Expert Opinion On Therapeutic Targets

Synthetic and natural Peroxisome Proliferator-Activated Receptor (PPAR) agonists as candidates for the therapy of the metabolic syndrome

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Abstract

Introduction

Peroxisome proliferator-activated receptors (PPARs) are the molecular targets of hypolipidemic and insulin-sensitizing drugs and implicated in a multitude of processes that fine-tune the functions of all organs in vertebrates. As transcription factors they sense endogenous and exogenous lipid signaling molecules and convert these signals into intricate gene responses that impact health and disease. The PPARs act as modulators of cellular, organ, and systemic processes, such as lipid and carbohydrate metabolism, making them valuable for understanding body homeostasis influenced by nutrition and exercise.

Areas covered

This review concentrates on synthetic and natural PPAR ligands and how they have helped reveal many aspects of the transcriptional control of complex processes important in health.

Expert opinion

The three PPARs have complementary roles in the fine-tuning of most fundamental body functions, especially energy metabolism. Understanding their inter-relatedness using ligands that simultaneously modulate the activity of more than one of these receptors is a major goal. This approach may provide essential knowledge for the development of dual or pan-PPAR agonists or antagonists as potential new health-promoting agents and for nutritional approaches to prevent metabolic diseases.

Keywords: agonists, insulin resistance, lipid metabolism, metabolic syndrome, PPARs, type 2 diabetes, phytochemicals

1. Introduction

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor family that function as ligand-inducible transcription factors in the regulation of genes involved in lipid and glucose metabolism [1]. There are three closely related isotypes: PPARα (NR1C1), PPARβ/δ (NR1C2), and PPARγ (NR1C3) [2]. The overarching structural features seen in all PPARs, as well as other members of the superfamily, are three major functional domains: an N-terminal domain (NTD) with constitutive activation function 1 (AF-1), a DNA-binding domain (DBD) containing two zinc finger motifs, and a C-terminal ligand-binding domain (LBD) with the ligand-dependent AF-2 (Figure 1A) [3]. Although PPARs share a high degree of structural homology, they are encoded in separate genes and exhibit both overlapping and distinct ligand specificities and tissue distribution, supporting a variety of systemic and cellular functions [4-6]. PPAR-mediated transcriptional regulation requires heterodimerization with the retinoid X receptor (RXR), which is also a member of the nuclear receptor superfamily (Figure 1B, C) [7, 8]. Ligand-dependent activation of the PPAR:RXR heterodimer triggers conformational changes that lead to the release of co-repressors (e.g., SMRT and NCoR) and the recruitment of co-activators (e.g., p300, CBP, SRC-1) (Figure 1B, C). The co-activator complex-bound PPAR:RXR heterodimer binds to a specific DNA sequence, termed the peroxisome proliferator response element (PPRE), within the regulatory region of its target genes (Figure 1B, C). Although PPARs exert their most prominent actions by enhancing target gene transcription in a ligand-dependent manner, studies have provided important insights regarding the basal activity of the receptor in the absence of ligand [9]. PPAR:RXR heterodimers have been found to bind

the PPREs of some PPAR target genes irrespective of ligand binding. However, whether the absence of exogenous ligands actually reflects a true ligand-independent action of the receptors or results from a weak concentration of endogenous native agonists is still debated. In the absence of ligand, the PPAR:RXR heterodimer remains bound to the nuclear receptor co-repressor (NCoR) and the silencing mediator of retinoid and thyroid hormone receptor (SMRT), resulting in the repression of certain target genes. Notably, post-translational modification of PPARs, including SUMOylation (Figure 1D), phosphorylation, acetylation, and ubiquitination (Figure 2), has been implicated in the modulation of their transcriptional regulatory activity [10]. For example, SUMOylation of PPARs, particularly PPARα and PPARγ, is involved in ligand-dependent transrepression of target genes through the recruitment of NCoR and histone deacetylases (HDACs) (Figure 1D) [11, 12].

Animal studies have yielded important information on the pleotropic cellular and systemic roles attributable to PPARs, reaching far beyond the stimulation of peroxisome proliferation in rodents for they were initially named [13]. PPARs are crucial in the regulation of cellular and systemic energy homeostasis by acting as critical mediators of internal or dietary stimuli (Figure 2). As lipid sensors, PPARs modulate metabolism in response to dietary lipid intake and govern lipid storage and metabolism [1]. PPARs also regulate glucose metabolism, insulin sensitivity, mitochondrial biogenesis, cell proliferation, differentiation, and survival, tumor development, inter-organ cross-talk during inflammation, and interactions with endocrine signaling pathways [9]. Natural ligands of PPARs are fatty acids (FAs), eicosanoids and phospholipids derived endogenously from cellular FA metabolism or exogenously from dietary lipids [13].

Among the synthetic ligands, the lipid-lowering fibrates and insulin-sensitizing thiazolidinediones (TZDs), which are PPARα and PPARγ agonists, respectively, underscore the potential of PPARs as therapeutic targets (Figure 3) [14-16]. With their multi-faceted functions in metabolism and energy homeostasis, PPARs have indeed emerged as potential therapeutic targets for the manifestations of metabolic syndrome (MetS), particularly obesity, insulin resistance (IR), type 2 diabetes mellitus (T2DM), nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), hypertension, and dyslipidemia (Figure 3) [17]. In light of the preponderance of evidence indicating the therapeutic roles of PPAR agonists in metabolic diseases, the latest preclinical findings on potential new drug candidates targeting PPARs are reviewed here. These agonists can activate PPARs in a selective, dual or pan receptor manner, or even using a dual receptor-type mechanism (Figure 4). Special emphasis is placed on the novel dual and pan-PPAR agonists.

2. Selective PPARα Agonists

PPARα is highly expressed in tissues with a high capacity for FA oxidation (FAO), such as liver, heart, skeletal muscle, brown adipose tissue, and kidney. PPARα plays a pivotal role in FA metabolism. Fasted PPARα-null mice exhibit hypoglycemia, hypoketonemia, hyperlipidemia, and hepatic steatosis, suggesting a role of PPARα in modulating these metabolic processes during fasting [18]. Adipocyte lipolysis was recently shown to stimulate steatosis in mice with hepatocyte-specific deletion of PPARα, identifying this receptor as a drug target in NAFLD [19].

Due to their clinical effectiveness, selective PPARα agonists (e.g., fibrates) have been used since the 1960s for the treatment of dyslipidemia due to their ability to lower plasma triglycerides, low density lipoprotein (LDL), and VLDL cholesterol levels, and to augment high density lipoprotein (HDL) cholesterol levels. A comprehensive discussion of the early clinical findings of existing fibrate drugs can be found elsewhere [20]. Fibrate drugs are generally prescribed to patients with primary hypercholesterolemia or mixed dyslipidemia and hypertriglyceridemia. PPARα agonists also possess anti-atherosclerotic and anti-inflammatory properties in experimental animal models [21]. Certain adverse effects, including increased incidence of gallstone formation and increased risk of acute kidney injury, rhabdomyolysis, and myopathy characterized by muscle pain with elevated serum creatine phosphokinase (CPK) levels, have been reported [22]. Given the indications for long-term or life-long use of fibrates during the past decade [22], continuing efforts must be made to monitor their long-term safety profiles. There is an unmet need for the development of improved therapeutic candidates that selectively target PPARα without these undesirable effects. The effects of fibrate drugs on insulin sensitivity were not clearly evident in humans, and only moderate efficacy was demonstrated for glucose control in animal models of T2DM at high fibrate doses [23, 24].

AVE8134, a novel full PPARα-dominated agonist was demonstrated to improve IR, lipid profile, and glucose metabolism in dyslipidemic mice and diabetic rats [25]. With its lipid-lowering and anti-diabetic effects in animal models, AVE8134 has the potential to improve the atherogenic lipid profile and optimize glucose homeostasis without the PPARγ-associated adverse side effects discussed below. In addition, a number of

chemicals previously not recognized as PPARα modulators were recently demonstrated to activate PPARα [26], including AP20187 (a synthetic dimerizer), benzofuran, galactosamine, the nuclear factor erythroid 2 related factor 2 (Nrf2) activator CDDO-imidazole, and the aryl hydrocarbon receptor activator 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). However, continuing efforts are required to delineate the mechanisms of action and therapeutic and/or adverse effects of these chemicals.

Over the past few decades, a growing body of evidence has demonstrated the agonistic effects of certain endogenous eicosanoids (e.g., 8-S-hydroxyeicosatetraenoic acid [8-S-HETE], leukotriene B_4 [LTB_4], 5-hydroperoxyeicosatetrenoic acid [5-HPETE], and epoxyeicosatrienoic acid) on PPARα [9, 27]. Furthermore, oleoylethanolamide (OEA) is a naturally occurring ethanolamide lipid produced by the small intestine, which is involved in peripheral regulation of feeding and body weight [28]. OEA has been shown to bind to PPARα with high affinity, inducing satiety and stimulating lipolysis [29, 30]. These studies have unraveled an unexpected role of PPARα in regulating eating behavior, raising the possibility of using selective PPARα agonists in the treatment of eating disorders. OEA was subsequently demonstrated to reduce body weight, neutral lipid content in the liver, and serum cholesterol and triglyceride levels in obese rats [31]. Interestingly, PPARα has also been shown to mediate the anti-obesity effects of oxytocin in diet-induced obese (DIO) rats via oxytocin-induced biosynthesis of OEA phospholipid precursor in adipose tissue [32]. The scalability and ease of OEA synthesis enables the study of the biological and nutritional functions of OEA in animals or human subjects with metabolic diseases.

In recent years, research efforts devoted to elucidating the effects of phytochemicals and traditional Chinese herbal medicines on PPARs increased. For example, the hypolipidemic and anti-obesogenic effects of tea have been attributed to its active compounds (i.e., epigallocatechin gallate and linalool) on PPARα [33]. In addition, we have provided evidence of the interactive roles of a combination of micronutrients comprising plant extracts and PPARα in body weight gain, hypertriglyceridemia, liver steatosis, and atherosclerosis in mice [34]. Furthermore, astragalus polysaccharides, the major bioactive component of *Astragalus propinquus,* was recently demonstrated to repress myocardial lipotoxicity and ameliorate lipotoxic cardiomyopathy in a PPARα-dependent manner *in vitro* and *in vivo*, indicative of its potential action as a novel PPARα activator whose molecular mechanism of action has not yet been elucidated [35].

