Peroxisome Proliferator-Activated Receptor α/γ Dual Agonists for the Treatment of Type 2 Diabetes

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Introduction

Type 2 diabetes is a complex metabolic disorder that affects between 6% and 20% of the population in Western industrialized societies. Type 2 diabetes is characterized by hyperglycemia, insulin resistance, and defects in insulin secretion and is usually associated with dyslipidemia, hypertension, and obesity. Although the detailed pathophysiology of this disease remains incompletely understood, metabolic defects in the liver, pancreatic *β*-cells, adipose tissue, and skeletal muscle all contribute to the development of type 2 diabetes. Though long thought to be mainly a disorder of carbohydrate metabolism, today a great deal of evidence suggests that abnormalities in fat metabolism play a central role in the pathogenesis of this disease.[1−3]

Peroxisome proliferator-activated receptors (PPARs) are orphan receptors belonging to the steroid/thyroid/retinoid receptor superfamily of ligand-activated transcription factors. Although cloned only slightly more than a decade ago,[4,5] the rapid progress in functional analysis of these receptors has established that the PPARs play a central role in regulating the storage and catabolism of lipids in both animals and humans. There are three PPAR subtypes, which are the products of distinct genes and are commonly designated PPARα, PPAR*γ*, and PPAR*δ*. The PPARs have a protein domain structure (Figure 1) common to other members of the nuclear receptor gene family. This consists of a variable N-terminal region that contains the transcriptional activation function 1 domain (AF-1), a highly conserved DNA-binding domain (DBD), and a ligand-binding domain (LBD) within which lies a C-terminal region that contains the transcriptional activation function 2 domain (AF-2). The LBD contains certain conserved amino acids that have been mapped to critical receptor functions involved in signal transduction. However, there is significant sequence variation in the residues that line the ligand-binding pocket,[6] which is reflected in the fact that each receptor subtype is pharmacologically distinct. The PPARs form functionally active heterodimers with another nuclear receptor, the 9-*cis*-retinoic acid receptor (RXR). These heterodimers regulate expression of target genes by binding to DNA sequence elements, termed PPAR response elements (PPREs, Figure 1). PPREs have been identified in the regulatory regions of a large number of genes, including many that encode proteins involved in lipid metabolism and energy balance, such as aP2, phosphoenolpyruvate carboxykinase (PEPCK), acyl-CoA synthetase, and lipoprotein lipase (LPL).

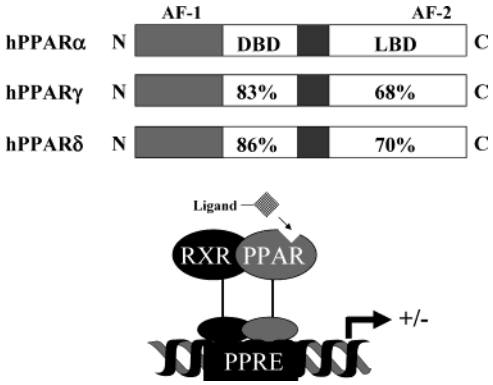

Figure 1. PPAR family of nuclear receptors. Top: comparison of human PPARs is shown. Numbers represent percent homology with PPARα: AF-1 = activation function 1; DBD = DNA-binding domain; LBD = ligand-binding domain; AF-2 = activation function 2. Bottom: the PPARs bind to DNA response elements in the regulatory regions of target genes as heterodimers with RXR. When an agonist binds to the PPAR receptor, recruitment of coactivator proteins (not shown) leads to transcriptional modulation: PPRE = PPAR response element.

A large body of evidence suggests that PPARα plays a pivotal role in the uptake and oxidation of fatty acids and also in lipoprotein metabolism.[7] PPARα is abundantly expressed in catabolically active tissues such as the liver, kidney, heart, and skeletal muscle. PPARα germ-line knockout and tissue-specific deletion/overexpression experiments have supported the cell-based data regarding the roles of PPARα. For example, PPARα$^{-/-}$ mice display considerable dysregulation in serum, hepatic, and adipose tissue lipid metabolism, with reduced expression of proteins involved in mitochondrial and lipoprotein-metabolizing enzymes.[8,9] Interestingly, PPARα$^{-/-}$ mice also show less insulin resistance when fed a high-fat diet than do normal mice.[10]

PPAR*γ* is predominantly expressed in adipose tissue, although it has been detected in other metabolically active tissues such as skeletal muscle, kidney, and intestine. PPAR*γ* was initially identified as a transcription factor involved in fat cell differentiation, and research to date has continued to support its role as a key modulator of adipocyte differentiation.[11] Additional studies have shown that PPAR*γ* activation is accompanied by the modulation of many genes encoding proteins involved in lipid metabolism as well as fat-derived hormones that affect whole body energy me-

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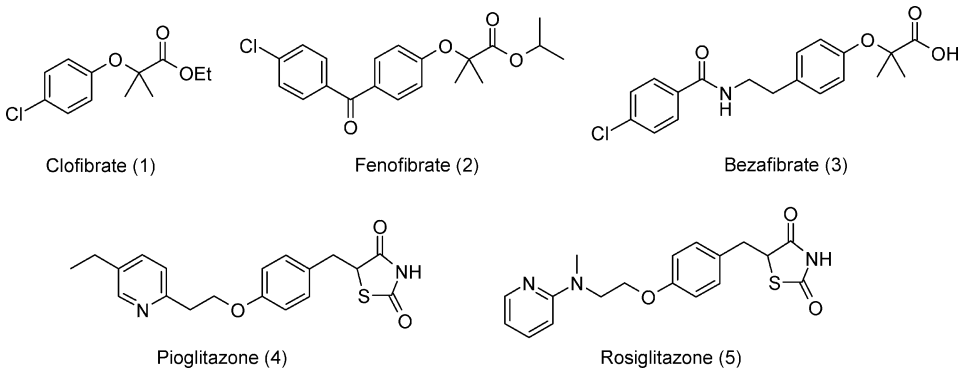

