PPARs as Targets for Metabolic and Cardiovascular Diseases

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Abstract: Peroxisome proliferator-activated receptors (PPARs) alpha, gamma and delta (beta) are ligand-activated transcription factors of the nuclear hormone receptor superfamily which have been shown to play key roles in maintaining glucose and lipid homeostasis. The physiological effects of several marketed drugs for the treatment of dyslipidemia (fenofibrate and gemfibrozil) and diabetes (rosiglitazone and pioglitazone) have now been shown to be mediated through PPARalpha and PPARgamma respectively. Over the past few years our understanding of how PPAR ligands and receptors modulate gene expression has greatly increased; this knowledge is being used to design even more potent and efficacious PPAR ligands for the treatment of diabetes, dyslipidemia, atherosclerosis and obesity. This review is a brief survey of the PPAR field which highlights recent progress, with an emphasis on new ligands with novel PPAR profiles, particularly compounds which are co-agonists of PPAα, γ and β (δ).

INTRODUCTION

Peroxisome Proliferator Activated Receptors (PPARs) are ligand-activated transcription factors and members of the nuclear hormone receptor superfamily. These receptors modulate gene expression and play a key role in maintaining glucose and lipid homeostasis. Several classes of drugs used in the treatment of dyslipidemia and diabetes, e.g., the fibrates and thiazolidinediones, are ligands for the various PPAR isoforms. Over the past several years our understanding of how these ligands and receptors modulate gene expression has improved. We can now use this knowledge to design more potent and efficacious compounds to treat diabetes, obesity and cardiovascular diseases. This review is a brief survey of the field which highlights key new findings with an emphasis on compounds with novel profiles, especially compounds which are dual ligands for PPARα and PPARγ.

The PPARs heterodimerize with the retinoid X receptors (RXR) and as such bind to distinct regions of DNA termed peroxisome proliferator response elements (PPREs) [1]. The PPREs are usually a direct repeat of the sequence GGTCA separated by a single nucleotide (DR-1) [1]. Many variations of this theme are present in promoters of PPAR responsive genes and are functional PPREs [2]. Typical examples include acyl coenzyme A oxidase (AOX or ACO), lipoprotein lipase (LPL) and fatty acid binding protein (aP2) [1-3].

The PPARs interact with an expanding list of intermediary molecules collectively known as corepressors and coactivators [4-7] which play an important role in the activity of these receptors. Some examples of coactivators are the members of the SRC/p160 and CBP/p300 family that have histone acetyl transferase activity (HAT); these cofactors destabilize the nucleosomal core by acetylation of lysine residues in the N-terminus of histones. Other examples of coactivators are PBP and PRIP [8] (which do not have HAT activity) and PGC-1, which has been shown to interact with the PPARs and plays an important role in mediating thermogenesis and gluconeogenesis [9-11]. The different steps leading to receptor activation are: 1) ligand binding and subsequent conformational change leading to increased receptor interaction with DNA, 2) dissociation of corepressors and recruitment of coactivators, 3) chromatin remodelling by the coactivator-associated histone acetyltransferase activity and 4) interaction with the basal transcription machinery and Polymerase II.

In addition to activating gene expression, the PPARs can also inhibit gene expression by negative regulation of the NFκB, AP-1 and STAT signaling pathways [12,13] *via* direct protein-protein interactions between PPAR and these transcription factors. [14]. Mechanistically, transrepression is very different from transactivation and is much less well characterized. The C-terminus (activating function [AF-2] domain) is absolutely required for ligand-dependent coactivator interaction and transactivation. However, neither DNA binding nor the AF2 function is required for transrepression [15].

PPARα

The mouse PPAR, later called mPPARα [16] was the first PPAR identified. Two other PPAR subtypes were subsequently cloned: PPARβ (also called NUC1, PPARδ, or FAAR [17-19] and PPARγ [17]). Sequence comparison indicates a remarkable homology within the PPAR subtypes in the DNA binding and ligand binding domains, as shown in (Fig. **1**) [20]. The N-terminus of the PPARs are, however, divergent, indicating that the transcription capabilities of the three PPAR subtypes are different. PPARα is expressed at high levels in liver, kidney, skeletal muscle and heart [21]. Recently, several splice variants (in the 5' untranslated region) have been identified [22].

Saturated and unsaturated fatty acids [23], eicosanoids (e.g 8(S)-HETEs) [24], the fibrate class of hypolipidemic drugs (fenofibrate, clofibrate, ciprofibrate, bezafibrate and gemfibrozil) and many other xenobiotics activate PPARα [25]. However, the fibrates are relatively low affinity ligands of PPARα [26]. High affinity synthetic fibrate PPARα ligands have now been identified, e.g. GW2331 [27] and the

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Fig. (1). Homology between the three PPAR subtypes, comparision of the amino acid sequences. The receptors are shown divided into domains (DBD, DNA binding domain, LBD, ligand binding domain). The numbers within the boxes represent the percent amino acid identity of that domain between the receptors. The numbers on top demarcate the domains.

more selective GW9578 [28]. The fibrates induce peroxisomal proliferation and hepatomegaly in mice and rats on prolonged exposure [29,30], but these effects have not been observed in either human hepatocyte cultures or in patients. PPARα has been demonstrated to be the molecular target for the fibrates by the lack of induction of fibrate-responsive genes in PPARα knockout mice [31]. This lack of induction may be attributed to a missing component in the human PPARα signaling pathway or to an attenuated response by human PPARα [32]. Another possible explanation is that the level of expression of the PPARα receptor in human liver is much lower than for mouse/rat liver [33]. A recent report showed that human PPARα is fully capable of supporting peroxisomal proliferation when introduced into mPPARα deficient mice [34].

What is the evidence that PPARα is the target of fibrates causing HDL elevation and triglyceride lowering in man? Fibrates lower triglyceride levels by increasing LPL-mediated lipoprotein lipolysis and by decreasing ApoCIII, a known inhibitor of VLDL clearance [35]. Recently, PPARα agonists have also been shown to induce another apolipoprotein, ApoAV [36], which apparently plays an important role in determining plasma triglyceride concentrations in humans. Fibrates also raise HDL cholesterol levels in humans, in part by increasing expression of ApoAI and ApoAII [35,37]. In the recent Veterans Affairs Cooperative Studies Program High-Density Lipoprotein Cholesterol Intervention Trial (VA-HIT), gemfibrozil therapy significantly reduced the risk of major cardiovascular events in patients with coronary disease whose primary lipid abnormalities was a low HDL cholesterol level [38]. Gemfibrozil raised HDL-C (6%) and lowered triglyceride levels (31%) in these patients who had normal LDL levels. However, in the Bezafibrate Infarction Prevention (BIP) Study in a different patient population (total cholesterol of 180 to 250 mg/dl and LDL cholesterol $\leq$ 180 mg/dl), bezafibrate, a more potent PPARα agonist compared to gemfibrozil**,** did not reduce the risk of cardiovascular events [39] although the lipid changes in the BIP study were more pronounced than in the VA-HIT study [40]. In a subgroup analysis, there was a decrease in the primary cardiovascular endpoints in patients with triglycerides above 200 mg/dl; however, this requires further confirmation.