Icariin, a flavonol glycoside isolated from *Epimedium*, has been recognized for its beneficial effects on MetS. Icariin treatment reduces the levels of serum total cholesterol and LDL cholesterol in rabbits fed a hypercholesterolemic diet and induces *Cyp4a14*, a PPARα-specific target gene in mouse livers [36, 37]. Icariin has also been implicated in the attenuation of the prothrombotic state in atherosclerotic rabbits [37], and in enhancing the expression of PGC-1α, PPARα, and NRF-1 during cardiomyocyte differentiation of murine embryonic stem cells *in vitro* [38]. Furthermore, icariin was recently shown to regulate the expression of PPARα target genes involved in mitochondrial and peroxisomal FAO, lipogenesis, and lipolysis [39]. Taken together, these results suggest that icariin is a novel PPARα-selective agonist and provides the molecular basis for its beneficial lipid-lowering effects. In addition to fibrates and their derivatives, the above-mentioned chemicals derived from either synthetic or naturally occurring compounds

represent alternative PPARα agonists, which may open new avenues for the development of novel therapeutic candidates that selectively target PPARα.

3. Selective PPARβ/δ Agonists

PPARβ was first discovered in *Xenopus* in 1992 [40] and was reported to be the ortholog of PPARδ discovered in mammals in 1994 [5], resulting in the alias PPARβ/δ. PPARβ/δ is expressed broadly and is particularly abundant in tissues associated with FA metabolism. Ligand binding assays revealed that unsaturated FAs, saturated FAs (much weaker), prostacyclin, 4-hydroxy-2-nonenal (4-HNE), 4-hydroxydodeca-(2E,6Z)-dienal (4-HDDE), and VLDL components are PPARβ/δ ligands [41, 42]. No PPARβ/δ compound has yet reached the market, but several have been developed and studied (Table 1). The physiological functions of PPARβ/δ in the adaptive response of skeletal muscle to increased demand for FAO first came to light with observations that its expression is markedly augmented upon fasting and exercise [43, 44]. PPARβ/δ is also crucial for the activation of FAO in skeletal muscle. Moreover, PPARβ/δ activation also ameliorates hepatic IR by reducing hepatic gluconeogenesis at the postprandial stage [45]. General knockout of PPARβ/δ in mice results in partial embryonic lethality, reduced adiposity, placental defects, growth retardation, and pronounced hypertriglyceridemia with decreased metabolic rate and glucose tolerance [46-48]. Transgenic models with skeletal muscle-specific overexpression of PPARβ/δ have shown that it promotes a myofiber type switch from glycolytic to oxidative fibers, augmenting muscle oxidative capacity and remarkably increasing exercise endurance [49]. Similar

results were obtained by expressing overactive PPARβ/δ fusion protein (PPARβ/δ-VP16) under the control of the α-actin promoter, which resulted in an average 2-fold increase in type I muscle fibers and a subsequent increase in muscle oxidative capacity, leading to a remarkable increase in running distance and time on a treadmill [50]. Notably, synthetic selective PPARβ/δ agonist GW501516 has been included since 2009 on the banned substance list of the World Anti-Doping Agency, which prohibits the use of GW501516 in athletes due to its exercise mimetic effects. In mice with selective deletion of PPARβ/δ in skeletal muscle, myocytes exhibit a switch toward fewer oxidative muscle fibers and have a reduced capacity to sustain running exercise. Furthermore, these mice develop age-dependent obesity and T2DM [51]. Further development of GW501516 for MetS was halted after preclinical studies reported that it could cause tumor development in several organs [52]. In a model of carbon tetrachloride-induced liver injury, GW501516-activated PPARβ/δ increased hepatic stellate cell proliferation in liver injuries, presumably via the p38 and c-Jun N-terminal kinase (JNK) MAPK signaling pathways, which would exacerbate fibrotic processes in injured livers [53]. In contrast, the PPARβ/δ agonist KD3010 has been shown to have hepatoprotective and antifibrotic effects when administered to mice with cholestasis- and carbon tetrachloride-induced liver injury [54]. The reason for the different effects of GW501516 and KD3010 in injured livers remains to be investigated.

In 2015, a phase II clinical trial of another PPARβ/δ-selective agonist, MBX8025, was initiated for the treatment of primary biliary cholangitis and cirrhosis. Treatment of dyslipidemic patients with MBX8025 also results in the selective depletion of pro-atherogenic dense LDL cholesterol particles, reduced plasma triglyceride levels,

decreased levels of high-sensitivity C-reactive protein (a biomarker of cardiovascular and systemic inflammation), and increased HDL [55].

In DIO mice, long-term (i.e., 11-13 weeks) PPARβ/δ activation by the agonist GW0742 mitigates a horde of metabolic conditions associated with MetS, including obesity, dyslipidemia, heart and kidney hypertrophy, IR, hypertension, vascular inflammation, endothelial dysfunction, vascular oxidative stress, and pro-inflammatory status (the latter two represent the early manifestations of atherosclerosis) [56]. These findings particularly highlight the cardioprotective role of PPARβ/δ.

In another study, the treatment of obese and diabetic *db*/*db* mice with L-165041, a selective PPARβ/δ agonist, at a relatively low dose increased plasma HDL cholesterol levels without impacting plasma glucose or triglyceride levels. L-165041 treatment in mice on alcohol was also shown to attenuate alcohol-induced hepatic IR and improve liver injury and repair [57]. A detailed review of other selective PPARβ/δ agonists was recently published elsewhere [58].

Plant-derived active compounds represent a rich and untapped resource of therapeutic candidates that selectively target PPARβ/δ. For example, *Artemisia iwayomogi*, a member of *Compositae*, has long been used for the treatment of diabetes, hepatitis, and hyperlipidemia, though the molecular mechanisms underlying its beneficial effects in metabolism were not yet understood. Recently, a 95% ethanol extract of *Artemisia* (95EEAI) was shown to directly activate PPARβ/δ and enhance FAO in skeletal muscle, thereby rendering protection against high-fat diet-induced obesity in mice [59]. In addition, a naturally occurring dimeric alkaloid isolated from *Picrasma quassioides,* picrasidine N, was identified as a novel PPARβ/δ agonist with isotype and

target gene selectivity, indicative of its potential as a lead compound for future drug development [60]. Flavonoid compounds have neurogenesis-inducing activities. One such compound, called Compound 4a (5,7-dimethoxy-8-(3-methyl-pent-2-enyl)-2-phenyl-chromen-4-one) has been shown to promote neuronal differentiation of embryonic stem cells by stimulating the expression of PPARβ/δ via a thus far unknown mechanism, which increased mitochondrial energy metabolism [61].

4. Selective PPARγ Agonists

PPARγ occurs in two isoforms, PPARγ1 and PPARγ2, that differ at their N-termini. The shorter isoform, PPARγ1, has a relatively broad expression pattern comprising immune and inflammatory cells, vascular cells, the gut, and brain. PPARγ2 is found at high levels mainly in adipose tissues, where it acts as a gene master regulator of adipocyte differentiation and lipid storage [1]. PPARγ also regulates key target genes involved in glucose and lipid metabolism, such as glucose and FA transporters and binding proteins [9]. Native ligands of PPARγ include unsaturated FAs (e.g., arachidonic acid), oxidized and nitrated FAs, 15-HETE, 9/13-HODEs, 13-oxo-ODE, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, phospholipid cyclic phosphatidic acid (CPA), and oxLDL components [62]. Generalized PPARγ-deficient mice are embryonically lethal, as PPARγ is necessary for terminal epithelial differentiation of the trophoblasts in the placenta [63]. PPARγ-null mice surviving to term were obtained using placental reconstitution and exhibited lipodystrophy, fatty liver, hemorrhage, and hypotension. These mice died during the first week of life [63].

Adipocyte-specific knockout of PPARγ results in abnormalities in the formation and function of adipose tissues. In response to high-fat diet, adipose-specific PPARγ knockout mice have diminished weight gain, do not exhibit glucose intolerance or IR, and have reduced serum levels of leptin and adiponectin [64]. Furthermore, PPARγ is necessary for the *in vivo* survival of mature adipocytes [65].

Thiazolidinediones (TZDs) are PPARγ agonists and currently the only antidiabetic drugs that function primarily by increasing insulin sensitivity and present clear benefits in glycemic control [15, 16]. TZDs also improve β-cell function and exert anti-atherogenic effects. However, clinical use of TZDs is associated with adverse effects, including body-weight gain, bone fractures, congestive heart failure, and possibly bladder cancer. How to harness the insulin-sensitizing effects of PPARγ for more effective T2DM treatment was recently discussed in depth elsewhere [15, 16].

There is a growing preponderance of evidence indicating that dietary components and plant extracts, such as dietary lipids, isoflavones, flavonoids, neolignans, amorfrutins, polyacetylenes, sesquiterpene lactones, and diterpenequinone derivatives, are PPARγ agonists [66]. For example, amorfrutin B isolated from the edible fruits of *Amorpha fruticosa* and roots of *Glycyrrhiza foetida* have been demonstrated to have strong binding affinity for PPARγ with relatively weaker binding affinity for PPARα and PPARβ/δ [67]. Amorfrutin B treatment in insulin-resistant, high-fat diet (HFD)-fed mice improved glucose tolerance, insulin sensitivity, and blood lipid profiles. Importantly, amorfrutin B, which also exhibits hepatoprotective properties, neither induces weight gain nor exhibits any adverse effects on osteoblastogenesis and fluid retention as observed in TZD treatment [67].

