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Review Article

Advances in antidiabetic agents targeting peroxisome proliferator-activated receptors (PPARs)

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CONTENTS

Abstract

One of the most promising approaches for the discovery of new antidiabetic agents is represented by the exploitation of peroxisome proliferator-activated receptor (PPAR) ligands. New agents that selectively target PPARs and molecules with combined activity on several PPARs (*i.e.*, PPARα and PPARγ) have been identified as potentially superior therapeutic agents for metabolic diseases. These include: 1) PPARα agonists, which exert significant antidyslipidemic and antiatherosclerotic effects; 2) PPARγ agonists, which are effective in treating type 2 diabetes; 3) selective partial PPARγ agonists with robust antidiabetic efficacy and fewer adverse effects than currently available agonists; 4) PPARγ antagonists, which are powerful tools to study PPARγ signaling pathways; 5) dual PPARα/γ agonists, which beneficially affect carbohydrate and lipid metabolism; 6) PPARδ agonists, which may have beneficial effects on circulating lipids and obesity; and 7) pan-PPARα/γ/δ agonists with excellent antidiabetic activity. The advances in antidiabetic agents targeting PPARs are summarized in this review.

Introduction

Type 2 diabetes, previously referred to as non-insulin-dependent diabetes mellitus (NIDDM), accounts for over 90% of the diabetes cases reported in the Western world. The global incidence of this disease is estimated to be 120 million at present and it is predicted to soar to over 200 million by the year 2010 (1).

The basic approach to treating type 2 diabetes focuses on the control of blood glucose levels. However, intensive glucose-lowering therapy is ineffective at reducing cardiovascular complications, despite decreasing microvascular complications such as retinopathy (2). These findings clearly indicate unmet clinical needs in lipid profile management among diabetics.

Among the various approaches being evaluated for the discovery of new agents, one of the most promising is represented by the exploitation of peroxisome proliferator-activated receptor (PPAR) ligands. The PPARs were discovered by Issemann and Green in 1990. They belong to the nuclear receptor superfamily of ligand-activated transcription factors that mediate the specific effects of small lipophilic compounds, such as steroids, retinoids and fatty acids (FAs), on DNA transcription. A few years after their discovery, three subtypes were identified, namely PPARα (NR1C1), PPARδ (NR1C2; also known as PPARβ, NUCI, FAAR) and PPARγ (NR1C3) (3).

The PPARα subtype is highly expressed in tissues that efficiently harvest energy from lipids, including liver and skeletal muscle. In these tissues, PPARα regulates the expression of numerous genes involved in lipid uptake, catabolism and homeostasis. The PPARδ subtype is ubiquitously expressed, but its function is not yet fully understood. The PPARγ receptor subtype is predominantly expressed in the adipose tissue and plays a pivotal role in adipocyte differentiation *in vitro*, suggesting that this subtype is an important component in the adipogenic signaling cascade and in lipid storage and utilization.

Following activation of the receptor by ligand binding, PPARs heterodimerize with the retinoid X receptor (RXR). Upon binding of the heterodimer receptor complex to peroxisome proliferator response elements (PPREs) located in the regulatory regions of the target genes, and cofactor recruitment, gene transcription of proteins involved in lipid metabolism and homeostasis is stimulated. PPREs have been identified in the promoter regions of several genes which encode proteins involved in lipid and lipoprotein metabolism, such as acyl-CoA oxidase (AOX), liver fatty acid-binding protein (L-FABP),

apolipoprotein C-III (apo C-III) and lipoprotein lipase (LPL).

The activation of PPAR/RXR heterodimers results in the specific induction of subsets of genes controlling lipids, carbohydrates and energy homeostasis. Thus, inappropriate activation or inactivation of PPARs can be directly linked to pathological processes, such as type 2 diabetes, cardiovascular diseases, obesity and dyslipidemia (4).

The ability of PPARs to mediate various metabolic and therapeutic actions has rendered them a central focus of pharmacological and genetic research for more than a decade. Ongoing pharmaceutical research seeks to identify PPAR ligands with superior therapeutic windows and more extensive metabolic actions.

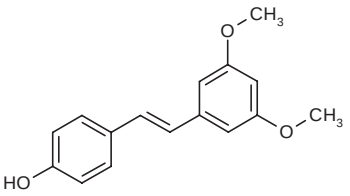
In parallel with these advances in basic pharmacology, new agents that selectively target PPARs and molecules with combined activity on several PPARs (*i.e.*, PPARα and PPARγ) have been identified as potentially superior therapeutic agents for metabolic diseases. These include: 1) PPARα agonists, which exert significant antidyslipidemic and antiatherosclerotic effects; 2) PPARγ agonists, which are effective in treating type 2 diabetes; 3) selective partial PPARγ agonists with robust antidiabetic efficacy and fewer adverse effects than currently available agonists; 4) PPARγ antagonists, which are powerful tools to study PPARγ signaling pathways; 5) dual PPARα/γ agonists, which beneficially alter carbohydrate and lipid metabolism; 6) PPARδ agonists, which may have beneficial effects on circulating lipids and obesity; and 7) pan-PPARα/γ/δ agonists with excellent antidiabetic activity (5, 6).

PPARα agonists

PPARα can be activated by a wide range of compounds, which include natural and synthetic agonists, such as pterostilbene, fibrates, α-substituted phenylpropanoic acid derivatives and isoxazolyl-serine-based compounds.

Natural PPARα agonists

Pterostilbene (**1**), an agonist for the PPARα subtype, gave 29% lower plasma low-density lipoprotein (LDL) cholesterol, 7% higher plasma high-density lipoprotein (HDL) cholesterol and 14% lower plasma glucose as compared to the control group in hypercholesterolemic hamsters. The LDL/HDL ratio was also statistically significantly lower for pterostilbene as compared to control animals at the same concentration in the diet. These studies demonstrated that pterostilbene possesses lipid- and glucose-lowering effects (7).



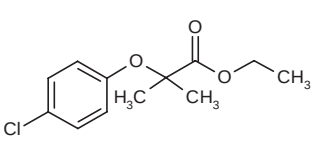
Pterostilbene (**1**)

Synthetic PPARα agonists

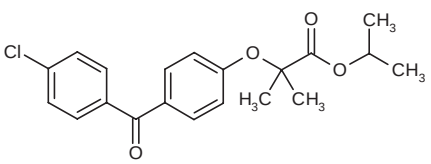
1. Fibrates and their analogues

For several years, fibrate-class drugs, such as clofibrate (**2**), fenofibrate (**3**) and bezafibrate (**4**) (Fig. 1), have been broadly used for the clinical treatment of dyslipidemia, and they continue to remain the treatment of choice for patients with severe hypertriglyceridemia. Fibrates effectively lower plasma triglyceride (TG) levels and modestly increase HDL cholesterol levels while lowering LDL cholesterol to a variable extent. Several studies have provided evidence that the hypolipidemic effect of this class of drugs is mediated by their agonist activity at the PPARα receptor. Although fibrates are ligands of PPARs, their affinity is very weak (high micromolar concentrations are needed to activate PPARα) and the PPAR subtype selectivity is poor. Consequently, in humans, fibrates must be used at very high doses (about 300-1200 mg/day) to achieve a sufficient lipid-lowering effect. Therefore, the need exists for more potent and subtype-selective human PPARα agonists. Such agents could provide a superior clinical profile for therapeutic intervention in dyslipidemia.

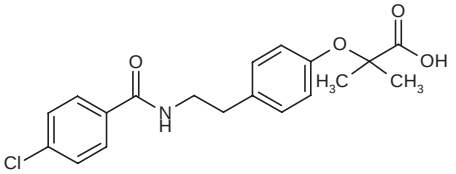
It has been reported that the stereochemistry of clofibrate analogues bearing a stereogenic center (**5**; Fig. 2) affects their pharmacological profile. A number of racemic and optically active acyclic (**5**; Fig. 2) and cyclic clofibrate (**6**-**8**; Fig. 2) analogues were synthesized and pharmacologically evaluated (8). Rigid analogues of clofibric acid



Clofibrate (**2**)



Fenofibrate (**3**)



Bezafibrate (**4**)

Fig. 1. Fibrate-class drugs.

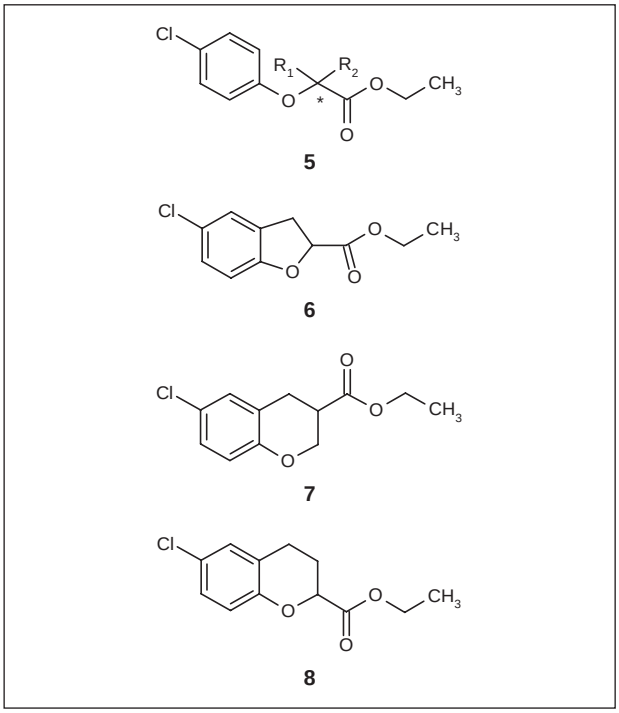


Fig. 2. Acyclic (**5**) and cyclic (**6-8**) analogues of clofibrate.

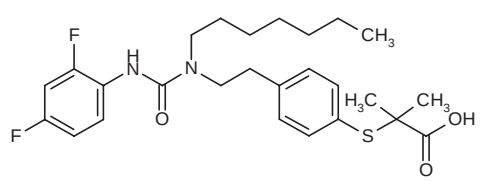
(Fig. 2) showed antilipolytic activity comparable to that of 2-alkyl-2-(4-chlorophenoxy)ethanoic acids (9).

GlaxoSmithKline identified GW-9578 (**9**) as a potent PPARα agonist with 300-fold selectivity for murine receptors and 20-fold selectivity for human receptors (10). The lipid-lowering activity of GW-9578 was due to its potent PPARα-agonist activity. In addition to its lipid-lowering effects, GW-9578 prevented weight gain and the development of hyperinsulinemia in insulin-resistant rats.

