected agonists with attenuated PPARγ activity showed to retain the beneficial effects while reducing the adverse effects, but none of them has progressed further than preclinical or phase II trials. This necessitates the development of additional pharmacophores for the activation of this PPAR subtype.

Natural stilbenes, chalcones, and some of their synthetic derivatives have shown to interact with PPARs, thus showing a potential as therapeutic antilipidemic and antidiabetic agents [14].

As a part of the ongoing research to find an effective PPAR-target based drug candidate, we have recently reported new PPARα agonists derived by the combination of antilipidemic drugs gemfibrozil and clofibric acid (the active metabolite of clofibrate) with natural α-asarone, stilbene, chalcone, and other bioisosteric modifications [15]. The highest agonistic activity was seen with an *E*-stilbene derivative of gemfibrozil and a phenyldiazene derivative of clofibric acid (Fig. 2).

In this study, we describe the synthesis and the evaluation of PPAR activity of the new tyrosine derivatives **1a**–**o** (Fig. 3), based on the combination of GW409544, a potent full agonist on both PPARα and PPARγ, and stilbene or phenyldiazene scaffolds. The tyrosine portion of GW409544 remained essentially unchanged, except two derivatives in which the hydrophobic appendix was modified by introducing a methyl in place of phenyl (**1n**) or inverting the position of the carbonyl and ethylenic groups of the vinylogous amide (**1o**), respectively. The phenyloxazole moiety was replaced with stilbene or its bioisoster phenyldiazene, spaced by an alkylphenoxy linker from the central phenyl ring. Noteworthy, most compounds were highly potent selective PPARγ agonists with efficacy variable in the 30–80% range compared to the reference compound rosiglitazone.

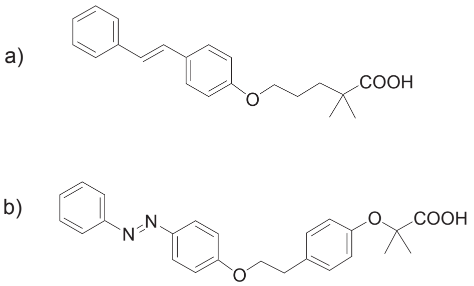

Fig. 2. a) Trans-stilbene derivative of gemfibrozil and b) phenyldiazene derivative of clofibric acid.

To gain more details on the interactions at a molecular level and to propose a binding mode explaining the SAR data, docking experiments were carried out. An isomerization process was hypothesized and confirmed by ^{1}H NMR studies to account for the lower activity of the phenyldiazene derivatives compared to the stilbene ones.

2. Results and discussion

2.1. Chemistry

Two series of compounds were synthesized: the *N*-vinylogous tyrosine derivatives (**1a**–**n**) and the *N*-amide tyrosine derivative **1o**. Some of them are stilbene derivatives (**1a**–**g** and **1n**–**o**), the others

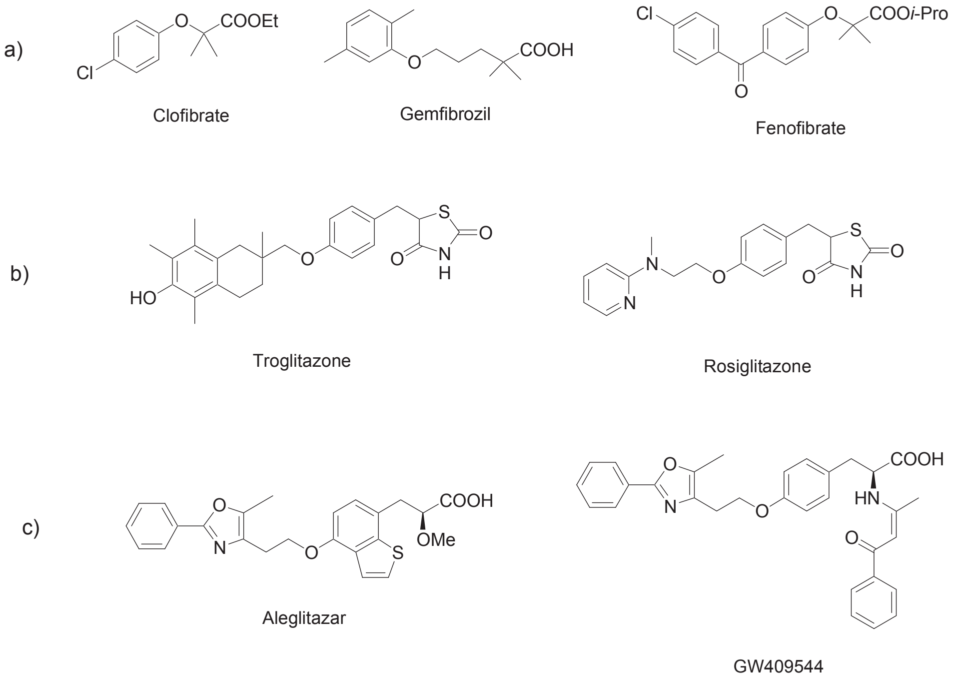

Fig. 1. Chemical structures of representative a) fibrates, b) thiazolidinediones, and c) dual PPARα/γ agonists.