Monascin is a mold-fermented product of *Monascus* that acts as an antioxidant and selective PPARγ agonist. Administration of monascin in rats treated with methylglyoxal, a toxic glucose metabolite and precursor of advanced glycation end products, improves insulin sensitivity and ameliorates hyperglycemia via simultaneous induction of PPARγ-mediated anti-diabetic and Nrf2-mediated anti-oxidative stress effects through increased glyoxalase-1 expression [68].

Analysis of oxygenated polyketides isolated from the marine sponge *Plakinastrella mamillaris* has identified gracilioether B and plakilactone C as selective PPARγ ligands. Both compounds covalently bind to the PPARγ LBD and regulate PPARγ-responsive genes in the liver, repressing the production of inflammatory mediators by macrophages [69].

5. Dual PPAR Agonists

As discussed earlier, PPARα is predominantly involved in lipid metabolism and its lipid-lowering properties justify the use of PPARα agonists for the treatment of dyslipidemia, especially hypertriglyceridemia. PPARγ plays a major role in glucose metabolism and insulin sensitivity, which correlate with the clinical use of TZDs for the treatment of T2DM, though this has adverse effects for some patients. PPARβ/δ regulates FAO in key metabolic tissues and plays an essential role in inflammation, but the use of PPARβ/δ agonists has yet to be fully realized in clinical trials. Therefore, the concept of combining the therapeutic benefits of at least two PPAR isotypes for the treatment of a larger subset of conditions related to MetS is an appealing idea and led to the synthesis and characterization of current dual PPAR agonists.

Unfortunately, many of these compounds (Table 2) have been discontinued because of the lack of positive benefit-to-risk outcomes rather than a lack of therapeutic efficacy and they will not be discussed further below. However, a series of noteworthy dual- and pan-agonists (Figure 4) are briefly described and the main effects of some of them are summarized in Figure 5.

5.1. PPARα/γ Dual Agonists

Finding the right candidate with the desired potency for PPARα and PPARγ that elicits maximal beneficial effects with minimal adverse effects has been an "Everest of a task". To date, only lobeglitazone (CKD-501) and saroglitazar have successfully made it to market as dual PPARα/γ agonists for the treatment of T2DM and diabetic dyslipidemia, respectively. Lobeglitazone was approved by the Ministry of Food and Drug Safety in Korea in 2013 [70-72]; it can be taken alone or in combination with metformin. Saroglitazar launched in India in September 2013, has upheld the expectations stemming from the concept of multi-targeted therapy in MetS. Saroglitazar has also had promising therapeutic effects in diabetic dyslipidemia [73, 74]. Saroglitazar has predominant PPARα and moderate PPARγ activity [75]. Phase III clinical trials are currently underway to investigate the therapeutic effects of saroglitazar in hypertriglyceridemia and lipodystrophy. Notably, no major serious side effects have been reported and the adverse effects identified with TZDs have not been observed with saroglitazar. The above successes illustrate the increasing momentum in the development of synthetic dual PPARα/γ agonists. Here are a few notable additional examples.

The dual agonist LT175 (*S*-enantiomer of an analog of clofibric acid) exhibits insulin-sensitizing properties and reduces body weight, most likely via a reduced capability to enhance the expression of genes involved in FA esterification and storage in adipocytes. L175 also activates PPARα in the liver, resulting in FA catabolism, which promotes beneficial lipid clearing [76].

TZD18 (5-[3-[3-[4-(phenoxy)-2-propylphenoxy]propoxy]phenyl]-1,3-thiazolidine-2,4-dione) demonstrates favorable effects on lipid homeostasis and its cholesterol lowering effect is additive with simvastatin in dogs [77, 78]. Either alone or in combination with imatinib, it inhibits human chronic myeloid leukemia (CML) cell proliferation [77]. This compound also activates an endoplasmic reticulum stress response, leading to growth arrest and apoptosis in breast cancer cells [73, 74, 79].

MHY908 (2-[4-(5-chloro-2-benzothiazolyl)phenoxy]-2-methyl-propanoic acid) is a new synthetic PPARα/γ agonist that has been shown to decrease liver triglyceride levels and alleviate hyperglycemia, hypertriglyceridemia, and hyperinsulinemia in aged rats. MHY908 also attenuates IR and the inflammatory response associated with aging [80, 81]. In parallel with the effects observed with Huangkui capsule (see below), MHY908-mediated JNK activation and reduced endoplasmic reticulum stress contribute to improved insulin signaling in the liver. In the kidneys, MHY908 elicits a potent anti-inflammatory effect mediated by inhibition of the Akt/IκB kinase signaling pathway and the suppression of NF-κB activation.

Another novel synthetic PPARα/γ agonist, DSP-8658, is efficacious in reducing plasma glucose, HbA1c, and triglyceride levels while increasing pancreatic production and secretion of insulin in a *db*/*db* mouse model of diabetes [82]. Administration of DSP-

8658 in DIO KK-A(y) mice (a diabetic obesity model) reduced the subcutaneous adipose tissue weight as a result of decreased lipid accumulation in the constituent adipocytes, accompanied by reduced plasma glucose and HbA1c levels. Unlike the selective PPARγ agonist pioglitazone, DSP-8658 ameliorates blood glucose without increasing adipogenesis in these diabetic obese mice.

Osthole, an O-methylated coumarin, is an active compound isolated from the medicinal plant *Cnidium monnieri*. Treatment of rats fed a high-fat, high-sucrose diet with osthole led to up-regulated expression of PPARα/γ-mediated target genes involved in lipid and glucose metabolism in the liver, adipose tissue, and skeletal muscle [83]. Osthole treatment also reduced serum and liver lipid levels, as well as fasting blood glucose levels, thereby ameliorating liver steatosis and IR in these animals [84, 85]. These findings suggest that osthole triggers dual PPARα/γ hypoglycemic and anti-hypertriglyceridemic effects.

Isolated from *Amorpha fructicosa*, 5,7-dihydroxy-6-geranylflavanone (DGF), has also been shown to exert dual PPARα/γ activation [86]. Upon treatment of 3T3-L1 pre-adipocytes with DGF, adipogenesis was markedly stimulated to a degree comparable with the effect of selective PPARγ agonist troglitazone, whereas FAO and glucose utilization were concomitantly enhanced through dual PPARα/γ agonism. Furthermore, DGF treatment in muscle cells was found to stimulate glucose uptake by enhancing insulin sensitivity.

Huangkui capsule, a Chinese traditional medicine extracted from *Abelmoschus manihot*, has also demonstrated dual PPARα/γ-stimulating effects in a diabetic nephropathic rat model [87]. The pathological conditions observed in these rodents,

including hypertriglyceridemia, hypercholesterolemia, liver steatosis, renal inflammation, and glomerular injury, were concomitantly ameliorated upon treatment with this compound. Strikingly, the protective effects of Huangkui capsule against renal injury were partly attributed to attenuated endoplasmic reticulum stress and JNK activation in the liver and kidneys in these rats [87].

Ankaflavin, a secondary metabolite isolated from an edible fungus used in traditional Chinese medicine (*Monascus* spp.), has been demonstrated to positively regulate a number of transcription factors involved in the prevention of MetS, such as PPARα, PPARγ, and Nrf2 [88]. Interestingly, ankaflavin has glucose-lowering properties and improves pancreatic function through PPARγ activation; its beneficial effects of increasing lipid metabolism are contributed by the activation of PPARα. The compound also exhibits Nrf2-mediated antioxidant effects.

Notably, the most promising synthetic PPARα/γ dual agonists have predominant PPARα and moderate PPARγ activity, which seems to minimize the undesirable side effects of PPARγ agonism.

5.2. PPARα/(β/δ) Dual Agonists

Elafibranor (GFT505; 2-[2,6 Dimethyl-4-[3-[4-(methylthio)phenyl]-3-oxo-1(E)-propenyl]phenoxyl]-2-methylpropanoic acid), a novel synthetic liver-targeting dual PPARα/(β/δ) agonist, is currently under fast-track development in phase III clinical trials for NASH treatment. In pre-clinical studies in rats, GFT505 showed protective hepatic effects on steatosis, inflammation and fibrosis. Studies are also underway to determine the therapeutic effects of elafibranor in hepatic fibrosis, NALFD, and primary biliary

cirrhosis [89, 90]. In addition, the efficacy and safety of elafibranor in treating multiple features of T2DM have been investigated in *db*/*db* mice and cynomolgus monkeys [89]. Elafibranor treatment has been demonstrated to improve dyslipidemia and hepatic and peripheral insulin sensitivity and reduce fasting glycemia and HbA1c levels. The glucose-lowering effect of elafibranor has been ascribed to decreased hepatic gluconeogenesis. In contrast to TZDs, elafibranor treatment does not cause adverse effects on systolic and diastolic blood pressure, heart rate, and weight. Importantly, evidence from long-term elafibranor treatment in monkeys has revealed that plasma creatinine and bone marrow differential cell count are not affected; these two parameters were linked with selective PPARα and PPARγ activation by fenofibrate and rosiglitazone, respectively.

γ-Mangostin, an active xanthone derived from the pericarp of *Garcinia mangostana* (purple mangosteen), has been evaluated as a therapeutic candidate for the treatment of MetS [91]; it exhibits strong PPARα/(β/δ)-activating potency but has weak stimulatory effects on PPARγ. This compound induces *Ucp-3* expression in L6 skeletal muscle cells, which is associated with energy expenditure and dissipation as heat. Treatment with γ-mangostin also up-regulates genes involved in lipid metabolism in HepG2 cells. These findings suggest the potential use of γ-mangostin as a preventive agent in MetS.

5.3. PPAR(β /δ)/γ Dual Agonists

Many compounds, including indolylacetic acids, indole carboxylic acid derivatives, benzoic acid analogs, indanylacetic acid, and propionic acids, have been reported to be dual PPAR(β/δ)/γ agonists with varying degrees of potency on PPARβ/δ

and PPARγ [92]. To date, none of these compounds have been validated in *in vivo* models of diseases associated with MetS, though dietary punicic acid has been reported to abrogate intestinal inflammation in mice via its dual agonistic actions on PPARβ/δ and PPARγ [93].