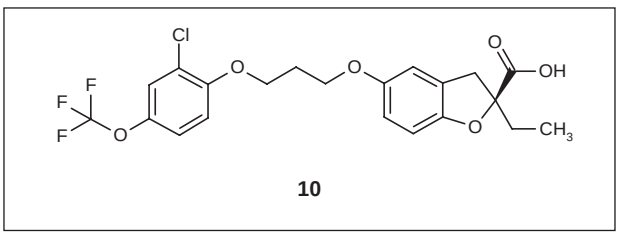
Based on the use of a 1,3-bis(oxy)propylidene linker to establish a connection between a PPARα subtype-selective acidic head group and a lipophilic tail, Merck & Co. identified a novel series of 2,3-dihydrobenzofuran-2-carboxylic acids as highly potent and subtype-selective PPARα agonists (11). This class of compounds displayed very high potency (EC_{50} < 10 nM) and subtype selectivity (> 1,000-fold). It is believed that the inherent selectivity of this class of compounds is primarily due to the conformational constraint rendered by the structurally unique 2,3-dihydrobenzofuran ring. In animal models of dyslipidemia using Syrian hamsters and male beagle dogs, compound **10** and related analogues displayed excellent cholesterol- and TG-lowering activity at doses much lower than fenofibrate.

Lilly and Ligand discovered a series of novel human PPARα agonists possessing a 2,4-dihydro-3*H*-1,2,4-triazol-3-one (triazolone) core. This work led to the discovery of LY-518674 (**11**) (12), which displays potent and selective binding affinity and functional activity at the human PPARα receptor subtype (EC_{50} = 42 nM). In co-transfection assays using the murine PPARα receptor, LY-518674 exhibited high efficacy (EC_{50} = 1052 ± 30 nM). After oral administration at 3 mg/kg once daily for 1 week, LY-518674 produced a 208% elevation in HDL cholesterol (ED_{50} = 0.3 mg/kg) and a 96% decrease in serum TGs (ED_{50} = 0.10 mg/kg) relative to controls. The ED_{50} values for both effects of LY-518674 are 2-3 orders of magnitude lower than the corresponding values for fenofibrate. LY-518674 has good oral bioavailability (> 50%), high exposure following oral dosing and a long plasma half-life. Based on this promising profile, LY-518674 was selected for clinical studies.

K-111 (**12**; formerly BM-17.0744), an ω-substituted alkylcarboxylic acid, was characterized recently by Meyer *et al.* (13) as a potent PPARα agonist that does not activate PPARγ. Those findings were confirmed independently by Wurch *et al.* (14), who also found no evidence of PPARδ activation. Treatment with K-111 normalized elevated plasma glucose, FA, triacylglycerol and insulin levels in diabetic mice (15). Subsequent research (16) revealed that K-111 is a potent antidiabetic and hypolipidemic drug in nonhuman primates.



GW-9578 (**9**)



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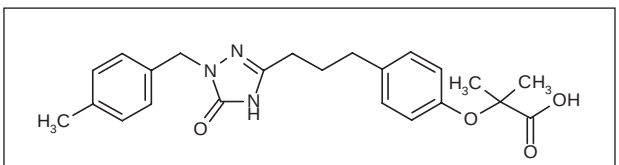
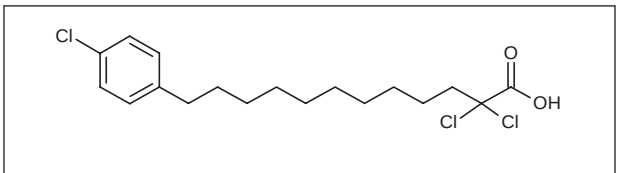


LY-518674 (**11**)



K-111 (**12**)

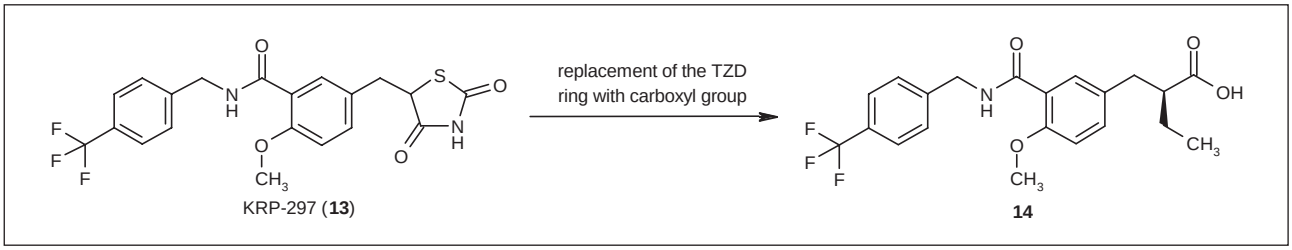


Fig. 3. The discovery of compound **14**.

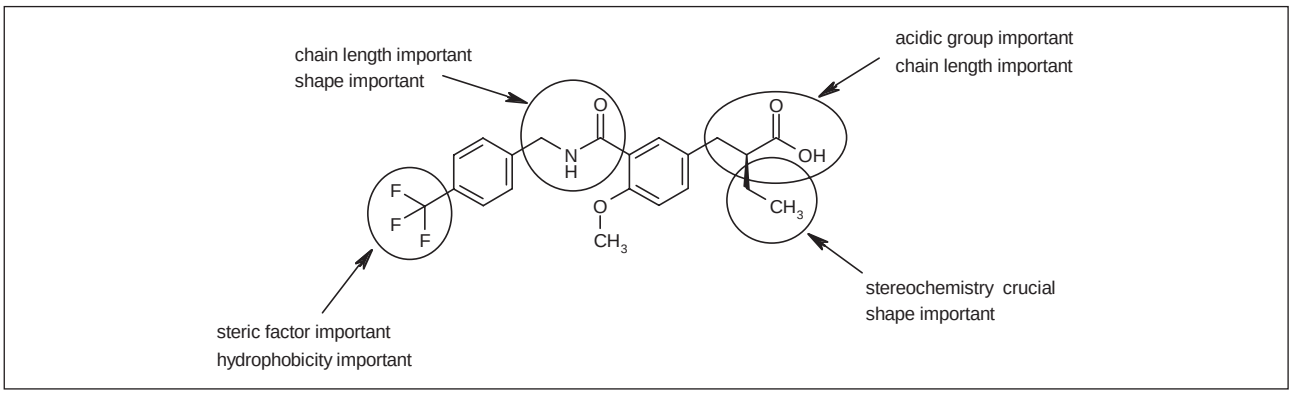


Fig. 4. Summary of the SAR of compound **14**.

2. α-Substituted phenylpropanoic acid derivatives

In an attempt to develop structurally novel human PPARα-selective agonists, Kyorin scientists identified KRP-297 (**13**), a dual PPARα/γ agonist with almost equal affinity for both subtypes, as a lead compound. They anticipated that replacement of the thiazolidine-2,4-dione (TZD) ring of KRP-297 with other acidic functionalities, such as the carboxyl group usually used in fibrates, would decrease the affinity for PPARγ and favor PPARα selectivity (Fig. 3). They developed a potent human PPARα agonist, **14**, an α-alkylphenylpropanoic acid derivative with higher selectivity for PPARα over PPARγ compared with the fibrates (17).

Structure-activity relationship (SAR) studies indicated that the nature and the stereochemistry of the substituent at the α-position of the head part containing the carboxyl group, the distance between the carboxyl group and the central benzene ring, the linking group between the central benzene ring and the distal benzene ring, and the substituent at the distal hydrophobic tail part of the molecule all play key roles in determining the potency and selectivity of PPAR subtype transactivation (Fig. 4).

In normal rats, compound **14** could reduce the serum levels of TG, free fatty acids (FFAs), total cholesterol and the sum of LDL cholesterol and very-low-density lipoprotein (VLDL) cholesterol. Further *in vivo* pharmacological evaluation of **14** and related compounds is currently under way (18).

3. Isoxazolyl-serine-based compounds

Most of the current synthetic PPARα, γ and δ agonists have several common elements: a polar "head" group "A" connected to an aromatic ring "C" through a short linker

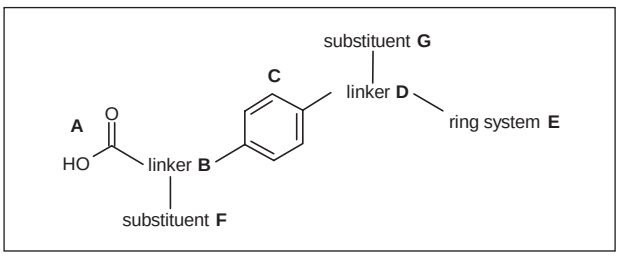


Fig. 5. General structure of most known synthetic PPARα, γ and δ agonists.

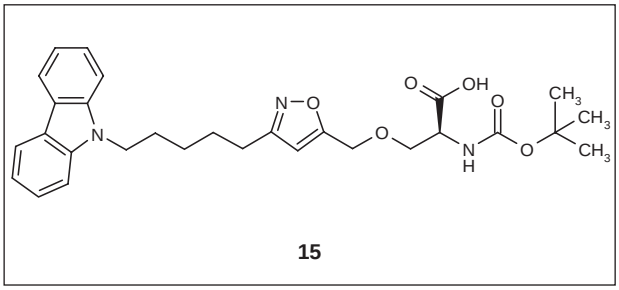
"B", and a linker "D" connecting the aromatic ring "C" to either an aromatic or an aliphatic ring system "E". The linkers "B" and "D" can also contain additional substituents "F" and "G" (Fig. 5). The reason for these structural characteristics is related to the idea that ligands should be able to adopt a U-shaped conformation in the case of both PPARα and γ, and an L-shaped conformation in the case of PPARδ.

On the basis of this description, a novel series of isoxazolyl-serine-based PPAR agonists with moderate affinities were designed and synthesized by Wei *et al.* (19). Among them, compound **15** was the most active, with an EC_{50} of 0.67 μM for PPARα. Further structural modification of these lead agonists is under way.

PPARγ agonists

Natural PPARγ agonists

A new class of natural agonists of PPARγ has been identified. These endogenous activators include mono-



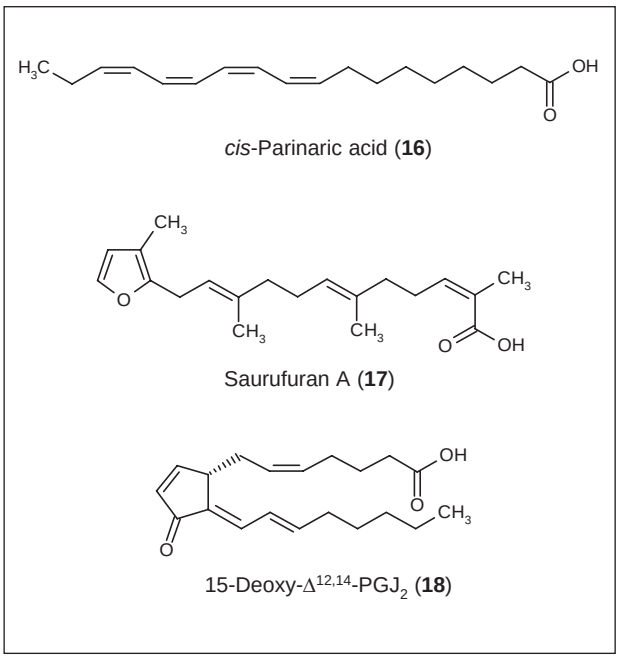
15

and polyunsaturated FAs, as well as eicosanoids, with EC_{50} values in the micromolar range.

