Bioorganic & Medicinal Chemistry Letters 19 (2009) 3550–3554

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Identification of a PPARδ agonist with partial agonistic activity on PPARγ

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ARTICLE INFO

Article history:
Received 26 February 2009
Revised 28 April 2009
Accepted 30 April 2009
Available online 9 May 2009

Keywords:
PPARd
PPARg
Agonist
Modulator

ABSTRACT

The discovery and optimization of a series of potent PPARδ full agonists with partial agonistic activity against PPARγ is described.

The peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor family of ligand-regulated transcription factors.[1] There are three subtypes: PPARα (NR1C1), PPARδ (NR1C2, also called PPARβ), and PPARγ (NR1C3). The PPARs are essentially dietary fat sensors that maintain lipid and glucose homeostasis through control of a network of genes involved in metabolism. Their endogenous ligands include a diverse array of fatty acids, eicosanoids and oxysterols.[2] PPARα is expressed mainly in the liver and, to a lesser degree, in muscle and heart tissue. Its principal function is control of fatty acid metabolism,[3] especially in response to a prolonged fast.[4] PPARγ is expressed primarily in adipose tissue, where it acts as a master regulator of adipocyte formation.[5] PPARδ is expressed in many tissues, albeit at low levels in the liver. Its primary functions are control of fatty acid catabolism and energy homeostasis.[6,7]

Synthetic ligands have helped elucidate the biology of PPAR receptors and revealed their utility as drug targets. Fenofibrate (Tricor™), a PPARα agonist that was developed as a hypolipidemic before the PPARs were discovered,[8–10] reduces triglycerides (TG) and free fatty acids (FFA) and raises high-density lipoprotein (HDL). Rosiglitazone (Avandia™), a selective agonist of PPARγ,[11] enhances insulin sensitivity, reduces plasma glucose an average of 60–80 mg/dL and exerts a moderate corrective effect on TGs, FFA and HDL in type 2 diabetes mellitus (T2DM) patients. GW501516, a selective agonist of PPARδ, decreases low-density lipoprotein (LDL), TGs and insulin by 29%, 56% and 48%, respectively, and increases HDL by 79% in obese rhesus monkeys.[12] Studies in rodents suggest that activation of PPARδ also has beneficial effects on obesity and insulin resistance.[13] Considerable research effort has focused on the development of selective PPAR agonists for the treatment of T2DM and metabolic syndrome.[1] However, the importance of controlling both lipid and glucose levels in T2DM has stimulated interest in PPARα/γ[14] and PPARδ/γ[15] dual agonists, as well as PPARα/δ/γ pan agonists.[16] As T2DM patients receiving rosiglitazone often encounter significant weight gain,[11] there has also been a drive to reduce this adverse effect. PPARα/γ dual agonists held promise in achieving this goal, owing to reports that fibrates reduce weight gain in rodents without impacting food intake.[17] Other workers have pursued PPARδ/γ dual agonists,[18] reasoning that the increased fatty acid oxidation attending PPARδ activation[19] might diminish weight gain. Prompted by evidence that PPARγ partial agonists show a reduced weight gain profile,[20] we have opted to combine PPARδ full agonism and PPARγ partial

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doi:10.1016/j.bmcl.2009.04.151

agonism into a single ligand. Thus, here we describe our effort to develop a PPARδ/γ agonist/modulator with acceptable pharmacokinetic properties for in vivo studies.

