

# Peroxisome Proliferator-Activated Receptors (PPARs): Choreographers of Metabolic Gene Transcription

Barry G. Shearer and William J. Hoekstra

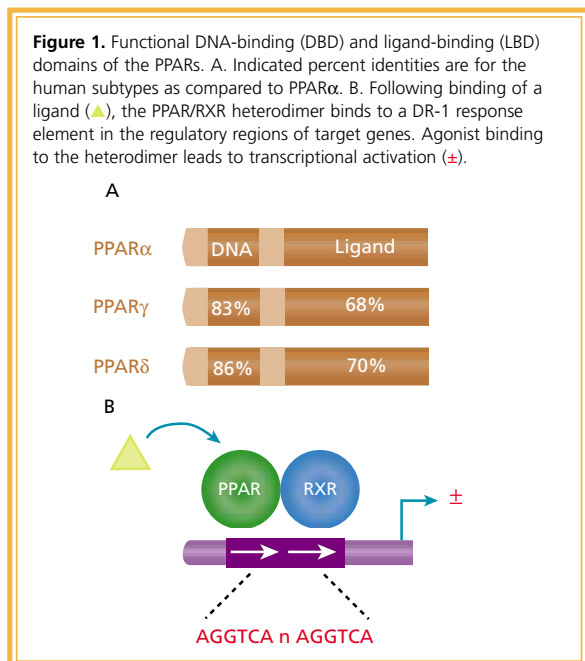
Nuclear receptors are ligand-activated transcription factors that regulate gene expression in response to small lipophilic compounds such as testosterone [androgen receptor (AR)], oxysterols [liver X receptor (LXR)], estrogen (ER), xenobiotics [pregnane X receptor (PXR)], retinoic acid (RAR), thyroid hormone (TR), bile acids [farnesoid X receptor (FXR)] and vitamin D (VDR). The peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor supergene family that play a central role in the regulation of storage and catabolism of dietary fats [1-4]. The three subtypes of PPAR (designated  $\alpha$ ,  $\delta$  and  $\gamma$ ) bind to fatty acids and fatty acid metabolites and regulate the expression of genes involved in the transport, metabolism and buffering of these ligands within cells. Each of the three PPAR subtypes exhibits a unique expression pattern within vertebrate tissues. In rats, PPAR $\alpha$  is most highly expressed in brown adipose tissue, followed by liver, kidney, heart and skeletal muscle [2]. PPAR $\gamma$  is most highly expressed in white and brown adipose tissue, but is also expressed in muscle, colon and liver. PPAR $\delta$  is expressed in all tissues studied to date.

The functional domains of the PPARs consist of N-terminal, DNA-binding (DBD) and ligand-binding (LBD) domains (Figure 1). Heterodimers of PPAR and retinoid X receptor (RXR) recognize DNA sequences

containing direct repeats of the hexanucleotide sequence AGGTCA present in promoter regions of their target genes, e.g. acyl-CoA oxidase (AOX) and adipocyte fatty acid-binding protein (aP2) [1]. Upon PPAR (or RXR) agonist binding, increases in the expression level of mRNAs encoded by these genes are observed. A comparison of the sequences of the PPAR DBDs shows a high percentage of conserved amino acids, while the LBDs exhibit slightly less conservation across subtypes (Figure 1). Notably, there is significant sequence variation in amino acids that line the ligand-binding pocket that is consistent with the observation that each receptor is pharmacologically distinct.

## PPAR $\alpha$

PPAR $\alpha$  plays an important role in the oxidation of fatty acids in the liver [5]. Receptor activation stimulates fatty acid oxidation, a crucial adaptive response to nutritional challenges such as fasting. Although the PPAR $\alpha$  DBDs are identical across a variety of species, the LBDs exhibit lower homology, possibly reflecting evolutionary adaptation to different dietary ligands. A comparison of human PPAR $\alpha$  to its murine counterpart reveals an 85% identity at the nucleotide level and a 91% identity at the amino acid level [1].



## About the Authors

**Barry G. Shearer** received his Ph.D. in organic chemistry from the University of South Carolina under the direction of Professor James A. Marshall. He joined NOVA Pharmaceutical in 1988 where he worked on inhibitors of protein kinase C. In 1991, he moved to Burroughs Wellcome (now GlaxoSmithKline) where his research in medicinal chemistry involved the design and synthesis of nitric oxide synthase inhibitors. More recently, he has focused on areas of metabolic diseases and his current research interests involve the discovery of orphan nuclear receptor ligands.

**William J. Hoekstra** received his Ph.D. in organic chemistry from Emory University in Atlanta, Georgia under the direction of Professor Dennis C. Liotta. He joined Merrell Dow Research Institute's Synthetic Process Chemistry Group in 1987 where he studied the synthesis of castanospermine esters and hirudin mimetics. In 1991, he moved to The R.W. Johnson Pharmaceutical Research Institute's Drug Discovery Department where he directed the design and synthesis of peptidomimetic antithrombotic agents including the Phase II GPIIb/IIIa antagonist elarofiban. Dr. Hoekstra joined GlaxoSmithKline in 2000 as chemistry coordinator of nuclear receptor modulator programs.