Palmer and Wolf identified *cis*-parinaric acid (CPA; **16**; Fig**.** 6) as a novel human PPARγ agonist, with a K_d of 669 nM (20). This report presented the first direct demonstration of FA binding to human PPARγ. The acyclic furanoditerpene compound, saurufuran A (**17**; Fig. 6), from the root of *Saururus chinensis,* was identified as a PPARγ agonist, with an EC_{50} of 16.7 μM (21). 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15-d-PGJ_2; **18**; Fig. 6), a terminal metabolite of the the most potent J series of cyclopentenone prostaglandins, has proven to be the most potent natural agonist, with an EC_{50} of 1-2 μM (22). Other natural agonists are considerably less potent than synthetic agonists.

Synthetic PPARγ agonists

Synthetic PPARγ agonists such as the marketed compounds rosiglitazone and pioglitazone have proven successful for glucose control and reducing glycosylated hemoglobin (HbA1c). Another compound, farglitazar, is in phase III clinical evaluation.



cis-Parinaric acid (**16**)

Saurufuran A (**17**)

15-Deoxy-$\Delta^{12,14}$-PGJ_2 (**18**)

Fig. 6. Natural PPARγ agonists.

1. Thiazolidinediones (TZDs)

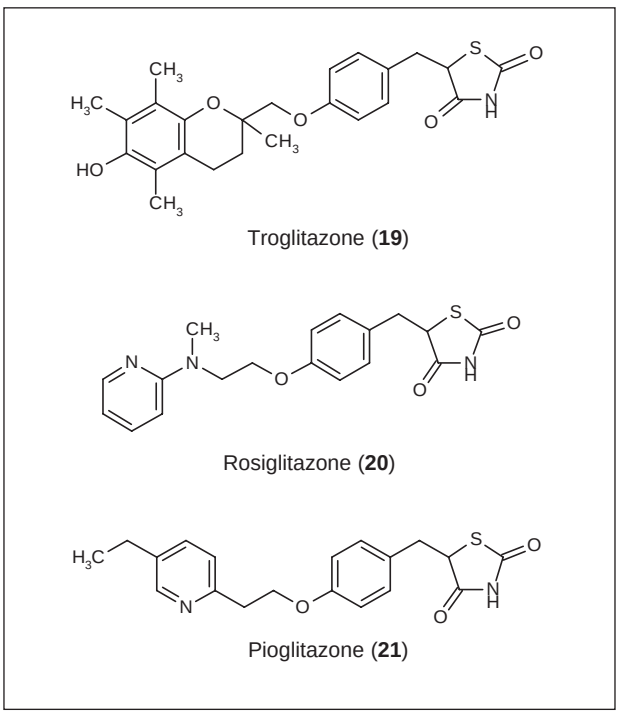
In the late 1990s, a new class of drugs called “glitazones” (or thiazolidinediones) was approved by the Food and Drug Administration (FDA) for the treatment of type 2 diabetes. These agents share a common partial chemical structure: thiazolidine-2,4-dione (TZD). Glitazones correct hyperglycemia by enhancing the insulin sensitivity of adipose, hepatic and skeletal muscle tissues. Because of this mode of action, glitazone treatment is not associated with the dangerous hypoglycemic incidents that have been observed with conventional sulfonylurea agents and insulin therapy.

In the U.S., troglitazone (Rezulin; **19**) was the first drug approved in this class, followed by rosiglitazone (Avandia; **20**) and pioglitazone (Actos; **21**) (Fig. 7). Concomitantly, in the mid-1990s, the molecular target of glitazones was discovered to be PPARγ.

Kurogi initially separated the possible pharmacophoric structure of TZDs, *i.e*., pioglitazone and rosiglitazone, into the binding and the effector site (23). The binding site is the thiazolidinedione moiety essential in all TZDs and the effector site is a secondary region that modifies the biological potency. There is also a linker between the binding and the effector sites, as shown in Fig. 8. Furthermore, investigations at GlaxoSmithKline showed that the SAR for PPARγ-agonist activity *in vitro* could accurately predict *in vivo* antihyperglycemic activity of TZDs in genetically obese and diabetic mice (24).

2. L-Tyrosine-based compounds

Compound **22** emerged as the most chemically useful from a set of several structurally novel agonists having



Troglitazone (**19**)

Rosiglitazone (**20**)

Pioglitazone (**21**)

Fig. 7. Thiazolidinediones currently on the market.

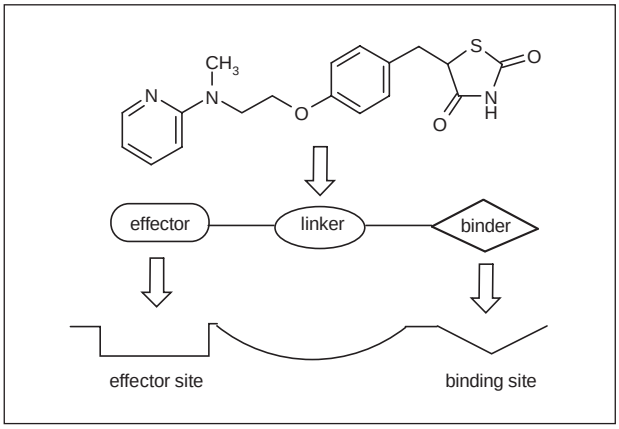


Fig. 8. Pharmacophoric structure of TZD-class PPAR agonists.

micromolar potency at the human PPARγ subtype. Starting from the screening lead **22**, GlaxoSmithKline scientists (25) identified **23** as a structurally novel PPARγ agonist (Fig. 9). Optimization of receptor potency and compound stability identified the 2-aminobenzophenone moiety as an optimal substituent for the chemically labile enaminone moiety in **23**, affording **24** (Fig**.** 9). Replacement of the benzyl group in **24** with substituents known to confer *in vivo* potency in the TZD class of antidiabetic agents afforded a series of potent and selective PPARγ agonists, exemplified by farglitazar (**25**) (Fig. 9).

Farglitazar showed a marked increase in *in vitro* functional potency and affinity for PPARγ receptors (pK_i = 8.94; pEC_{50} = 9.47) when compared to the corresponding TZD antidiabetic agents. The difference in potency and affinity between TZDs and farglitazar can be explained as shown in Fig. 10. There is a pocket in the bottom left of the active site where the 2-benzoylphenylamino moiety of farglitazar can plug in (Fig. 10), and this results in more potent binding affinity (mainly through a hydrophobic action) between farglitazar and PPARγ. TZDs do not have such a group to plug into this pocket. In addition, the β-phenylpropionic acid moiety functions as a suitable isosteric replacement for the benzyl 2,4-thiazolidinedione ring (Fig. 10) (26). Farglitazar demonstrated antihyperglycemic and antihyperlipidemic activity in two rodent models of type 2 diabetes and has progressed to phase III clinical trials.

Company researchers then expanded the SAR around the phenylalkyl ether moiety. Replacement of the phenyl ring of the phenyloxazole moiety with more polar groups, such as a 4-pyridyl group to give **26** (pK_i = 8.85; pEC_{50} = 8.74) or a 4-methylpiperazine to give **27** (pK_i = 8.66; pEC_{50} = 8.89), provided two potent and selective PPARγ agonists with increased solubility as compared to farglitazar (Fig. 9) (27).

3. Benzoxazinone derivatives

Rybczynski *et al.* (28) discovered a series of benzoxazinones devoid of the TZD moiety, and then tested them for PPARγ-agonist activity. The SAR of this PPARγ agonist series has been developed. Previous work determined the optimal location for the carboxylic acid in the phenyl ether and that substitution of the benzoxazinone aryl ring was not tolerated. This work demonstrated that compounds with aliphatic side-chains at the nitrogen of the benzoxazinone provided the most potent agonists, and the optimal chain length was 5-8 atoms. Substitution on the amide of the heterocyclic ring offered enhancement of receptor activation, while generally maintaining bioavailability and resistance to oxidative metabolism.

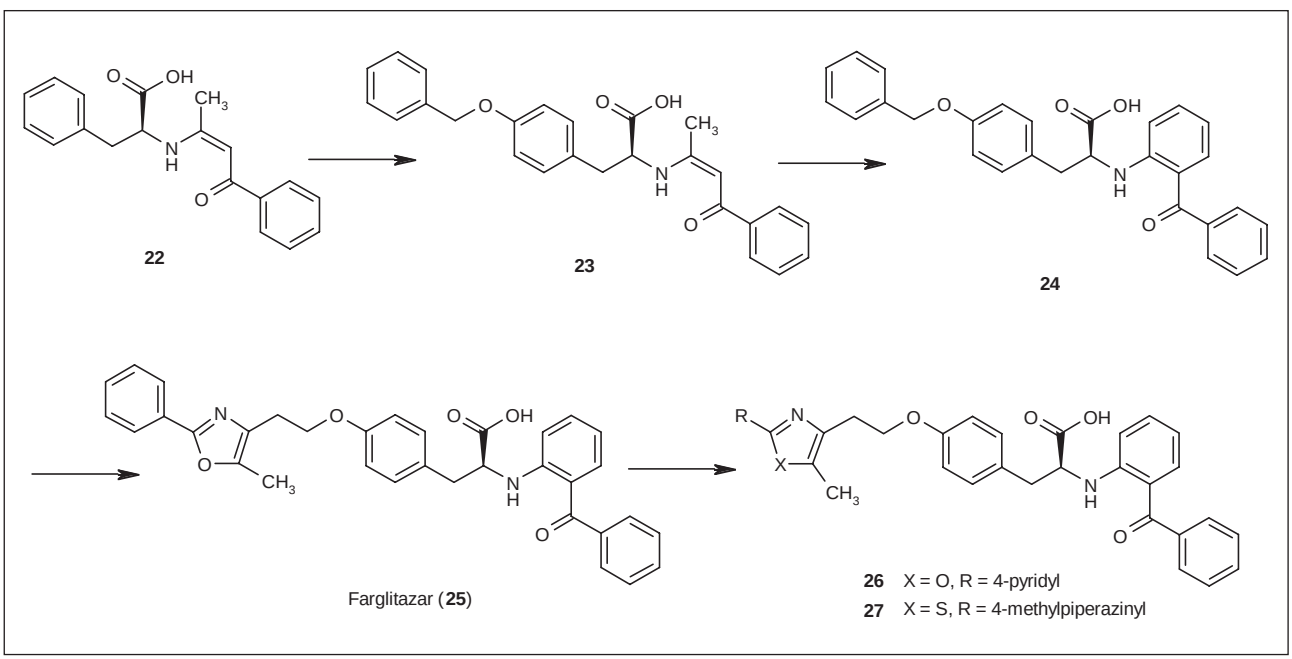


Fig. 9. The discovery of farglitazar and its derivatives.