Part of this apparent discrepancy in the clinical trial results could be due to the fact that fibrates, in addition to raising HDL-C levels, also inhibit anti-inflammatory processes occurring directly at the vascular wall. PPARα agonists have been shown to transcriptionally repress (transrepress) expression of adhesion molecules (VCAM-1) in human vascular endothelial cells [41]. Fenofibrate reduced the inflammatory response in patients with coronary artery disease by decreasing the levels of IL-6, fibrinogen and C-reactive protein [13]. However, all fibrates may not have the same efficacy in transrepression since this process is very different from transcriptional activation (transactivation). A thorough analysis of the effect of different fibrates on repressing the inflammatory processes at the vessel wall should help to clarify this issue.

Obesity is a major risk factor for insulin resistance, diabetes and cardiovascular diseases. PPARα-selective activators improve insulin sensitivity and reduce adiposity and body weight gain without influencing caloric intake in rodent models of insulin resistance [42]. In obese hypertriglyceridemic, hyperinsulinemic rhesus monkeys, fenofibrate improved the lipid profile and decreased insulin levels without causing changes in body weight or food consumption [43]. This profile is clearly different from that of known PPARγ ligands, particularly the thiazolidinediones, which improve insulin sensitivity but also increase adiposity and body weight gain in rodents and humans. If PPARα selective agonists can be shown to improve insulin sensitivity and control obesity (in addition to their anti-dyslipidemic effects) in humans, they should be extremely effective agents for the treatment of metabolic syndrome.

Hence, PPARα ligands have several effects *in vivo.* They improve dyslipidemia, control inflammation and adhesion of monocytes in the vessel wall and modulate insulin

sensitivity and obesity. It is of interest to note that high affinity PPARα agonists have not yet reached the market, although it has been ten years since the fibrate class of drugs were discovered to be PPARα ligands. PPARα agonists have been shown to regulate bile acid synthesis, resulting in increased cholesterol saturation of bile with increased risk of gallstone formation [44]. Fenofibrate raised homocysteine levels, an emerging cardiovascular risk factor, in humans [45]. Increase in homocysteine levels was not seen with gemfibrozil [46] suggesting important differences in the activity of PPARα ligands. The side effects of the fibrates may be limiting the clinical dosage, thus preventing maximal efficacy of these drugs to protect against cardiovascular disease. However, it is possible that these side effects are specific to the fibrate chemotype, and may not necessarily apply to other, structurally different PPARα agonists.

A more complete understanding of how PPARα modulates genes that control these pathways will allow us to develop more efficacious drugs for the treatment of dyslipidemia and cardiovascular disease while limiting the side effects. In addition, based on rodent *in vivo* data [42], some researchers believe that potent PPARα agonists may also have anti-obesity effects in humans. This has not been demonstrated with the current fibrates so far, however, whether this is observed with a more potent compound remains to be seen. In a recent report K-111, a potent PPARα agonist, demonstrated insulin sensitization and decreased body weight gain in obese, insulin resistant rhesus monkeys [47].

*2.1.2. PPAR*β (δ)

The PPARβ subtype in humans is homologous to Xenopus PPARβ [17]. However, this receptor is sufficiently divergent from Xenopus PPARβ to warrant reclassification as an additional PPAR subtype, PPARδ/β [20]. This ubiquitously expressed receptor is the least well understood of all the PPAR subtypes, partly because selective, high affinity PPARβ ligands have been identified only recently [48].

PPARβ has been shown to play an important role in lipid metabolism. The PPARβ selective agonist L-165041, at doses which had no effect on plasma glucose and triglyceride levels, raised cholesterol levels in db/db mice [48]. The increased plasma total cholesterol was primarily associated with HDL particles [49]. Of particular interest is the recent report that a high affinity, selective PPARβ agonist (GW501516) raised HDL-C levels (80%) and decreased triglyceride levels (50%) in obese rhesus monkeys [50]. Recently, PPARβ has been shown to be an important regulator of fatty acid oxidation and energy uncoupling in fat and muscle; thus it may play a role in regulating adipose tissue levels and obesity [51]. PPARβ also regulates the inflammatory status of the macrophage by a switching mechanism involving association and dissociation of corepressors [52]. Hence, PPARβ and its ligands are attractive targets for the treatment of inflammatory disease and atherosclerosis.

*2.1.3. PPAR*γ

The third PPAR subtype is PPARγ, which has been extensively studied because the thiazolidinediones (TZDs) used for the treatment of type 2 diabetes were discovered to be ligands for this receptor [53]. PPARγ is a key regulator of adipogenesis and therefore has a role in the pathogenesis of obesity, insulin resistance and diabetes [54]. There are three isoforms of PPARγ: PPARγ1, PPARγ2 and PPARγ3, which are all products of differential promoter usage and splicing. PPARγ1 and PPARγ3 have the same amino acid sequence while PPARγ2 contains 30 additional amino acids at the N-terminus. [3,55]. PPARγ1 and PPARγ2 give similar transcriptional response to PPAR agonists [55]. In comparison to PPARα and PPARβ, PPARγ has a more restricted tissue distribution, with high receptor levels in fat, spleen, colon and macrophages and low (but detectable) receptor levels in liver, skeletal muscle and pancreas [55-57]. The roles of the three isoforms of PPARγ are currently under investigation.

It is well known that the vast majority of insulin-stimulated glucose disposal occurs in skeletal muscle. However, since PPARγ is expressed at very high levels in adipocytes, many view adipose tissue as the primary site of action of PPARγ agonists, particularly the TZD class of antidiabetic drugs. The TZDs may induce a paracrine signal from fat to muscle, thereby mediating insulin sensitization. However, the possibility of a direct effect on muscle cannot be ruled out. Recently, transgenic mice with fat cells selectively ablated have been generated [58,59]. The study of these mice should help in deciphering if fat is the primary target tissue for the thiazolidinediones and other PPARγ agonists.

Although the endogenous ligand(s) for PPARγ (particularly those that modulate insulin sensitivity and adipocyte diffferentiation) have not been identified, strong candidates include polyunsaturated fatty acids (e.g linoleic acid, arachidonic acid and eicosapentaenoic acid), metabolites of linoleic acid, (e.g. 9-HODE and 13-HODE) [60] and prostaglandins, in particular 15-deoxy-$\Delta^{12,14}$-prostaglandin J2 [61,62]. All these compounds are low affinity ligands for the PPARγ receptor and activate PPARγ at micromolar concentrations. PPARγ has recently been shown to regulate the expression of key proteins involved in signaling; one such molecule is adiponectin, which is selectively and abundantly expressed in adipose tissue [63]. Adiponectin levels are decreased in the obese and diabetic state in both animals and humans. The thiazolidinediones induce a robust increase in circulating adiponectin levels in patients and thus adiponectin can serve as a convenient biomarker for PPARγ-mediated effects [64]. Another secreted protein from fat cells, resistin, was shown to increase insulin resistance in mice; TZDs decreased the secretion of resistin [65]. However, this conclusion has been disputed by another report [66].

The thiazolidinedione class of antidiabetic drugs (e.g. troglitazone, rosiglitazone and pioglitazone) are synthetic ligands and agonists of PPARγ [53]. They increase the transcriptional response of PPARγ in cotransfection assays [53] and increase cofactor recruitment in FRET assays [67]. It is important to realize that these compounds were already in development for the treatment of type 2 diabetes before they were identified as PPARγ ligands. What then is the evidence that PPARγ is truly the primary pharmacological target for the thiazolidinediones? First, there is a correlation

between *in vitro* potency (EC_{50} from cotransfection assays) and the minimum effective dose (MED) for antihyperglycemic activity [68]. Second, RXR selective ligands (rexinoids) targeting the PPARγ/RXR heterodimer also function as insulin sensitisers in rodent models of insulin resistance [69]. These data indicate that PPARγ (or the PPARγ/RXR heterodimer) is the target for the thiazolidinediones. The major side effects of TZDs are increased weight gain (adipogenesis) and peripheral edema [70].