Figure 2. Chemical structures of marketed fibrates and glitazones.

tabolism such as leptin, TNF-α, and adiponectin. Tissue-specific overexpression/deletion studies and studies with heterozygous PPAR$\gamma^{+/-}$ mice have also led to an increased understanding of the role of PPARγ in insulin resistance and lipid storage. While the PPARγ null mutation is embryonic lethal, the heterozygous PPAR$\gamma^{+/-}$ mice develop normally and show no metabolic defects. Unexpectedly, PPAR$\gamma^{+/-}$ mice are more insulin-sensitive than wild-type controls.[12,13] Furthermore, upon challenge with a high-fat diet, the PPAR$\gamma^{+/-}$ mice were partially protected from weight gain and the development of insulin resistance. This surprising result was explained by noting that the adipocytes of the PPAR$\gamma^{+/-}$ mice were less hypertrophic, the circulating levels of leptin and adiponectin were higher, and the circulating levels of fatty acids and TNF-α were lower than in the wild-type mice. Paradoxically, treatment of PPAR$\gamma^{+/-}$ mice with a PPARγ agonist reverses this insulin-sensitive phenotype. It appears that both heterozygous PPAR deficiency and administration of PPARγ agonists each induce the formation of small adipocytes leading to increased insulin sensitivity and protection from insulin resistance induced by a high-fat diet but through different mechanisms. This dichotomy may reflect the roll of PPARγ in metabolic thrift. It is possible that what was once a "thrifty" gene evolved to aid the storage of fat during times of relative famine now predisposes modern humans on high-fat diets to obesity and type 2 diabetes.[14] Finally, although PPARδ is not the focus of this review, it is important to note that this receptor also appears to play an important role in the regulation of lipid metabolism and cholesterol efflux. The detailed physiological role of the PPAR family in the regulation of lipid metabolism and storage has been the subject of several recent reviews.[15−19]

Role of PPARα and PPARγ Agonists in Type 2 Diabetes

The role of PPARα and PPARγ activation in ameliorating the hyperglycemia and hyperlipidemia associated with type 2 diabetes originates with two classes of compounds, the fibrates and the glitazones or thiazolidinediones (TZDs), which were empirically discovered via rodent pharmacology to have antihyperlipidemic and antihyperglycemic activity, respectively. The fibrates (e.g., clofibrate (**1**), fenofibrate (**2**), and bezafibrate (**3**); Figure 2) are drugs that have long been shown to effectively reduce triglycerides (TG) and free fatty acids (FFA) and increase high-density lipoprotein cholesterol (HDL) in both rodents and man.[19] The discovery that these compounds are weak activators of PPARα[5,20] suggested that this receptor may be the primary molecular target of this class of drugs. This hypothesis has been reinforced by the discovery of more potent and selective ligands for PPARα that display improved lipid-lowering activity compared to the fibrates.[21] In addition, fibrates have also been shown to improve glucose tolerance in type 2 diabetic patients,[22] although this activity may not be attributable to activation of PPARα because some these compounds also have appreciable PPARγ activity.[19] Fibrates are generally well-tolerated drugs; however, they are associated with a number of side effects, the most common of which are gastrointestinal side effects such as nausea and diarrhea, and elevations in liver enzymes.[23,24] Skeletal myopathy and acute rhabdomyolysis have also been reported during treatment with all the currently marketed fibrates. It is not clear whether the effects on muscle are mediated by PPARα, but it will be important to carefully monitor these side effects with the more potent, selective PPARα agonists currently in clinical development. In addition, fibrates are excreted via the kidneys and thus should be avoided in patients with renal failure. Finally, fibrates have a propensity to cause drug−drug interactions because of their inhibition of cytochrome P450 enzymes and thus must be used with great caution in combination with other lipid-lowering drugs, particularly statins. While the combination of stains and fibrates has shown improved control of lipoprotein risk factors relative to either agent alone, this combination has shown an increase in renal failure, myopathy, and severe rhabodomyolysis.

The TZDs have been shown to enhance the sensitivity of target tissues to insulin and to reduce plasma glucose, lipid, and insulin levels in animal models of type 2 diabetes and in humans.[25] The TZDs pioglitazone (Actos (4)) and rosiglitazone (Avandia (**5**)) are currently marketed for the treatment of type 2 diabetes and represent important agents in the treatment of this disease both as monotherapy or in combination with existing therapies. These drugs display significant glucose-lowering efficacy, generally achieving mean decreases in hemoglobin A_{1C} (HbA_{1C}) of approximately 1−1.5% and mean decreases in fasting glucose of 60−80 mg/dL in type 2 diabetic patients. These drugs also display modest beneficial effects on TGs, FFAs, and HDL cholesterol.[25] As was the case with fibrates and PPARα, the discovery that the TZDs are potent, selective agonists of PPARγ[26,27] provided a key link to understanding the molecular mechanism of these drugs. These discoveries have also

