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

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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

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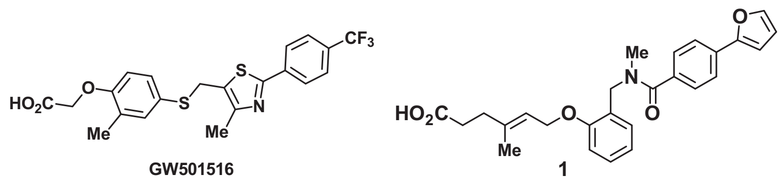

Fig. 1. GW501516 and Compound **1**.

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

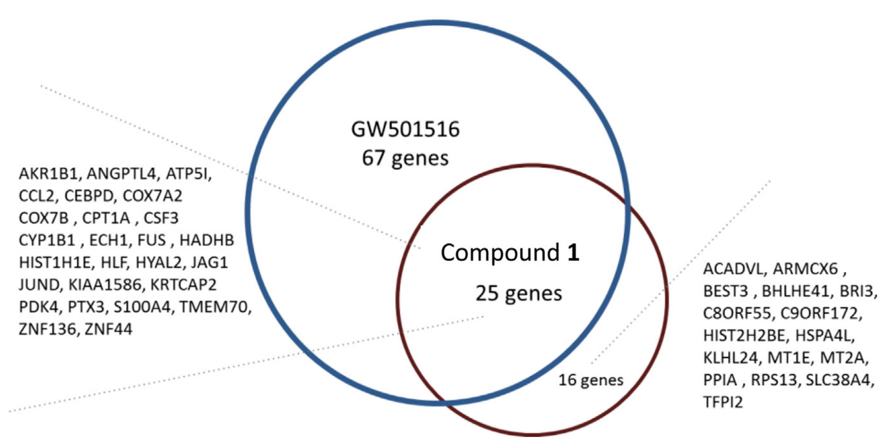

Fig. 2. Restricted gene expression profile observed with Compound **1** compared to GW501516 in primary human muscle cells.

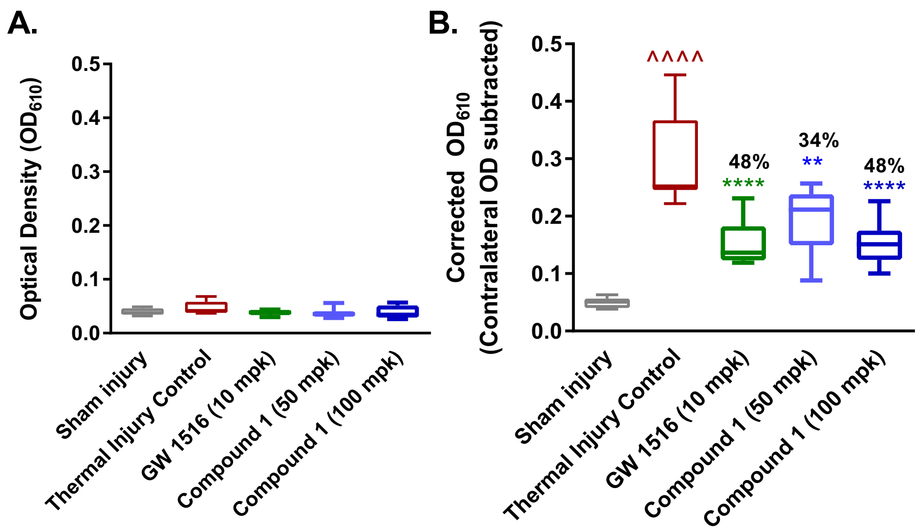

Fig. 3. Recovery from thermal injury in mice treated with Compound **1** compared to GW501516. (A) Un-injured contralateral control muscle and (B) injured muscle. ^^^^ p < .0001 vs untreated thermal injury control by Student's t-test. ** p < .01; **** p < .0001 by One-way ANOVA with Dunnett's post hoc multiple comparison test versus untreated thermal injury control. Sample size, n = 8/group.

compound **1** showed equivalent pharmacological activity at comparable (10 mpk dose of GW501516 and 50 mpk of compound **1**) plasma exposure or at a higher exposure of compound **1** (100 mpk dose). The doses reported here are not to be interpreted as minimum efficacious doses for the two compounds.

Though compound **1** and GW501516 showed similar profile in the pharmacological model, the known tumorigenic effects could be related to the unique set of genes affected by GW501516. This hypothesis was tested by monitoring the proliferation marker Ki-67[15] in 14-day rat safety studies with GW501516 and compound **1**. The results are summarized in Table 2. Ki-67 staining has been utilized by many labs to assess cell proliferation in gastric cancer,[16] breast cancer,[17] prostate cancer[18] and urinary bladder neoplasia.[19]

The animals treated with GW501516 showed significant toxicity at the highest dose (Due to significant drop in body weight and food intake, the 300 mg/kg dose was reduced to 200 mg/kg after day 6. All the surviving animals needed to be sacrificed due to deteriorating clinical condition on day 9). Statistically significant increases in Ki-67-positive proliferating cells in non-glandular stomach were observed for animals dosed with GW501516 at 100 mg/kg and 300 mg/kg doses (Table 2 and Fig. 4). After dosing compound **1** at 300 mg/kg dose, only a marginal increase (not statistically significant) in Ki-67-positive proliferating cells in non-glandular stomach was observed (Table 2 and Fig. 5). It is important to note that while the plasma exposure of GW501516 was 10-fold higher than compound **1** (Table 3), the toxicological effects observed in the non-glandular stomach are likely local and independent of the systemic exposure.[20] But even at a comparable plasma exposure (30 mg/kg for GW501516 versus 100 mg/kg for Compound **1**), significantly higher cell proliferation is observed in the animals treated with GW501516 than compound **1**. Longer