### PPAR $\alpha$ Ligands

PPAR $\alpha$  binds to a diverse set of ligands, namely, arachidonic acid metabolites (prostaglandins and leukotrienes) and plasticizers and synthetic fibrate drugs (see Figure 2), including clofibrate (Prod. No. [C 6643](#)), fenofibrate (Prod. No. [F 6020](#)) and bezafibrate (Prod. No. [B 7273](#)) [5]. Given that no single high affinity natural ligand has been identified for PPAR $\alpha$ , it has been proposed that a physiological role of the receptor may be to sense the total flux of dietary fatty acids in key tissues. Many PPAR $\alpha$  ligands, including most of the common fibrate ligands, show only modest selectivity over the other PPAR subtypes. However, a potent thioisobutyric acid GW7647 (Figure 2) has been identified that shows excellent selectivity for both murine and human PPAR $\alpha$  [6,7].

### PPAR $\alpha$ in Disease

#### PPAR $\alpha$ and Dyslipidemia

The fibrates are a class of triglyceride lowering drugs that mediate their clinical effects through activation of PPAR $\alpha$  [8,9]. The marketed agents of this class are clofibrate, fenofibrate, bezafibrate, ciprofibrate (Prod. No. [C 0330](#)), gemfibrozil (Prod. No. [G 9518](#)) and bezafibrate [1]. All of these drugs were discovered prior to the cloning of PPAR $\alpha$  and without knowledge of their mechanism of action. Recently, chemical libraries of ureidothioisobutyric acids have been synthesized on solid support and PPAR $\alpha$  agonists have been identified with improved lipid lowering activity compared to fenofibrate, e.g. GW7647 and GW9578 (see Figure 2) [10].

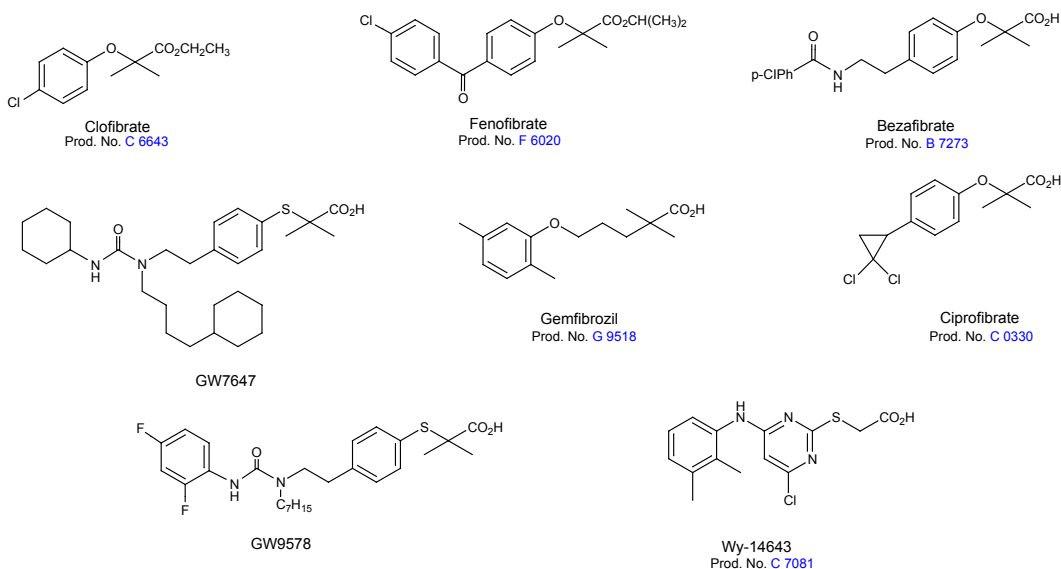
### PPAR $\alpha$ and Other Indications

PPAR $\alpha$  agonists affect a range of biological processes that contribute to the etiology of coronary artery disease. For instance, expression of vascular cell adhesion molecule-1 (VCAM-1), an adhesion molecule that recruits monocytes to endothelial cells at sites of atherosclerotic lesions, has been shown to be down-regulated by PPAR $\alpha$  agonists in endothelial cell studies [11]. This process can be inhibited by control of proatherogenic gene transcription induced by nuclear factor- $\kappa$ B (NF- $\kappa$ B). To this end, the murine PPAR $\alpha$  agonist Wy-14643 (Prod. No. [C 7081](#); Figure 2) has been reported to correct the abnormal expression of genes under the control of NF- $\kappa$ B in aged mice [12]. Obesity is a risk factor in the development of diabetes, and fibrate treatment has been reported to reduce weight gain in rodents, possibly through increased energy expenditure or fatty acid catabolism. Induction of the genes involved in these processes has been demonstrated by bezafibrate [13], Wy-14643 [14] and other agents [15,16].