Insulin resistance is associated with hypertriglyceridemia and low HDL-C levels. PPARγ agonists have been shown to inhibit the development of atherosclerosis in LDL receptor knockout mice [71]. Since the majority of type 2 diabetic patients eventually die from cardiovascular complications, the concept of a PPARγ/PPARα dual agonist to treat type 2 diabetes and the associated dyslipidemia is very attractive. Several such PPARα/γ coagonists have been identified (e.g. GW2331, KRP-297 and AZ 242) [27,72,73], and some of these compounds are currently in clinical development. We will deal with this important class of compound in greater detail later.

Recently, data obtained from PPARγ knockout mice presented an unexpected twist in our understanding of PPARγ and insulin resistance. The PPARγ null (-/-) mutation is embryonically lethal. The embryos die around day 10-11 p.c. due to placental dysfunction. Embryonic fibroblasts from PPARγ -/- mice could not be induced to differentiate into adipocytes, thus demonstrating that PPARγ is essential for adipocyte differentiation [74]. Using the tetraploid rescue technique, one homozygous mutant mouse was brought to term. This pup showed an almost complete absence of fat [75].

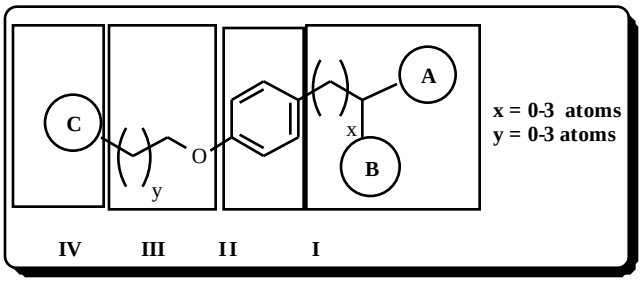
Interestingly, high fat-fed PPARγ (+/-) heterozygous knockout mice were more sensitive to insulin compared to their wild type counterparts. The PPARγ +/- mice had less white adipose tissue (WAT), smaller adipocytes, lower glucose and insulin levels, higher body temperature and higher circulating leptin levels compared to the wild type mice on a high fat diet [74]. This shows that in this animal model lowering total PPARγ activity in the face of a high fat challenge may improve insulin sensitivity and strongly suggests the investigation of PPARγ antagonists for the treatment of obesity and obesity-linked insulin resistance. Two mutations identified in the human PPARγ gene support this hypothesis [76,77].

The activity of PPARγ can be downregulated with either a PPARγ antagonist or a PPARγ partial agonist. This type of pharmacological approach may address the underlying disorder, insulin resistance, without the associated weight gain observed clinically with the thiazolidinediones. MCC-555 is a partial agonist of PPARγ in certain functional assays, yet its antihyperglycemic efficacy in KK-A^y mice is greater than many thiazolidinediones, including rosiglitazone [78].

Hence, it may be possible to identify partial agonists/antagonists of PPARγ that would be equal or superior in their anti-diabetic efficacy compared to the full PPARγ agonists rosiglitazone and/or pioglitazone. These partial agonists/antagonists may be selective modulators of PPARγ activity, in analogy to the selective estrogen receptor modulators (SERMs). The challenge would be to identify promoter and tissue selective modulators which maintain the anti-diabetic activity of the TZDs without the associated increase in weight and edema. Indeed, a selective PPARγ modulator LG100641 has been described [79]. LG100641 is a PPARγ ligand which promotes basal and insulin stimulated glucose uptake, but does not induce 3T3-L1 adipocyte differentiation. Potentiation of glucose uptake by the TZDs in adipocytes was maintained in the presence of a dominant negative mutant of PPARγ [80]. Finally, some selective PPARγ antagonists have shown to decrease diet-induced obesity and improve glucose tolerance and insulin action in mice [81,82]. Therefore, selective PPARγ modulators have the potential of becoming one of the drugs of the future in the treatment of diabetes, obesity and cardiovascular disease.

We now discuss the chemistry of the various PPAR ligands which have been used in research and others which are currently in development.

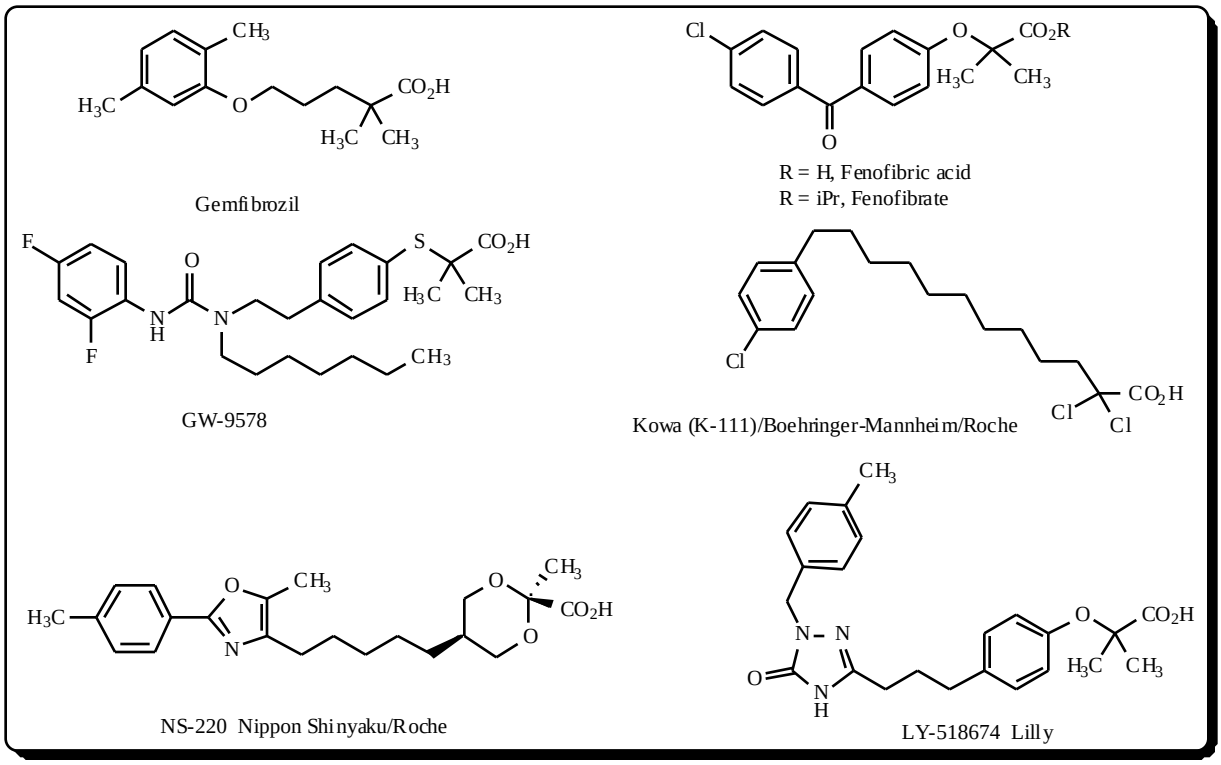
A generalized overall pharmacophore for PPAR ligands [26] can be expressed by the schematic structure shown. A typical PPAR ligand contains an acidic functionality I (e.g. A = a carboxylic acid, B = a hydrophobic group for tyrosine-derived ligands such as farglitazar or ragaglitazar, A + B = thiazolidinedione for TZD ligands such as rosiglitazone), central aromatic ring(s) II and a linker III to other aromatic/heteroaromatic ring(s) IV which in general contain a hydrogen bond acceptor (e.g. C = substituted oxazole). This general pharmacophore for synthetic PPAR ligands correlates well with the current hypothesis that the endogenous substrates for the PPAR receptors are likely to be structurally related fatty acids/eicosanoids or their metabolites (e.g. 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, 9-HODE, 13-HODE, etc) [26,83]. The focus of this review will be on synthetic PPAR ligands.