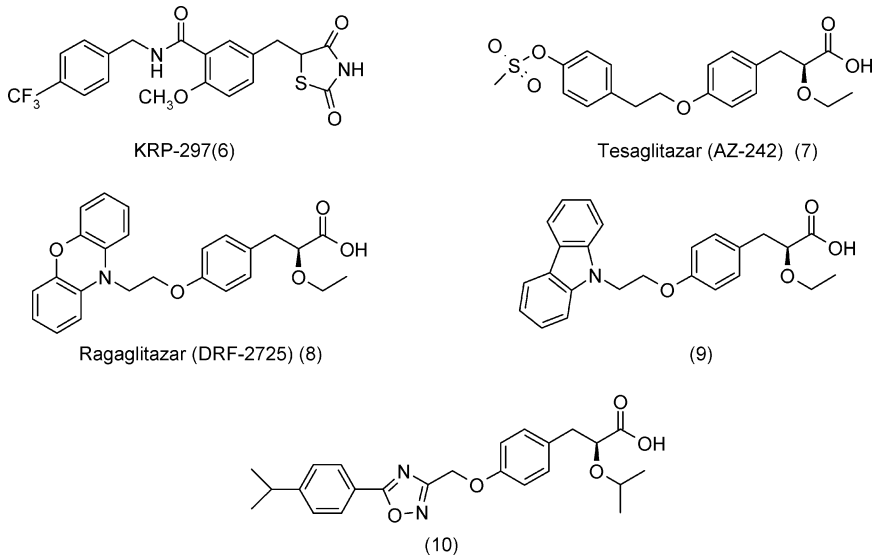

Figure 3. Chemical structures of selected PPARα/γ dual agonists.

provided the opportunity for target-directed approaches in the effort to optimize selective PPARγ agonists as effective antidiabetic agents and selective PPARα agonists as antihyperlipidemic agents.

While rosiglitazone and pioglitazone have many beneficial effects in type 2 diabetics, they also have some undesirable effects. A gain in weight of 3−5 kg is seen in most patients, with a minority gaining an inordinate amount of weight.[28] This weight gain is accompanied by an increase in subcutaneous fat mass. The clinical significance of this weight gain requires further evaluation, but in a treatment population that generally is already overweight this can minimally lead to negative psychological effects. In some patients this weight gain is accompanied by an increase in plasma volume leading to edema that is often resistant to diuretic therapy. This plasma volume expansion may precipitate or exacerbate congestive heart failure, and hence the currently marketed TZDs are not recommended in diabetic patients with NYHA class III and class IV cardiac status and should be used with caution in class I and class II patients. While the mechanism of the fluid retention seen with the marketed TZDs has not been elucidated, it is likely to be a PPARγ-mediated effect because structurally unrelated selective PPARγ agonists also promote fluid retention.

Given the importance of controlling both glucose and lipid levels in type 2 diabetes, the concept of identifying ligands that bind and activate both PPARα and PPARγ represents a logical continuation in the field of PPAR research. In addition to their benefit on lipids, reports in the literature that fibrates reduce body weight gain in rodents without affecting food intake[29,30] offer hope that activation of PPARα may mitigate the weight gain induced by PPARγ activation seen in humans. The hypothesis that PPARα/γ dual agonism should provide additive, and possibly synergistic, pharmacology has resulted in an intensive effort within the pharmaceutical industry to develop and evaluate these agents.

PPARα/γ Dual Agonists

A number of PPARα/γ dual agonists have appeared in the literature, and their structures are depicted in Figures 3 and 4. Many more dual agonists have been claimed in patent applications; however, because of both space limitations within this Miniperspective and lack of supporting biological data in many of the patents, these molecules will not be discussed here. Table 1 lists the potency at both PPARα and PPARγ in receptor binding and/or cell-based functional (transactivation of a reporter gene) assays for the reported PPARα/γ dual agonists.

The first literature report of a "balanced" PPARα/γ dual agonist was KRP-297 (MK-767, **6**), a TZD derivative that was reported to bind PPARα and PPARγ with an affinity of approximately 0.230 and 0.330 μM, respectively (Table 1), and to transactivate PPARα and PPARγ with potencies of 1.0 and 0.8 μM, respectively.[31] The synthesis and brief SAR study of this series of compounds have been reported, but PPAR activity data were only reported for KRP-297.[32] This compound has demonstrated classical PPARγ-mediated and PPARα-mediated pharmacological effects in vitro and in vivo. KRP-297 induced the expression of acyl-CoA oxidase mRNA (a PPARα-regulated gene) in primary rat hepatocytes and in livers of obese rats and also induced the expression of aP2 mRNA (a PPARγ-regulated gene) in adipose tissue of obese rats.[30,31] Treatment with KRP-297 resulted in significantly less weight gain relative to the selective PPARγ agonists pioglitazone and rosiglitazone in both fatty Zucker rats[30] and db/db mice,[33] with the blunting in body weight gain in db/db mice accompanied by a reduction in food intake. Interestingly, KRP-297 also increased glucose-stimulated insulin secretion to levels above that seen with pioglitazone in db/db mice.[33] Recent results from phase I clinical trials indicate KRP-297 is well-tolerated and showed dose-dependent lowering of plasma TG, FFA, and cholesterol.[34] KRP-297 was jointly developed by Kyorin Pharmaceuticals and Merck & Co. but recently was terminated during phase III clinical trials for toxicological reasons. It is unclear whether the toxicity observed is PPAR-mediated.

