Bioorganic & Medicinal Chemistry Letters 24 (2014) 2267–2272

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Identification of the first potent, selective and bioavailable PPARα antagonist

Yalda Bravo *, Christopher S. Baccei, Alex Broadhead, Richard Bundey, Austin Chen, Ryan Clark, Lucia Correa, Jason D. Jacintho, Daniel S. Lorrain, Davorka Messmer, Karin Stebbins, Peppi Prasit, Nicholas Stock

Inception Sciences, 5871 Oberlin Drive Suite 100, San Diego, CA 92121, USA

ARTICLE INFO

Article history:
Received 20 January 2014
Revised 25 March 2014
Accepted 26 March 2014
Available online 4 April 2014

Keywords:
Antagonist
PPAR alpha
Nuclear hormone receptor
Fatty acid oxidation
Cancer

ABSTRACT

The discovery and SAR of a novel series of potent and selective PPARα antagonists are herein described. Exploration of replacements for the labile acyl sulfonamide linker led to a biaryl sulfonamide series of which compound **33** proved to be suitable for further profiling in vivo. Compound **33** demonstrated excellent potency, selectivity against other nuclear hormone receptors, and good pharmacokinetics in mouse.

Cancer cells are known to exhibit distinct characteristics from their 'normal' counterparts with regards to their energy requirements and metabolism. This divergence in turn, presents researchers with a variety of approaches to selectively apply metabolic stress on cancerous cells without adversely affecting healthy cells. Warburg observed that most cancer cells are programmed to increase glucose uptake in order to provide the necessary energy for their proliferative processes. Subsequently, much of the research into aberrant cancer metabolism has, thus far, been focused on targeting the glycolysis pathway, leaving the other metabolic pathways largely unexplored.[1] Recently, the contribution of fatty acid oxidation (FAO) to cancer cell function has gained more attention from the research community.[2–4] It has been shown that there are specific cancer cell types; including prostate, ovarian and renal cell carcinoma, that are more reliant on fatty acids to satisfy their metabolic needs.[5] It has also been demonstrated that following detachment of cancer cells from their extracellular matrix (i.e., the initial step of cancer metastasis), a metabolic switch towards increased fatty acid utilization for ATP generation can be seen even in the most glycolytic cancer cell types.[2] Finally, new research suggests that leukemia-initiating cancer (LIC) stem cells may be reliant on FAO for their maintenance and function, hinting at the possibility of eradicating leukemia through the exhaustion of the chemo-resistant LIC pool via inhibition of FAO.[6]

Peroxisome proliferator-activated receptors (PPARs) are a group of DNA-binding transcription factors within which three distinct isoforms (i.e., α, β/δ and γ) have been identified.[7] When activated by their respective endogenous ligands such as free fatty acids and eicosanoids, PPARs undergo a series of conformational changes to accommodate the recruitment of a suitable scaffolding co-activator protein and to facilitate their hetero-dimerization with 9-*cis* retinoic acid receptor (RXR). The resulting ternary complex then binds to the appropriate peroxisome proliferator response element (PPRE) on DNA and initiates the transcription and expression of its target genes. PPARα is implicated primarily in the regulation of lipid metabolism and as such, its activation leads to an increase in uptake and catabolism of fatty acids. This phenotype is achieved via the up-regulation of genes involved in the binding and transport of fatty acids (i.e., CPT1/2, CACT, and others) for oxidative processing. Consequently, it follows that *antagonism* of the PPARα receptor, coupled with the knowledge that certain malignant cells rely on FAO, represents a novel paradigm to stop the proliferative and metastatic tendencies of these cancer cells.