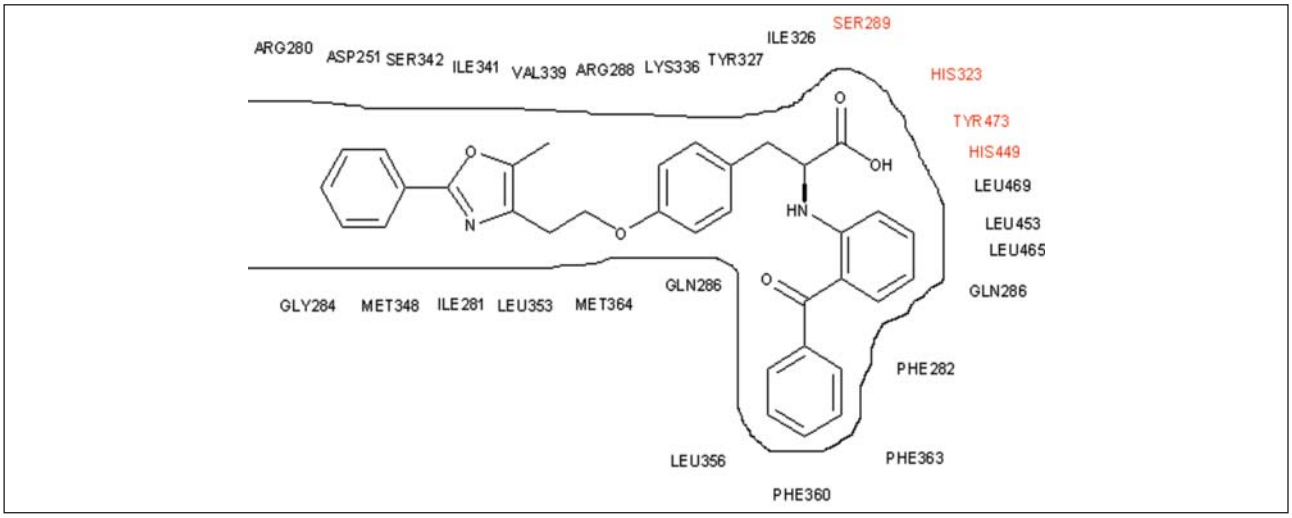


Fig. 10. The relevant amino acids surrounding the binding sites of PPARγ. The catalytic quadrant is formed by SER289, HIS323, TYR473 and HIS449 (in red). Farglitazar is exemplified as the agonist.

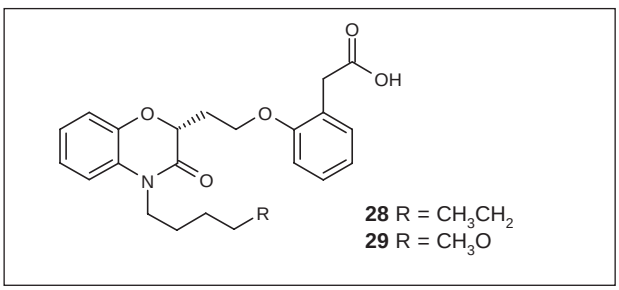
These chains could be substituted with hydroxy, fluorine, carbonyl or oxime groups. However, carboxylic acids and amides were not tolerated. Sulfur and oxygen could be successfully introduced as a member of the chain. The stereochemistry of the compound was critical to potency. The preferred stereochemistry was inferred to be (*R*) by virtue of the route used to obtain enantiomerically pure compounds. *In vivo* analysis with **28** and **29** demonstrated that these compounds, with EC_{50} values at PPARγ receptors of 110 and 274 nM, respectively, have *in vivo* glucose-lowering efficacy in a *db*/*db* mouse model of type 2 diabetes.

4. Diaryl ether carboxylic acid derivatives

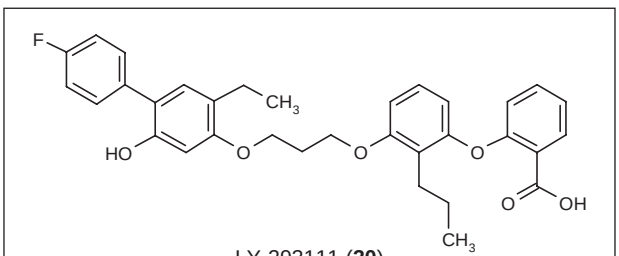
LY-293111 (etalocib; **30**), a novel diaryl ether carboxylic acid derivative, was demonstrated to have activity consistent with PPARγ agonism in the Zucker diabetic fatty (ZDF) rat diabetes model (ED_{50} for glucose reduction = 33 mg/kg). The EC_{50} for adipocyte differentiation in 3T3-L1 cells was 0.5 μM. LY-293111 can be safely administered by continuous oral therapy. The toxicities observed to date are mild and manageable (29). LY-293111 is presently in phase II studies.

5. Indole-based compounds

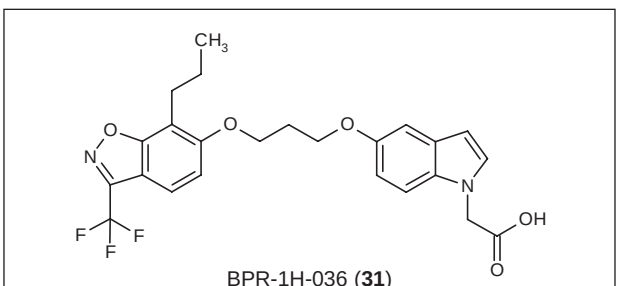
A novel indole-based series of PPAR agonists was synthesized by Mahindroo *et al.* (30) and the SAR were elucidated on the basis of binding and functional activities at PPARγ. The *n*-propyl or *n*-butyl linker, the 5-substituted indole and the acetic acid head with a benzisoxazole tail were most appropriate for potent PPARγ binding and functional activity. The compounds with a 7-propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-ol and 7-propyl-3-phenylbenzo[*d*]isoxazol-6-ol tail showed the most potent activity. *In vitro* evaluation led to the lead compound BPR-1H-036 (**31**). In *in vivo* studies in KKA^{y} mice, BPR-1H-036 demonstrated highly effective glucose-lowering activity and an excellent pharmacokinetic profile. Structural biology studies of BPR-1H-036 revealed that the indole ring contributed strong hydrophobic interactions with PPARγ and could be an important moiety for binding to the protein.



28 R = CH_3CH_2
29 R = CH_3O



LY-293111 (**30**)



BPR-1H-036 (**31**)

Partial PPARγ agonists

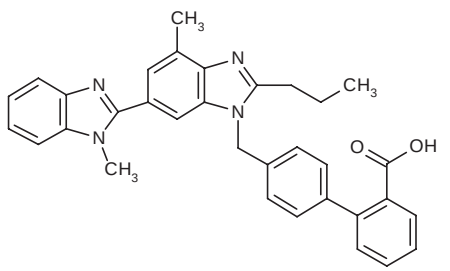
Recent preclinical studies indicated that the angiotensin II receptor blocker (ARB) telmisartan (**32**) acts as a selective partial PPARγ agonist when tested at concentrations that might be achievable with the oral doses recommended for the treatment of hypertension; this property does not appear to be shared by other ARBs (31). In cellular transactivation assays, telmisartan functioned as a partial PPARγ agonist and achieved 25-30% of the maximal receptor activation attained with conventional PPARγ agonists. Preclinical and clinical studies indicated that the administration of telmisartan can improve carbohydrate and lipid metabolism without causing the side effects associated with full PPARγ agonists. If the preliminary data are supported by the results of ongoing large-scale clinical studies, telmisartan could have a central role in the prevention and treatment of metabolic syndrome, diabetes and atherosclerosis.

PPARγ antagonists

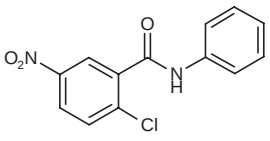
Study of the role of PPARγ in multiple cellular processes has been facilitated by the availability of high-affinity ligands. The diverse classes of ligands mentioned above are all agonists of PPARγ. Identification of PPARγ antagonists would provide powerful tools to study PPARγ signaling pathways.

In the course of a high-throughput screening to search for ligands for PPARγ, GlaxoSmithKline identified GW-9662 (**33**), a potent PPARγ antagonist with a nanomolar IC_{50} value for PPARγ and 10- and 600-fold lower potency in binding experiments using PPARα and PPARδ, respectively (32).

In cell-based reporter assays, GW-9662 was a potent and selective antagonist of full-length PPARγ. The functional activity of GW-9662 as a PPARγ antagonist was confirmed in an adipocyte differentiation assay. GW-9662 showed essentially no effect on transcription when tested using both full-length PPARδ and PPARα. The selective and irreversible nature of GW-9662 treatment suggests that this compound may be a useful tool for elucidating the role of PPARγ in biological processes.



Telmisartan (**32**)



GW-9662 (**33**)

Dual PPARα/γ agonists

Recent studies have indicated that dual PPARα/γ agonists could be of great interest in medicinal chemistry. For instance, in insulin-resistant animal models, dual PPARα/γ agonists decreased free plasma TG concentrations, which can be associated with an increase in the activity of adipocyte LPL and an increase in β-oxidation. In addition, dual PPARα/γ agonists increase plasma HDL concentrations. These effects on glycemia and insulin sensitivity are comparable to those of the TZD class of PPARγ agonists. This phenomenon is associated with an increase in insulin sensitivity in rodents, but this should be a consequence of the decrease in FFA concentration. Another advantage of PPARα/γ co-activation is a reduction in the side effects observed with TZDs, such as increase in weight and/or edema.

Natural dual PPARα/γ agonists

The arachidonic acid metabolite 8-(*S*)-HETE (**34**) is a strong agonist at PPARα receptors (EC_{50} = 100 nM) and also exhibits partial activity at PPARγ. Therefore, Caijo *et al.* (33) selected 8-(*S*)-HETE as the lead for the design of new analogues (Fig. 11). Some of these products exhibit very promising activity as dual PPARα/γ agonists. Moreover, the most active compounds, such as the quinoline-derived product **35**, present a relatively unusual structural characteristic, with a triple bond in positions 5-6. Compound **35** has high activity at PPARα receptors, with an EC_{50} value of 114 nM and a transactivation response 287% that of Wy-14643; furthermore, it also showed activity at the PPARγ subtype, with an EC_{50} of 617 nM and 72% transactivation. It was therefore selected as the lead compound for future studies.