PPARα Agonists

The first PPARα ligands to be identified were the fibrate class of anti-dyslipidemic drugs, which were discovered by *in vivo* screening in rodent models. These compounds are characterized by the presence of the aryloxyacetic acid moiety and include the marketed drugs gemfibrozil (Lopid®) and fenofibrate (Tricor®) [84]. As with the thiazolidinedione PPARγ agonists, the fibrates had already been extensively studied in both animals and in human clinical trials prior to the discovery of the PPARα receptor.

As mentioned previously, treatment of patients with fibrates results in decreases in triglycerides and increases in HDL-cholesterol. The data from the VA-HIT clinical trial [85] with gemfibrozil showed that lowering triglycerides and

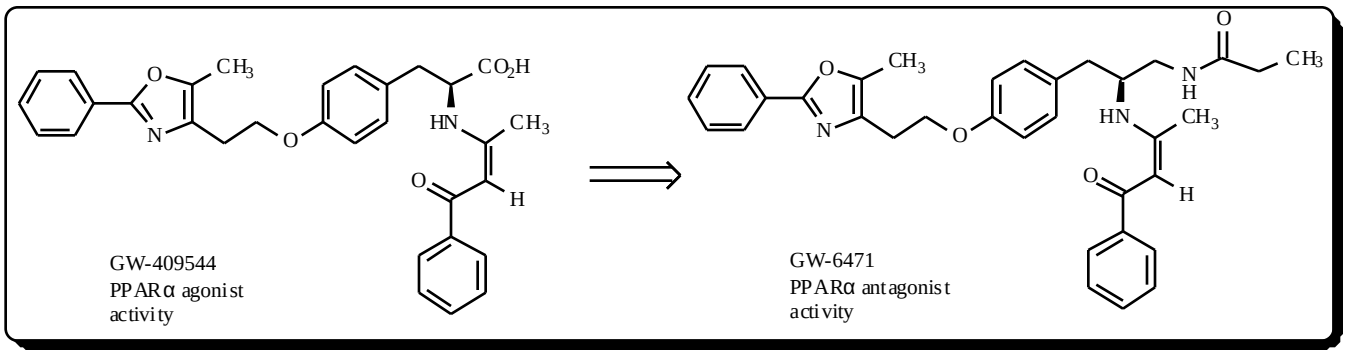


modestly increasing HDL-cholesterol result in a reduction of cardiac events in dyslipidemic patients with normal LDL cholesterol levels. The Diabetes Atherosclerosis Intervention Study (DAIS) [86,87] showed that fenofibrate reduces the angiographic progression of coronary-artery disease in type 2 diabetic patients. Fenofibric acid (the parent acid of the prodrug fenofibrate) and gemfibrozil are relatively weak PPARα agonists, with transactivation EC_{50} values in the high micromolar range, which is reflected in their relatively high clinical doses (200 and 1200 mg/day respectively). Clearly the development of more potent and efficacious PPARα agonists are of significant interest for the treatment of dyslipidemia as well as atherosclerosis.

The development of PPARα receptor binding and cell based functional assays have allowed for the screening and identification of other chemotypes as PPARα agonists. Optimization of the fibrate chemotype at GlaxoSmithKline led to the discovery of the very potent ureido-fibrate PPARα agonist GW-9578 [28]. This compound shows selective activity at PPARα (PPARα EC_{50} = 0.05 μM *vs* PPARγ EC_{50} = 1.0 μM and PPARδ EC_{50} = 1.4 μM) *in vitro*. In the cholesterol/cholic acid-fed rat model, GW-9578 reduced total cholesterol by 60% at 1.0 mg/kg (dosed twice daily for 4 days) versus a 50% decrease achieved with fenofibrate at 30 mg/kg under the same conditions. Maximal reductions of serum apoC-III of 30% were achieved with fenofibrate versus 80% with GW-9578.

Currently, a number of PPARα agonists are reported to be in clinical trials or late preclinical development; among these are K-111 (Kowa), GW-590735, NS-220 (Nippon Shinyaku/Roche) and LY-518674 (Lilly) [88].

Interestingly, one of the first examples of a PPARα antagonist, GW-6471 (from GlaxoSmithKline) has recently been reported [89]. This compound was structurally derived from the potent PPARα/γ dual agonist GW-409544. In a cell-based reporter assay, GW-6471 completely inhibited GW-409544-induced activation of PPARα with an IC_{50} of 0.24 μM. The proposed mechanism of antagonism (supported by X-ray crystal structures of a ternary complex of GW-6471 bound to PPARα LBD and a peptide containing a portion of the SMRT corepressor) is a 3-turn α-helix conformation assumed by the SMRT peptide that prevents the carboxy-terminal activation (AF-2) helix from



assuming the active (agonist) conformation. The binding of the co-repressor motif to the PPARα LBD is stabilized by the PPARα antagonist.

PPARδ(β) Agonists

To date there have been no marketed drugs which are known to be PPARδ ligands. Again, the development of appropriate binding and functional assays for PPARδ have allowed for the identification and characterization of PPARδ agonists which may serve to elucidate the pharmacology of this receptor. A number of potent PPARδ agonists *in vitro* have been described in the literature, as exemplified by L-165041 from Merck. L-165041 has been shown to regulate cholesterol metabolism in *db/db* mice [49]. The first PPARδ agonist to show significant anti-dyslipidemic efficacy in an animal model is GW-501516 [50] from GlaxoSmithKline, which is currently undergoing clinical trials. This compound has been shown to be a selective PPARδ agonist in a cell-based transactivation assay (EC_{50} = 0.001 μM *vs* PPARα EC_{50} = 1.1 μM and PPARγ EC_{50} = 0.85 μM). GW-501516 was shown to increase expression of ATP-binding cassette A1 (ABC-A1), the transporter which mediates cholesterol efflux in macrophages and intestinal cells. Moreover, it induced apoA-1-mediated cholesterol efflux in THP-1 macrophages. In studies in insulin-resistant obese rhesus monkeys, GW501516 treatment resulted in significant dose-dependent increases in serum high density lipoprotein (HDL) cholesterol while decreasing the levels of small-dense low density lipoprotein (LDLC), fasting triglycerides, and fasting insulin. These encouraging initial efficacy data for a selective PPARδ agonist indicates that this approach may serve as an effective treatment for the cardiovascular disease associated with the metabolic syndrome (in particular by increasing reverse cholesterol transport). However, it is important to note that the safety aspects (i.e. any toxicological side-effects) of long-term treatment with a PPARδ agonist are currently unknown. This is particularly important with respect to drug development due to the ubiquitous expression of the PPARδ receptor in various tissues and organs and also the uncertainty of the role of the PPARδ in these tissues.