Several groups have reported phenylpropanoic acid-based PPARα/γ dual agonists. The α-ethoxy-β-phenylpropanoic acid dual agonist tesaglitazar (Galida,

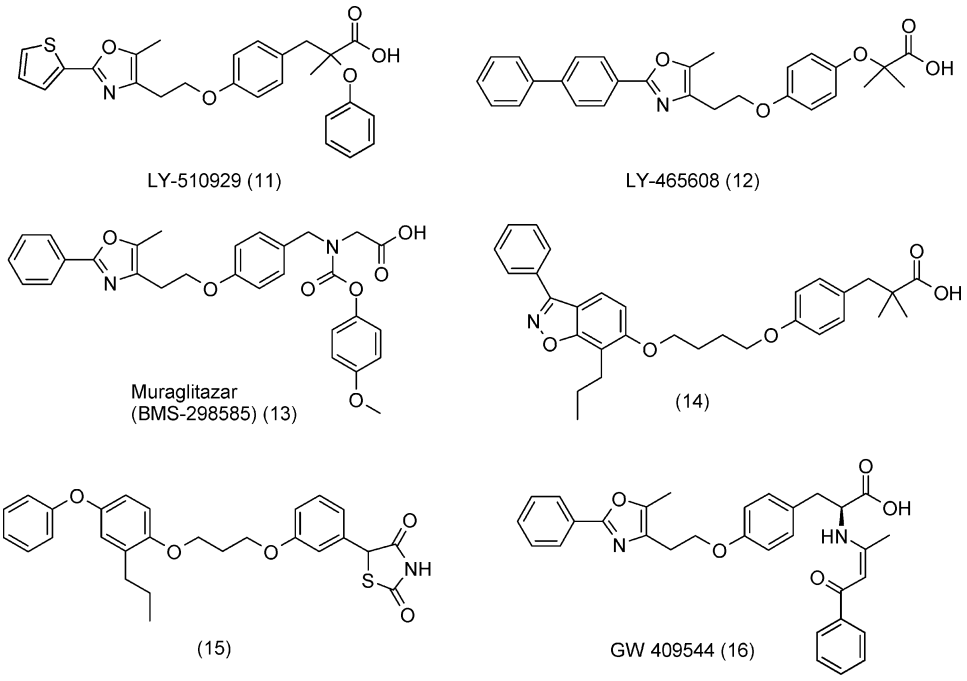

Figure 4. Chemical structures of selected PPARα/γ dual agonists.

Table 1. Activity of PPARα/γ Agonists in Binding and Cell-Based Functional Assays

	binding (μM)[a]		cell-based EC_{50} (μM)[b]		
compd	PPARα	PPARγ	PPARα	PPARγ	ref
clofibrate[c]	nr	nr	55	~500	19
fenofibrate[c]	nr	nr	30	300	19
bezafibrate	nr	nr	50	60	19
rosiglitazone	ia	0.05	ia	0.04	57
pioglitazone	ia	1.2	ia	0.58	57
6	0.23	0.33	1.0	0.80	31
7	1.0	0.20[d]	1.7	0.25[d]	35, 36
8	0.98	0.09	3.2	0.57	38, 46
9	nr	nr	0.36	0.17	38
10	nr	nr	0.013	0.004	39
11	0.004	0.003	0.009	0.004	40
12	0.17	0.55	0.15	0.88	41
13	nr	nr	0.240	0.120	43
14	0.01	0.02	0.004	0.02	45
15	0.03	0.06	0.03	0.01	44
16	0.002	0.001	0.002	0.0002	6

[a] All data were generated using hPPAR LBD as reported in ref 57. Data reported are either K_i or IC_{50} values in which $K_i = IC_{50}/(1 + [L]/K_d)$ (where IC_{50} is the concentration of test compound required to inhibit 50% of the specific binding of the radioligand, [L] is the concentration of the radioligand used, and K_d is the dissociation constant for the radioligand at the receptor). Refer to the original reference to determine whether reported values is K_i or IC_{50}; nr = not reported. [b] All data were generated using the hPPAR-GAL4 transactivation assay as detailed in ref 57; ia = inactive at 10 μM or the highest concentration tested; nr = not reported. [c] Data are for active metabolites. [d] Data are for murine receptors.

AZ-242, **7**) is the most advanced clinically. Tesaglitazar is reported to be about 10-fold more potent at PPARγ than at PPARα (Table 1).[35,36] This compound also displays in vivo pharmacology associated with both PPARα and PPARγ activation. Oral administration of tesaglitazar to ob/ob mice improved the hyperglycemia, hyperinsulinemia, and hypertriglyceridemia in a dose-dependent fashion. In addition, euglycemic hyperinsulinemic clamp studies in Zucker fatty rats showed tesaglitazar restored insulin sensitivity to that of lean controls and decreased basal insulin secretion.[35] Tesaglitazar is reportedly in phase III clinical development by AstraZeneca.

Scientists at Dr. Reddy's Research Foundation also disclosed a series of α-ethoxy-β-phenylpropanoic acid derivatives exemplified by ragaglitazar (DRF-2725, **8**, Figure 3), which has a phenoxazine group as the lipophilic tail portion of the molecule.[37] Ragaglitazar has a binding affinity of 0.98 μM at hPPARα and 0.092 μM at hPPARγ and transactivates PPARα and PPARγ with EC_{50} values of 3.2 and 0.570 μM, respectively (Table 1). This compound also displays good in vivo antidiabetic activity in db/db mice and is reported to have 77% oral bioavailability in Wistar rats. Ragaglitazar was colicensed by Novo Nordisk and completed phase II clinical trials; however, the clinical development of ragaglitazar has been terminated because of an incidence of bladder tumors in rodents. As with KRP-297 toxicity, it is unclear whether the observed tumorigenicity is mechanistically related to PPAR activation. A subsequent SAR investigation of the phenoxazine portion of this molecule has recently been disclosed by Sauerberg et al.[38] whereby excision of the oxygen atom of the phenoxazine ring to form the corresponding carbazole analogue **9** (Figure 3) increased potency at both PPARγ and PPARα relative to ragaglitazar (Table 1). This compound displays excellent pharmacokinetic properties in rodents and was efficacious in lowering glucose and TGs in db/db mice and lowering cholesterol and TGs in a high-cholesterol fed rat model. We also recently reported a series of PPARγ/α dual agonists that are α-alkoxy-β-phenylpropanoic acids containing a lipophilic phenyloxadiazole tail. The series is exemplified by compound **10** (Figure 3), which has an EC_{50} = 0.013 μM at PPARα and an EC_{50} = 0.004 μM at PPARγ.[39] Interestingly, some of these compounds also showed weak partial agonist activity at PPARδ.