Synthetic dual PPARα/γ agonists

Synthetic compounds developed as dual PPARα/γ agonists include tesaglitazar (**36**), ragaglitazar (**38**), muraglitazar (**43**), KRP-297 (**13**) and naveglitazar (**41**).

1. α-Substituted phenylpropanoic acid derivatives

New dual PPARα/γ agonists designed to combine the beneficial effects of both insulin sensitizers and fibrates have received increased attention. A few β-aryl α-substituted propanoic acids, their derivatives and analogues have been reported to be useful in the treatment of hyperglycemia and hyperlipidemia.

Tesaglitazar (**36**), a novel dihydrocinnamate derivative, is a dual PPARα/γ agonist. Tesaglitazar was well tolerated and produced significant, dose-dependent

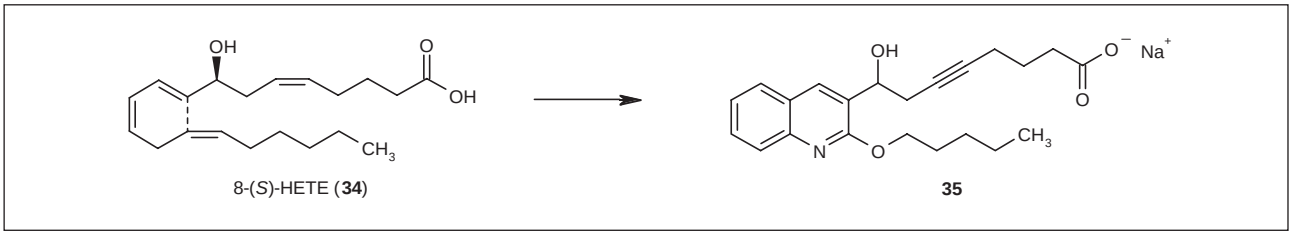
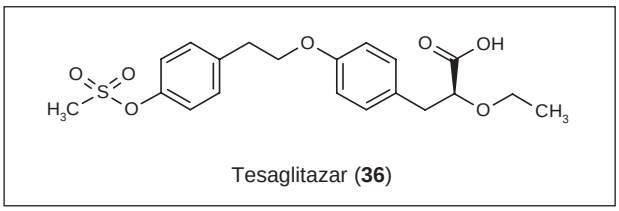


Fig. 11. 8-(*S*)-HETE and its quinoline-derived product **35**.



improvements in lipid and glucose metabolism and insulin sensitivity (34). However, development of the compound, which had reached phase III clinical evaluation, was recently discontinued by GlaxoSmithKline as the overall benefit/risk profile was not considered to offer a significant advantage over currently available therapies.

The methylaminobenzoxazole group of SB-213068 (**37**) was substituted by Lohray *et al.* (35) with different tricyclic ring systems. This led to the discovery of ragaglitazar (**38**), a phenoxazine analogue of phenylpropanoic acid (Fig. 12). Ragaglitazar is a dual human PPARα/γ agonist with EC_{50} values of 0.98 and 0.092 μM, respectively. In *db/db* mice, 9 days' treatment with ragaglitazar induced a 56% reduction in plasma glucose and a 62% reduction in TG as compared to a 33% reduction in glucose and a 16% reduction in TG for rosiglitazone at the same dose. Pharmacokinetic studies of this molecule in male Wistar rats showed very good oral bioavailability and impressive pharmacokinetic characteristics (36). No further development of this compound has been reported since Novo Nordisk suspended clinical trials in 2002.

By combining structural elements from rosiglitazone, the ethoxypropionic acid moiety and tricycles, together with SAR and computer modeling, Novo Nordisk identified the novel carbazole α-ethoxyphenylpropionic acid derivative **39**, with dual agonist activity at PPARα (EC_{50} = 0.36 μM) and PPARγ receptors (EC_{50} = 0.17 μM) *in vitro* (37). After treatment of *db/db* mice with compound **39** for 10 days, the improvement in insulin sensitivity was superior to that seen with both pioglitazone and rosiglitazone, suggesting *in vivo* PPARγ activity. Furthermore, the compound lowered plasma TGs and cholesterol after 4 days of treatment in high-cholesterol-fed rats, clearly indicating *in vivo* PPARα activity.

LY-510929 (**40**) was also identified as a balanced dual PPARα/γ agonist by Lilly and Ligand (38). LY-510929 possesses a potent dual human PPARα/γ agonist profile (IC_{50} = 28 and 10 nM, respectively; EC_{50} = 9 and 4 nM, respectively) and potent agonist activity in cell-based co-transfection assays. In preclinical studies, LY-510929 remarkably improved insulin sensitivity and potently reversed diabetic hyperglycemia, while significantly improving overall lipid homeostasis. However, LY-510929 was distinctly less potent in elevating HDL cholesterol, reflecting the relative lack of cross-species consistency for PPARα activation. These results support the hypothesis that LY-510929 has the potential to stimulate both PPARα and PPARγ at similar plasma exposures in the clinical setting, thus providing optimal control of both hyperglycemia and dyslipidemia. LY-510929 was therefore selected for advancement to clinical trials for the treatment of type 2 diabetes, although development is currently on hold.

From SAR studies around the 2-alkoxydihydrocinnamate scaffold that incorporates a phenoxyphenyl ether tailpiece, Lilly identified a series of compounds with high affinity for PPARγ and *in vivo* potency in ZDF rats. Changes in the *para*-substituent of the terminal phenyl ring of the tailpiece and conformational restriction in the linker were found to modulate PPAR potency and selectivity. Naveglitazar (**41**) is a high-affinity ligand for PPARγ that behaved as an agonist in a cell-based efficacy assay. Naveglitazar is also a weak human PPARα agonist, which could lead to a better cholesterol profile in humans.

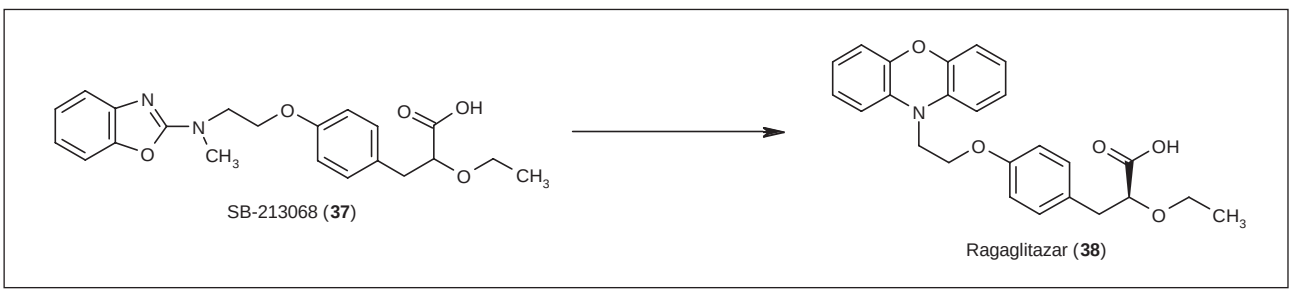
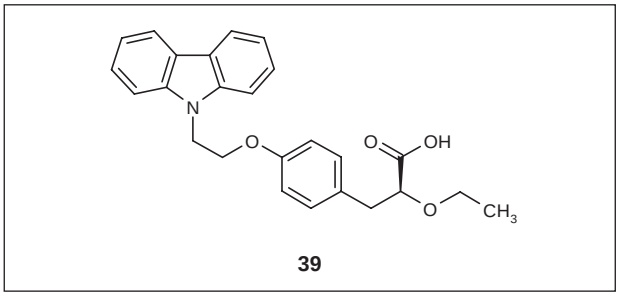


Fig. 12. The discovery of ragaglitazar.



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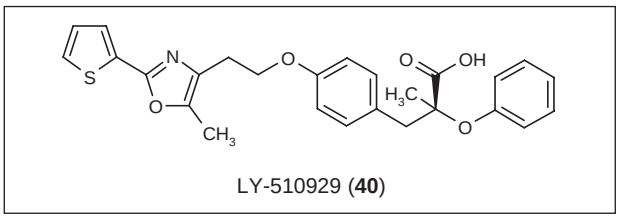
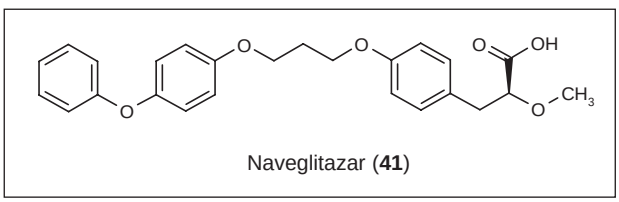


LY-510929 (**40**)



Naveglitazar (**41**)

In vivo studies in the ZDF rat showed that naveglitazar is effective at low doses in normalizing plasma glucose and reducing plasma TG (39). As a PPARγ-dominant dual agonist with beneficial PPARα activity, naveglitazar was selected for clinical evaluation for the treatment of type 2 diabetes and is currently in phase II.

Drawn from known α-alkoxy and α-aminoarylpropanoic acid PPAR agonists, an initial lead, the oxybenzylglycine **42**, was conceptually derived via homologation of a novel azaisostere structure by Bristol-Myers Squibb. Significant advantages gained from this change include the elimination of a chiral center, simplification of synthesis, and opportunities for rapid generation of diversity from the lead structure. Thus, exploration of the replacement of the *N*-benzyl moiety of **42** was carried out in an effort to optimize the relatively weak *in vitro* potency of **42**. Several of these carbamate acids showed promising oral activity *in vivo*, and muraglitazar (**43**) was identified from this effort as a non-TZD dual PPARα/γ agonist (Fig. 13). Muraglitazar showed potent activity *in vitro* at human PPARα (EC_{50} = 320 nM) and PPARγ (EC_{50} = 110 nM) receptors. Muraglitazar demonstrated high efficacy in lowering glucose, insulin, TGs and FFAs in genetically obese, severely diabetic *db*/*db* mice and had a favorable pharmacokinetic profile (40).

Buse *et al.* (41) evaluated the efficacy and safety of muraglitazar in adult patients with type 2 diabetes. In this study, 24 weeks of treatment with muraglitazar 2.5 or 5 mg was an effective treatment option for these patients. Muraglitazar was associated with HbA1c-lowering effects and clinically meaningful improvements in other glycemic parameters, including favorable effects on fasting plasma glucose (FPG) and insulin levels. Treatment with muraglitazar favorably affected the lipid abnormalities associated with diabetic dyslipidemia, producing significant decreases in TG, apolipoprotein B (apo B), and non-HDL cholesterol levels, and significant increases in HDL cholesterol levels. Muraglitazar was generally well tolerated. However, Bristol-Myers Squibb decided to discontinue further development for commercial reasons.