6. Pan-PPAR Agonists

The beneficial effects observed in clinical trials with dual PPARα/γ and PPARα/(β/δ) agonists and the promising efficacy of certain selective PPARβ/δ agonists in targeting dyslipidemia and alleviating weight gain have prompted the development of singular compounds and combined treatments targeting all three PPAR isotypes (Table 3). This approach stems from the concept that pan-PPAR agonists may circumvent some of the limitations and adverse effects associated with current singular agonists, such as weight gain and cardiovascular risks [94]. The anti-diabetic drugs currently on the market, including TZDs, fail to ameliorate CVD. By combining the therapeutic benefits of all three PPAR subtypes, multiple metabolic anomalies of MetS could be treated simultaneously. For example, bezafibrate, a weak pan-PPAR agonist currently on the market, has been shown to improve hyperlipidemia and T2DM and reduce the incidence of myocardial infarction in patients with MetS. However, further development of a large number of structurally diverse pan-PPAR agonists has been terminated due to serious safety concerns (Table 3).

Only IVA337 and tetradecylthioacetic acid (TTA) have successfully progressed to phase II clinical trials for NASH, T2DM, and dyslipidemia, respectively, and chiglitazar is currently undergoing phase III clinical trial for the treatment of T2DM.

Notably, IVA337 was shown to exert synergistic anti-inflammatory and anti-fibrotic properties without interfering with wound healing in mice with bleomycin-induced dermal fibrosis [95]. The beneficial effects of IVA337 highlight its potential as a therapeutic candidate to be used in clinical trials of systemic sclerosis and NASH, a severe inflammatory and pre-fibrotic condition associated with NAFLD. In rats, TTA has antioxidant capacity, reduces inflammation, decreases plasma cholesterol levels, affects plasma fatty acid composition, hepatic lipids, and expression of relevant genes in the liver [95-97].

Telmisartan, a marketed antihypertensive drug, has been shown to induce the expression of all PPAR isotypes in white and brown adipose tissue in DIO mice [98]. In turn, telmisartan exerts (i) anti-obesity effects through the induction of sustained PPARβ/δ-mediated sympathetic activation of the β3-adrenergic receptor in white adipose tissue, (ii) PPARα-mediated activation of uncoupling protein 1 in brown adipose tissue, and (iii) non-shivering thermogenesis.

RXR ligands can activate PPAR:RXR heterodimers bound to a PPRE [7, 8] (Figure 4). CNX-013-B2, a potent and highly selective rexinoid that specifically binds and activates RXRs, has been demonstrated to induce pan-PPAR activation effects via the activated PPAR:RXR heterodimer. Not surprisingly, CNX-013-B2 also modulates the activity of other nuclear receptors, such as liver X receptor (LXR), thyroid hormone receptor (THR), and farnesoid X receptor (FXR), which all form heterodimers with RXR [99]. Importantly, CNX-013-B2 controls multiple risk factors for MetS, including IR and dyslipidemia, without the risk of hypertriglyceridemia, hepatomegaly, and body weight gain in DIO and *ob*/*ob* mice.

Interestingly, a recent study demonstrated that GW625019-induced pan-PPAR activation leads to liver enlargement accompanied by lipidomic remodeling, oxidative stress, and increased pro-inflammatory eicosanoid production [100]. This discrepancy in the hepatotoxic effects of certain pan-PPAR agonists may be explained by the differential PPAR isotypic activation profiles between CNX-013-B2 and GW625019.

Furthermore, ZBH201102 [(E)-5-(3-methoxy-5-(4-methoxystyryl) phenoxy)-2, 2-dimethylpentanoic acid] was recently constructed by incorporating the key pharmacophore of fibrates into the natural scaffold of resveratrol [101]. ZBH201102 preserves approximately one-third of the resveratrol-mediated activation of sirtuin 1 (SIRT1). PPAR binding and transactivation assays have shown that ZBH201102 directly binds and activates all three PPAR subtypes with 21-fold more efficient binding to PPARα than bezafibrate. Treatment of HFD-induced hyperlipidemic hamsters with ZBH201102 for 5 weeks ameliorates hyperlipidemia and IR without signs of liver damage.

Lyso-7, an indole-thiazolidine with both pan-PPAR agonistic and cyclooxygenase inhibitory properties, represents another interesting therapeutic candidate that may improve the cardiovascular events associated with MetS by controlling neutrophil influx and vascular inflammation [102].

Treatment of DIO mice with GW4148, a pan-PPAR agonist with a greater potency for PPARα and PPARβ/δ, results in a more sustained weight loss through the suppression of food intake and increased energy expenditure than GW9135, a pan-PPAR agonist with greater potency for PPARα than PPARβ/δ [103]. This study highlights the critical role of both PPARα and PPARβ/δ activation in the effective induction of weight loss. In view of

these findings, the recent discovery of a new class of pan-PPAR agonists known as isoflavones, which show promising efficacy, particularly towards PPARα and PPARβ/δ, may represent future leads for MetS treatment [94].

Such pan-PPAR agonists with a potent PPARα and PPARβ/δ co-stimulation profile have also been found in plants. Bavachinin, a prenylated isoflavone extracted from the fruit of the traditional Chinese glucose-lowering herb Malaytea Scurfpea, for example, exhibits synergistic glucose- and lipid-lowering effects without weight gain or hepatotoxicity in *db*/*db* and DIO mice [104].

In a study evaluating the activation of PPARs by cembranoid diterpenoids from the soft coral *Lobophytum crassum*, three of the compounds affected the transactivation of all three PPAR types in a dose-dependent manner, suggesting that these diterpenoid constituents may be used to develop products against inflammatory and metabolic diseases [105].

7. Dual Receptor-type Agonists

Interestingly, some natural agents as agonists for both PPAR and RXR (Figure 4). The vitamin A metabolite all-*trans*-retinoic acid (atRA) binds to and activates both PPARβ/δ and the retinoic acid receptors [106]. The activation of these two receptor types by the same ligand is regulated by intracellular FA transporters CRABP2 (also called CRABP-II) and FABP5. FABP5 and CRABP2 translocate to the nucleus in response to RA and interact with their corresponding receptors, PPARβ/δ and RAR, respectively, enabling the direct transfer of atRA [106, 107]. The FABP5/CRABP2 expression ratio is critical to the efficient activation of PPARβ/δ or RARs. Therefore, the relative expression

levels of the two transporters under different physiological circumstances have important functional consequences [107]. This proposition of direct PPARβ/δ activation by atRA has been challenged by data suggesting that atRA does not function as a ligand of PPARβ/δ in different assays, and that the previously reported atRA effect is due to a thus far unknown indirect mechanism [108].

The neolignans magnolol and honokiol are ligands for both PPARγ and RXR. Magnolol, which targets both RXRα and PPARγ, enhances adipocyte differentiation and glucose uptake [109] and ameliorates circulating glucose levels, preventing the development of diabetic nephropathy. Magnolol exhibits a biased agonism on PPARγ:RXRα heterodimer, instead of the RXRα:RXRα homodimer. Honokiol stimulates basal glucose uptake similar to pioglitazone but does not induce adipogenesis. The application of honokiol in diabetic KKAy mice prevents hyperglycemia and suppresses weight gain [110]. These phytochemicals are potential new leads or may be envisioned as dietary supplements for metabolic disease due to their beneficial effects in diabetic rodent models, with reduced adverse effects compared to TZD agonists [66].

8. Endocrine Disruptors

Endocrine disruptors are exogenous substances that alter the endocrine system and cause adverse health effects. These compounds can inhibit or activate members of the nuclear receptor superfamily, including PPARs. The interactions between endocrine disruptors and PPARs were recently reviewed elsewhere [111] and will not be extensively discussed here. Briefly, phthalates and perfluorinated compounds activate PPARα and PPARγ, and organotins and halogenated biophenols activate PPARγ.

9. Conclusion

Since their discovery in the early 1990s [40, 112], PPARs have been the subject of relentless investigation. The interest in PPARs was initially fueled by their role in FA β-oxidation [40]. Soon after this discovery, fibrates, which had been used clinically since the late 1960s, were found to activate PPARα. Fibrates is indicated for disorders with increased triglyceride levels. Then, the TZDs, which were reported to be insulin-sensitizing drugs in 1983 [113], were found to be ligands for PPARγ [114]. Thus, since the mid-1990s, PPARs have been studied intensively as targets for many conditions, particularly those associated with lipid and glucose metabolism [3, 15, 115]. PPAR-activating agents have sparked concerns over side effects and safer, more effective, more targeted, possibly even personalized, treatments are now being studied.

These synthetic ligands have been helpful tools for exploring a multitude of PPAR functions. One of most fascinating and challenging facets of PPAR biology is their implication in so many processes. Within the very complex web of interconnected physiological and metabolic pathways forming a coherent ensemble of integrated processes at all stages of life, it is difficult to find one in which there is no PPAR input.

The broad diversity of PPAR functions is only matched by the variety of natural compounds, including n-3 and n-6 FAs, eicosanoids, and a few endocannabinoids and phospholipids, that have been identified as PPAR ligands [62]. The distribution of these ligands in the body and the combination in which they occur in a given tissue depends on many factors, such as physiological (e.g., food, physical activity, biological rhythms) and

health-related conditions (e.g., chronic low grade inflammation, hyperlipidemia, atherosclerosis, hypertension, diabetes, cancer). This situation amplifies the difficulty analyzing the role of each as a PPAR ligand. In addition to the activator cocktail, the levels and combinations of the three PPARs in a cell nucleus, as well as those of co-activators and co-repressors, will determine the final responses that can be monitored and that modulate health and disease in the whole organism. Complex responses also depend on organ-organ crosstalk and host-microbiome interactions, which also have an impact on PPARs [13, 116]. These responses illustrate today's challenge of digging deeper into the functions of these receptors, which is necessary, not least of all to design novel interventions based on their actions and novel pharmaceutical agents to fine-tune their activity.

Since the discovery of PPARs, analytical and discovery tools have evolved considerably. Such tools can now be applied to the field, including genetic approaches to cell type-specific *in vivo* loss and gain of function (gene deletion, gene overexpression), high-resolution gene expression profiling, high-precision proteomics and metabolomics approaches.