A collaborative effort between Eli Lilly and Ligand Pharmaceuticals has produced novel PPARα/γ dual agonists exemplified by LY510929 (**11**, Figure 4).

LY510929 is equipotent at both receptor subtypes in vitro (Table 1) and has demonstrated very potent glucose-lowering activity in ZDF rats, with an ED_{50} for glucose normalization of 0.004 mg/kg.[40] Another report from these labs contains molecules that are hybrids of known PPARα and PPARγ ligands.[41] These analogues contain the phenoxyisobutyric acid "headgroup" common to many fibrates attached to a modified 2-phenyl-5-methyloxazole moiety common to several potent PPARγ ligands.[19] Substitution at the para position of the 2-phenyloxazole group provided a boost in potency at both receptor subtypes. An example from this series is the biphenyl derivative LY465608 (**12**, Figure 4), which binds to PPARγ with an $IC_{50} = 0.548\ \mu M$ and to PPARα with an $IC_{50} = 0.174\ \mu M$. In addition to showing good antidiabetic and anthyperlipidemic efficacy in db/db mice and ZDF rats, LY465608 also lowered plasma TGs and raised HDL cholesterol in human apoA-I transgenic mice[42] and lowered glucose and TGs in apoA-I transgenic mice rendered diabetic by dietary manipulation and low-dose streptozotocin treatment.[41] However, this molecule also has considerable agonist activity at PPARδ ($EC_{50} = 171$ nM)[40] and thus is more properly classified as a PPAR "pan-agonist" (i.e., a molecule that displays potent and balanced activation of all three PPAR subtypes) rather than a PPARα/γ dual agonist as originally reported.

Cheng et al. disclosed muraglitazar (BMS-298585, **13**, Figure 4), an oxybenzylglycine derivative that displays potent activity in vitro at both PPARα and PPARγ (Table 1).[43] This compound displays good pharmacokinetics in rats and monkeys and has demonstrated antidiabetic and antihyperlipidemic activity in both rodents and hamsters on a high-fat diet. Muraglitazar is currently reported to be in phase II clinical trials. Scientists at Merck have recently reported two series of dual PPARα/γ agoinsts exemplified by compounds **14** and **15** (Figure 4).[44,45] Compounds in the benzisoxazole series generally also have some affinity for PPARδ, while the meta-substituted TZD series analogues appear to have no affinity for PPARδ. Compounds from both series have shown good oral antidiabetic activity in preclincial models. Finally, we have described the α-amino-β-phenylpropanoic acid derivative GW409544 (**16**, Figure 4), a close structural analogue of the selective PPARγ agonist farglitazar. In vitro this compound is the most potent dual PPARα/γ agonist described to date, with a PPARα $EC_{50} = 0.002\ \mu M$ and a PPARγ $EC_{50} = 0.0002\ \mu M$.[6]

Structural Studies of PPARα/γ Dual Agonists

X-ray crystal structure elucidation of both apo and ligand-bound structures of the LBDs from all three PPAR subtypes have provided a great deal of insight into understanding the structural basis for both agonist activation and the subtype selectivity of various PPAR ligands. All three PPAR subtypes are predominantly α-helical in nature with a small section of β-sheet strands, with an overall fold similar to those of other nuclear receptors. A large Y-shaped pocket of approximately 1300−1440 $Å^3$ accommodates the ligand. The increased size of the ligand-binding pocket relative to that of other nuclear receptors (by comparison, the binding pocket of RXRα is ∼470 $Å^3$) accounts for the ability of the PPARs to accommodate a wide variety of ligands. Comparison of the three PPAR LBDs reveals that while the overall sizes of the ligand-binding pockets are similar, there are significant differences in the detailed topology of the LBDs.[6] Crystal structures of TZD-based and carboxylic acid-based agonists complexed with LBDs from all three PPAR subtypes reveal a common binding mode in which the acidic moiety forms a conserved hydrogen-bonding network with several residues lining this area of the pocket. Contained within this network is a direct hydrogen bond to a key tyrosine residue located in the C-terminal AF-2 helix that appears to be crucial for achieving a conformation conducive to coactivator recruitment and subsequent DNA binding (Figure 5).

