INTRODUCTION

1 INTRODUCTION

1.1 Type 2 diabetes mellitus (T2DM)

Type 2 diabetes mellitus (T2DM) affect a large population worldwide. T2DM is a disease often associated with a cluster of metabolic disturbances and cardiovascular disease (CVD) risk factors such as obesity, insulin resistance, hyperilpidemia, hypertension, atherosclerosis, a prothrombotic state, and endothelial dysfunction, collectively referred as the metabolic syndrome (MS).

T2DM is characterized by fasting and postprandial hyperglycemia, due to peripheral insulin resistance and impaired insulin secretion by pancreatic β-cells.[1] Insulin resistance can be defined by the fact that the insulin-sensitive tissues lose their ability to respond to insulin stimulation. In skeletal muscle and adipose tissue, insulin resistance can be defined as the inability of the cells to uptake and use glucose after insulin stimulation. T2DM is mainly associated with obesity and it is thought that the inappropriate deposition of lipids in liver and muscles contributes to reduction in an insulin sensitivity, which in turn, places a burden on the beta cell (β cell) to secrete more insulin to achieve normoglycemia. Over an extended period of time, this can result in β cell failure and diminished glycimic control. In long run, these metabolic perturbations are associated with chronic hyperglycaemia and lead to microvascular and macrovascular complications such as retinopathy, nephropathy, neuropathy and coronary heart disease. The prevalence of metabolic syndrome is projected to increase with sedentary lifestyles and high caloric diet.

Many primary and secondary preventation trials in diabetes have shown that lipid lowering therapies are often associated with CVD risk. Beyond glucose control, management of lipid metabolism is being considered in the treatment of T2DM.

1.1.1 Hyperlipidemia (Dyslipidemia):

Hyperlipidemia in type 2 diabetes is characterised by high levels of plasma triglycerides (particularly post prandially), low levels of the athero-protective high-

density lipoprotein cholesterol (HDL-C) and the appearance of small and dense low-density lipoprotein cholesterol (sdLDL-C). The prevalence of dyslipidemia in any population varies by genetic traits, dietary intake and composition, physical activity and insulin resistance.

1.1.2 Mechanisms of Lipoprotein abnormalities associated with type 2 diabetes

Diabetic dyslipidemia potentially precedes the type 2 diabetes by several years, indicating that the disturbance of lipid metabolism appears to be an early event in the development of type 2 diabetes.[2] In addition, the lipid abnormalities in type 2 diabetes are not isolated abnormalities but believed to be metabolically linked to each other.[3,4] The lipid abnormalities associated with insulin resistance do not differ substantially from those of individuals without type 2 diabetes (**Figure 1**).[3-6] In addition, triglyceride-rich lipoproteins, remnant lipoproteins, ApoB100, small and dense LDL particles have also been shown to be increased in type 2 diabetic patients.[7] In type 2 diabetic patients, small and dense LDL particles are carry less cholesterol per particle; therefore, at any given LDL-C concentration, there are more LDL particles present in an individual with the disease as compared to an individual without the disease. Small dense LDL particles contribute to damage arterial wall in patients with type 2 diabetes.[8]

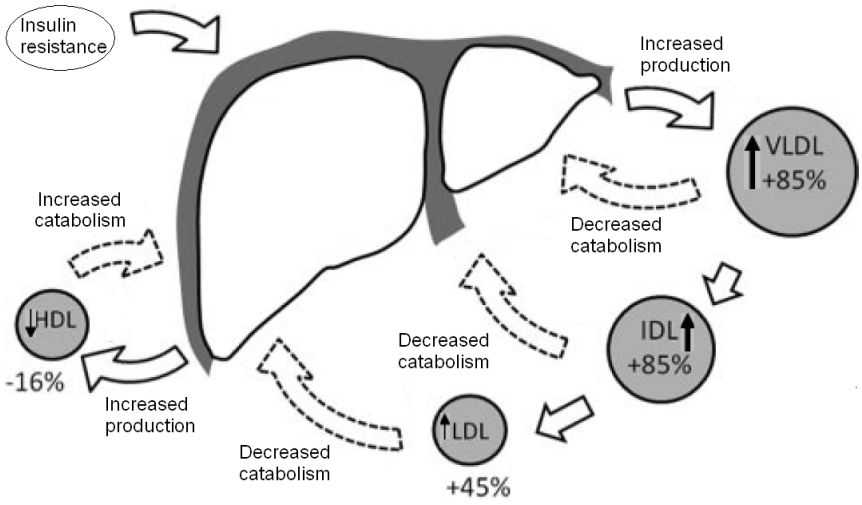


Figure 1: Changes in lipoprotein metabolism associated with type 2 diabetes mellitus and the metabolic syndrome.[9]