10. Expert Opinion

Currently known natural PPAR ligands define the PPAR receptors as lipid sensors. These ligands can be food-derived and endogenously produced by different tissues in relation to physiological or pathophysiological conditions. The modulation of PPAR activities by these ligands fine-tunes lipid and glucose metabolism and multiple processes associated with cellular, organ, or whole organism energy management.

Therefore, it is not surprising that PPARs are often implicated in processes that impact health or, if deregulated, disease. Fine-tuning these processes relies on low-grade responses and work over time. Interestingly, the fibrate and TZD classes were used or identified for the treatment of risk factors associated with hyperlipidemia and the prevention of T2DM, respectively, before the discovery of the targets through which they act (i.e., PPARs).

A relatively important part of this article is devoted to phytochemicals that modulate PPAR activity, which is in line with the important notion of fine-tuning biological processes. Natural products are a rich source of ligands for nuclear receptors and promising for the development of therapeutic agents that can be used in clinical practice [117]. A potentially promising avenue may be to identify low-affinity compounds from plant or marine sources that are associated with foods. Plants in particular provide an abundant source of biologically active molecules that play critical roles in pharmacology, as many of them have demonstrated beneficial medicinal attributes. For some of the plant extracts analyzed thus far, the active compound/s has not yet been formally identified. When the active compounds have been identified, it has not always been proven that it is these compounds that directly bind to PPARs rather than derivatives thereof. It will be important in the future to analyze the molecular interactions of such agents with the PPARs in detail, including the interaction strength between ligands and residues in PPARs. In depth analysis of the molecular interactions of the same molecule with different PPAR isotypes would allow optimization of the selectivity ratio between the different isotypes. Other factors, such as cofactor affinities, are also thought to contribute to the physiological behavior of molecules. Such investigations

should allow the selection of new PPAR agonists with improved efficacy and safety profiles that would promote differential regulation of an ensemble of genes providing beneficial effects in lipid and glucose homeostasis without adverse side effects [118].

To date little is known about the relative efficiency with which synthetic (e.g. drugs) and natural (for instance phytoingredients) agonists reached their target PPAR(s) in different tissues. How are these distinct entities impacted by the microbiome and/or influence it composition; are these interactions influencing the crossing of the intestinal barrier? There is little doubt that a better knowledge of the underlying mechanisms of events occurring in the intestinal compartment before the agonists reach the body interior will help to design better pharmacological and nutritional interventions.

The development of dual PPAR agonists has met with success, with lobeglitazone and saroglitazar reaching the market in Korea and India, respectively, and others, such as elafibranor [89], reaching phase 3 studies. The underlying theme is that agents with specificity for at least two PPAR isotypes (e.g., PPARα/γ, PPARα/(β/δ), and PPAR(β/δ)/γ) or that exhibit some specificity at the tissue level would be more efficacious and have relatively fewer negative side effects than current agonists with specificity towards a single PPAR isotype. Finally, the development and availability of specific agonists targeting two or all three PPAR isoforms (dual and pan agonists) would further expand treatment options, such as for NASH, which remains one of several unmet needs in metabolic diseases.

Because of the broad cell-type expression of PPARs and the complexity of metabolic diseases, tissue-targeted delivery of agonists would represent a big advantage. This is a global challenge in drug-based interventions and occupies a large body of

researchers around the world. The hunt for efficient and specific carriers is open. Alternatively, the association of advanced chemical synthesis with deep knowledge of cell physiology may allow the shaping of molecules that accumulate in a tissue-specific manner and could be used at low concentrations to avoid unwanted side effects. Finally the still emerging but rapidly progressing unveiling of the physiological effects of natural food derived ingredients by nutrigenomics and systems biology approaches should lead to design of healthier diets shaped to have beneficial impact on PPAR activity. Combined with reasonable well conceived exercise programs this approach may lead to significant amelioration of the metabolic syndrome.

Article highlights

- PPARs are transcription factors activated by fatty acids and fatty acid derivatives with a broad range of cellular (proliferation, differentiation) and metabolic regulatory functions.
- PPARs convert lipid signaling into appropriate gene responses, influencing cellular and whole-organism energy homeostasis.
- Ligands specific to each of the three PPARs have contributed to unveiling their respective functions.
- Dual and pan agonists help explore the complementary roles of PPARs.
- Natural plant-derived molecules represent a rich source of PPAR ligands and possible lead compounds for the development of therapeutic agents.
- Dual and pan agonists are promising leads for the development of safer drugs.
- PPARs are key mediators of the health effects of nutrition and exercise.

Financial and competing interests disclosure

The authors are supported by a Start-Up Grant from the Lee Kong Chain School of Medicine, Nanyang Technological University, Singapore, and a Tier 1 Singapore Ministry of Education grant awarded to W. Wahli. W. Wahli is a consultant for Zydus/Cadila Healthcare Limited, Ahmedabad, India. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

List of Abbreviations

A

AF-1	Activation function 1
atRA	all-*trans*-retinoic acid

C

CAD	Coronary artery disease
CML	Chronic myeloid leukemia
CPA	Cyclic phosphatidic acid
CPK	Creatine phosphokinase
CVD	Cardiovascular disease

D

DBD	DNA-binding domain
DGF	5,7-dihydroxy-6-geranylflavanone
DIO	Diet-induced obesity

F

FAs	Fatty acids
FAO	FA oxidation
FXR	Farnesoid X receptor

G

GABPs	GA binding proteins
GFT505	Elafibranor
GW677954	Sodelglitazar

H

HDACs	Histone deacetylases
4-HDDE	4-hydroxydodeca-(2E,6Z)-dienal

HDL	High density lipoprotein
8-S-HETE	8-S-hydroxyeicosatetraenoic acid
HFD	High fat diet
4-HNE	4-hydroxy-2-nonenal
5-HPETE	5-hydroperoxyeicosatetrenoic acid
I	
IR	Insulin resistance
J	
JNK	c-Jun N-terminal kinase
L	
LBD	Ligand-binding domain
LDL	Low density lipoprotein
LPL	Lipoprotein lipase
LTB_4	Leukotriene B_4
LXR	Liver X receptor
M	
MC555	Netoglitazone / isaglitazone
MetS	Metabolic syndrome
MI	Myocardial infarction
N	
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
NCoR	Nuclear receptor co-repressor 1
Nrf2	Nuclear factor erythroid 2 related factor 2
NTD	N-terminal domain

O

OEA	Oleoylethanolamide

P

PGC-1α alpha	Peroxisome proliferator-activated receptor gamma co-activator 1-
PGX510	Netoglitazone / isaglitazone
PLX204	Indeglitazar
PPARs	Peroxisome proliferator-activated receptors
PPM204	Indeglitazar
PPRE	Peroxisome proliferator response element

R

RXR	Retinoid X receptor

S

SMRT	Silencing mediator for retinoid or thyroid-hormone receptors
SIRT1	Sirtuin 1
SRC-1	Steroid receptor coactivator-1

T

TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
T2DM	Type 2 diabetes mellitus
THR	Thyroid hormone receptor
TTA	Tetradecylthioacetic acid
TZDs	Thiazolidinediones

V

VLDL	Very low density lipoprotei

Table 1. Selective PPARβ/δ agonists

Selective PPARβ/δ agonist	Class	Authors	Development phase	Treatment conditions	Exp model
95EEAI	Herbaceutical	Cho et al. [59]	Pre-clinical	High-fat diet-induced obesity	In vitro In vivo
GW0742	Piperazine	Toral et al. [56]	Pre-clinical	Hypertension Vascular inflammation Oxidative stress Endothelial dysfunction DIO	In vivo
L165041	Phenyloxyacetate	Pang et al. [57]	Pre-clinical	Alcohol-induced liver insulin resistance Liver injury	In vivo
Picrasidine N	Dimeric alkaloid picrasidine	Zhao et al. [60]	Pre-clinical	ND	In vitro
MBX8025	Acetate Thiazole	Bays et al. [55]	Pre-clinical Phase II	Atherogenic dyslipidemia Primary biliary cholangitis Cirrhosis	
KD3010	Anti-obesity Anti-hyperglycemic Anti-fibrotic Anti-hyperlipidemic	Iwaisako et al. [54]	Pre-clinical Phase II, completed	Obesity NASH T2DM Liver injury Dyslipidemia	
GW501516	Thiazole Anti-hyperlipidemia	Geiger et al. [52]	Phase II, discontinued	Hyperlipidemia	

This list is not exhaustive and comprises only agents discussed in the present review.