### PPAR $\gamma$

PPAR $\gamma$  has been the most extensively studied PPAR subtype to date. Two distinct N-terminal isoforms, termed PPAR $\gamma$ 1 and PPAR $\gamma$ 2, have been identified in mice and humans [17]. PPAR $\gamma$  is a pivotal transcription factor in the regulation of adipocyte gene expression and differentiation. The regulation of adipocyte differentiation by PPAR $\gamma$  takes place through a coordinated signaling cascade involving other families of transcription factors and has been reviewed [18].

Figure 2. Structures of PPAR $\alpha$  Agonists



## Peroxisome Proliferator-Activated Receptors (PPARs)...(continued)

In addition to adipogenic effects, PPAR $\gamma$  has been shown to be an important regulator of target genes involved in glucose and lipid metabolism [1]. PPAR $\gamma$  agonists are efficacious antidiabetic agents and represent a novel class of successfully marketed diabetes drugs. PPAR $\gamma$  agonists may also have therapeutic utility in the treatment of other disease states such as atherosclerosis and cancer [1].

### PPAR $\gamma$ Ligands

#### Natural Ligands

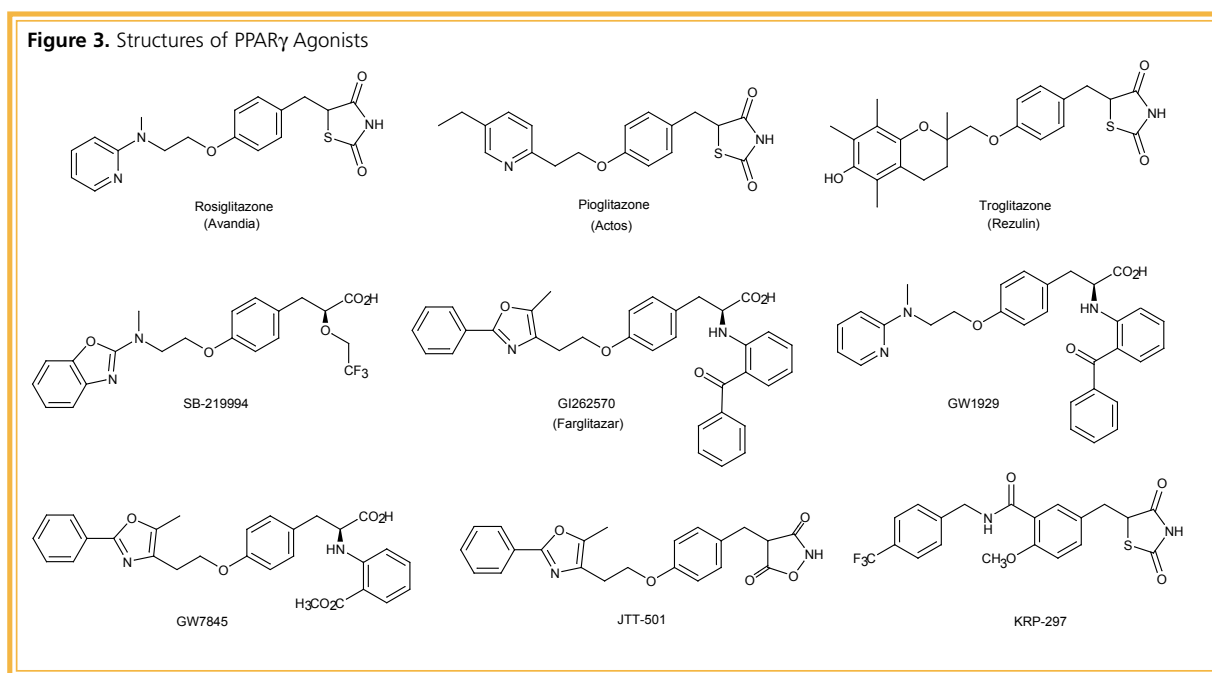
While significant debate continues over the identity of the natural ligand(s) for PPAR $\gamma$ , studies have shown numerous naturally occurring fatty acids, eicosanoids, prostaglandins and their metabolites to be weak endogenous activators of PPAR $\gamma$ . The concentrations of fatty acids required for PPAR $\gamma$  activation are consistent with those found in human serum. PPAR $\gamma$  exhibits modest preference for essential polyunsaturated fatty acids (PUFAs) including linoleic acid (Prod. No. [L 1268](#)), linolenic acid (Prod. No. [L 2376](#)), arachidonic acid (Prod. No. [A 9673](#)) and eicosapentaenoic acid (Prod. No. [E 7006](#)) [20]. Certain oxidized metabolites of PUFAs such as 9- or 13-hydroxyoctadecadienoic acid (9-HODE or 13-HODE; Prod. No. [H 9146](#)) and 15-deoxy-12,14-prostaglandin J<sub>2</sub> (15d-PGJ<sub>2</sub>), have been demonstrated to result in increased PPAR $\gamma$ -mediated transactivation [21,22]. Thus, PPAR $\gamma$  may serve as a generalized fatty acid sensor that couples changes in overall PUFA concentrations with the target genes associated with lipid and glucose homeostasis. The possibility that a yet to be identified natural high affinity PPAR $\gamma$  ligand exists, however, cannot be excluded.