The association of hyperglycemia with microvascular complications in type 2 diabetes is unequivocal.[10,11] However, in diabetic dyslipidemia, cardiovascular complications may correlate more directly with the lipid abnormalties[12] and mechanistic evidence is suggests that cardiovascular risk is more lipocentric versus glucocentric in patients with type 2 diabetes.[7,13] Insulin resistance is central to the pathogenesis of lipid abnormalities in type 2 diabetes.[6] Insulin resistance is associated with increased levels of insulin in plasma and depletion of β-cells and resulting in impaired regulation of circulating lipoprotein and glucose levels.[3,13] Insulin resistance alters the metabolisim of triglyceride rich lipoprotein and increases the hepatic secretion of very low density lipoprotein (VLDL), retarded clearence of large VLDL increased the production of small and dense LDL particles and intestinally derived chylomicrons.[3,4,9,13]

Impaired action of insulin on adipocyte is believed to result in reduced suppression of intracellular hydrolysis of stored triglycerides with the greater release of non esterified fatty acids (NEFAs) into the circulation.[6] The increased influx of NEFAs delivery to the liver increases TG production and the assembly, which in turn drive the secretion of hepatic VLDL, this resulting impaired lipoprotein lipase activity and increase in the level of plasma VLDL (hypertriglyceridemia) and postprandial hyperlipidemia. Hyperlipidemia can activate thrombogenic modifications in the coagulation system. In hypertriglyceridaemic state, HDL particles tend to be small and dense, so they are more likely to undergo catabolism, so that they can reduce the levels of cardioprotective HDL-C. The reduction of HDL-C levels is accompanied by a reduction in antioxidant and antiatherogenic activities. Hypertriglyceridemia and increased CETP activity increase the level of triglyceride enriched LDL particles and the large VLDL particle convert to small and denser particles by the triglyceride lipase activity of hepatic lipase, whereas large buoyant LDL particles are cleared rapidly by LDL receptor, small and dense LDL particles are removed slowly.[14] When LDL particles become small and dense, they are more prone to oxidation, particularly in type 2 diabetes by glycation and, more readily adhere to

and subsequently invade the arterial wall, contributing to atherosclerosis; these small and dense LDL particles are therefore regarded as more atherogenic.[6]

1.1.3 Global prevalence of Type 2 diabetes

According to International Diabetes Federation (IDF), the T2DM now affects a staggering population (382 million people) worldwide, with 46% of all those affected in the 40–59 age group, 80% of them live in low- and middle-income countries. Its incidence is increasing rapidly and this number of affected people will increase by 55% (592 million) in 2035, if nothing is done. The IDF Diabetes Atlas' latest data (**Table 1**) provides a worrying indication of the future impact of diabetes as a major threat to global development. (IDF DIABETES ATLAS Sixth edition.)

Table 1. Top 10 countries for number of people with diabetes (Age 20-79 years)

Countries	2013(Millions)	2035(Millions)
China	98.4	142.7
India	65.1	109.0
USA	24.4	29.7
Brazil	11.9	19.2
Russian Federation	10.9	15.7
Mexico	8.7	14.1
Indonesia	8.5	13.1
Germany	7.6	12.8
Egypt	7.5	11.8
Japan	7.2	11.2

Type 2 Diabetes mellitus and associated cardiovascular disease imposes a large economic burden on individuals and families, national health systems, and countries. Global health is spending at least USD 548 billion (10.8% of total health expenditure worldwide) in 2013 to treat diabetes and manage complications. By 2035, this number is projected to exceed USD 627 billion.