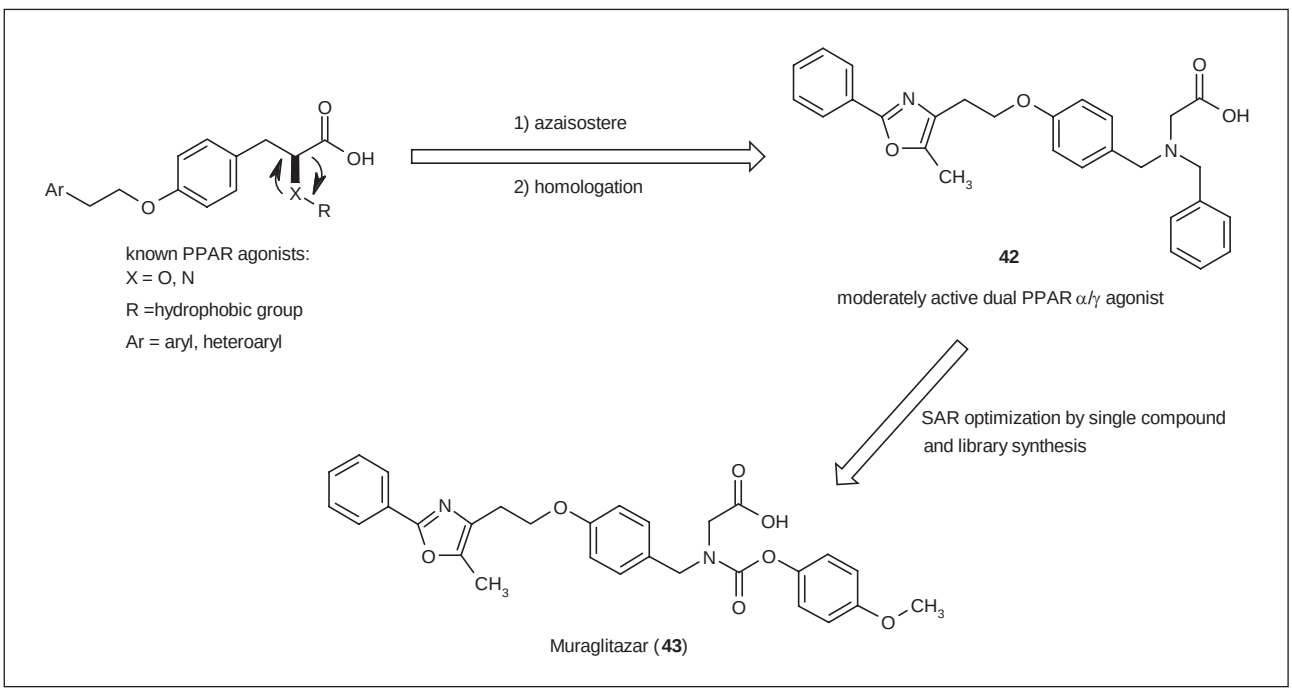


Fig. 13. The discovery of muraglitazar.

2. Fibrate analogues

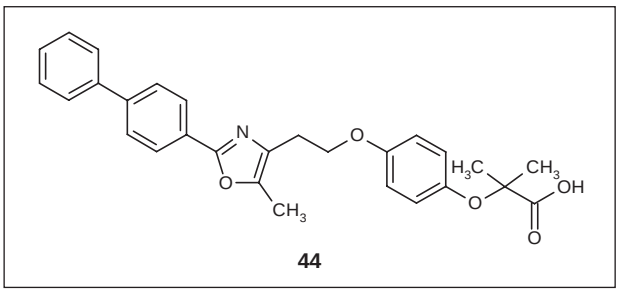
The propionic acid derivative **44** was designed and synthesized by Lilly and Ligand combining the putative pharmacophores of known PPARγ- and PPARα-selective agents in a single molecule (42). Compound **44** exhibited potent dual PPARα/γ-agonist activity. Further preclinical evaluation of **44** on multiple facets of type 2 diabetes and associated cardiovascular risks is under way (43).

Glitazones have a chiral center at the 5-positon of the TZD ring and only the (*S*)-enantiomer carries selective PPARγ activity. Fibric acid (2-phenoxyisobutyric acid) is a common substructure of fibrates, which act as weak PPARα agonists. Thus, by combining the cyclized fibrate concept, TZD as structurally similar to the carboxylic acid moiety and side-chains from previous studies, Merck & Co. synthesized and evaluated a series of structurally novel antidiabetic (2*R*)-chromane-2-carboxylic acids for PPAR-agonist activities (Fig. 14).

They successfully incorporated both PPARα and PPARγ activities into one structure. Using SAR studies for dual PPARα/γ agonism, compound **45** was discovered as a potent and selective dual PPARα/γ agonist. Compound **45** exhibited comparable antihyperglycemic activity to rosiglitazone when orally administered in the *db*/*db* mouse. In addition, the lipid-lowering activities of **45** were amply demonstrated when orally administered in a Syrian hamster model and a dog model. Compound **45** has desirable pharmacokinetic profiles in dogs and rhesus monkeys. Moreover, **45** is a single enantiomer that is not vulnerable to racemization (44).

The unique *O*-arylmandelic acid PPAR agonist **46** (Fig. 15) was discovered by Merck & Co. as a PPAR ligand with micromolar human PPARγ affinity and nanomolar affinity for human PPARα (K_i = 63 nM) (45). Compound **46** proved to be remarkably selective for human PPARγ and α over human PPARδ, showing no or trace displacement in the analogous human PPARδ binding assay at up to 50 mM. Compound **46** and related analogues, such as compound **47** (Fig. 15), showed excellent antihyperglycemic efficacy in a *db/db* mouse model of type 2 diabetes. These PPARα-weighted agonists do not show the typical PPARγ-associated side effects in a rat tolerability assay.

For the structural design of novel PPAR compounds, company scientists combined the isobutyric acid head group of fibrates and the lipophilic aryloxy moiety of the mandelate lead **47**, the putative pharmacophores of these two classes of compounds, and generated the 2-aryloxy-2-methylpropionic acid compound **48** (Fig. 15). Compound **48** was identified as a dual PPARα/γ agonist with potent PPARα-agonist activity and weak PPARγ-activating activity. Compound **48** lowered both plasma glucose and lipids in animal models without causing an increase in body weight, an adverse effect frequently associated with glitazone PPARγ agonists. The PPARα activity of compound **48** appeared to play a significant role in lowering glucose levels in *db/db* mice (46, 47). On



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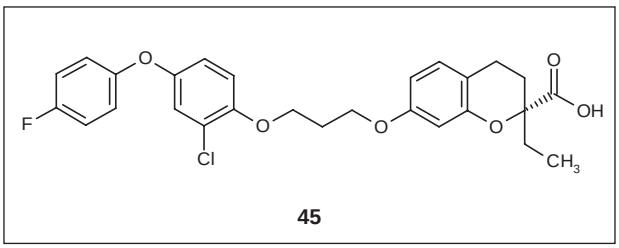

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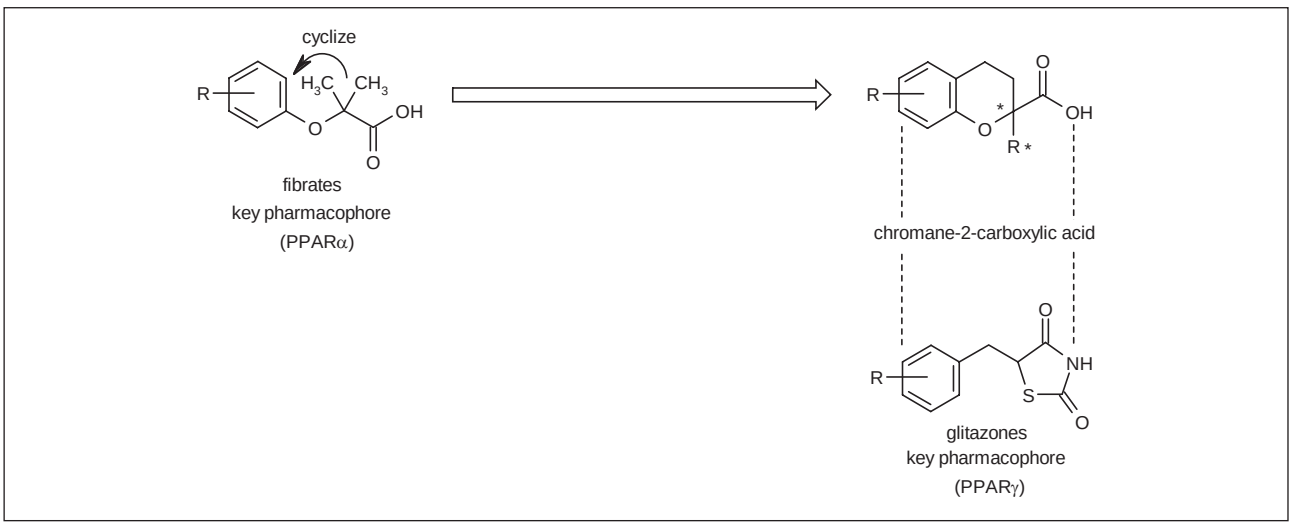


Fig. 14**.** Cyclized fibrate concept.

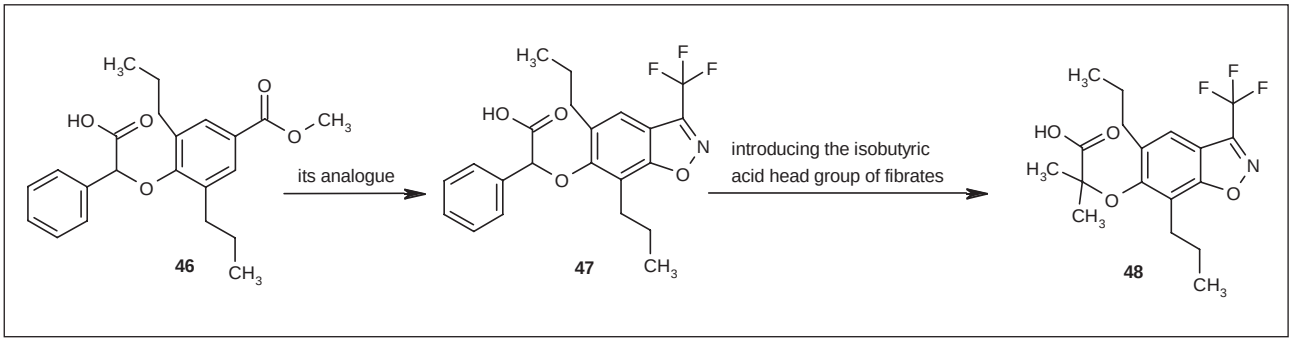
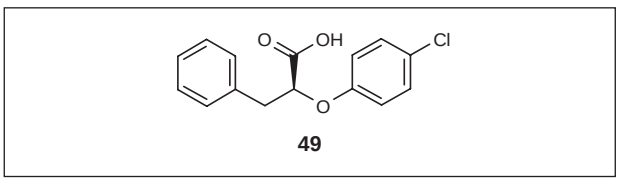


Fig. 15. The discovery of compound **48**.



the basis of its *in vitro* and *in vivo* profile, **48** was selected for further evaluation in man.