Table 2. Dual PPAR agonists

Dual PPAR agonists	Class	Company/ Authors	Development phase	Targeted conditions	Exp model
PPARα/γ agonist					
Ankaflavin	Nrf2 activator	Hsu and Pan [88]	Pre-clinical	MetS	In vivo
DGF	Geranyl flavanone Insulin sensitizer	Lee et al. [86]	Pre-clinical	ND	In vitro
DSP8658	Anti-hyperglycemic Anti-obesity	Goto et al. [82]	Pre-clinical	Hyperglycemia Obesity	In vitro In vivo
Huangkui capsule	Flavonoid Anti-hyperlipidemia	Ge et al. [87]	Pre-clinical	Diabetic nephropathy Hyperlipidemia NAFLD	In vitro In vivo
MHY908	Propionic acid Insulin sensitizer Anti-inflammatory	Park et al. [80]	Pre-clinical	Insulin resistance Inflammation	In vitro In vivo
Osthole	Coumarin Anti-hyperglycemic Anti-hyperlipidemic	Qi et al. [83]	Pre-clinical	Insulin resistance Hyperglycemia Hypertriglyceridemia NAFLD	In vivo
LT175	Clofibric acid analog	Gilardi et al. [76]	Pre-clinical	Adipocyte culture Diet-induced insulin resistance DIO	In vitro In vivo
TZD18	Thiazolidinedione	Zang et al. [77]	Pre-clinical	Hyperglycemia Hyperlipidemia	In vitro
*AVE0847	Anti-hyperglycemic	Sanofi-Aventis	Phase II, discontinued	T2DM Lipid metabolism disorders	
*AZD6610	Anti-hyperlipidemic	AstraZeneca	Phase II, discontinued	T2DM Lipid metabolism disorders	
Cevoglitazar	Carboxylic acid Indole Oxazoles Sulfonamide	Novartis	Phase II, discontinued	T2DM Lipid metabolism disorders	
*MK0767 (KRP297)	Thiazole Thiazolidinedione Anti-hyperglycemic	Kyorin Pharma Banyu	Phase II, discontinued	T2DM Hyperlipidemia	
Muraglitazar	Oxazole Anti-hyperglycemic	Bristol-Myers Squib	Phase II, discontinued	T2DM Lipid metabolism disorders	
Naveglitazar	Phenylpropionate Anti-hyperglycemic	Eli Lily Ligand Pharma	Phase II, discontinued	T2DM	
Aleglitazar	Oxazole Propionic acid Thiophene Anti-hyperglycemic	Roche	Phase III, discontinued	T2DM Cardiovascular diseases	
Farglitazar	Oxazole Anti-hyperglycemic	GlaxoSmithKline	Phase III, discontinued	T2DM	
Imiglitazar	Anti-hyperglycemic	Takeda Pharma	Phase III, discontinued	T2DM	
Ragaglitazar	Oxazine Phenylpriopionate Anti-hyperlipidemic Anti-hyperglycemic	Dr. Reddys Research Foundation Novo Nordisk	Phase III, discontinued	T2DM	
Tesaglitazar	Alkansulfonate Phenylpriopionate Anti-hyperlipidemic	AstraZeneca	Phase III, discontinued	Insulin resistance T1/2DM Heart arrhythmia MetS	
Lobeglitazone	Anti-hyperglycemic Pyrimidine Thiazolidinedione	Kim et al. [71]	Marketed	T2DM	
Saroglitazar	Anti-hyperglycemic	Jani et al. [73, 74]	Marketed	Diabetic dyslipidemia	

	Anti-hyperlipidemic		Phase III	Hypertriglyceridemia Lipodystrophy NASH	
PPARα/(β/δ) agonist					
γ-Mangostin	Nutraceutical Xanthone	Matsuura et al. [91]	Pre-clinical	ND	In vitro
Elafibranor (GFT505)	Anti-inflammatory Anti-fibrotic Anti-hyperglycemic Anti-hyperlipidemic	Hanf et al. [89]	Pre-clinical Phase I Phase III	Hepatic fibrosis NAFLD Primary biliary cirrhosis NASH	
PPAR(β/δ)/γ agonist					
Punicic acid	Nutraceutical	Bassaganya-Riera et al. [93]	Pre-clinical	Inflammatory bowel disease	In vivo

MetS: metabolic syndrome, ND: not determined, NAFLD: non-alcoholic fatty liver disease, T1/2DM: type 1 or 2 diabetes mellitus, NASH: non-alcoholic steatohepatitis.

*Mentioned in the text but not discussed
This list of dual agonists is not exhaustive.

Table 3. Pan-PPAR agonist

Pan-PPAR agonist	Class	Company/ Authors	Development phase	Targeted conditions	Exp model
Bavachinin	Natural prenylated isoflavone	Feng et al. [104]	Pre-clinical	Hypertriglyceridemia Hyperglycemia	In vitro In vivo
CNX-013-B2	Rexinoid Insulin sensitizer Anti-hyperlipidemic	Sadasivuni et al. [99]	Pre-clinical	Insulin resistance T2DM Dyslipidemia	In vitro In vivo
GW4148	Anti-obesity Anti-hyperlipidemic Insulin sensitizer	Harrington et al. [103]	Pre-clinical	Obesity Hypertriglyceridemia Hyperinsulinemia	In vitro In vivo
GW9135	Anti-hyperlipidemic Insulin sensitizer	Harrington et al. [103]	Pre-clinical	Hypertriglyceridemia Hyperinsulinemia	In vitro In vivo
Isoflavone	Synthetic isoflavones	Matin et al. [94]	Pre-clinical	ND	In vitro
Lyso-7	Indole-thiazolidine Cyclooxygenase inhibitor	Santin et al. [102]	Pre-clinical	Vascular inflammation and damage	In vivo
*LY465608	Carboxylic acid	Eli Lilly	Pre-clinical	Insulin resistance T2DM	In vivo
*PLX134	Anti-hyperglycemic	Plexxikon	Pre-clinical	T2DM	In vivo
ZBH201102	α-alkyl-substituted aryloxyalkanoic acid Anti-hyperlipidemic	Chen et al. [101]	Pre-clinical	Insulin resistance Hyperlipidemia	In vitro In vivo
*DRL11605	Anti-hyperglycemic Anti-hyperlipidemic Anti-obesity	Perlecan Pharma	Phase I, discontinued	T2DM Dyslipidemia Obesity	
*GW625019	Anti-hyperglycemic	GlaxoSmithKline	Phase I, discontinued	MetS T2DM	
IVA337	Anti-fibrosis Anti-inflammation	Ruzehaji et al. [95]	Phase I Phase IIb	Systemic sclerosis NASH	
*Indeglitazar (PLX204, PPM204)	Carboxylic acid Indole	Plexxikon and Wyeth	Phase II, discontinued	T2DM	
*Netoglitazone/ isaglitazone (PGX510, MC555)	Thiazolidinedione Anti-hyperglycemic Anti-hyperlipidemic	Mitsubishi Pharma Perlegen Sciences	Phase II, discontinued	T2DM	
*Sipoglitazar	Carboxylic acid insulin sensitizer	Takeda Pharma	Phase II, discontinued	T2DM	
*Sodelglitazar (GW677954)	Thiazol Anti-hyperglycemic Insulin sensitizer	GlaxoSmithKline	Phase II, discontinued	Hyperlipidemia MetS T2DM	
*Tetradecylthio-acetic acid (TTA)	Anti-hyperglycemic Anti-hyperlipidemic	Haukeland University Hospital	Phase II, completed	T2DM Dyslipidemia	
*Chiglitazar	Carbazole Propionic acid Anti-hyperglycemic	Chipscreen Biosciences	Phase III	T2DM	
Bezafibrate	Benzamide Fibric acid derivative Phenylbutyrate Anti-hyperlipidemic	Boehringer Mannheim Teikyo University School of Medicine, Japan Instituto Mexicano del Seguro Social, Mexico	Marketed Phase I, completed Phase IV, completed	Hyperlipidemia Hyperglycemia Myocardial infarction	
Telmisartan	Benzimidazole Benzoate Anti-hypertensive Anti-obesity	Abbott Laboratories Astellas Pharma Boehringer Ingelheim GlaxoSmithKline Glenmark Pharma	Marketed Pre-clinical	Hypertension Obesity	

T2DM: type 2 diabetes mellitus, ND: not determined, MetS: metabolic syndrome, NASH: non-alcoholic steatohepatitis

* Compounds not discussed in this review.

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

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Figure 1. Modes of action of PPARs. A) Structural and functional domain organization of nuclear receptors. B) The antagonist-activated PPAR forms a PPAR:RXR heterodimer bound to co-repressors, which associates with histone deacetylases (HDACs) that maintain histone tails in a hypoacetylated state and repress gene expression when bound to a PPRE. C) The binding of an agonist triggers the clearance of co-repressors, which allows efficient coactivator recruitment and transcriptional activation [9]. D) SUMOylated PPARα and PPARγ repress gene expression. A model of PPARα-induced repression of *Cyp7b1* in female mice is shown. SUMOylated PPARα interacts with DNA-bound GA binding proteins (GABPs; TTCC), inducing the recruitment of NCoR, HDACs, and histone and DNA methyltransferases. These events result in histone H3 deacetylation, trimethylation, and DNA methylation at the Sp1 binding site (GGCGG). These events displace Sp1, which leads to down-regulation of *Cyp7b1* expression [12]. AF1: activation function 1, AF2: activation function 2, LBD: ligand-binding domain, NCoR: nuclear receptor corepressor, SMRT: silencing mediator for retinoid and thyroid receptors, HDACs: histone deacetylases, CBP: CREB-binding protein, SRC-1: steroid receptor coactivator 1, SUMO: small ubiquitin-like modifier protein, GABP: GA-binding protein, Dnmt: DNA methyltransferase; Sp1: specificity protein 1; H3-K9 tri-me: histone 3 lysine 9 tri-methylation.

Figure 2. PPAR ligands are natural or synthetic compounds. The levels of fatty acids and eicosanoids available depend on physiological and pathophysiological conditions, such as those listed. Ligands also depend on nutrition, comprising fatty acids and plant- and marine organism-derived compounds. Synthetic compounds, such as drugs and endocrine