1.1.4 Treatment and management of Type 2 diabetes

The keystone of treatment of diabetes and associated cardiovascular diseases is lifestyle modification through increased physical activity and attention to food intake. When lifestyle modifications do not result in normalization or near

to normalization of metabolic abnormalties, pharmacologic therapy is required. So far, diabetes, dyslipidemia and cardiovascular diseases (CVDs) are treated separately with specific monomodal compounds. However, the rising epidemics of type 2 diabetes is a call for a global treatment strategy to control the supplementary perturbations in glucose, lipid metabolism and global cardiovascular risk. The present strategies in the treatment of these diseases are based on the improvement of parameters used for their diagnosis (e.g., glucose in diabetes).

1.1.4.1 Hyperglycemia and insulin reisistance

Present therapeutic strategies for the treatment of diabetes are aimed at improving the hyperglycemia by increasing plasma insulin levels or decreasing insulin resistance. For that purpose, different classes of drugs are available. (**Table 2**)

Table 2. Current marketed drugs for the treatment of hyperglycemia and insulin resistance

Compounds	Action	Side effects	Example
Secretagogues (sulfonylureas, nonsulfonylureas)	Increase of insulin secretion	Hypoglycemia, hyperinsulinaemia, weight gain, vomiting	Glibenclamide, Meglitinide
Biguanides	Decrease of hepatic glucose output	Diarrhoea, vomiting, lactic acidose	Metformin
α-Glucosidase inhibitors	Inhibition of carbohydrate absorption	Flatulence, diarrhoea, abdominal pain	Acarbose miglitol
GLP1, GIP, DPP IV	Incretin effect improvement of β-cell function	Vomiting, nausea, upper respiratory tract infections	Liraglutide, CJ-1131, Exenatide, LAF-237, Sitagliptin, Linagliptin
Thiazolidinediones	PPAR-γ activation	Weight gain, edema	Pioglitazone, Rosiglitazone, Troglitazone

GLP-1: Glucagon-like peptide 1; GIP: Glucose-dependent insulinotropic polypeptide; DPP IV: Dipeptidyl peptidase IV.

1.1.4.2 Dyslipidemia

LDL-cholesterol play an important role in the cardiovascular risk factors and the aggresive lowering of LDL chloesterol is associated with a significant risk reduction of CHD death.Current lipid-modulating agents aim at reducing LDL-c. New therapeutic approaches target also low HDL levels. However, desipite the significant risk reduction, there was still a significant risk of CHD events and death in the treatement group of these studies. Lipid lowering agents include several classes of drugs that differ in mechanism of action and magnitude of lipid lowering (**Table 3**).

Table 3. Current therapy for the treatment of dyslipidemia.

Compounds	Action	Side effects	Example
Statin	Decrease of cholesterol synthesis by inhibition of HMG-CoA reductase	Myopathy, rhabdomyolysis	Pravastatin, Rosuvastatin, Simvastatin, Atorvastatin Lovastatin, Pitavastatin
CETP inhibitor	Increase of HDL level		Torcetrapib JTT-705
Niacin/nicotinic acid	Inhibition of lipolysis, decreased triglycerides, increased HDL	Hepatotoxicity, flushing	Niaspan
NPC1L1 blocker	Inhibition of intestine cholesterol uptake		Ezetimibe
Fibrate	PPAR-α activation	Myositis, myopathy, hepatitis, pancreatitis	Bezafibrate, Gemfibrozil, Fenofibrate, Clofibrate, Ciprofibrate

CETP: Cholesterol ester transfer protein; HMG-CoA: 3-Hydroxy-3-methylglutaryl coenzyme A; NPC1L1: Niemann-Pick C1-like 1.