#### Synthetic Agonists

The thiazolidinedione (TZD) class of antidiabetic agents, commonly referred to as "glitazones", represent the first compounds identified as high affinity PPAR $\gamma$  agonists. The glitazones were discovered through empirical compound screening in rodent models of type 2 diabetes [23]. Two glitazones, rosiglitazone (Avandia) and pioglitazone (Actos), are currently marketed for the treatment of type 2 diabetes (Figure 3). Troglitazone (Rezulin), a third approved glitazone, was withdrawn from the marketplace due to liver toxicity (Figure 3).

The TZDs undergo racemization at C-5 under physiological conditions, resulting in the pursuit of PPAR $\gamma$  agonists with alternative head groups that are less prone to racemization. A series of  $\alpha$ -alkoxy- $\beta$ -phenylpropanoic acids, exemplified by SB-219994 (Figure 3), display agonist activity at both PPAR $\gamma$  and PPAR $\alpha$  [24]. Higher binding affinities and functional activity for PPAR $\gamma$  were observed for the (S)-enantiomers of this series.

A series of tyrosine-based PPAR $\gamma$  agonists, exemplified by GI262570 (Farglitazar), GW1929 and GW7845, have also been developed (see Figure 3) [25-27]. These compounds represent the first antidiabetic agents to be optimized based upon their activity on human PPAR $\gamma$ . This class of PPAR $\gamma$  agonists also represents some of the most potent agonists reported to date. The (S)-enantiomers have been shown to possess greater binding affinity and functional activity at PPAR $\gamma$  than the corresponding (R)-enantiomers [26]. In cell based transactivation



## Peroxisome Proliferator-Activated Receptors (PPARs)...(continued)

assays, these analogs exhibit up to 1000-fold selectivity for PPAR $\gamma$  over the PPAR $\alpha$  and PPAR $\delta$  subtypes. GW1929 (Figure 3) possesses potent and efficacious antihyperglycemic activity in ZDF rats [26,28]. Farglitazar has shown potent reduction of glucose activity, reduction of triglycerides and elevation of HDL cholesterol in diabetic patients in Phase II studies [28,29]. The positive lipid effects of farglitazar may be due to residual PPAR $\alpha$  activity in the compound.

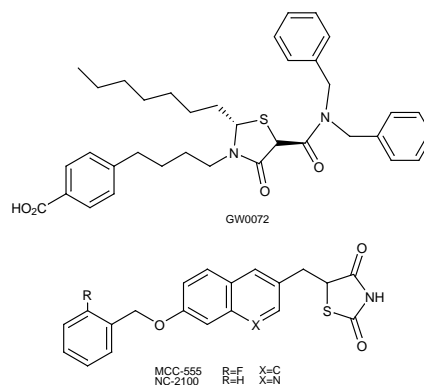
The isoxazolidinedione JTT-501 (Figure 3) is a PPAR $\gamma$  agonist that possesses some PPAR $\alpha$  activity [30]. Its activity is believed to be mediated through a malonic amide metabolite derived from hydrolysis of the heterocyclic head group. The TZD analog KRP-297 (Figure 3) is a PPAR $\gamma$  agonist with similar affinity for PPAR $\alpha$ . *In vivo*, KRP-297 has been reported to improve abnormal lipid metabolism in liver and elicit hypoglycemic, hypoinsulinemic and hypolipidemic conditions in obese rats [31].

### Selective PPAR $\gamma$ Modulators

While the clinical benefits of PPAR $\gamma$  agonists in treating type 2 diabetes have been clearly demonstrated, the current generation of glitazone drugs is associated with undesired side effects such as weight gain and edema. Thus, there is significant interest in the design of novel PPAR $\gamma$  modulating drugs that retain efficacious insulin sensitizing properties while minimizing potential adverse side effects.

