Bioorganic & Medicinal Chemistry Letters 28 (2018) 533–536

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Highly selective peroxisome proliferator-activated receptor δ (PPARδ) modulator demonstrates improved safety profile compared to GW501516

Bharat Lagu [a,*], Arthur F. Kluge [a], Matthew M. Goddeeris [a], Effie Tozzo [a], Ross A. Fredenburg [a], Shekar Chellur [b], Ramesh S. Senaiar [b], Mahaboobi Jaleel [b], D. Ravi Krishna Babu [b], Nirbhay K. Tiwari [b], Taisuke Takahashi [c], Michael A. Patane [a]

[a] Mitobridge, Inc., 1030 Massachusetts Ave., Cambridge, MA 02138, United States
[b] Aurigene Discovery Technologies, Ltd., Bengaluru, Karnataka, India
[c] Astellas Pharma, Tsukuba, Japan

ARTICLE INFO

Article history:
Received 4 October 2017
Revised 30 October 2017
Accepted 2 November 2017
Available online 4 November 2017

Keywords:
PPARδ modulator
GW501516
Gene expression
Thermal injury model
Ki-67

ABSTRACT

Compound **1** regulates significantly fewer genes than the PPARδ modulator, GW501516. Both compounds are efficacious in a thermal injury model of muscle regeneration. The restricted gene profile of **1** relative to GW501516 suggests that **1** may be pharmacoequivalent to GW501516 with fewer PPAR-related safety concerns.

Marketed modulators of PPARα (fibrates), and PPARγ (thiazolidinones) as well as dual PPARα/PPARγ agonists like Muraglitazar have been associated with class-related side effects.[1–4] Selective PPARδ modulators may offer therapeutic value without the undesirable activities associated with the modulators of PPARα and PPARγ.[5] PPARδ is ubiquitously expressed and is found to be highly expressed in liver, skeletal muscle, intestine and adipose tissue.[6] Therefore, selective PPARδ modulators could potentially be useful as treatments for metabolic disorders and conditions that would benefit from muscle regeneration.[7,8] Clinical trials with a well-studied PPARδ modulator, GW501516[9] (Fig. 1) were discontinued due to tumorigenic potential that was observed in rats.[10]

Recently, Evans and co-workers have described structurally distinct and highly selective PPARδ modulators.[5] The authors suggest that a PPARδ modulator with improved isoform selectivity could have greater efficacy and improved side effect profile than predecessor compounds. In part, this hypothesis is based on data demonstrating that PPARδ modulators reach the same E_{max} *in vitro* and *in vivo* for gene regulation products regardless of their concentration (i.e., 10×, 100× or 1000× EC_{50} values). Hence, gene regulation appears to saturate and is either "on" (activated) or "off" (repressed) when the concentrations exceed EC_{90} levels. Raising the levels of compounds does not increase the expression of mRNA or protein above the E_{max}.levels.

The improvement in the safety profile may be attributable to a restricted gene regulation signature for such compounds. In order to test this hypothesis *in vivo*, a compound with pharmacokinetic properties suitable for oral dosing was required. In the preceding paper, we have described the structure-activity relationship work that led to identification of a potent and selective PPARδ modulator, **1** (Fig. 1).[11] Herein, we describe the results of gene regulation and safety studies for compound **1** and GW501516 in addition to the *in vivo* efficacy data in thermal injury model of muscle regeneration.

Compound **1** is highly potent for human PPARδ and displays subtype selectivity over human PPARα (>160-fold) and human PPARγ (>270-fold) in transactivation assays.[11] For **1**, the potency for mouse PPARδ receptor was about 7-fold lower than for the human PPARδ receptor; a trend that has been noted for GW501516. Compound **1** was screened against 68 receptors and transporters in a panel

* Corresponding author.
E-mail address: blagu@mitobridge.com (B. Lagu).

https://doi.org/10.1016/j.bmcl.2017.11.006
0960-894X/

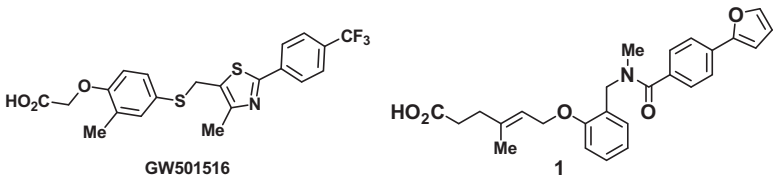

Fig. 1. GW501516 and Compound **1**.

Table 1
Potency, selectivity and Safety data for **1** and selected data for GW501516.

