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Isoxazolyl-Serine-Based Agonists of Peroxisome Proliferator-Activated Receptor: Design, Synthesis, and Effects on Cardiomyocyte Differentiation

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The peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily that plays key roles in lipid, carbohydrate, and cholesterol metabolism.[1] Three subtypes, PPARα, γ, and δ (or β), have been identified from *Xenopus* to humans, each forming a functional heterodimer complex with the 9-*cis*-retinoic acid receptor (RXR). PPARα is predominantly expressed in several tissues that have high lipid catabolism activity. PPARγ is highly enriched in adipocytes, while the δ subtype is ubiquitously expressed. PPARs are important molecular targets for the development of drugs for the treatment of human metabolic diseases, inflammation, and cancer,[1,2] as demonstrated by the thiazolidinediones (TZDs) and fibrates.

PPARs are known to be activated by a variety of structurally diverse compounds, including naturally occurring fatty acids and synthetic ligands (Figure 1).[1,3] Structures of the PPAR ligand binding domains (LBDs) in the absence and presence of ligands have been solved by X-ray crystallography.[4] Each of the PPAR subtypes possesses a large Y-shaped binding pocket (~1300 Å^3) located within the lower half of the LBD. Because of the large size of the ligand-binding pockets, PPARs are capable of binding a number of structurally diverse ligands. Despite differences in their gross chemical structure, small molecule PPAR agonists share a common binding mode.[4f] The structural characteristics of PPARs have provided molecular insights into the role ligands play in regulating PPAR activity on one hand; they also provide key insights for the design of new PPAR ligands with altered binding characteristics and modified receptor pharmacology on the other hand. We present herein the identification of novel PPAR ligands using a structure-based drug design approach.

Our structure-based design efforts were based in part on the SAR available for the known PPAR ligands. Most of the known current synthetic PPARα, γ, and δ ligands have several common elements (Figure 1): a polar "head" group "A" connected to an aromatic ring "C" through a short linker "B", and a linker "D" connecting the aromatic ring "C" to either an aromatic or aliphatic ring system "E". The linkers B and D can also contain additional substituents F and G. The reason for these structural characteristics relates to the idea that ligands should be able to adopt a U-shaped conformation in case of both PPARα and γ, and an L-shaped conformation in case of PPARδ.[4b] On the basis of this description, a novel isoxazolyl-serine-based PPAR agonist **1** (Chart 1) was designed using the de novo/rational approach in combination with our chemical knowledge.

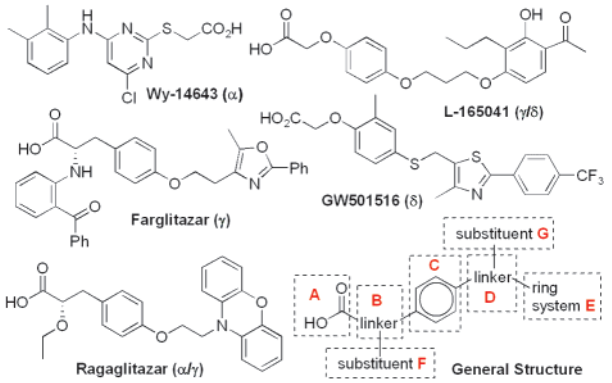
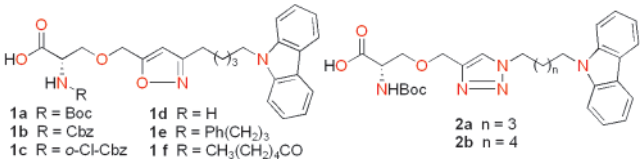


Figure 1. Some known PPAR agonists and the general structure.

Chart 1. Novel isoxazolyl/triazolyl-serine-based PPAR agonists.



A virtual combinatorial library based on the isoxazolyl-serine ligand was created and docked to the LBD of PPARγ as identified in the crystal structure of PPARγ-GW409544 complex.[4b] The docking and scoring were performed using the FlexX and CScore modules available in Sybyl 6.91. Several compounds from the combinatorial library were identified as potential ligands. Our docking studies suggest that the isoxazolyl-serine-based ligands have modes of binding similar to that seen in the crystal structure of PPARγ with GW409544 (Figure 2). The phenyl moiety of **1b** occupies the hydrophobic, phenylalanine-rich subpocket (Phe282, Phe360, and Phe363).

A common feature of many PPAR ligands is the presence of a carboxyl group. Reported crystal structures have shown that the carboxyl group is important for stabilizing the C-terminal helix in an active conformation and that it is coordinated by two histidines (His323, His449) and a tyrosine (Tyr473) in the AF2 helix. Superposition of the PPARγ-GW409544 crystal structure with the modeled complex formed from PPARγ and the docked ligand **1b** shows that the carboxyl of **1b** is positioned in precisely the same manner as is the carboxyl of GW409544 in the crystal structure.