Although numerous examples of potent and selective PPARα agonists can be found in the literature; including those that have been approved clinically for the treatment of hypercholesterolemia, hypertriglyceridemia and other related metabolic disorders,[8–12] there are only a few isolated reports of confirmed

* Corresponding author. Tel.: +1 858 354 9160.
E-mail address: yaldabravo47@gmail.com (Y. Bravo).

http://dx.doi.org/10.1016/j.bmcl.2014.03.090
0960-894X/

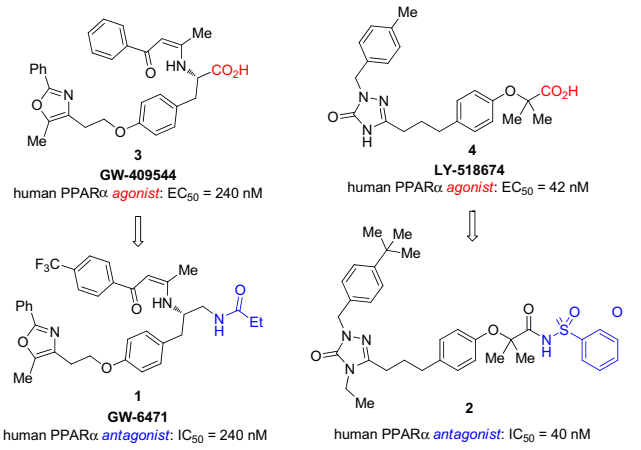


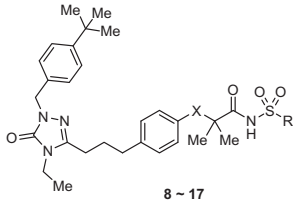
Figure 1. Previously reported PPARα modulators.[15]

PPARα antagonists. These inhibitors (compounds **1** and **2**, Fig. 1) were reported to be discovered serendipitously, following independent SAR campaigns carried out on their respective PPARα agonist precursors (i.e., compounds **3** and **4**). The reported initial program goal was to identify a suitable replacement for their shared carboxylic acid warhead (highlighted in red). In both cases, replacement of the carboxylic acid group by either an inverse amide (i.e., GW6471, compound **1**) or an acyl sulfonamide (i.e., compound **2**) triggered an unanticipated agonist-to-antagonist switch that resulted from the lifting of the PPARα C-terminal AF-2 helix, which, in turn, favored the recruitment of a co-repressor peptide such as SMRT.

Both antagonists were found to dose-dependently inhibit the activation of PPARα-driven luciferase expression by GW7647 (a known PPARα agonist) in a cell-based functional assay[13] we employed to drive our SAR. Unfortunately, neither compound proved suitable for assessing whether the antagonism of PPARα would be efficacious in our murine cancer models. Specifically, when mice were orally administered compound **1**, no measurable drug levels could be detected in the mouse plasma regardless of when the blood was collected after dose. Although this lack of oral drug exposure could be mitigated via an alternative mode of administration (i.e., intra-peritoneal injection), compound **1**'s lack of solubility in conventional dosing vehicles necessitated that the compound be eventually formulated in neat DMSO. However, this vehicle choice complicated the interpretation of the resulting in vivo data. The DMSO vehicle arm was found to exhibit a significant anti-metastatic effect when compared to conventional vehicles such as 0.5% aqueous methocel or saline (data not shown).[14] While compound **2** was found to possess physicochemical properties amenable for conventional formulation, we quickly discovered that this compound was very unstable in murine plasma and underwent an almost instantaneous enzyme-mediated hydrolytic cleavage to the acid; a potent PPARα *agonist*. As a result, we initiated separate SAR campaigns on both of these scaffolds with the aim of addressing their respective key liabilities that prohibited their profiling in vivo. This manuscript will focus on our efforts at improving the metabolic stability of compound **2** towards hydrolysis, while preserving its potency and selectivity against PPARα. Our effort around compound **1** will be disclosed separately in due course.