1.2 Peroxisome proliferator activated receptors (PPARs)

Peroxisome proliferator activated receptors (PPARs) are transducer proteins belonging to the nuclear receptor super family. These receptors were identified in the 1990s in rodents and named after their property of peroxisome proliferation. PPARs control expression of various genes that are crucial for lipid, glucose and cholesterol metabolism, apparently making them ideal targets for the development of oral agents for treating metabolic syndrome.[15,16]

1.2.1 PPAR subtypes

Three distinct receptor subtypes are: PPARα (NR1C1), PPARγ (NR1C3) and PPARδ or β (NR1C2) have been identified[17] and cloned in most of the rodent and mammalian species. These three subtypes share a high level of sequence and structural homology and yet have distinct physiological functions and each PPAR subtype exhibits unique tissue expression pattern.[18]

1.2.2 Structural features of PPARs

As a member of the nuclear receptor super family, all the three PPAR subtypes share structural and functional organization, similar to that of other nuclear receptors. Four major domains have been identified, called A/B, C, D and E/F (**Figure 2**). The *N*-terminal A/B domain differs in both length and predicted amino acid sequence and contains a ligand-independent transcriptional activation function 1 (AF-1). This domain plays an important role in regulating PPAR activity through both phosphorylation and interdomain communication.[19] The DNA binding domain (DBD) or C domain promotes the binding of PPAR to the peroxisome proliferator response element (PPRE) in the promoter region of the target genes. It is a highly conserved domain containing two zinc fingers.[20] The D domain or so called “hinge domain” links DBD to a ligand-binding domain (LBD). The D site is a docking domain for cofactors. The E/F, C terminal region contains two important domains that are moderately conservered in sequence and highly conserved in structure among the various nuclear receptors. One is the LBD which is responsible for ligand specificity and activation of PPAR binding

to the PPRE, resulting in the modulation of gene expression.[21] This region also has been found to play an important role in the dimerization and nuclear localization. The other is Ligand-Dependent Activation Function 2 (AF-2), which is located in C terminal, *α*-helix 12 and is critical for both ligand binding and recruitment of PPAR cofactors.[21-23]

Figure 2 : Schematic representation of the functional domains of PPARs.

1.2.3 Protein structure of PPARs

Crystal structures of the LBD of human PPAR*α*, PPARγ and PPAR*β*/δ have been solved in complex with several ligands and have revealed a common three dimensional fold structure among LBDs. The molecules fold into a single domain that contains a bundle of 13 helices and a small four-stranded β-sheet (**Figure 3**). The ligand-binding site is a very large cavity within the protein with a total volume of 1300 to 1400 Å, which is substantially larger than those found in other nuclear receptors (NRs).[24,25] The three PPAR subtypes have a common large Y-shaped binding site and consist of an entrance extending from the surface of the protein, then branching off to two pockets: Arm I, extending toward the AF-2 helix H12; Arm II which is situated between helix H3 and the *ß*-sheet. Each arm is approximately 12 Å in length. Arm I, is the only substantially polar PPAR cavity. Arm II is mainly hydrophobic, which is not surprising given the hydrophobic nature of the natural ligands.The loop between helices H2′ and H3, situated at the entrance of the binding site, is very flexible in all PPAR structures and is even disordered in some structures. This suggests that the binding site entrance can potentially adapt, allowing large ligands to enter the binding pocket without significantly changing the overall structure of the LBD.[26,27] The amino acid residues that are in approximity to binding site form hydrogen-bonding network involving the carboxylate group of natural ligands like fatty acids and Eicosanoic acids[27,28] and carboxylic acid or thiazolidinedione (TZD) of synthetic ligands.[29-31] The key amino acid residues in the LBD of each PPAR isotype involved in the hydrogen-bonding with ligands are listed below in **Table 4**. This

conserved a network of protein–ligand interactions, that involves the surrounding amino acid residues, that help in holding the AF2-helix in the active conformation, thus promoting the binding of the co-activator proteins.[27,29] Natural and synthetic ligands are generally composed of a polar head (carboxylate function or TZD group) and a hydrophobic tail. For most agonists, the polar head exchanges hydrogen-bonds with four side chains of arm I, including a residue of the AF2-helix, as described above. The hydrophobic tail generally occupies the hydrophobic arm II, and the hydrophobic part of the entrance. Due to the large size of the binding site cavity, it has been observed for some ligands, like an Eicosanoic acid[27] or Ragaglitazar[32] that the hydrophobic tail is equilibrium between the different positions.