A novel PPAR $\gamma$  modulator GW0072 (Figure 4) was identified from a combinatorial library of non-TZD thiazolidine acetamides [32]. GW0072 profiles as a partial agonist in transactivation assays with only 15-20% of the efficacy of rosiglitazone. It antagonizes rosiglitazone in this assay to the level of its own partial agonist activity, possessing an IC<sub>50</sub> value of 150 nM. GW0072 behaves as a full antagonist of rosiglitazone-induced adipocyte differentiation. However, when insulin (Prod. Nos. [I 0259](#), [I 2643](#)) was used to promote adipocyte differentiation, GW0072 no longer profiled as an antagonist, but appeared to function as an insulin sensitizer [33]. Thus, GW0072 is a PPAR $\gamma$  modulator that can inhibit the adipogenic effects of rosiglitazone, but not of insulin. Interestingly, dosing GW0072 in insulin-resistant Zucker diabetic rats resulted in the lowering of plasma insulin and triglycerides to nearly the same level as a full PPAR $\gamma$  agonist without inducing as much weight gain [33]. These promising results suggest that PPAR $\gamma$  modulators can activate insulin-sensitizing pathways without all of the associated side effects.

Figure 4. Structures of PPAR $\gamma$  Modulators



The glitazones MCC-555 and NC-2100 (Figure 4) represent a second class of PPAR $\gamma$  modulators [34,35]. These structurally related glitazones profile as weak-binding full agonists in cell-based reporter assays. In contrast to GW0072, both MCC-555 and NC-2100 promote adipocyte differentiation in cell culture. MCC-555 and NC-2100 each possess *in vivo* activity in obese insulin-resistant mice comparable to rosiglitazone despite their weak agonist profiles [34,35]. In mice, NC-2100 produced less weight gain compared to other glitazones when similar mice maintained comparable levels of glycemic control.

### Antagonists

GW9662 (Figure 5) was recently identified as a potent irreversible PPAR $\gamma$  ligand that profiles as a functionally selective PPAR $\gamma$  antagonist at concentrations of 1-10  $\mu$ M in cell-based assays [20]. GW9662 binds covalently to Cys286 located on helix 3 of the PPAR $\gamma$  ligand binding domain. Despite the fact that this cysteine residue is conserved in all three PPAR subtypes, GW9662 displays greater affinity for PPAR $\gamma$  than for PPAR $\alpha$  or PPAR $\delta$ . GW9662 antagonizes PPAR $\gamma$  activation in multiple cell types including adipocytes, macrophages and hepatic stellate cells [36]. Recently, a structurally related ligand, T0070907 (Figure 5), was described as a potent and selective PPAR $\gamma$  antagonist [37].

PD 068235 (Figure 5) has been reported to be a PPAR $\gamma$  antagonist that inhibits transcriptional activity and cofactor association induced by rosiglitazone [38]. In addition, high  $\mu$ M concentrations of PD 06823 block adipogenesis induced by either rosiglitazone or insulin. The ligand LG100641 (Figure 5) has been described as a specific antagonist that inhibits rosiglitazone-induced adipocyte differentiation, but stimulates insulin-mediated glucose uptake in adipocytes [39]. LG100641 also prevents TZD-induced cofactor recruitment and antagonizes

## Peroxisome Proliferator-Activated Receptors (PPARs)...(continued)

**Table 1. Activity of PPAR Agonists in Cell-Based Transactivation Assays. <sup>a</sup>**

Compound	Human receptor EC <sub>50</sub> (μM)			Murine receptor EC <sub>50</sub> (μM)		
	PPAR $\alpha$	PPAR $\gamma$	PPAR $\delta$	PPAR $\alpha$	PPAR $\gamma$	PPAR $\delta$
Wy-14643	5.0	60	35	0.63	32	IA @ 100
Fenofibrate	30	300	IA @ 100	18	250	IA @ 100
GW7647 <sup>b</sup>	0.0061	1.0	8.0	0.0018	1.5	IA
Pioglitazone	IA	0.58	IA	IA	0.55	IA
Rosiglitazone	IA	0.043	IA	IA	0.076	IA
KRP-297	0.85	0.083	9.1	10	0.14	7.2
GI262570	0.45	0.00034	IA	IA	0.00035	IA
GW1929	IA	0.0062	IA	IA	0.013	IA
GW7845	3.5	0.00071	IA	IA	0.0012	IA
GW501516 <sup>c</sup>	1.0	0.80	0.0012	1.5	0.80	0.024

<sup>a</sup> Data as reported in reference [1] unless otherwise noted. All data were generated using the PPAR-GAL4 transactivation assay.

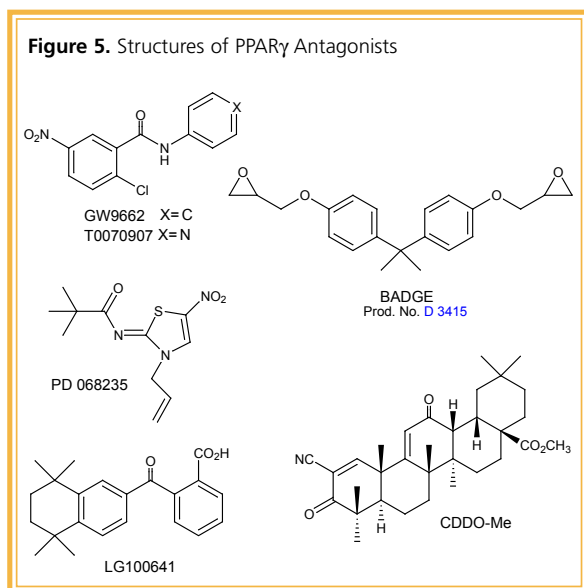
IA = inactive at 10 μM or the indicated concentration.