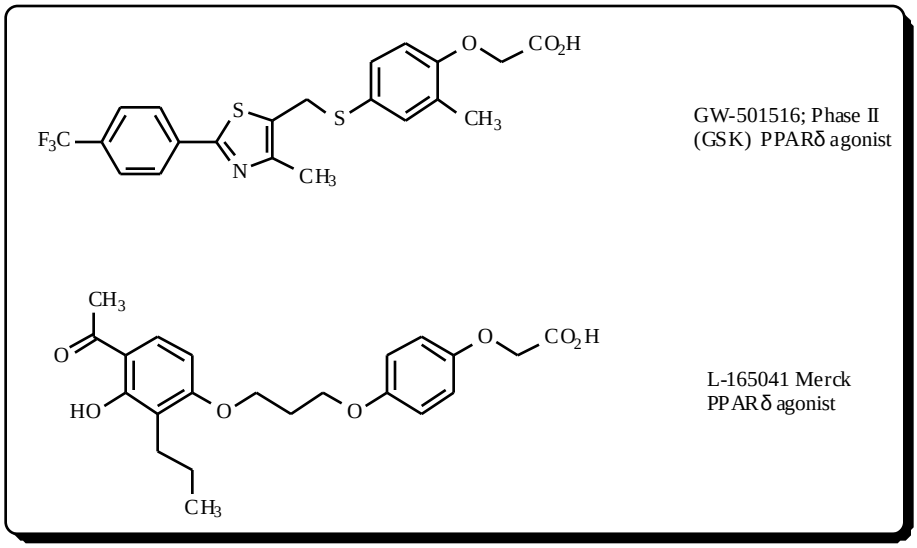
PPARγ Agonists

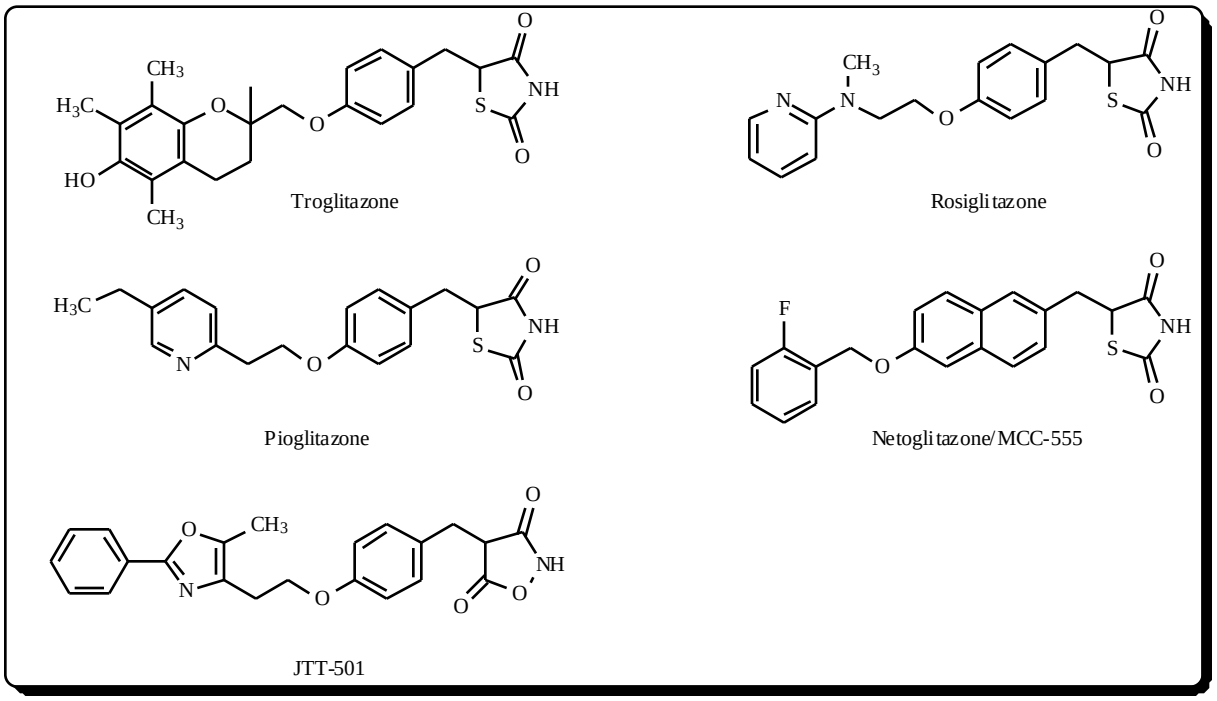
The first PPARγ ligands to be identified were the thiazolidinediones (TZDs), also known as the "glitazones". This class of compounds had already been extensively studied in both animals and in human clinical trials prior to the discovery of the PPARγ receptor. The structure-activity relationships of the thiazolidinedione class of PPARγ agonists has been extensively reviewed [68]. Rosiglitazone [90] (Avandia®, GlaxoSmithKline) and pioglitazone [91] (Actos®, Takeda/Lilly), which are the only two currently marketed PPARγ agonists, are both thiazolidinediones. Troglitazone [92], which was withdrawn in 1999 due to idiosyncratic occurrence of hepatotoxicity, is also from this structural class.

These compounds show generally good anti-hyperglycemic activity in standard animal models of type 2 diabetes (e.g. the *db/db* mouse). The rank order of their *in vitro* PPARγ transcriptional activity (EC_{50}) parallels that of their *in vivo* antidiabetic activity (minimal effective dose) [68]. This is reflected in their clinical doses: rosiglitazone (4- 8 mg/day) > pioglitazone (15-45 mg/day) > troglitazone (200-600 mg/day) [68]. Moreover, emerging preclinical and clinical data show that PPARγ agonists also have positive impact on many cardiovascular risk factors associated with type 2 diabetes, including dyslipidemia, redistribution of fat, hypertension, inflammation and atherosclerosis [93].

Other thiazolidinedione-class PPARγ agonists which have been/are in clinical development include MCC-555/netoglitazone [78] (Mitsubishi/Johnson & Johnson), balaglitazone (Novo-Nordisk/Dr. Reddy's Foundation) and rivoglitazone (CS-011, R-119702, Sankyo/Pfizer) [94]. JTT-501 (Japan Tobacco/Pharmacia) [95] is a very closely related isoxazolidinedione which has previously undergone clinical trials.

The development of PPARγ receptor binding and functional assays allowed for the screening and identification of other chemotypes as PPARγ agonists. Prominent among these new chemotypes are the α-alkoxy-β-arylpropanoic acids (exemplified by SB-236636) [26] and the related tyrosine-based agonists (exemplified by the very potent PPARγ agonist farglitazar/GI-262570 [96] from