In a recent patent,[21] we disclosed a class of compounds possessing potent PPARδ agonist activity that increases HDL levels in high-fat-fed Sprague–Dawley rats. Compound **1** (Fig. 1) was selected from this series as our lead because of its favorable properties as a PPARδ/γ agonist/modulator. Most notably, **1** exhibits high affinity for PPARδ (K_i = 0.007 μM) and full agonism in the PPARδ transactivation assay (Gal4 EC_{50} = 0.018 μM, 93% efficacy relative to GW501516[12]). Its affinity for PPARγ is weaker (K_i = 0.42 μM) and it acts as a partial agonist in the cell based assay (Gal4 EC_{50} = 0.46, 20% relative to rosiglitazone[11]). It is selective for PPARδ and PPARγ versus PPARα (PPARα K_i = 1.1 μM, Gal4 EC_{50} >10 μM). Finally, **1** exhibits acceptable PK properties, with a clearance in rat of 0.06 L/h/kg and a Vdss of 0.7 L/kg following 0.5 mg/kg iv dosing, and an oral bioavailability of 99% after 5.0 mg/kg po dosing. Our strategy for optimizing **1** as a PPARδ/γ agonist/modulator was to probe the effect of substitutes for the Cl atom on the central ring, and when improved central ring analogs were identified, to prepare libraries in which the CF_3 aryl is diversified.

The affinity of compounds towards PPARδ was evaluated using a scintillation proximity assay, while their affinity for PPARγ was assessed using a filtration assay.[22] The selectivity of compounds was evaluated using a PPARα scintillation proximity assay as a counter-screen. Compounds exhibiting K_i values less than 0.1 μM in the ligand binding assays were assessed for agonist activity in a transactivation assay in CV-1 cells, using a PPAR ligand binding domain/GAL4 DNA binding domain expression construct and luciferase reporter gene.[23]

The general synthetic route to the core structure of **1** is described in Scheme 1.[24] Ethyl aryloxyacetate **3** was obtained on treatment of 2,3-dimethylphenol with ethylbromoacetate in DMF/Cs_2CO_3. Chlorosulfonation of **3** followed by Sn reduction yielded mercaptan **5**, which was then converted to aldehyde **6** on treatment with 4-fluoro-3-methoxymethyleneoxybenzaldehyde[25] and Cs_2CO_3 in DMF. Compound **7** was then obtained via $NaBH_4$ reduction of aldehyde **6**, followed by formation of the benzyl chloride with MsCl/TEA. S_N2 displacement of benzyl chloride **7** with 4-hydroxybenzotrifluoride in DMF/Cs_2CO_3 furnished compound **8**. In the final steps, the MOM protecting group in **8** was removed with 4 N HCl in dioxane, the nascent phenol was alkylated with an appropriate alkyl halide in DMF/Cs_2CO_3 and the ethyl ester was saponified in NaOH/EtOH.

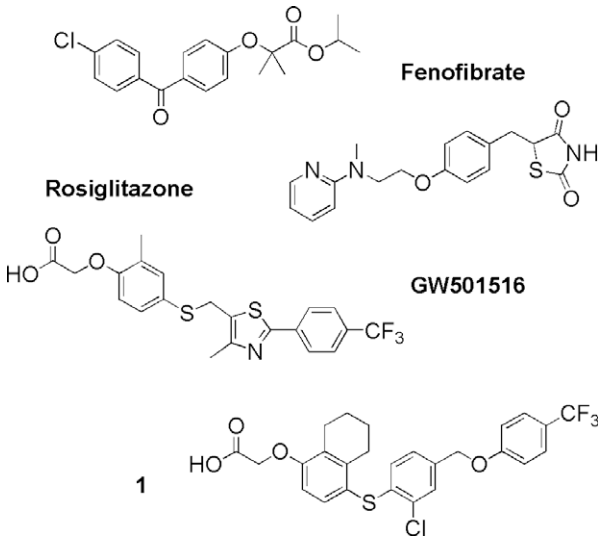
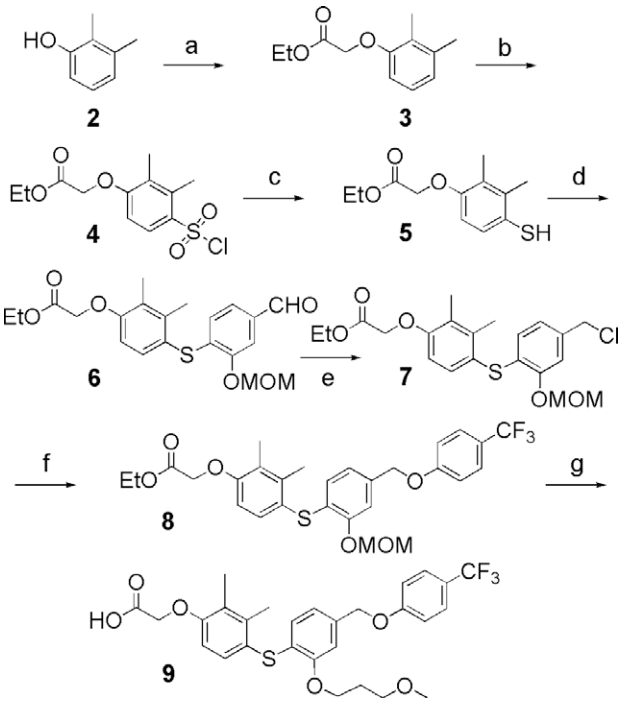