Another compound, **49**, was designed and synthesized by Pinelli *et al.* (48) from structural simplification of β-aryl-α-oxy-substituted propanoic acids, which possess very potent dual PPARα/γ-agonist activity. Compound **49** showed dual PPARα/γ activity, and its stereochemistry was crucial in receptor activation. Interestingly, **49** exhibited only a modest ability to induce differentiation of murine 3T3-L1 fibroblasts to adipocytes compared to rosiglitazone, a well-known PPARγ agonist, suggesting that it is a PPARγ modulator (49).

3. TZDs

As previously mentioned, a group of Japanese scientists disclosed KRP-297 (**13**), the first published example of a dual PPARγ and PPARα agonist. Results from cell-based assays indicated that KRP-297 is a robust submicromolar dual human PPARα/γ agonist with a γ:α potency ratio of ~2. The mechanism by which it activates both PPARα and PPARγ involves ligand-stimulated co-activator recruitment to the receptor ligand binding domain. In a comparative study in *ob/ob* mice, KRP-297 normalized hyperglycemia and hyperinsulinemia with equal or greater potency and efficacy than pioglitazone. When tested in hamsters, KRP-297 showed impressive hypolipidemic activity, lowering both TGs and total cholesterol. In dogs, KRP-297 reduced serum cholesterol levels with a potency more than 10-fold greater than simvastatin, a cholesterol-lowering drug used to inhibit the production of cholesterol by the liver (50). Kyorin and partner Merck & Co. (MK-767) halted the development of this compound after it was shown to cause a rare type of cancer in mice.

Merck & Co. identified a novel series of 5-aryl-TZD-based dual PPARα/γ agonists. These compounds have the TZD ring directly attached to the phenyl ring. Compounds with the *para* relationship between the TZD ring and the 3-carbon methylene linker (such as **50**) are PPARγ-selective agonists. Changing the point of attachment of the TZD ring to the phenyl ring from the *para* to the *meta* orientation with respect to the 3-carbon methylene tether (such as **51**) transformed PPARγ-selective agonists into dual PPARα/γ agonists (Fig. 16). Efficacy study results of some *meta*-linked TZD analogues in *db/db* mice showed activity superior to rosiglitazone in correcting hyperglycemia and hypertriglyceridemia.

In earlier SAR studies, company scientists had established the need for the 3-carbon methylene tether and *n*-propyl group as the optimal substituent at the C-2′ position for potency and selectivity. They extended these efforts to include the synthesis of oxazolidine-2,4-diones (OZDs; **52**: X = O) as bioisosteric replacements for the corresponding TZD ring (Fig. 16) (51).

Further SAR studies identified the C-4′ position as an important site for additional structural modifications. This led to the identification of the potent, orally active dual PPARα/γ agonist **53** (Fig. 16). Compound **53** was potent in both binding affinity and functional activity at PPARα and γ. In *db/db* mice, compound **53** produced a 76% glucose correction and lowered TGs by 77%, reflecting the correlation between *in vitro* potency and *in vivo* efficacy (52).

PPARδ agonists

In contrast to PPARα and γ, there are no marketed drugs that target PPARδ and the physiological role of PPARδ remains largely mysterious, due in part to the lack of selective ligands as tools to study its pharmacology. Thus, identification of potent and selective ligands is essential for elucidating the function of PPARδ. However, most of the ligands published to date either have low affinity for PPARδ or lack selectivity over the other PPAR isoforms.

Starting with high-throughput screening, followed by combinatorial chemistry to develop small focused libraries containing lipophilic carboxylic acids, and finally structure-guided lead optimization, GlaxoSmithKline developed the first truly selective PPARδ agonists, GW-501516 (**54**) and its analogue GW-0742 (**55**) (53). Both GW-501516 and GW-0742 showed an EC_{50} value of 1.1

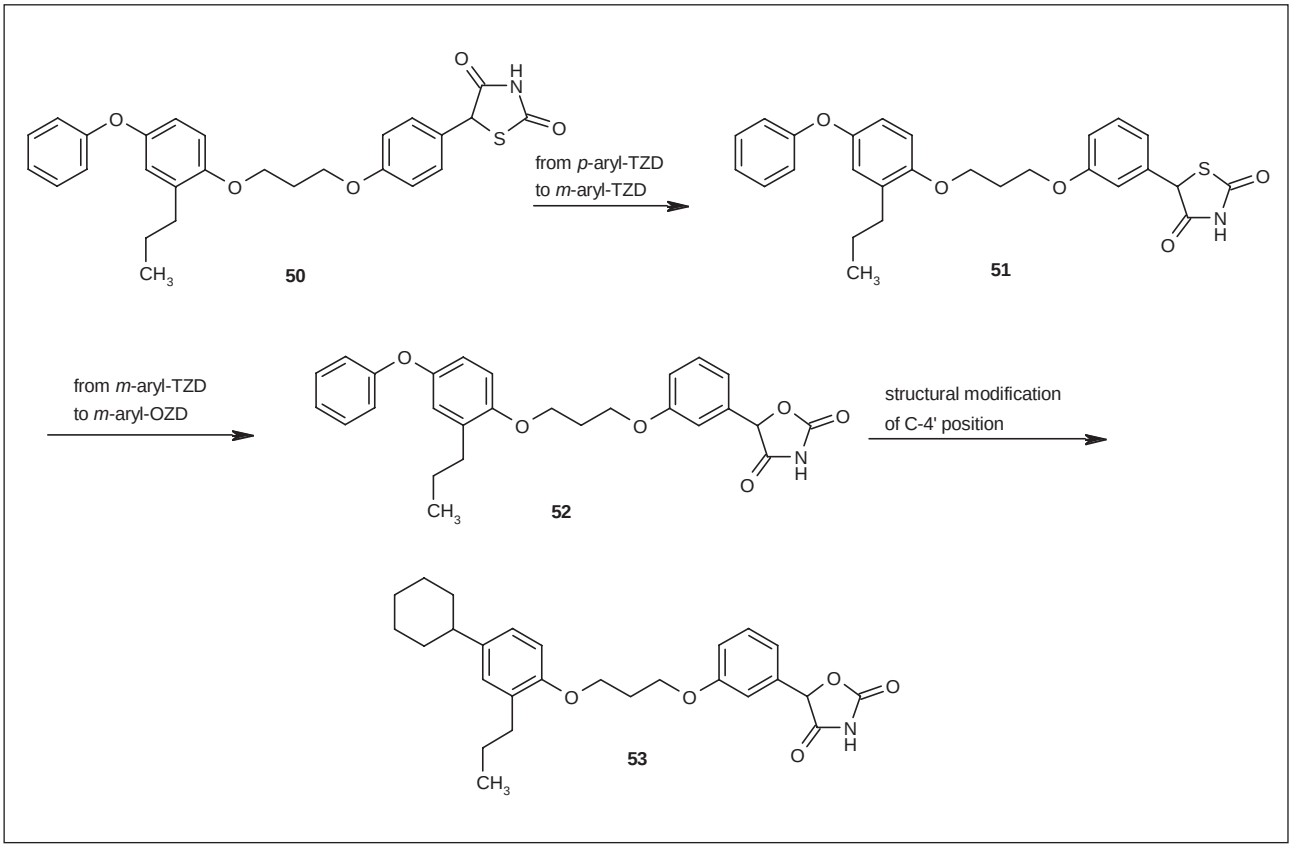
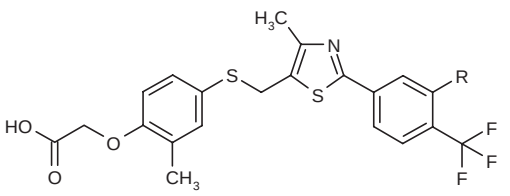


Fig. 16. SAR studies and structural optimization of 5-aryl-TZD-based dual PPARα/γ agonists.

nM at PPARδ and 1,000-fold selectivity over the other human subtypes. In macrophages, fibroblasts and intestinal cells, GW-501516 induced apolipoprotein A1 (apo A1)-specific cholesterol efflux, in part by increasing expression of the reverse cholesterol transporter ATP-binding cassette A1. When administered to insulin-resistant middle-aged obese rhesus monkeys, GW-501516 produced a marked, dose-dependent rise in serum HDL cholesterol while decreasing levels of small dense LDL, fasting TGs and fasting insulin (54).

These compounds are expected to eventually prove to be valuable tools for studying the function of PPARδ and potential therapeutic agents for the treatment of diseases associated with elevated serum TGs and low levels of HDL cholesterol.



GW-501516 (**54**) R = H
GW-0742 (**55**) R = F

Pan-PPAR$\alpha/\gamma/\delta$ agonists

Amphipathic compounds

Merck & Co. reported a series of benzisoxazole pan-PPAR$\alpha/\gamma/\delta$ agonists led by the amphipathic carboxylate compound **56**. The phenylacetic acid **57** is a potent but nonselective PPAR$\alpha/\gamma/\delta$ agonist and an effective insulin sensitizer in insulin-resistant *db/db* mice. Retaining the lipophilic tail of compound **57**, the optimization of acid proximal structure for *in vitro* and *in vivo* potency produced **58** (Fig. 17), with apparent K_i values of 3.1, 0.3 and 7.26 nM, respectively, for PPARγ, δ and α, and EC_{50} values of 6, 20 and 5 nM, respectively, in transfected COS-1 cell (African green monkey kidney fibroblasts). When administered to *db/db* mice, compound **58** showed efficacy equal or superior to rosiglitazone in correcting hyperglycemia and hypertriglyceridemia. In addition, compound **58** exhibited promising pharmacokinetic parameters, with 76% bioavailability and a dose-normalized area under the curve (AUC) of 4.2 mM.h for a dose of 2 mg/kg p.o. as the sodium salt in rats (55).