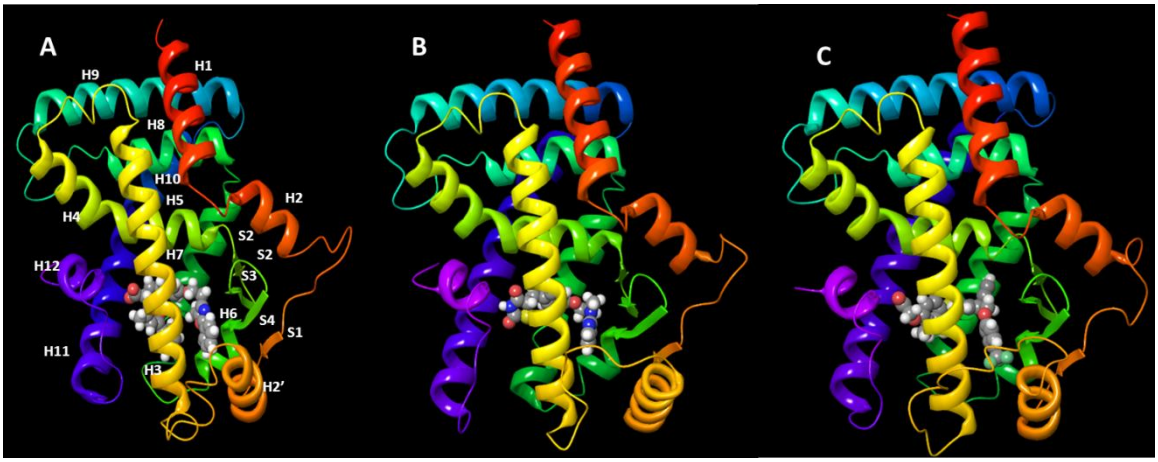


Figure 3: A. PPARα cocrystallized with GW-409544 (PDB entry no: IK7L); **B.** PPARγ cocrystallized with Rosiglitazone (PDB entry no: 2PRG); **C.** PPARδ cocrystallized with D32 (PDB entry no: 3GZ9)

1.2.4 Molecular mechanism of gene transcriptional activation of PPARs.

The gene transcription mechanism is identical in all PPAR subtypes. The process of transcription begins with the binding of ligand (endogenous or exogenous) to the PPAR receptor. Ligand-bound PPAR heterodimerises with RXR.[33] This heterodimer complex binds to peroxisome proliferator response element (PPRE) which is located in the regulatory (promoter) region of the target genes.[34] PPRE consists of direct repeat (DR)-1 elements of two hexanucleotides with the AGGTCA sequence separated by a single nucleotide spacer.[35,36] The

DR-1 pattern is specific for PPAR–RXR heterodimer, which distinguishes it from the DR-3, DR-4 patterns of other nuclear receptor responsive element patterns. Upon binding of the PPAR–RXR heterodimer complex to PPREs, and of cofactor, gene transcription of proteins involved in lipid and glucose metabolism and energy homeostasis is stimulated. Cofactors (coactivators or corepressors) are proteins which mediate the ability of nuclear receptors to initiate or suppress the transcription process. They interact with nuclear receptors in a ligand-dependent manner.[36] In the unligated state, heterodimerised nuclear receptor associates with multicomponent co-repressors containing histone deacetylase activity, such as nuclear receptor co-repressor (NCoR) and the silencing mediator for retinoid and thyroid hormone receptor (SMRT).[37,38] The deacylated state of histone inhibits the transcription.[39] Alternatively, coactivators such as steroid receptor co-activator (SRC)-1 and the PPAR binding protein (PBP) with histone acetylase activity[40,41] initiate a sequence of events, which induces the gene transcription process upon the ligand binding (**Figure 4**). This results in increase in transcription activities of various genes, involved in the diverse biological processes.