Table 2
Proliferation index Ki-67 staining of non-glandular stomach of rats after 14 day dosing (6 animals per group) with GW501516 and Compound **1**.*

Dose	GW501516	Compound **1**
0 mg/kg (Vehicle)	35.7 ± 4.6	49.2 ± 4.3
30 mg/kg	47.8 ± 3.0 (34%↑)	NT
100 mg/kg	64.7 ± 4.8 (81%↑)	48.1 ± 3.8 (2%↓)
300 mg/kg**	87.2 ± 16.9 (190%↑)	63.0 ± 6.1 (28%↑)

* Proliferating cells (# at 40× magnification), mean of 3 areas are shown above. Changes shown in parenthesis are % over control. The up or down arrows indicate increase or decrease respectively.
** For GW501516, the dose was reduced to 200 mg/kg after 6 days. All the surviving animals needed to be sacrificed moribund on day 9 in this group. NT = Not Tested.

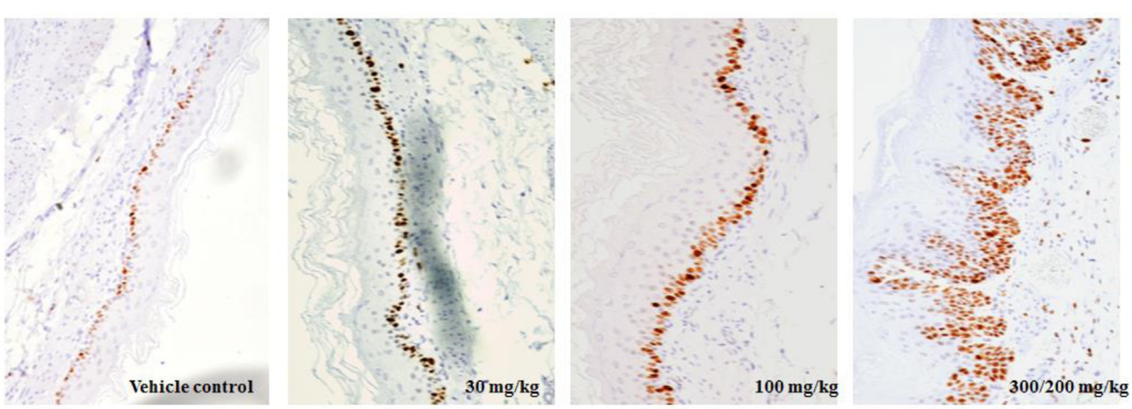

Fig. 4. Histopathology slides of non-glandular stomach with Ki-67 staining for GW501516 (40× magnification). The red-brown color indicates the Ki-67 stain.

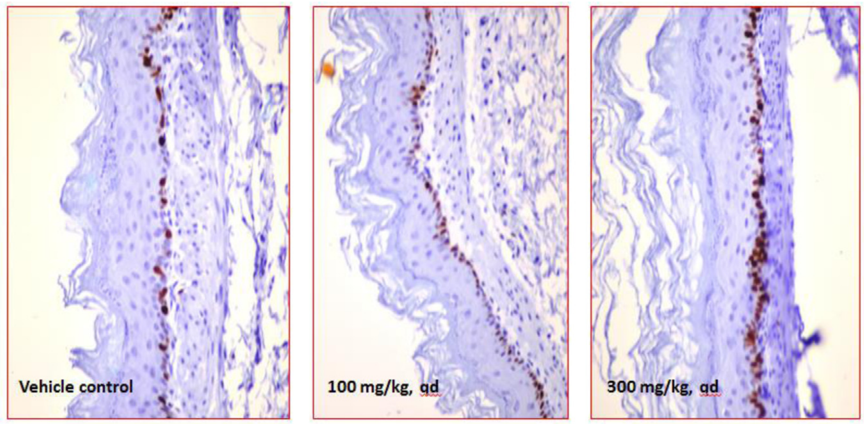

Fig. 5. Histopathology slides of non-glandular stomach with Ki-67 staining for Compound **1** (40X magnification). The red-brown color indicates the Ki-67 stain.

Table 3
Plasma exposure in rats shown as area under curve (AUC) for GW501516 and Compound **1**.

Dose	AUC (0-last) (ng * h/mL)	
	GW501516	Compound **1**
30 mg/kg	72,000	NT
100 mg/kg	613,000	48,000
300 mg/kg	2,270,000[*]	201,000

NT = Not tested.
[*] Plasma exposure on day 8.

toxicity studies in rats will be needed to confirm the results from 14-day toxicity studies. It is important to point out that the 14-day toxicity study has not been used to calculate a therapeutic index since: 1) the pharmacological effect has been shown in a mouse model whereas rats were used for the 14-day toxicity experiment, 2) the local exposure rather than the systemic AUCs may be more relevant in the cell proliferation assay and 3) as mentioned before, minimum efficacious dose was not identified for compound **1** in the thermal injury model.

In summary, we have demonstrated that compound **1** is efficacious in a thermal injury model in mice. The compound may be safer than GW501516 by nature of the fact that it affects fewer genes, a hypothesis that is supported by 14-day toxicological study.

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