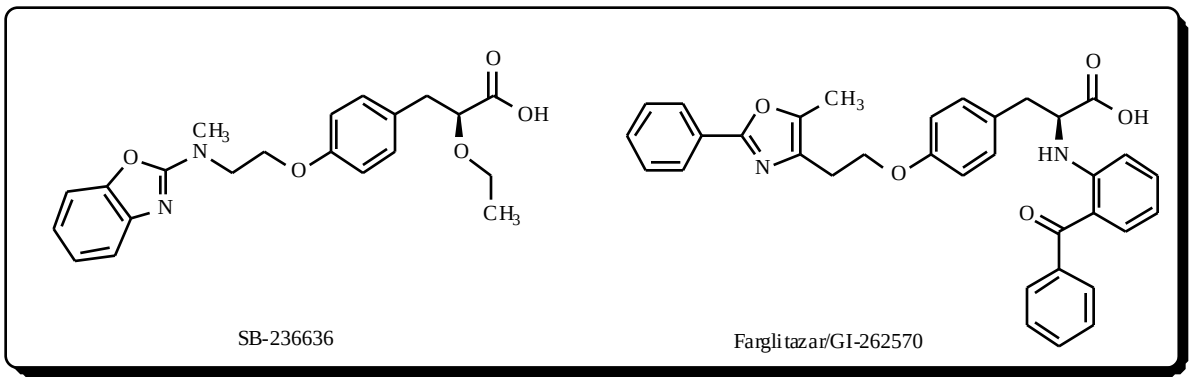


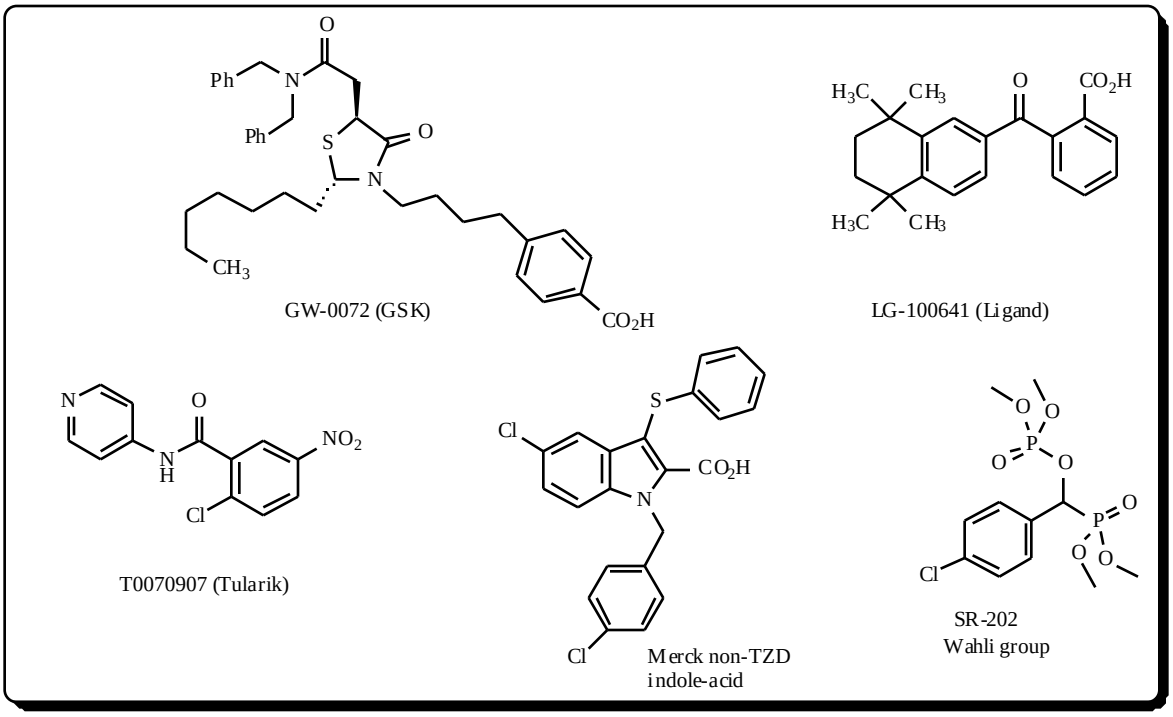
GlaxoSmithKline, which reached Phase III clinical trials). It is notable that the S-enantiomer is preferred for these series of compounds.

In clinical trials thus far, the PPARγ agonists show a number of what appear to be mechanism-based side effects (since they are observed with several structurally different classes of PPARγ agonists). Weight gain and edema (fluid retention) are the principal adverse effects observed upon treatment with PPARγ agonists, particularly in combination with insulin [97]. The idiosyncratic hepatotoxicity observed with the PPARγ agonist troglitazone appears to be compound/structure-specific as there has been no evidence reported of this occurring with other TZDs, either rosiglitazone or pioglitazone [98].

PPARγ "Modulators"- Partial Agonists and Antagonists

A number of PPARγ ligands have recently been identified as "partial agonists" or "antagonists" of the PPARγ receptor. These include various irreversible ligands, such as T0070907 [99], as well as non-covalent ligands such as the benzophenone-acid LG100641 [79] and an indole-based non-TZD partial agonist from Merck [100]. A characteristic compound, such as GW-0072 [101], reduced the ability of a PPARγ agonist (generally a TZD, e.g. rosiglitazone) to promote the differentiation of pre-adipocytes (e.g. 3T3-L1 mouse pre-adipocytes) to adipocytes (a characteristic activity of synthetic PPARγ agonists). A recent report [81] from Wahli's group showed that their selective phosphonophosphate PPARγ antagonist SR-202 not only inhibits TZD-induced, but also hormone-mediated (insulin/dexamethasone/IBMX cocktail) pre-adipocyte differentiation. Other PPARγ "antagonists", such as LG10064, do not inhibit insulin-mediated pre-adipocyte differentiation. SR-202 also shows intriguing *in vivo* activity in *ob/ob* mice (improvement in glucose disposal and insulin sensitivity). A PPARγ "antagonist" that prevents adipocyte differentiation/hypertrophy (and presumably obesity) and improves insulin resistance could be of interest for the treatment of diabetes/obesity/metabolic syndrome. However, the concept/definition of a PPARγ "antagonist" remains nebulous and at present individual compounds can likely only be fully characterized by correlating their *in vitro*/*in vivo* activities with appropriate gene expression profiling in different tissues.





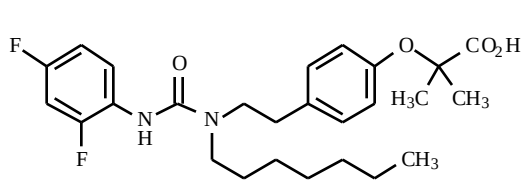
PPARα/γ Dual Agonists

There is now a wealth of positive clinical data on the use of PPARγ agonists for the treatment of diabetes as well as the use of PPARα agonists for the treatment of dyslipidemia and coronary heart disease. It therefore appears very likely that a dual PPARα/γ agonist that improves insulin sensitivity, lowers glucose and corrects lipoprotein abnormalities would be of great interest as a drug for the treatment of type 2 diabetes and the associated dyslipidemia and cardiovascular risk factors [93,94]. In particular, simultaneous activation of PPARα (resulting in triglyceride lowering and HDL cholesterol elevation) and PPARγ (resulting in decrease of hyperglycemia and insulin resistance) may provide a unique opportunity for the treatment of the multiple components of the increasingly prevalent metabolic syndrome worldwide (insulin resistance, abnormalities in triglyceride and HDL cholesterol metabolism, hypertension and obesity) [102]. There has been a significant amount of research in this area over the past few years and a number of PPARα/γ dual agonists (with varying degrees of α- and γ- selectivity) are currently in clinical development. Some structures of representative PPARα/γ dual agonists are shown below. It has been a general observation that very slight differences in structure can lead to very significant differences in PPARα:PPARγ functional activity (e.g. GW-2331, a PPARα/γ dual agonist, differs from GW-9578, a PPARα-selective agonist, only by a single atom transformation from oxygen to sulfur). These compounds all show varying degrees of PPARα and PPARγ binding affinities and functional activities. It should be noted that the EC_{50} values obtained in PPAR transactivation assays can vary depending on the cell-line, the particular PPAR response element and the reporter used in the particular assay. There are a number of issues to be considered when determining the appropriate "optimal" balance between PPARα and PPARγ *in vitro* functional activity. First, the significant interspecies differences between the human and rodent PPAR receptors (particularly the well-documented differences in the PPARα ligand binding domain) complicates the correlation of *in vitro* potency (generally using human PPAR receptors) to any assessment of *in vivo* efficacy in rodent efficacy models. A "balanced" coagonist in human PPAR *in vitro* assays (i.e. with similar EC_{50}s and efficacies/activities at human PPARα/γ receptors) thus may not show "balanced" activity in a mouse efficacy model. Similar complications may arise in correlating *in vivo* efficacy in animal models with clinical data. Secondly, the PPARα and PPARγ subtypes are preferentially expressed in different tissues, PPARα being highly expressed in liver and skeletal muscle, whereas PPARγ is expressed in adipose and spleen and at low levels in skeletal muscle and liver. As a result, even if one is fortunate enough to obtain an optimal *in vitro* "ratio" of PPARα:PPARγ functional activity for a given compound, the *in vivo* activity/efficacy may be highly influenced by the relative tissue distribution of a PPARα/γ dual agonist *in vivo.* For instance, if a balanced PPARα/γ dual agonist happens to be mainly localized in the liver (with minimal drug levels in adipose), it may show *in vivo* efficacy very similar to a PPARα-selective compound. In spite of these difficulties in identifying and profiling a compound with optimal PPARα/γ activity, a number of PPARα/γ dual agonists have been advanced into clinical development over the past few years. There are currently two PPARα/γ dual agonists in late stage development:

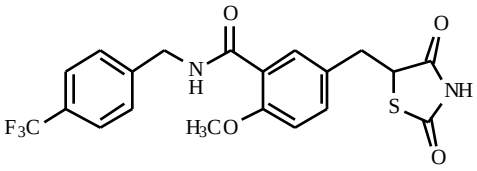
1) BMS-298585 (muraglitazar, from Bristol-Myers Squibb) [103-105].

2) AZ-242 (tesaglitazar, from Astra-Zeneca) [73].

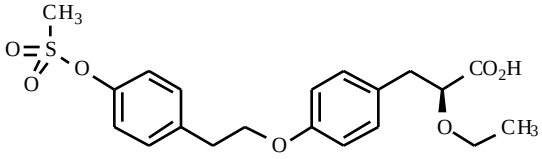
The development of two other compounds which had completed Phase II clinical trials, NN-622 (ragaglitazar from Dr. Reddy's Foundation/Novo-Nordisk) [106] and the thiazolidinedione KRP-297/MK-767 (Merck/Kyorin) [72,107], were recently halted reportedly due to the detection



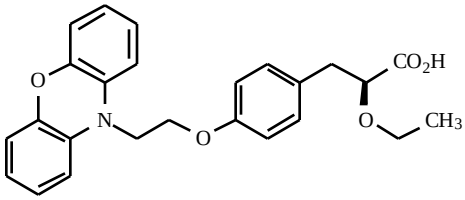
GW-2331
Glaxo SmithKline



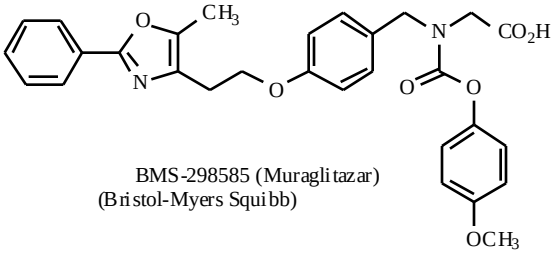
KRP-297/MK-767
Kyorin/Merck



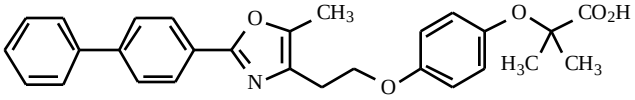
AZ-242; Tesaglitazar
Astra-Zeneca



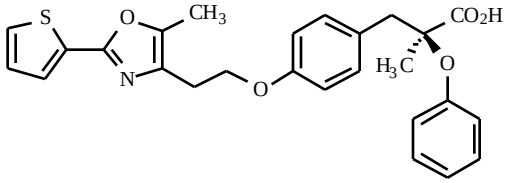
(-) DRF 2725/ NNC 61-0029/
NN-622; ragaglitazar
Dr. Reddy/Novo Nordisk



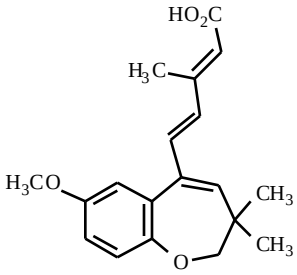
BMS-298585 (Muraglitazar)
(Bristol-Myers Squibb)



LY465608
(Lilly) / Ligand



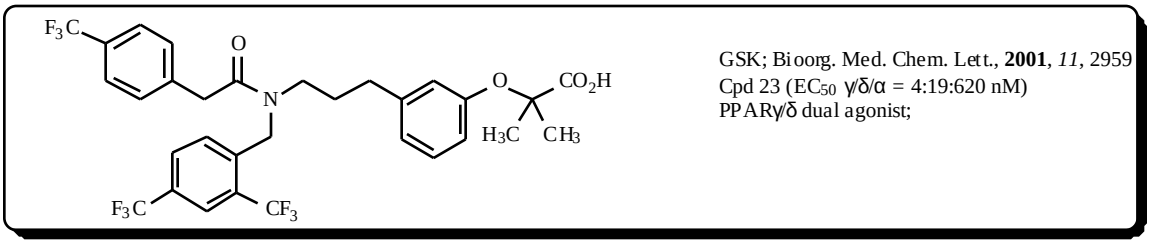
LY510929 (Lilly)



LM 4156 (E. Merck)

of bladder tumors and hemangiosarcomas respectively in long-term carcinogenicity studies in rodents. However, it should be noted that, thus far, there have been no reports of bladder tumors or hemangiosarcomas in clinical trials with PPAR agonists.

Interestingly, all these compounds are derived from several different chemotypes. GW-2331 (GlaxoSmithKline) [27,108], the first relatively balanced and potent PPARα/γ dual agonist reported in the literature, is derived from SAR work on the ureido-fibrates. The Lilly compound LY-465608 [109,110] combines the pharmacophores of known PPARα agonists (the fibrate phenyloxyacetic acid) and PPARγ agonists (the phenyloxazole moiety of JTT-501 and other thiazolidinediones). Another Lilly PPARα/γ dual agonist, LY-510929, features a thiophenyl substituted oxazole and an α-aryloxy β-aryl propionic acid. KRP-297/MK-767 is a thiazolidinedione, while tesaglitazar and ragaglitazar are both α-ethoxy β-aryl propionic acid derivatives; interestingly, LM-4156 (Merck KGa) has a unique oxepin dienoic acid structure. Finally, BMS-298585 is an oxybenzylglycine derivative. These compounds have all been demonstrated to have excellent anti-hyperglycemic and anti-dyslipidemic activities in appropriate preclinical animal models (e.g. *db/db* mice and high fat diet-fed hamsters). The preliminary clinical efficacy profile of ragaglitazar was recently disclosed (Phase II clinical data), which showed that the compound had relatively good glycemic efficacy accompanied by a reasonable lipid-lowering profile (triglycerides) [111]. However, ragaglitazar did show the characteristic side-effects of a PPARγ agonist, i.e. dose-dependent increases in weight gain as well as high incidence of edema [111]. Overall, the current preclinical and clinical data for PPARα/γ dual agonists indicate that these agents show great promise for the treatment of diabetes and the associated dyslipidemia, as well as being potentially uniquely suited for the treatment of dysmetabolic syndrome in terms of glycemic efficacy and lipid efficacy.