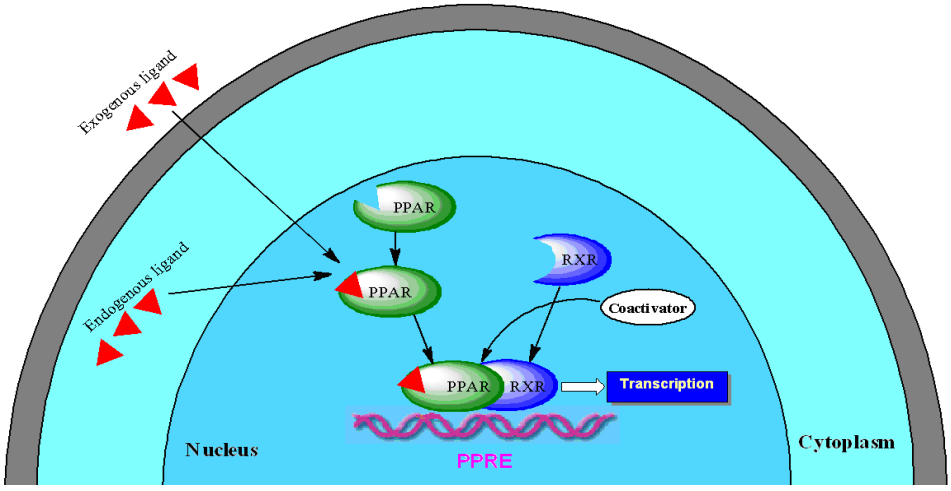


Figure 4: Molecular mechanism for transcriptional activation by PPAR isoforms

1.2.5 PPARα

The human PPARα gene has been mapped to chromosome 22q12-q13.1 by somatic cell hybridization and linkage analysis.[42] Analysis of PPARα tissue

distribution in rodents and humans has revealed high levels of expression in metabolically active tissues, such as liver, heart, kidney, muscle and brown tissue.[43,44] Although the DNA binding domains (DBDs) are identical across a variety of species, the ligand binding domains (LBDs) exhibit lower homology, which may reflect evolutionary adaptation to different dietary ligands. Comparison of human PPARα to the murine PPARα shows 85% identity at the nucleotide level and 91% identity at the amino acid level.[45] Activation of PPARα leads to the proliferation of peroxisomes and hepatomegaly in rodents.[46] However, this has not been observed in nonrodent species including human.[47] The molecular basis of this species-specific response may be due to differences in the function of PPARα in rodents and humans. There are differences in the hepatic expression of PPARα across the species. The wild-type PPARα is expressed in rodent liver at 10 times higher levels than in human liver.[48] In addition to the differences in the expression levels of PPARα, it has been also reported that the DR-1 response elements of key peroxisomal genes are not conserved between rodent and human.[49] Thus, the physiological role of PPARα as a regulator of peroxisome function appears to be restricted to rodents, and the common designation of this receptor does not reflect its biological function in humans.

1.2.5.1 Therapeutic significance of PPARα

1.2.5.1.1 Dyslipidemia

PPARα plays a crucial role in dyslipidemia as it regulates the expression of several genes involved in the several aspects of lipid and lipoprotein metabolism. PPARα upregulates the genes that are coded for fatty acid transporter protein (FATP), AcylCoA synthase, carnitine palmitoyltransferase (CPT) I and II. This results in an increased cellular uptake, intracellular esterification, and mitochondrial *β*-oxidation of fatty acids, thereby reducing plasma free fatty acids.[50-53] PPARα activation also stimulates the expression of two major apolipoproteins of HDL-C, namely ApoAI and ApoAII resulting in the

elevation of plasma HDL-C levels through reverse cholesterol transport *via* cholesterol efflux from peripheral tissues and subsequent uptake of cholesterol by the liver as well as the release of new HDL particles into the circulation. The lowering of plasma triglycerides (TG) and LDL-C by PPAR*α* activation is mediated through upregulation of lipoprotein lipase.[54] Upregulation of lipoprotein lipase also results in the lipolysis of VLDL and reduction in their production leading to an increased intracellular fatty acid oxidation and cellular fatty acid uptake.[53] All together, PPARα activation contributes to a less atherogenic lipid profile and favors a healthier lipid homeostasis (**Table 5**).