Figure 1. Published PPAR ligants and lead compound **1**.



Scheme 1. Reagents and conditions: (a) ethylbromoacetate, Cs_2C0_3, DMF, 50 °C, 4 h, 95%; (b) chlorosulfonic acid, 0 °C–rt, 4 h, 85%; (c) Sn, EtOH, HCl/dioxane, 0–80 °C, 4 h, 80%; (d) 4-fluoro-3-methoxymethyleneoxybenzaldehyde, Cs_2C0_3, DMF, 50 °C; 16 h, 60% (e) (1) $NaBH_4$/THF 0 °C–rt, 4 h, 95%; (2) MsCl/TEA, 0 °C–rt, 16 h, 95%; (f) 4-hydroxybenzotn-fluonde, Cs_2C0_3, DMF, 60 °C, 16 h, 60%; (g) (1) 4NHC1/dioxane, 0 °C, l h, 95%; (2) $Br(CH_2)_3OMe$, Cs_2C0_3, DMF, 60 °C, 4 h, 80%; (3) NaOH/EtOH, 0 °C–rt, 2 h, 95%.

The first series of compounds examined the effect of replacing the Cl atom of **1** with ethers (Table 1). As compound **1** is already appreciably lipophilic, preference was given to low molecular weight and polar central ring substitutents. In a further effort to reduce cLog *P*, the 2,3-dimethyl phenyl mercaptan was used in the synthesis instead of the tetrahydronaphthyl headpiece. Previous experience with **1** suggested that the 2,3-dimethyl headpiece would be tolerated but would likely modulate PPARα/γ selectivity.[21] As the data in Table 1 show, ligands with heteroatom-containing substituents on the central ring generally have lower activity in the δGal4 functional assay. While compounds **9**, **11**, **12** and **16** all showed strong binding to PPARδ, only weak activity was observed in the δGal4 cell assay. The data in Table 1 also show a correlation between the basicity of nitrogens present in central ring substituents, PPARδ affinity and δGal4 activity. Pyridines **14** and **15,** for instance, both showed low-nM affinity for PPARδ, while the morpholine analog **17** was 5-fold less potent and the tertiary amine **18** was inactive. All four compounds showed lower activity in the δGal4 assay, while the most basic ligands **17** and **18** were inactive. Encouragingly, compound **10** stood out for its high affinity for PPARδ, its activity in the PPARδ cell-based assay, and its binding affinity for PPARγ. The cocrystal of **10** in PPARδ[26] revealed a full agonist binding mode,[27] in which the three contiguous aryl rings wrap around helix-3 (H3), the carboxylate interacts with Y473, H323 and H449, and the CF_3-aryl is positioned in close proximity to C285 (Fig. 2). The propargyl substituent projects into the central ligand binding pocket (LBP), aligned along the H7 backbone towards H5. The acetylene bond is parallel to the F368 amide carbonyl and within pi-stacking distance of the side chain phenyl.