<sup>b</sup> see reference [10]

<sup>c</sup> see reference [57]

adipogenic gene expression. However, it should be noted that LG100641 exhibits higher binding affinity for RXR than for PPAR $\gamma$ .

The plasticizer bisphenol A diglycidyl ether (BADGE; Prod. No. [D 3415](#); Figure 5) is a low affinity ( $K_i$  ~100 μM) PPAR $\gamma$  ligand that has been reported to be a PPAR $\gamma$  antagonist [40]. BADGE blocks both rosiglitazone- and insulin-induced adipogenesis, but requires concentrations that approach its limit of solubility. The PPAR $\gamma$  ligand CDDO-Me ( $K_i$  =100 μM; Figure 5) is a synthetic triterpenoid that has been shown to inhibit adipocyte differentiation at concentrations below 1 μM [41]. However, CDDO-Me is toxic to cells at concentrations above 1 μM due to interaction with PPAR $\gamma$ -independent pathways.



### PPAR $\gamma$ In Disease

#### PPAR $\gamma$ and Diabetes

The treatment of type 2 diabetes is the most widely studied therapeutic utility for PPAR $\gamma$  agonists. PPAR $\gamma$  agonists reduce plasma glucose, lipids and insulin levels in type 2 diabetic patients [42,43]. The glitazones are known to improve glucose utilization in skeletal muscle which raises the paradoxical question, "How does a receptor expressed predominantly in adipose tissue improve glucose metabolism in muscle?" Furthermore, it seems counterintuitive that a receptor that promotes adipocyte differentiation would be an effective diabetes target given that obesity is a major risk factor for the development of type 2 diabetes. Several explanations have been postulated. First, PPAR $\gamma$  activation in adipocytes represses the secretion of endocrine signaling molecules such as TNF $\alpha$  (Prod. Nos. [T 5944](#), [T 7539](#), [T 6674](#)) and leptin (Prod. Nos. [L 3772](#), [L 5037](#) and [L 4146](#)) that influence insulin resistance [44]. Second, PPAR $\gamma$  agonists decrease circulating free fatty acid levels and may thereby indirectly affect glucose levels by invoking the Randle cycle [45]. In addition, PPAR $\gamma$  activation in rodents has been shown to increase the number of small adipocytes while decreasing the number of large adipocytes [46,47]. Smaller adipocytes typically have greater insulin sensitivity, take up more glucose and have lower rates of lipolysis compared to large adipocytes.

In insulin-resistant ZDF rats, PPAR $\gamma$  agonist treatment results in an increase in the expression of numerous genes involved in lipogenesis and fatty acid transport, storage and oxidation in adipose tissue [48]. In contrast, many of the same fatty acid metabolism genes are decreased in skeletal muscle. Decreased

## Peroxisome Proliferator-Activated Receptors (PPARs)...(continued)

expression of genes required for gluconeogenesis also occurred in the liver. This is consistent with PPAR $\gamma$  agonists promoting a flux of glucose and fatty acids into adipose tissue and away from muscle with a net result of increased glucose utilization in muscle and decreased gluconeogenesis in liver. Finally, PPAR $\gamma$  is expressed in skeletal muscle and liver, albeit at significantly lower levels than in adipocytes [49,50]. It is therefore plausible that some of the antidiabetic effects of PPAR $\gamma$  agonists are derived through receptor activation in muscle and liver.

### PPAR $\gamma$ and Atherosclerosis

The progression of atherosclerosis involves the accumulation of foam cells below the arterial wall endothelium. Foam cells are cholesterol-laden macrophages which result from the internalization of oxLDL particles by lipid transporters such as CD36, scavenger receptor-A and others [51]. The abundant expression of PPAR $\gamma$  in macrophages and foam cells led to the speculation that PPAR $\gamma$  might be proatherogenic by promoting foam cell formation. However, using a standard model of atherosclerosis, treatment of LDL receptor-deficient mice with rosiglitazone or GW7845 was shown to prevent the formation of atherosclerotic lesions despite increasing CD36 expression [52]. In addition, current clinical data indicate that PPAR $\gamma$  agonists actually protect type 2 diabetes patients from atherosclerosis [53].