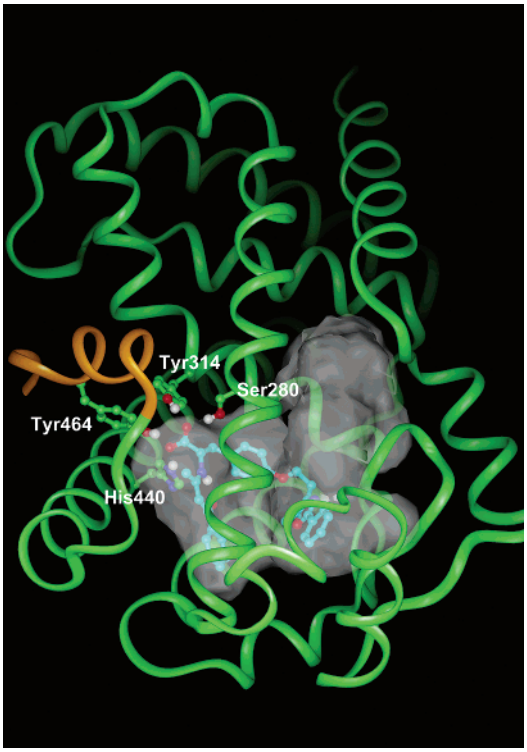

Figure 5. X-ray cocrystal structure of GW409544X (blue carbon atoms) in the PPARα ligand-binding pocket (gray surface). The PPARα protein is indicated by a green ribbon, with the AF-2 helix region indicated by the orange ribbon. Key amino acid residues Tyr^{464}, Tyr^{314}, His^{440}, and Ser^{280} involved in binding the acidic headgroups are indicated.

There are four reports in the literature describing X-ray crystal structures with the PPARα/γ dual agonists GW409544, tesaglitazar, ragaglitazar, and compound **9** bound to the LBDs of PPARα and PPARγ.[6,36,38,46] These studies reveal that the PPARγ and PPARα binding pockets are very similar in size and shape to each other. However, evidence from X-ray crystallographic, NMR, and receptor point mutation studies[6] suggest that one key determinant of subtype selectivity is the substitution of Tyr^{314} in PPARα for His^{323} in PPARγ (Figure 6). This amino acid is one of four important residues that make up the hydrogen-bonding network the PPAR proteins form with the acidic headgroup of PPAR ligands. The data indicate that ligands such as TZDs that contain bulky headgroups should encounter more steric interaction with the larger Tyr^{314} in PPARα relative to the sterically smaller His^{323} in PPARγ, thus disrupting the H-bonding network between the head-

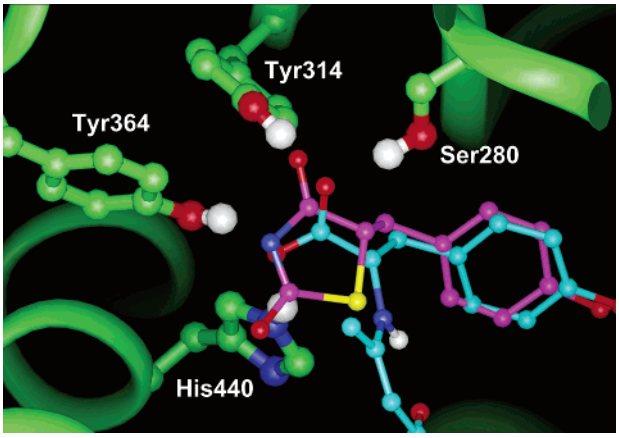

Figure 6. X-ray cocrystal structure overlay of the headgroup portions of rosiglitazone (purple carbon atoms) and GW 409544X (blue carbon atoms) in the PPARα ligand-binding pocket. The PPARα protein is indicated by a green ribbon. The rosiglitazone structure overlay was generated from the published cocrystal structure complex of rosiglitazone/PPARγ/9-*cis*-retinoic acid/RXRα/SRC-1 peptide. Key amino acid residues Tyr^{464}, Tyr^{314}, His^{440}, and Ser^{280} involved in binding the acidic headgroups are indicated. Reprinted with permission from *Molecular Cell*.[63] Copyright 2000 Elsevier.

group and the other three key amino acid residues. This may explain the observation that most of the PPARγ/α dual agonists reported to date contain sterically smaller carboxylic acid headgroups whereas the TZDs are generally selective for PPARγ. However, the observation that some TZDs such as KRP-297 can bind both PPARα and PPARγ indicates that the seemingly inherent selectivity for PPARγ that the 2,4-thiazolidinedione ring provides can be overcome by an appropriate disposition of substituents. Molecular modeling suggests that the meta substitution of the TZD and side chain on the central phenyl ring in KRP-297 may allow an improved fit in the PPARα protein.

PPARα/γ Dual Agonists in the Treatment of Diabetes and Its Complications: Challenges and Outlook

As alluded to above, preclinical data in rodent models of type 2 diabetes clearly show that PPARα/γ dual agonists are effective antidiabetic and antihyperlipidemic agents. Furthermore, research has shown that these dual agonists display pharmacology associated with both PPARα and PPARγ activation in vivo, which supports the concept that a dual agonist should provide additional benefit on glucose and/or lipid control relative to a single PPAR subtype-selective agent. Treatment of obese insulin-resistant, hyperlipidemic rhesus monkeys with the dual agonist GW409544X lowered serum insulin, TGs, and non-HDLc and also raised HDLc, providing additional confidence that PPARα/γ dual agonists will display the combined pharmacology in humans.[47] Given the clinical experience with the subtype-selective agents, there seems little doubt that PPAR α/γ dual agonists will prove to be efficacious in humans. Nevertheless, a number of questions and issues remain in the development of PPARα/γ dual agonists as antihyperglycemic and antihyperlipidemic drugs.