GSK; Bioorg. Med. Chem. Lett., **2001**, *11*, 2959
Cpd 23 (EC_{50} γ/δ/α = 4:19:620 nM)
PPARγ/δ dual agonist;

PPARγ/δ Agonists

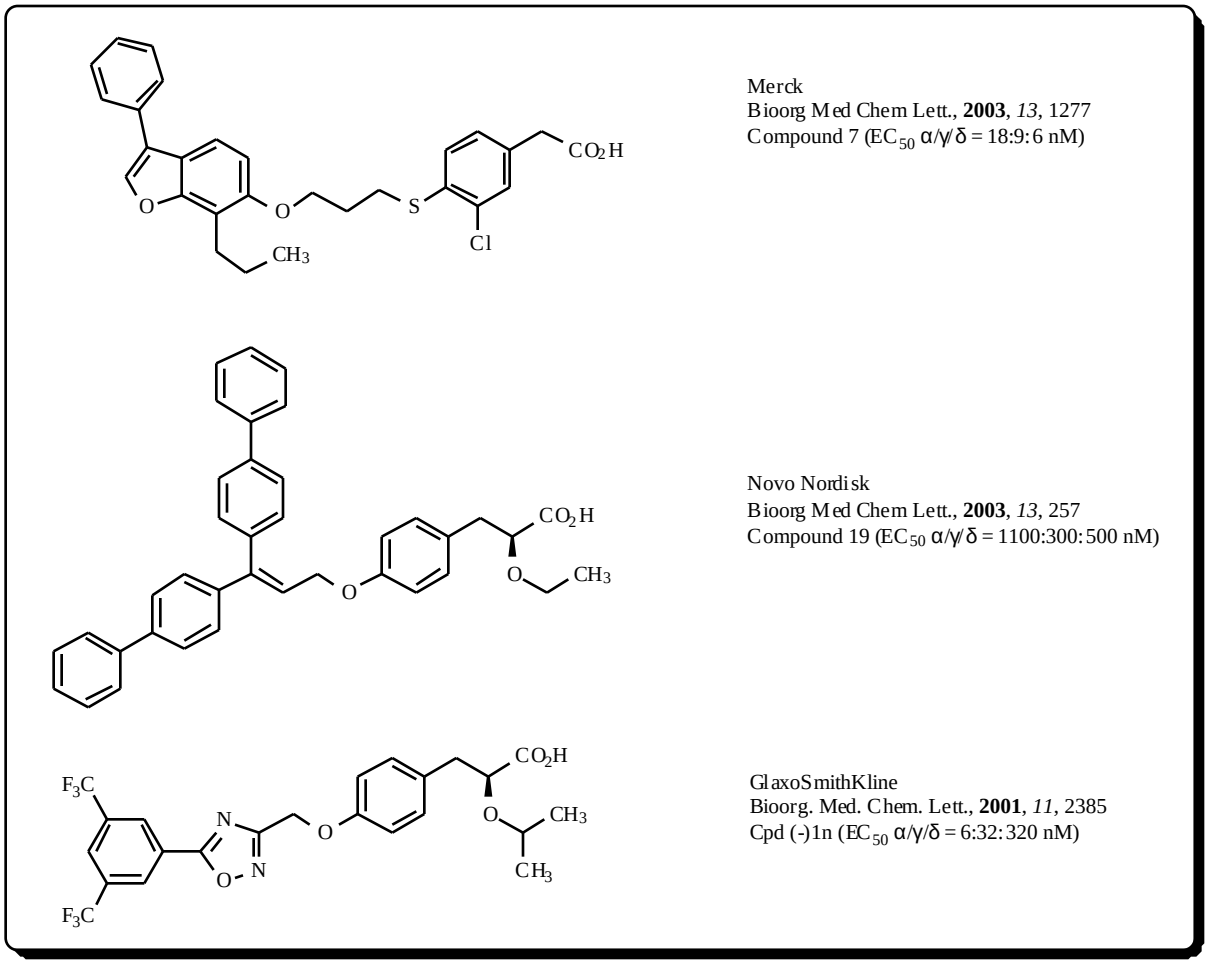
Based on their success in the discovery of PPARδ agonists, the GlaxoSmithKline group has also reported the discovery of selective PPARγ/δ agonists, as exemplified by the compound shown below [112], through the synthesis of a solid-phase library based on the fibrate phenyloxyacetic acid substructure. The specified compound, when dosed orally at 30 mg/kg twice a day for 7 days in male ZDF rats, significantly reduced plasma glucose (-47%) and serum triglycerides (-51%) with a concomitant increase in HDL-cholesterol (24%). This represents another approach to PPAR agonists which control type 2 diabetes and the associated dyslipidemia.

PPARα/γ/δ Pan Agonists

The first PPARα/γ/δ pan agonists from Merck [113], Novo-Nordisk [114] and GlaxoSmithKline [115] have now been reported. The Merck compound at a 30 mg/kg/day dose for 11 days showed glycemic and triglyceride lowering efficacy in *db/db* mice equal to rosiglitazone, but showed less efficacy in the Zucker Diabetic Fatty rat [113]. Similarly, treatment of male *db/db* mice with the Novo-Nordisk compound (dosed at 1 mg/kg/day) for 7 days resulted in significant reductions in blood glucose (47%) and plasma insulin (71%) respectively. In comparison, treatment of *db/db* mice with the PPARα/γ dual agonist ragaglitazar under the same conditions (but at a 3 mg/kg/day dose) resulted in a 51% decrease in blood glucose and a 79% decrease in plasma insulin.[31b] Clearly, further studies will be needed to determine if there is any added advantage to the use of PPARα/γ/δ pan agonists versus PPARα/γ dual agonists for the treatment of various metabolic diseases.

CONCLUSIONS

PPARs have now been shown to be fertile molecular targets for drug discovery. Remarkable advances have been



Merck
Bioorg Med Chem Lett., **2003**, *13*, 1277
Compound 7 (EC_{50} α/γ/δ = 18:9:6 nM)

Novo Nordisk
Bioorg Med Chem Lett., **2003**, *13*, 257
Compound 19 (EC_{50} α/γ/δ = 1100:300:500 nM)

GlaxoSmithKline
Bioorg. Med. Chem. Lett., **2001**, *11*, 2385
Cpd (-)1n (EC_{50} α/γ/δ = 6:32:320 nM)

made in our understanding of the mechanism of action of these receptors over the last ten years. Nevertheless, more questions remain to be addressed.

Would more potent and efficacious PPARα agonists be more effective at raising HDL-C and lowering triglyceride levels in humans compared to the existing fibrates and will they be useful for the treatment of obesity? Would PPARβ selective agonists also raise HDL-C levels and lower triglycerides in humans and if so, what would be their mechanism of action? It is interesting that the lowering of triglyceride levels in obese rhesus monkeys by the PPARβ(δ)-selective agonist GW-501516 was accompanied by an increase in ApoCIII levels. [50] This is different from the effects observed with the PPARα agonists (fibrates), which lower both triglycerides and ApoCIII levels. Would selective PPARγ modulators be able to decrease insulin resistance in patients and if so, would it be possible to combine these agents with PPARα and/or PPARβ agonists to increase glycemic and anti-dyslipidemic efficacy and thus treat a broader patient population? Finally, would potent PPARα/γ dual agonists be superior to the present PPARγ agonists (TZDs) and fibrates in treating diabetes and the associated cardiovascular diseases? The answers to these questions will determine the ultimate clinical utility of all these classes of PPAR ligands.

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