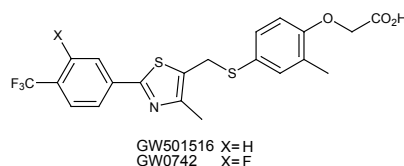
A recent study has provided insights into the antiatherogenic effect of PPAR $\gamma$ . ABCA1 is a member of the ATP binding cassette (ABC) family of energy-dependent transporter proteins that regulate cholesterol efflux from macrophages. ABCA1 gene expression is regulated by the nuclear oxysterol receptor LXR [54]. PPAR $\gamma$  activation induces the expression of LXR $\alpha$  which promotes ABCA1 expression and ultimately cholesterol efflux [55]. Thus, PPAR $\gamma$  may be antiatherogenic *in vivo* by enhancing cholesterol efflux from macrophages and endothelial cells.

### PPAR $\delta$

Although human PPAR $\delta$  (also known as PPAR $\beta$ ) was cloned in the early 1990s [56], an understanding of the physiological function of this subtype has lagged behind the others. A systematic study of fatty acid binding to PPAR $\delta$  has found that both saturated and unsaturated acids bind to the receptor [19]. Recent studies with a potent, selective PPAR $\delta$  agonist, GW501516 (Figure 6), have indicated that activation of this subtype can induce reverse cholesterol trans-

port and rectify lipoprotein profiles and triglyceride levels in obese rhesus monkeys [57]. Significantly, GW501516 raised HDLc levels more than fenofibrate in this model [58]. PPAR $\delta$  has also exhibited a potential role in placentation, adiposity, colorectal cancer, and diabetic factors [59-61]. GW0742 (Figure 6) is a closely related analog of GW501516 and shows equivalent potency and selectivity for PPAR $\delta$ .

Figure 6. Structures of PPAR $\delta$  Agonists



### PPAR Structural Studies

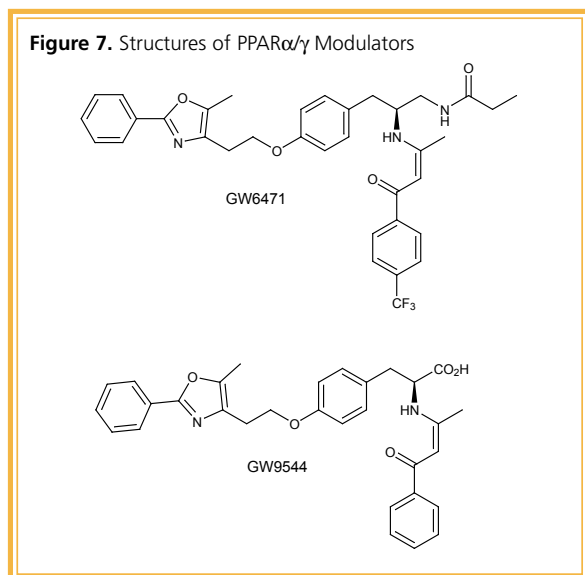
Structures of the ligand binding domain (LBD) of the PPARs in the absence and presence of ligands have been solved by x-ray crystallography [7,19,33,62-65]. The apo-PPAR $\gamma$  LBD crystal structure shows the receptor consists of a bundle of 13  $\alpha$ -helices and a small four-stranded  $\beta$ -sheet arranged in a canonical helical sandwich fold commonly observed for other nuclear receptors [62]. Each of the PPAR subtypes possesses a similar Y-shaped binding pocket located within the lower half of the LBD that extends from the C-terminal AF-2 helix to the  $\beta$ -sheet between helices H3 and H6. However, marked differences in the detailed topology of the binding pockets allow for selective ligand binding. The solved structures for ligand-bound PPARs reveal that agonists, such as TZDs, fibrates and fatty acids, share a common binding mode in which their acidic head groups participate in an intricate hydrogen bonding network within the ligand binding pocket that includes a key tyrosine residue located in the C-terminal AF-2 helix [62,64]. These interactions form a charge clamp that effectively stabilizes the receptor into a conformation permissive to the recruitment of coactivator proteins, such as SRC-1 required for transcriptional activation [62,64]. This is illustrated by the structures of the PPAR $\gamma$ /farglitazar/SRC-1 and PPAR $\alpha$ /GW9544/SRC-1 ternary complexes [62,65].

The PPAR $\gamma$  partial agonist GW0072 (Figure 4) also contains a carboxylic acid. However, X-ray crystallography has shown that this ligand fails to contact the AF-2 helix [32]. The receptor adopts a conformation similar to the apo-PPAR $\gamma$  with GW0072 occupying the ligand binding pocket in a region distal from the

## Peroxisome Proliferator-Activated Receptors (PPARs)...(continued)

residues involved in agonist stabilization of the AF-2 helix. In this structure, the charge clamp is not stabilized by the ligand which may account for its weak agonist activity.