One of the multifaceted challenges is identifying the "optimal" ratio of PPARα and PPARγ agonist activity within a given ligand. Conceptually one would like a PPARα/γ drug to have the ability to activate each receptor sufficiently to provide maximum efficacy on those target genes affecting glucose and lipids without overstimulation, which might lead to undesired side effects. On the most basic level, comparison of receptor binding affinities provides one measure of relative activity for a given dual agonist at PPARα and PPARγ but clearly is too simplistic a method for determination of the "optimal" ratio. Cell-based functional assays provide a more accurate pharmacological picture because they allow measurement of both potency and level of activation of a given ligand on each receptor in a more biologically relevant setting, complete with proteins such as RXR and various cofactors necessary for gene transcription. As the data in Table 1 suggest, medicinal chemists have been able to generate ligands with a range of potencies at each receptor, leading to dual agonists with modest to considerable functional selectivity for either PPARα or PPARγ in addition to agonists that are essentially equipotent on both PPAR subtypes.

The greater challenge then becomes translation of the in vitro profile at each receptor into in vivo pharmacological profiles. For a variety of practical, economical, and biological reasons, rodent models are used in the majority of preclinical studies to evaluate the efficacy of PPAR agonists. A number of variables conspire to make the extrapolation of in vitro to rodent and ultimately to human pharmacology challenging in the case of the PPARα and PPARγ receptors. First, there is a species differential in the case of PPARα resulting in many ligands being much less potent at murine PPARα relative to the human orthologue.[19,48] This does not appear to be the case with PPARγ, where the potency of the known ligands against the human and murine receptors is similar. There are also differences in expression level of these receptors in rodents vs humans. For example, PPARα has a 10-fold higher protein expression in rodent livers than in human livers.[49] In addition, humans have a PPARα splice variant that represents 20−50% of the total mRNA in the liver and codes for a truncated form of the receptor that shows dominant negative activity under certain conditions.[50] This truncated PPARα protein is not present in rodent liver. Finally, there are species differences in the regulation of some PPAR-responsive genes. For example, the acyl-CoA oxidase gene promoter is responsive to PPARα activation in rodent but not in humans.[51] These observed differences have been cited as rationale for why peroxisomal proliferation and hepatomegaly driving subsequent nongenotoxic carcinogenesis appear to be a rodent-specific phenomenon.[52]

Of more importance in evaluating the efficacy of PPARα/γ dual agonists, there are significant differences in lipid and lipoprotein metabolism between rodents and humans. For example, mice carry most of their cholesterol via HDL rather than LDL partly because of the absence of cholesterol ester transfer protein (CETP), a key enzyme for cholesterol transport. Furthermore, PPARα in humans increases expression of apolipoprotein A-I (apoA-I), the major protein constituent of HDL cholesterol, whereas in rodents PPARα activation leads to a suppression of apoA-I expression via up-regulation of Rev-erbα.[53] Although no examples of specific differentially regulated genes between rodents and humans

have been reported in the case of PPARγ, the degree of triglyceride lowering in animal models far exceeds that seen in the clinic, which may suggest species differences in PPARγ-mediated lipid metabolism and storage. Transgenic mice have been developed and utilized by some groups as models for evaluating PPARα/γ dual agonists in order to circumvent these species-specificity issues.[41,42]

Other variables also can confound efforts to identify an "optimal" PPARα/γ ratio for clinical evaluation. The tissue distribution of PPARα and PPARγ receptors suggests that PPARα, which is highly expressed in the liver, is likely to see higher concentrations of an orally dosed PPAR agonist than PPARγ. However, the relative exposure to the two receptors in vivo will be a function of the physical and pharmacokinetic properties of the particular compound. Thus, a highly lipophilic compound might be more likely to have relatively higher and/or longer exposure to adipose tissue and have more pronounced effects on PPARγ-mediated gene transcription than a more polar analogue having the same in vitro receptor potency ratio. The difficulty in accurately measuring drug levels in liver and adipose tissue further complicates attempts to correlate the in vitro PPARα/γ ratio to an optimal in vivo pharmacology. Finally, the issue of active drug metabolites with differing potencies at the two PPARs relative to the parent compound could also influence the in vivo pharmacology. Pharmaceutical companies intent on developing optimal PPARα/γ dual agonists should be prepared to survey a number of molecules in the clinical setting.

In addition, the important question of whether an acceptable safety profile can be achieved with these dual agonists has not yet been answered. As mentioned above, both fibrates and TZDs have side effects associated with their use. Because of the complexity of metabolic events regulated by PPARα and PPARγ, it is difficult to predict whether simultaneous activation of both receptors by a single agent will result in additive, synergistic, reduced, or even new side effects relative to those seen with the selective PPAR agents. In particular, the potential for induction of peroxisomal proliferation in the liver with these agents will need to be closely monitored. PPARα agonists such as fibrates are known to cause the proliferation of peroxisomes and subsequent hepatomegaly and hepatocellular proliferation in rodents; however, years of clinical experience with fibrates in humans have not led to evidence of peroxisome proliferation, as determined by morphological examination of liver biopsies, or to an increased incidence of liver cancer.[19] Despite the comfort that this clinical experience provides, the data obtained are with compounds that have been shown to be fairly weak activators of PPARα. In addition, while nonrodent species are clearly less responsive to peroxisomal proliferation via activation of PPARα, they are not completely refractory.[54,55] Thus, it is not yet clear whether a drug with potent PPARα activating ability will cause peroxisomal proliferation at levels that are a concern with respect to human safety.