Several compounds containing a carbazole moiety have been tested previously against all three PPAR subtypes, and a crystal structure of one of these carbazole-based compounds with PPARγ has been solved.[5] The carbazole moiety of this ligand binds to a

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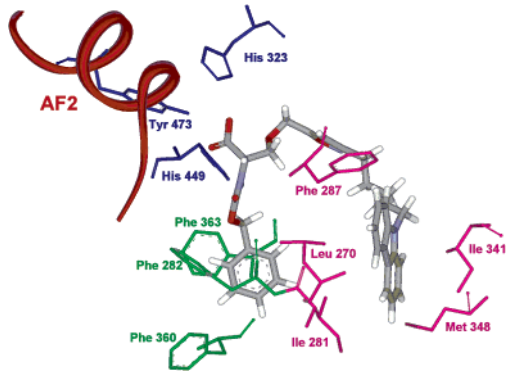

Figure 2. Modeled binding of **1b** to PPARγ.

Table 1. Transactivation of PPARα by Wy-14643 and **1a**−**c**

ligand	EC_{50} (μM)	SEM
Wy-14643	0.47	±0.06
1a	0.67	±0.07
1b	1.05	±0.08
1c	1.65	−

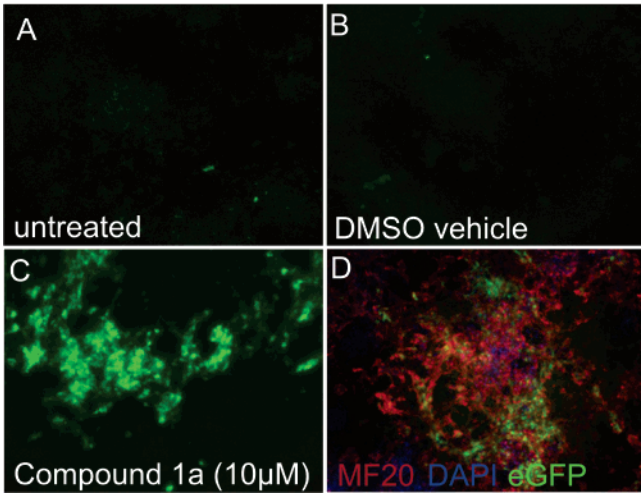

Figure 3. **1a** stimulated cardiomyocyte differentiation. Murine CGR8 ES cells with an αMHC-GFP transgene[6] were plated onto gelatin-coated dishes (3200 cells/cm^2) under conditions permissive for differentiation (DMEM supplemented with 10% FBS, 2 mM glutamine, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 0.1 mM βME, 100 units/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin). Cells were exposed to compounds between days 2−6, and eGFP fluorescence was scored at day 8. Untreated (A) or diluted vehicle (DMSO) (B) stimulated no or minimal differentiation, whereas addition of **1a** consistently stimulated eGFP-positive cells that beat rhythmically (C). Anti-myosin antibody (MF20 co-immunostaining (red) localized with GFP; blue nuclear counterstain is DAPI (D).

hydrophobic pocket formed by residues Leu270, Ile281, Phe287, Ile341, and Met348. The lack of well-defined electron densities indicates that the binding is not tightly constrained. In our compounds, linker D (Figure 1) is longer than the linker of the ligand employed in the crystal structure study.[5] Therefore, the carbazole moiety in our ligands is pushed toward the distal end of the binding pocket. Moreover, by docking our ligands to PPARα, we found that the carbazole moiety occupies a different binding site, one adjacent to the hydrophobic pocket defined for PPARγ, while the remainder of the ligand maintains the same interactions with the protein.

The isoxazolyl-serine-based ligands were readily prepared by use of an intermolecular nitrile oxide cycloaddition reaction as the key step (see Supporting Information). Human PPAR binding assays show that **1a**−**c** and **1f** possess moderate binding affinities for some or all of the three hPPAR subtypes (see Supporting Information). The mouse PPAR-Gal4 assays show that ligands **1a**−**c** are PPARα selective (inactive at 5 μM for PPARγ/δ) (Table 1). Two triazole analogues **2a** and **2b** (Chart 1) were also prepared by use of click chemistry.[7] Neither shows any significant PPAR activity.

PPAR agonists regulate cardiomyocyte gene expression and might modulate hypertrophy.[8] Therefore, the novel PPAR ligands were screened for their ability to stimulate cardiomyocyte differentiation from murine ES cells. Ligand **1a** was the most active one tested at concentrations between 1.25 to 20 μM between days 2−6, coinciding with the period when mesodermal cells can be recruited to become cardiomyocytes (Figure 3). Compounds **1d**−**f** and **2a**−**b** were also active. Notably, the PPARα agonists fenofibrate and Wy-14643 were inactive in this assay, as were the PPARγ agonists rosiglitazone and GW1929 and PPARδ agonist GW501516. Moreover, of the compounds that induced cardiomyogenesis, only **1a** and **1f** bound PPARs. Thus, we conclude that the PPAR activation is not the primary mechanism for cardiomyocyte differentiation.

In summary, we have designed and synthesized a series of isoxazolyl-serine-based PPAR ligands with moderate affinities. Further structural modification of these lead ligands aimed at improving upon their binding affinities and the identification of the primary target for our ligand-induced cardiomyocyte differentiation are underway.

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Supporting Information Available: Methods of biological assays, schemes, and detailed experimental procedures with spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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