A crystal structure of PPAR $\alpha$  bound to the antagonist GW6471 (Figure 7) and a SMRT corepressor motif was recently published [7]. The antagonist GW6471 binds in a configuration similar to the agonist GW9544 (Figure 7). However, the modified amide head group of GW6471 is unable to form the critical hydrogen bond with the AF-2 helix and creates an unfavorable steric interaction with the AF-2 helix. The AF-2 helix is displaced from an active conformation creating a larger pocket for cofactor binding. The SMRT corepressor motif binds to the receptor and extends into the voided AF-2 helix space, thereby maintaining the receptor in an inactive conformation. These structures have therefore provided molecular insights into the role ligands play in regulating receptor activity. They also provide insights for the design of improved PPAR ligands.



### Conclusions

The PPAR family of nuclear receptors function to regulate a broad range of genes in many metabolically active tissues. These receptors participate in the systemic regulation of lipid metabolism serving as sensors for fatty acids, eicosanoids, prostaglandins and related metabolites. The PPARs have emerged as therapeutic targets with widespread impact on the treatment of metabolic disorders. PPAR agonists

### PPAR Reagents Available from Sigma-RBI

<a href="#">B 7273</a>	<b>Bezafibrate</b>
<a href="#">C 0330</a>	<b>Ciprofibrate</b>
<a href="#">C 3974</a>	<b>Ciglitazone</b>
<a href="#">C 6643</a>	<b>Clofibrate</b>
<a href="#">C 7081</a>	<b>Wy-14643</b>
<a href="#">19,777-7</a>	<b>Clofibric acid</b>
<a href="#">D 3415</a>	<b>Bisphenol A diglycidyl ether (BADGE)</b>
<a href="#">F 0259</a>	<b>FMOC-leucine</b>
<a href="#">F 6020</a>	<b>Fenofibrate</b>
<a href="#">G 9518</a>	<b>Gemfibrozil</b>
<a href="#">H 4019</a>	<b>8[S]-HETE</b>
<a href="#">H 7779</a>	<b>N-(4-Hydroxyphenyl)retinamide</b>
<a href="#">H 9146</a>	<b>13-Hydroxyoctadecadienoic acid (13(S)-HODE)</b>
<a href="#">L 0517</a>	<b>Leukotriene B<sub>4</sub></b>
<a href="#">L 5408</a>	<b>LY-171,883</b>
<a href="#">P-249</a>	<b>Anti-Peroxisome proliferator activated receptor</b>
<a href="#">P-250</a>	<b>Anti-Peroxisome proliferator activated receptor (<math>\gamma</math>2 isoform)</b>
<a href="#">P 4547</a>	<b>Prostaglandin A<sub>2</sub></b>
<a href="#">S 8139</a>	<b>Sulindac</b>

have established therapeutic benefits in treating diabetes and cardiovascular diseases. Understanding the role of the PPARs in other diseases remains an area of active research. The discovery of receptor-specific ligands has led to significant advancement in our understanding of the structure of the PPARs and the molecular mechanism of their ligand-dependent activation. Finally, the potential to develop PPAR modulating drugs with improved efficacy and safety profiles appears promising.

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## New Product Highlights

### Tetanus toxin and its C-fragment conjugates: Useful tools for mapping the central nervous system

Tetanus toxin (Prod. No. [T 3194](#)), which is produced by the bacterium *Clostridium tetani*, induces the spastic paralysis of tetanus by blocking neurotransmitter release from inhibitory spinal neurons [1]. It also possesses unique properties that make it a valuable tool for elucidating and tracing neuronal connections within the central nervous system (CNS). This potent neurotoxin exhibits absolute specificity for neuronal cells as a result of binding to gangliosides that are present in the presynaptic membrane of all peripheral neuronal cell types (motor, sensory and autonomic). The bound toxin is then internalized and transported axoplasmically to the CNS, where it undergoes transsynaptic retrograde transport [1].

Structurally, tetanus toxin (150 kDa) is comprised of two polypeptide chains, a heavy chain (100 kDa) and a light chain (50 kDa). One disulfide bridge connects these two polypeptides and the heavy chain contains the toxin's binding domain [2,3].

An important fragment, the tetanus toxin C-fragment (Prod. No. [T 3694](#)), is generated when the

toxin is enzymatically cleaved by papain [4]. The C-fragment (50 kDa) contains the C-terminus of the heavy chain. The C-fragment is useful because it retains the binding, internalization and transsynaptic transport capabilities of the whole toxin, but is non-toxic without disrupting any neuronal processes [5]. Tetanus toxin C-fragment horseradish peroxidase conjugate (Prod. No. [T 3944](#)) has been demonstrated to be transported from motor neurons to presynaptic terminals in the spinal cord [5]. Tetanus toxin C-fragment fluorescein isothiocyanate (FITC) conjugate (Prod. No. [T 3819](#)) has been utilized to visualize mouse neuromuscular junctions [6]. Thus, the C-fragment and its conjugates are important neuronal tracer dyes for studying synaptic innervation in the central and peripheral nervous systems.

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