Perhaps the key factor in determining the potential value of PPARα/γ dual agonists as a new antidiabetic therapy is whether the weight gain and fluid retention that is observed upon treatment with selective PPARγ agonists can be mitigated by activation of PPARα in humans. As noted above, PPARα/γ dual agonists appear to alleviate most if not all of the weight gain associated with selective PPARγ agonists in several rodent models of type 2 diabetes. There has been little data presented on the effects of PPARα/γ dual agonists on body weight and fluid retention from the clinical trials conducted to date. Data from phase II clinical trials with ragaglitazar indicated that this compound showed increases in body weight and rates of edema that were similar to those seen with selective PPARγ agonists.[56] Farglitazar, which is an extremely potent PPARγ agonist that has appreciable human PPARα activity (ca. 1000-fold selective for PPARγ in vitro),[57] showed rates of weight gain and edema similar to those of rosiglitazone despite peak plasma levels that were above the EC_{50} for activation of PPARα.[58] These studies suggest that dual agonists that are more potent on PPARγ relative to PPARα do not possess a profile where the PPARγ-mediated side effects observed in humans are effectively mitigated by PPARα activation at clinically efficacious doses. Would a more "evenly balanced" dual agonist possess sufficient antihyperglycemic efficacy and a better side effect profile? Selective PPARα agonists have shown improvement of insulin action on glucose utilization in two insulin-resistant rodent models, perhaps due to increased fatty acid metabolism.[59] If this PPARα-mediated activity translates to humans, it might be possible to achieve robust insulin sensitization at lower levels of PPARγ activation and perhaps decrease PPARγ-mediated side effects. Clinical data on weight gain and edema from the recently completed phase II studies with KRP-297 and tesaglitazar, which are more "evenly balanced" dual agonists with respect to their in vitro potencies at the two PPAR subtypes, will be important in determining whether different relative magnitudes of PPARα activation can modulate the severity of these PPARγ-induced side effects.

In addition to having beneficial effects on plasma glucose and lipids in type 2 diabetics, PPARα/γ dual agonists may also be useful in treating other diseases. Perhaps the most promising of these additional therapeutic indications lies in the treatment of atherosclerosis and other inflammatory diseases. Fibrates have been shown clinically to reduce the progression of atherosclerosis and to reduce cardiovascular events as judged by data from multiple studies.[28] In studies such as the Helsinki heart study and the VA-HIT study, which have cardiovascular endpoints, chronic treatment with fibrates showed a significant risk reduction in the combined incidence of nonfatal MI and CHD death. In addition, several large clinical studies in which angiographic endpoints were evaluated have shown that treatment with fibrates significantly reduced the progression of segmental coronary lesion narrowing. In vitro data also suggest that PPARα activation has beneficial effects on the vessel wall and in downregulation of proinflammatory cytokines.[19] Most of the data obtained thus far in both mice and humans suggest that selective PPARγ agonists also have antiatherogenic properties. Treatment of LDL receptor deficient mice fed a high-fat diet with selective PPARγ agonists reduced the number and size of atheromatous lesions despite an increase in CD36 expression.[60] Subsequent

studies showed a similar antiatherogenic effect in apolipoprotein-E knockout mice.[61] Rosiglitazone was recently shown to reduce the atherosclerotic plaque area in apolipoprotein-E knockout mice made diabetic by treatment with streptozotocin.[62] Importantly, rosiglitazone did not affect plasma glucose levels in this late stage model of diabetes, and yet it reduced the correlation coefficient between plasma glucose and the degree of atherosclerosis, suggesting that rosiglitazone has direct effects on atherogenesis independent of any effects mediated by improvement of insulin resistance. Limited human clinical studies with troglitazone and pioglitazone have also demonstrated an antiatherosclerotic effect with these drugs.[28] Although the value of PPAR α/γ dual agonists as antiatherosclerotic drugs has yet to be established, the hypothesis and preliminary data are encouraging. Research with selective PPAR agonists is being undertaken in several other therapeutic areas such as cancer, hypertension, Alzheimer's disease, and autoimmune diseases; however, it is currently unclear whether a PPARα/γ dual agonist would have utility in the treatment of any of these diseases.

The discovery and development of molecules that serve as dual agonists for PPARα and PPARγ have provided scientists with a tremendous range of molecular tools for studying the integrated biology of these two PPAR subtypes. This has increased both the complexity of the underlying pharmacology associated with simultaneous activation of two receptors and also the opportunity of finding agents capable of delivering increased efficacy and/or having a reduced side effect profile relative to the currently marketed selective PPAR agonists. Successful development of this concept will require striking a careful balance between the beneficial pharmacology and the detrimental side effect profile associated with activation of each receptor subtype, an approach that may require a commitment to advance multiple clinical candidates. The data obtained in preclinical models of diabetes and dyslipidemia indicate that PPARα/γ dual agonists provide additive and possibly synergistic efficacy relative to selective activation of either subtype alone, along with a reduction in magnitude of undesired PPARγ-mediated side effects. The limited clinical data suggest that while the antihyperglycemic and antihyperlipidemic efficacy of these dual agonists is impressive, the side effect profile may not be improved relative to currently marketed selective PPARγ agonists; additional clinical data from compounds having differing relative potency levels are needed to firmly answer this question. Nevertheless, these agents have the potential to address a wider spectrum of the multiple metabolic abnormalities associated with type 2 diabetes than do existing PPAR-based therapies and should prove to be a useful addition to the pharmaceutical regimen available for the treatment of this disease.

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Biography

Brad R. Henke received his Ph.D. in Organic Chemistry from the University of Illinois in 1989 under the supervision of Prof. Scott E. Denmark. After postdoctoral work at the University of California, Berkeley, in the laboratories of Prof. Clayton H. Heathcock, he joined the Glaxo Research Institute (now part of GlaxoSmithKline) in 1991. He is currently Department Director, Medicinal Chemistry, within the Metabolic and Viral Diseases Drug Discovery group at GlaxoSmithKline, Research Triangle Park, NC.

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