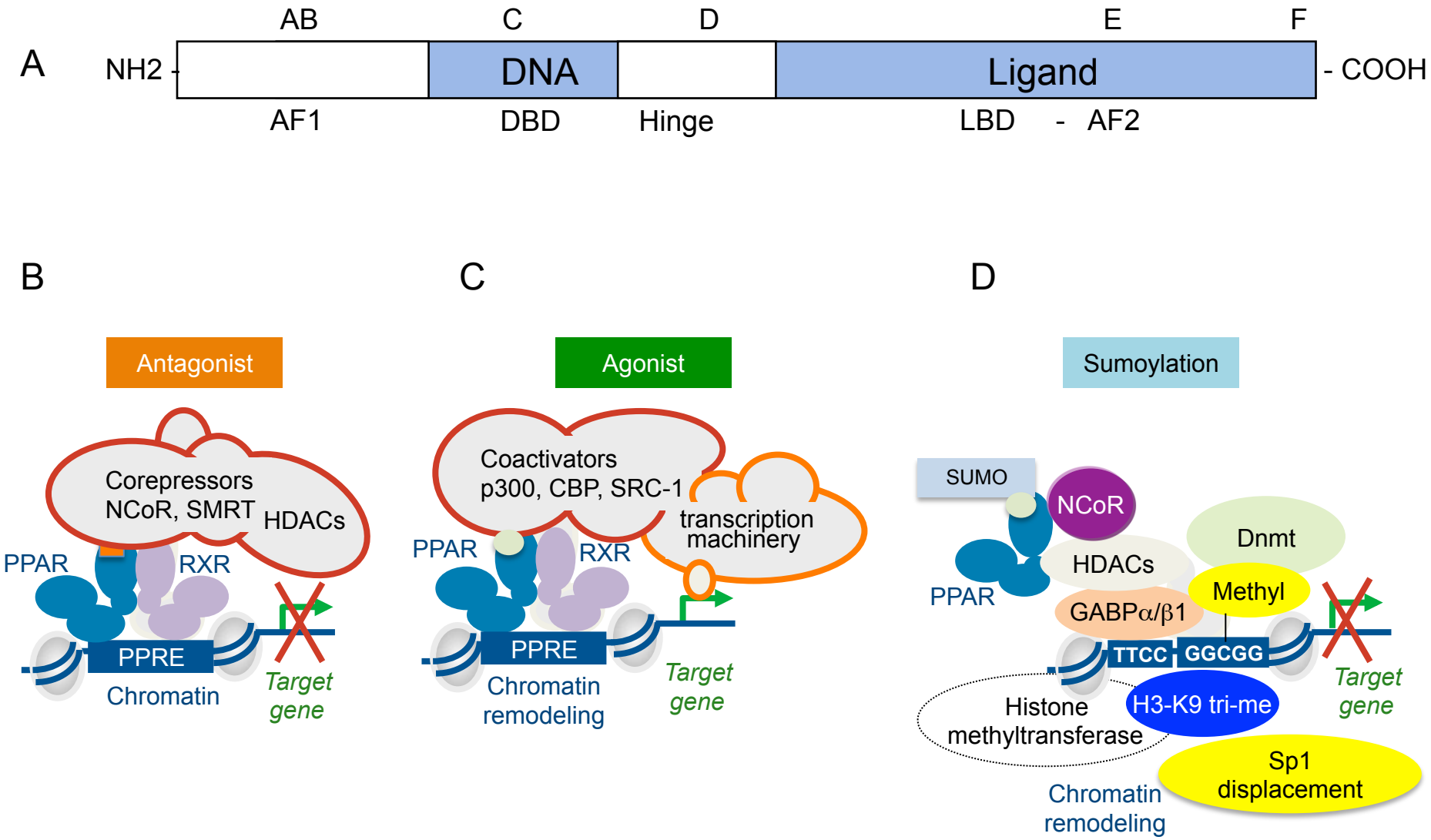
disruptors, can also activate or inhibit the transcriptional activity of PPARs. At a given time point a complex mix of ligands will be present in cell nuclei and this mix can be anticipated to change (circadian rhythm) depending on internal and external conditions. Given the variety and distribution pattern in the body of fatty acids and fatty acid derivatives with a wide range of affinity for PPARs, it has been difficult to thoroughly evaluate the contribution of each of these endogenous ligands to the biology of PPARs in a given situation. PPAR activity is also regulated by posttranslational modifications. Major intracellular signaling cascades integrate signals coming from the plasma membrane to modulate PPAR activity through phosphorylation. Phosphorylation events may influence the ubiquitination and degradation of PPARs.

Figure 3. Dual PPARα/γ agonists in metabolic syndrome. In metabolic syndrome, atherogenic risk factors combine with underlying insulin resistance. Several key features are now recognized, including hyperinsulinemia, insulin resistance, abnormal glucose metabolism (i.e., impaired glucose tolerance or diabetes), hypertension, dyslipidemia (low HDL cholesterol, high triglycerides), high ApoB, and obesity (especially visceral) with chronic low-grade inflammation. PPARα drugs (fibrates) and PPARγ drugs (thiazolidinediones) have complementary effects, with the fibrates acting mainly as hypolipidemic agents and the thiazolididiones as insulin sensitizers. Metabolic syndrome accumulates perturbations of lipid and glucose metabolism, leading to interest in developing dual PPARα/γ agonists.

Figure legends

Figure 4. Modes of PPAR activation of by agonists. Some agonists are receptor-selective binding and activating either PPARα, PPARβ/δ or PPARγ; some others can bind and activate two receptor isotypes, PPARα and β/δ, PPARα and γ, or PPARβ/δ and γ. Finally, activation of both partners of the PPAR:RXR partners is also observed.

Figure 5. Comparative presentation of the effects of dual and pan PPAR agonists. ↓, decrease; ↑, increase; =, no effect; TG, triglycerides, LDLc, low-density lipoprotein cholesterol; Tc, total cholesterol, HDLc, high density lipoprotein cholesterol; PG, plasma glucose; HbA1c, glycated haemoglobin; ALT, alanine aminotransferase; IR, insulin resistance, INS; plasma insulin; Ste, steatosis; Infl, inflammation; Adi, adipocyte/adipose tissue mass; apM1, adiponectin, BW, body weight; Spp, species; m, mouse; r, rat; ha, hamster; d, dog; mo, monkey; h, human; Ref, reference.


A
AB
C
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- COOH
AF1
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Hinge
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Antagonist
Corepressors
NCoR, SMRT
HDACs
PPAR
RXR
PPRE
Chromatin
Target gene
C
Agonist
Coactivators
p300, CBP, SRC-1
transcription machinery
PPAR
RXR
PPRE
Chromatin remodeling
Target gene
D
Sumoylation
SUMO
NCoR
HDACs
PPAR
Dnmt
Methyl
GABPα/β1
TTCC
GGCGG
Target gene
Histone methyltransferase
H3-K9 tri-me
Sp1 displacement
Chromatin remodeling

Figure 1

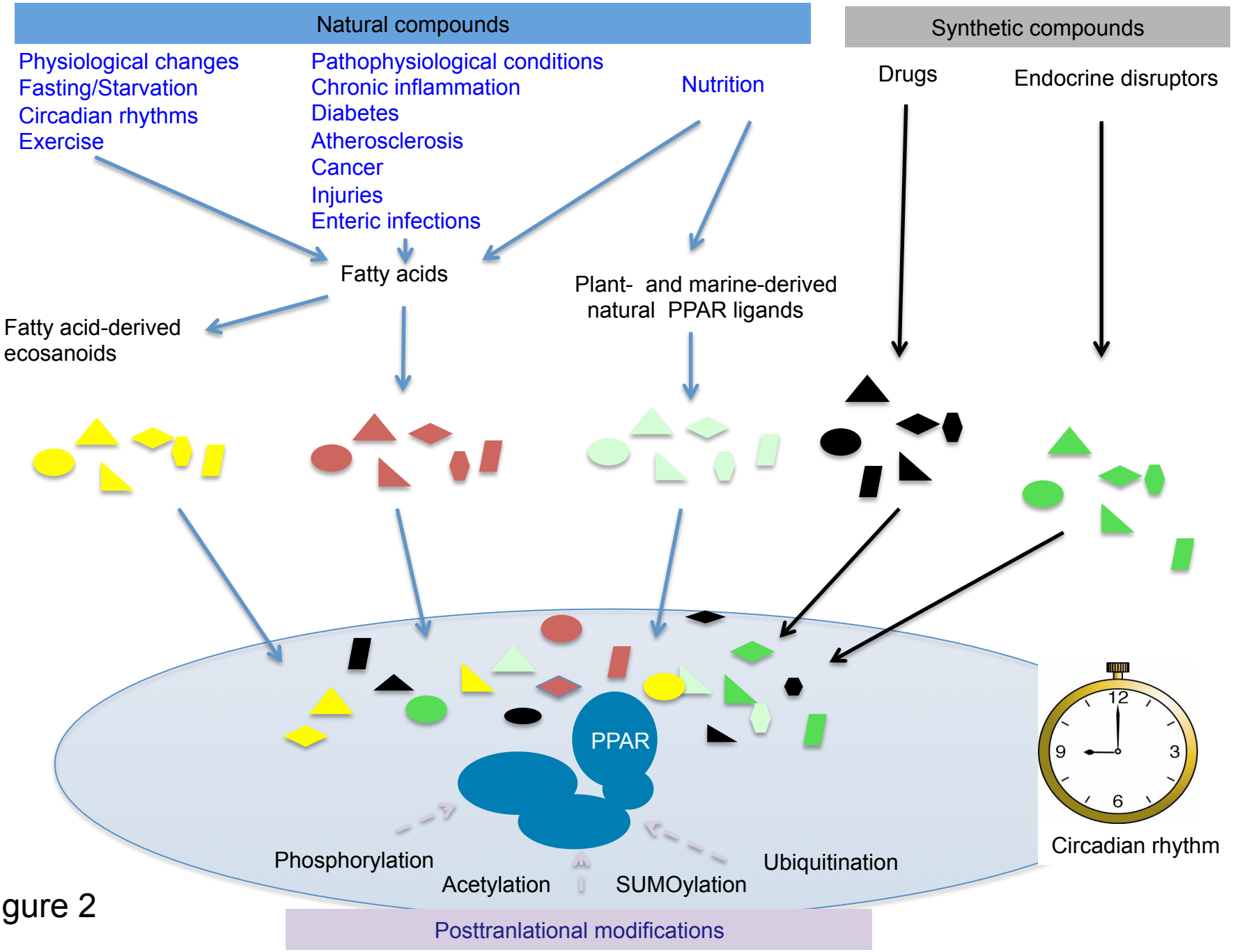
Natural compounds
Synthetic compounds
Physiological changes
Fasting/Starvation
Circadian rhythms
Exercise
Pathophysiological conditions
Chronic inflammation
Diabetes
Atherosclerosis
Cancer
Injuries
Enteric infections
Nutrition
Drugs
Endocrine disruptors
Fatty acids
Fatty acid-derived ecosanoids
Plant- and marine-derived natural PPAR ligands
PPAR
Phosphorylation
Acetylation
SUMOylation
Ubiquitination
Circadian rhythm
Posttranlational modifications