It was initially hypothesized that modification of the steric and/or the electronic environment adjacent to the cleavage site in compound **2** might afford compounds that are more resistant towards hydrolysis. In this regard, we first evaluated the impact of the sulfonyl substituent on both stability in murine plasma (after a

Table 1
SAR of acyl sulfonamides

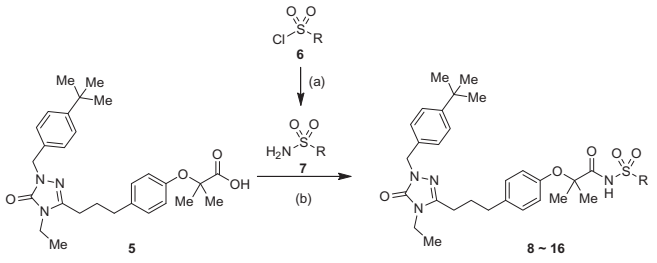


Compound	X	R:	PPARα IC_{50}[a] (nM)	% Parent compound remaining[b] 30 min	60 min	24 h
2	O	Ph	2.7 ± 0.9	6.7 ± 0.6	0.7 ± 0.1	
8	O	4-Me-Ph	2.8 ± 1.9	73.1 ± 1.4	63.7 ± 3.4	
9	O	4-^{i}Pr-Ph	13 ± 0	89.5 ± 2.0	81.9 ± 3.3	2.7 ± 0.1
10	O	4-CF_3-Ph	34 ± 18	102.7 ± 1.9	105.3 ± 3.1	53.7 ± 3.5
11	O	3-Pyridyl	80 ± 18	82.4 ± 2.6	75.2 ± 1.1	0.2 ± 0.1
12	O	Cyclohexyl	3 ± 0	60.5 ± 2.0	43.6 ± 2.0	
13	O	Cyclopentyl	4.6 ± 1.0	21.0 ± 0.8	7.0 ± 0.3	
14	O	Cyclopropyl	6.6 ± 3.0	1.7 ± 0.2	0.1 ± 0.04	
15	O	3-Furan	78 ± 0	75.4 ± 1.0	66.6 ± 2.0	
16	O	4-Pyran	42 ± 14	86.9 ± 2.9	86.5 ± 3.3	
17	C	Ph	60 ± 10	80.0 ± 2.8	62.3 ± 2.5	

[a] Values are the mean of at least three experiments.
[b] For experimental procedure see Ref. 20.

30 min and 60 min incubation period) and potency against PPARα in our luciferase assay (Table 1). These compounds were readily accessed through EDC-mediated condensation of acid **5** and sulfonamide **7** in the presence of DMAP.[16] Sulfonamides that were not commercially available were themselves synthesized from the corresponding sulfonyl chloride and liquid ammonia (Scheme 1).

The incorporation of an aliphatic group such as methyl (**8**) or isopropyl (**9**) at the *para* position of the terminal phenyl ring delivered PPARα antagonists of comparable potency to compound **2**. Both modifications led to a significant improvement in plasma stability, with the larger isopropyl group being more resistant towards hydrolysis (82% of **9** remained after 60 min of incubation with murine plasma vs 64% of **8**). Unfortunately, the vast majority of acyl sulfonamide **9** (>97%) was still cleaved to PPARα agonist **5** after a 24 h incubation period. Although switching the isopropyl residue in **9** for its known electron-withdrawing isostere CF_3 (**10**) further improved the resulting compound's resistance towards hydrolytic cleavage at a cost of only a small drop in PPARα potency, compound **10** could not survive the more rigorous 24 h incubation protocol unscathed. Replacement of the terminal benzene ring in compound **2** with a heteroaromatic plate such as 3-pyridyl (**11**) proved to be highly detrimental for PPARα antagonism. On the other hand, reducing it to the fully saturated cyclohexane (**12**) was tolerated in terms of potency against PPARα. These observations combine to reveal that the phenyl group in **2** occupies a large, hydrophobic pocket in the PPARα ligand binding domain but is



Scheme 1. Reagents and conditions: (a) liquid NH_3, DCM, −78 °C, 90–95%. (b) EDC, DMAP, DCM, 40–80%.