Assay	Compound 1	GW501516
Human PPARδ[a]	EC_{50} = 37 ± 5 nM	EC_{50} = 2.6 ± 0.5 nM
Human PPARα[a]	EC_{50} = 6100 nM	EC_{50} = 7700 nM
Human PPARγ[a]	EC_{50} > 10,000 nM	EC_{50} > 10,000 nM
Mouse PPARδ[b]	EC_{50} = 270 nM	EC_{50} = 70 nM
Selectivity	No activity in Eurofin PanLabs LeadProfilingScreen® of 68 molecular targets up to 10 μM. No activity (up to 10 μM) for androgen, progesterone or glucocorticoid receptors	NA
Thermodynamic solubility	190 μM	250 μM
Caco-2 permeability	A to B = 4.58E-05; B to A = 1.03E-04 (Efflux ratio 2.24)	NA
CYP450 inhibition	>10 μM for CYPs 3A4, 2C9, 2C19, 2D6, 1A2	NA
hERG (patch clamp)	1% inhibition at 30 μM	NA
Mutagenicity	Non-mutagenic in mini-Ames test	NA

NA = Not available.
[a] Transactivation assay.
[b] Assay carried out at Indigo Bioscience.

of Eurofins Panlabs assays and no significant binding (<20%) was observed at 10 μM. The results are summarized in Table 1.

In *in vitro* safety assays, compound **1** did not show ancillary activities. Compound **1** displayed good ADME profile and good oral availability in mice, rats and monkeys.

Gene expression data was obtained in human muscle cells treated with compound **1** and GW501516 at their EC_{50} concentrations for 24 h. Both compounds engage a core set of genes known to be responsive to PPARδ modulation (e.g., CPT1A, ANGPTL4, PDK4). Compound **1** affected significantly fewer genes than GW501516 (Fig. 2) among a panel of known PPAR-responsive genes. This selectivity could lead to different pharmacological and/or toxicological outcomes than GW501516.

Pharmacology of **1** was assessed using the thermal injury mouse model for muscle regeneration reported by Evans and coworkers.[12] In this model, C57BL/6 mice were dosed with the compound once-a-day via oral gavage for 10 days (Day 0–9).[13,14] On day 4, thermal injury was caused by placing a 1 g weight that was cooled to dry ice temperature onto the exposed tibialis anterior (TA) muscle of left leg for 10 s. The damaged muscle proceeds through phases of degeneration, inflammation, regeneration and remodeling that accompany recovery from muscle injury. Effects on repair efficiency were evaluated by measuring the retention of Evans blue dye (EBD), injected on day 8, in the injured muscle. Evans blue dye is retained in injured muscle fibers until the cell is completely removed by the inflammatory response, so in this model increased EBD retention is an indication of incomplete or delayed muscle regeneration. On Day 9 animals were sacrificed, TA muscles removed and EBD retention evaluated after extraction. As anticipated, no change in optical density (OD) was observed for the contralateral (non-injured) TA muscle (Fig. 3A). TAs exposed to thermal injury showed significant increase in EBD compared to values from the non-injured (contralateral) and sham injury groups (Fig. 3B). Compound **1** demonstrated statistically significant reduction in OD at 50 mg/kg and 100 mg/kg doses and comparable to the reduction in OD observed for GW501516 dosed at 10 mg/kg. It is important to note that the thermal injury model was used only to demonstrate a pharmacological effect. Both GW501516 and

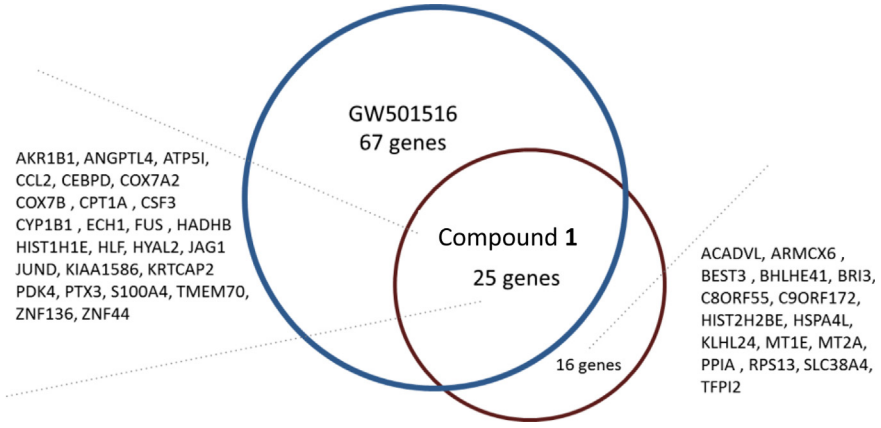

Fig. 2. Restricted gene expression profile observed with Compound **1** compared to GW501516 in primary human muscle cells.