α-Substituted phenylpropanoic acid and its dimeric analogues

Novo Nordisk identified a group of pan-PPAR$\alpha/\gamma/\delta$ agonists (**59**) using the dual PPARα/γ agonist **39** as a

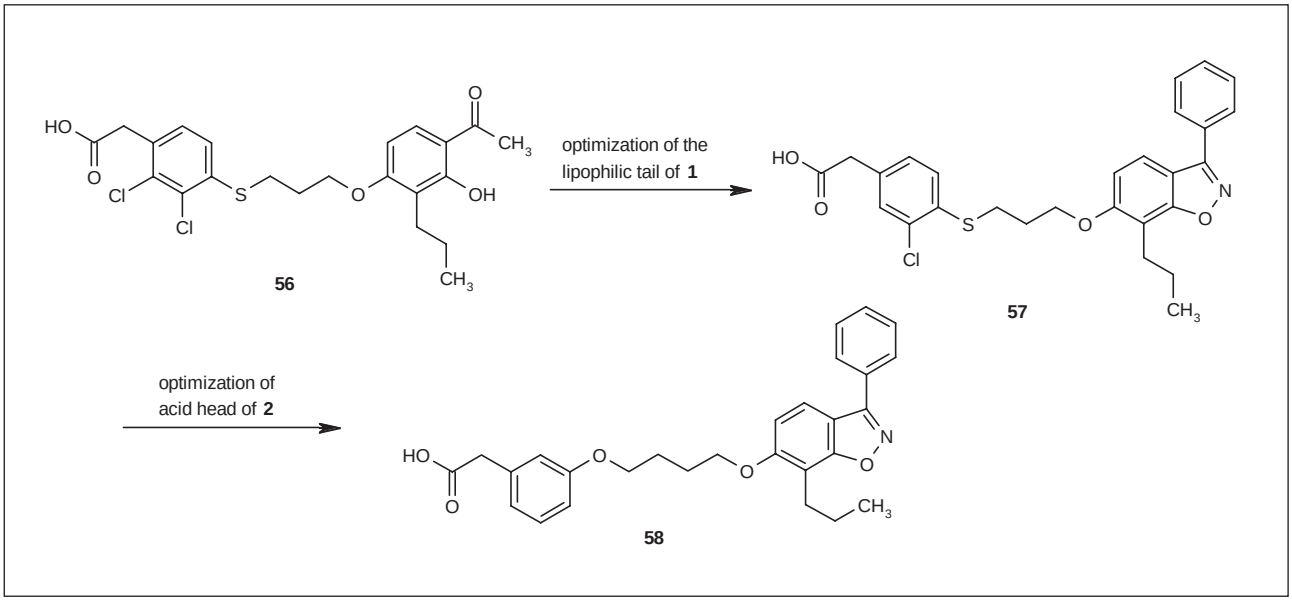


Fig. 17. Optimization of amphipathic compounds as pan-PPARα/γ/δ agonists.

structural template. By extending the structural knowledge of this group of pan-PPARα/γ/δ agonists, the PPAR agonists **60** and NNC-61-4655 (**61**), as well as their dimeric analogues **62**, **63a** and **63b,** were designed and synthesized (Fig. 18). *In vitro* PPARα, γ and δ transactivation data showed that **60** and NNC-61-4655 are potent, nonselective but PPARα-preferring agonists, while the three dimeric ligands showed agonist activity at all three PPAR subtypes, although with different profiles than the monomers **60** and NNC-61-4655.

NNC-61-4655 (EC_{50} = 0.0067, 1.13 and 6.90 μM, respectively, at PPARα, γ and δ) has excellent pharmacokinetic properties and was shown to be more effective *in vivo* in male *db/db* mice than either rosiglitazone or pioglitazone. Due to the unique PPAR subtype profile, together with impressive pharmacological properties, NNC-61-4655 was chosen as a promising antidiabetic drug candidate (56).

X-ray crystal structure and modeling experiments suggested that the dimers interacted with the activation function 2 (AF-2) helix, as well as with amino acid residues in the lipophilic pocket close to the receptor surface. Despite breaking all the “rule of five” criteria, the dimers had excellent oral bioavailability and pharmacokinetic properties, resulting in good *in vivo* efficacy in *db/db* mice. The dimeric PPAR agonists may be suitable drug candidates, further suggesting that the principle of dimeric agonists could be applied more broadly than seen until now (57).

Netoglitazone

Netoglitazone (**64**) is a novel insulin sensitizer that activates not only PPARγ but also PPARα and δ *in vitro*. Investigation at Mitsubishi Pharma using both macrophages and foamed macrophages showed that netoglitazone concentration-dependently decreased tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) secretion, increased apo A1-mediated cholesterol efflux and apo A1 secretion, and inhibited TNF-α-induced vascular cell adhesion molecule-1 (VCAM-1) expression and monocyte chemoattractant protein-1 (MCP-1) secretion from human artificial episomal chromosomes (HAEC). These data suggest that netoglitazone may have beneficial effects on macrophages and foamed macrophages in early stages of atherosclerosis. Netoglitazone has successfully progressed to phase II clinical trials (58, 59).

Conclusions and perspectives

It has been nearly a decade since the first PPAR subtype was reported as an orphan member of the nuclear receptor gene family. The ability of PPARs to mediate many metabolic and therapeutic actions has made them therapeutic targets with widespread impact in the treatment of human metabolic diseases.

The fibrate and glitazone drugs were developed by a succession of pharmaceutical companies over a period of 40 years using empirical medicinal chemistry and rodent pharmacology. Although their cellular targets were unknown, these drugs were successfully employed in the treatment of hypertriglyceridemia and type 2 diabetes in humans. The demonstration that PPARα and PPARγ are the receptors mediating the biological activity of the fibrate and glitazone drugs has led to a renaissance in nuclear receptor research to develop drugs for diabetes and cardiovascular disease.

More potent PPARα and PPARγ agonists, PPARδ agonists and dual PPARα/γ agonists are currently in development or in human trials. PPARα agonists provide significant antidyslipidemic and antiatherosclerotic effects. PPARγ agonists improve insulin sensitivity, lower glucose levels and lower plasma TG and FFA levels by

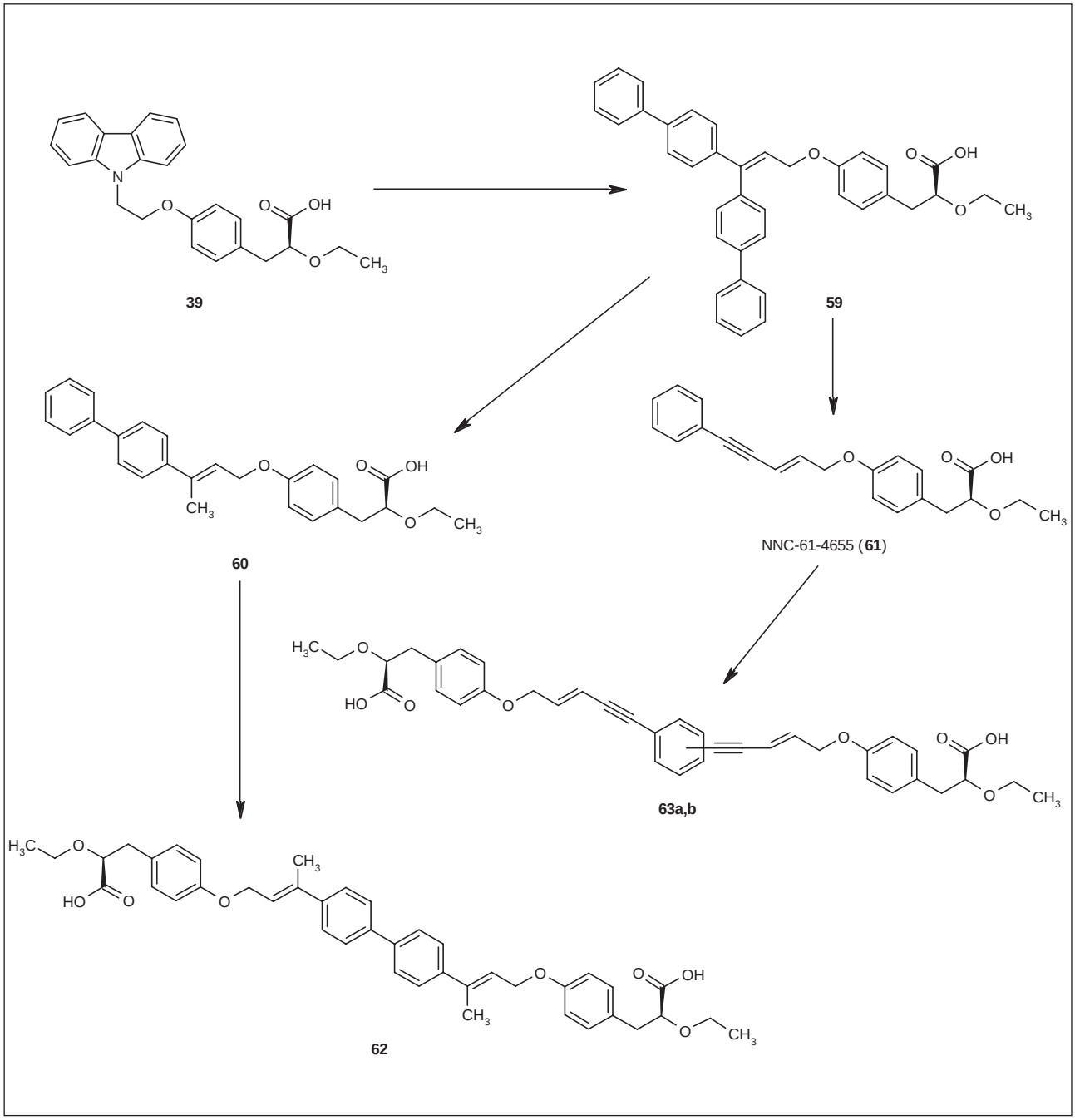
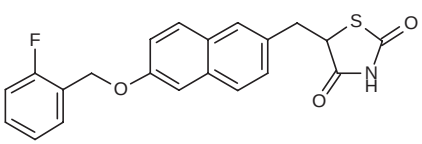


Fig. 18. The discovery of α-substituted phenylpropanoic acid and its dimeric analogues as pan-PPARα/γ/δ agonists.

enhancing their uptake into adipocytes. Recent preclinical data indicate that PPARδ ligands might have beneficial effects on circulating lipids and obesity. Also, dual PPARα/γ agonists show promise in the simultaneous treatment of diabetic hyperglycemia and dyslipidemia.

Continuing efforts to delineate the physiology, pharmacology and functional genomics of PPARs will increase our understanding of the beneficial and adverse effects of modulating PPAR activity and should provide superior ligands with increased therapeutic impact.



Netoglitazone (**64**)

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