Figure 2

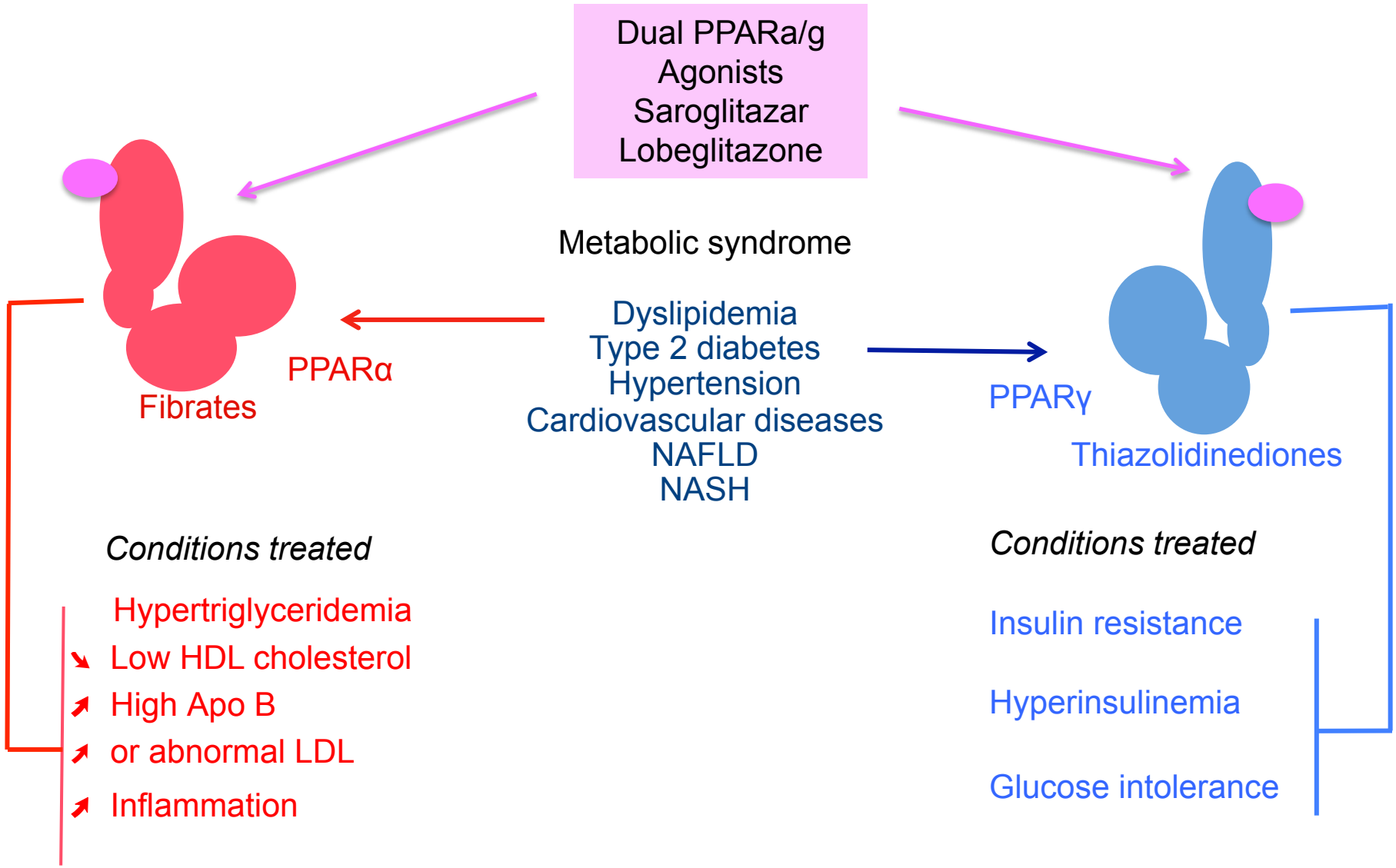
Dual PPARa/g
Agonists
Saroglitazar
Lobeglitazone
Metabolic syndrome
Dyslipidemia
Type 2 diabetes
Hypertension
Cardiovascular diseases
NAFLD
NASH
PPARα
Fibrates
PPARγ
Thiazolidinediones
Conditions treated
Hypertriglyceridemia
Low HDL cholesterol
High Apo B
or abnormal LDL
Inflammation
Conditions treated
Insulin resistance
Hyperinsulinemia
Glucose intolerance

Figure 3

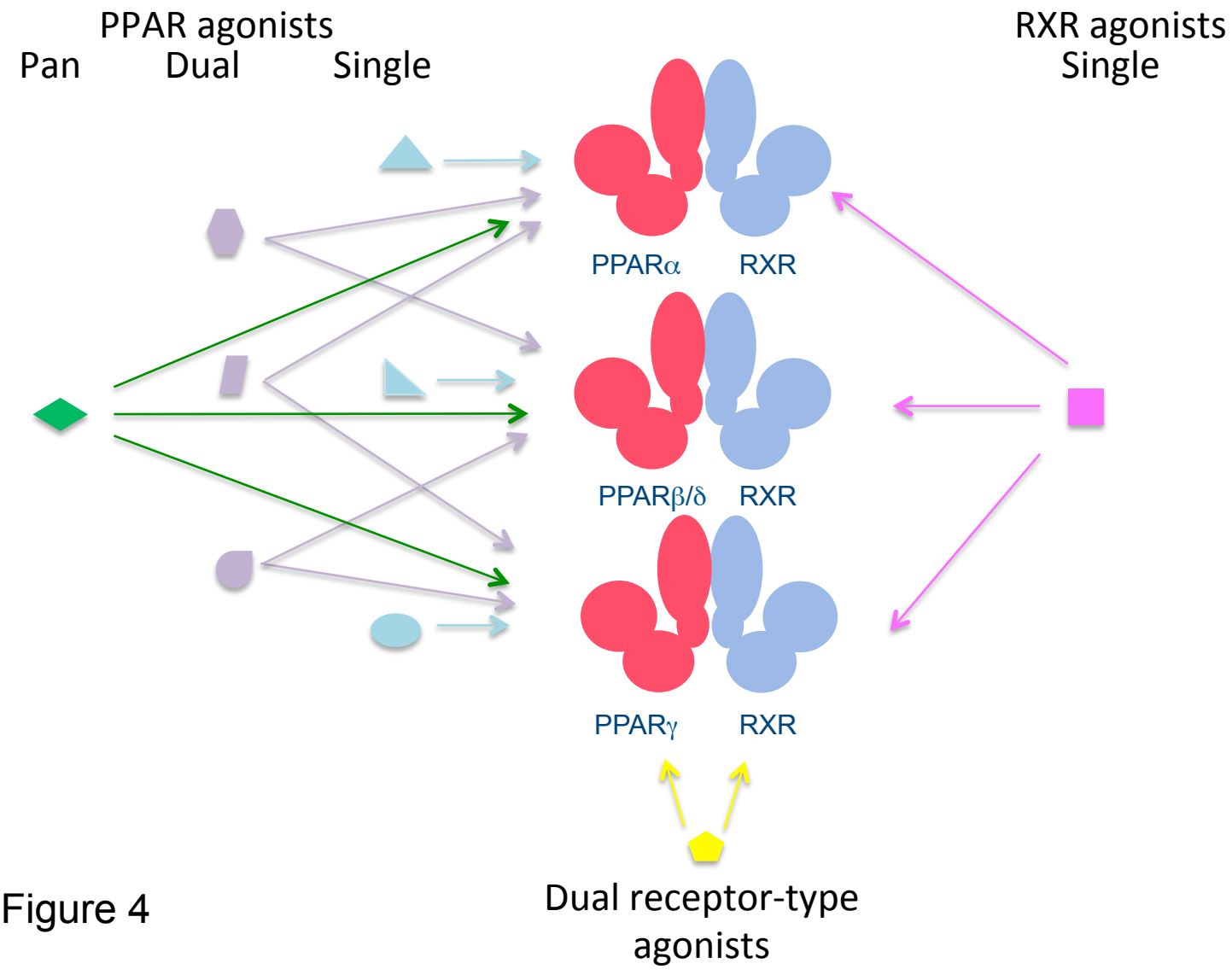
PPAR agonists
Pan
Dual
Single
RXR agonists
Single
PPARα
RXR
PPARβ/δ
RXR
PPARγ
RXR
Dual receptor-type agonists

Figure 4

Dual or Pan agonism	EFFECTS ON														Spp.	Ref
	TG	LDLc	Tc	HDLc	PG	HbA1c	ALT	IR	INS	Ste	Infl	Adi	apM1	BW		
PPARα/γ																
Saroglitazar	↓	↓	↓	↑	↓	↓	↓	↓	↓	↓			↑	↓	h,ha,m,mo,r	[73,74]
Lobeglitazone	↓	*↑	↑	↑	↓	↓			↓			↑	↑		h	[72]
LT175	↓		↓	↓	↓			↓	↓			↓	↑	↓	m	[76]
TZD18	↓	↓	↓	↓	↓										d,ha,m,r	[78]
MHY908	↓				↓			↓	↓	↓	↓	↓	↑	=	m,r	[80,81]
DSP-8658	↓				↓	↓			↑			↓			m	[82]
Osthole	↓		↓		↓			↓	↓	↓	↓		↑	↓	r	[83]
Huangkui	↓	↓	↓	↑						↓	↓		↑	↑	r	[87]
Ankaflavin	↓	↓	↓	↑	↓			↓		↓		↓			ha,m,r	[88]
PPARα/(β/δ)																
GFT505	↓	↓	↓	↑	↓	↓	↓	↓		↓	↓		=		h,m,mo,r	[89,90]
Pan-PPAR																
TTA	↓		↓								↓			↓	r	[96,97]
Telmisartan					↓			↓	↓	↓	↓	↓	↑	↓	m	[98]
ZBH201102	↓	↓	↓	↑	↓		=	↓	↓	↓		↓		↓	ha	[101]
GW4148	↓		↑	↑	↓				↓			↓		↓	m	[103]

* % of small dense LDL cholesterol is reduced

Figure 5