1.2.5.1.2 Inflammation and atherosclerosis

Factors other than dyslipidemia contribute to the development of atherosclerosis. PPAR*α* activation affects a range of biological processes which contribute to the etiology of coronary artery disease. For instance, expression of VCAM-I (Vascular Cell Adhesion Molecule-I), an adhesive protein which recruits monocytes to endothelial cells at sites of vascular inflammation or atherosclerotic lesions, is down regulated by PPAR*α* activation in endothelial cells.[55] This inflammatory process can be inhibited by control of proatherogenic gene transcription induced by NFκB (Nuclear Factor– kappa B).[56] PPAR-*α* activators inhibit the production of inflammatory response markers such as endothelin-1, vascular adhesion molecule-1 (VCAM-1), interleukin (IL)-6 and tissue factors in endothelial cells, smooth muscle cells and macrophages. In patients with dyslipidemia, PPAR-*α* activators reduce the plasma levels of inflammatory markers such as IL-6, fibronogen, C-reactive protein, possibly through negative regulation of NFκB and AP-1 by PPAR*α*.[57] Activation of PPAR*α* also reduces levels of serum amyloid A, plasminogen, *α*2-macroglobin, interferon-γ, IL-2, tumor necrosis factor-*α* and IL-1β. These effects of PPAR*α* activation on the vessel wall may explain their cardiovascular protective effects that extend beyond their lipid lowering effects.[58]

1.2.5.1.3 Obesity and diabetes

Obesity is a major risk factor for type 2 diabetes and cardiovascular disease. The major evidence showing the role of PPARα in obesity is the observation of increased accumulation of more body fat in PPAR null mice.[59,60] PPARα agonist fibrate treatment has been reported to reduce weight gain with no effect on food intake in rodents.[61-64] These data suggest the role of PPARα in the regulation of genes coded for uncoupling proteins (UCP-1, UCP-1 and UCP-3) involved in fatty acid catabolism or energy expenditure.[65] Uncoupling proteins (UCPs) are mitochondrial membrane transporters those allow conversion of fuel into heat by uncoupling substrate oxidation from adinosine triphosphate (ATP) synthesis.[66] UCPs are important regulators of body weight regulation and thermogenesis. Experimental results reveal that the treatment with PPARα agonist increases the levels of UCP1 in white adipose tissue (WAT) and that of UCP3 in WAT and skeletal muscle.[67] An interesting observation that the mRNA levels of UCP3 are reduced in type 2 diabetes[68], and the upregulation of this UCP3 by the activation of PPARα revels the role of PPARα in diabetes.[69]

Table 4. Summary of structure, homology and tissue expression of PPARs.

	PPARα	**PPARγ**	**PPARδ**
Tissue distribution	Brown adipose tissue, followed by liver, kidney, heart, and skeletal muscle	Highest expression in adipose tissue. Lower in skeletal muscle, spleen, heart and liver.	Maximal levels in placenta and skeletal muscle.
Length	468 amino acids	505 amino acids	441 amino acids
Molecular weight	52225 Da	576205 Da	49903 Da
Homology	93% with Mouse/Rat PPARα 84% with Human PPARδ 76% with Human PPARγ	94% with Mouse/Rat PPARγ 77% with Human PPARδ	84% Homology with Human PPARα 77% with Human PPARγ
Active site amino acids	Ser 280, Tyr 314, Tyr 464 and His 440	Ser 289, His 323, Tyr 473 and His 440.	His 449/His 323, Tyr 473 and Thr 289

1.2.6 PPARγ

PPARγ is the most extensively investigated of the three PPAR subtypes so far. The receptor has been cloned from various species, including mice[70,71] , hamsters[72], rhesus monkeys[73] and humans.[74-76] The PPARγ protein shows marked conservation across all the species examined; for example, the human protein is homologous to the murine protein, with 95% identity at the amino acid level. This high level of conservation may reflect that PPARγ functions as an essential regulator of glucose and lipid homeostasis across all the species. The human PPARγ gene has nine exons that extend over more than 100 kb of genomic DNA and has been mapped to human chromosome 3p25 by somatic cell hybridization and linkage analysis.[75,77]

1.2.6.1 Therapeutic significance of PPARγ

1.2.6.1.1 Adipogenesis

PPARγ plays a crucial role in the process of adipogenesis and insulin sensitization. Several evidence supports the critical role of PPARγ in adipogenesis *in vivo*. PPARγ +/– mice are characterized by decreased adipose tissue mass.[78] The study on wild type chimeric mice and PPARγ null cells showed that the injection of PPARγ –/– embryonic stem cells (ES) fail to contribute to adipose tissues, whereas most organs did not require PPARγ for proper development.[79] These data demonstrating that PPARγ is essential for development of adipose tissue.

These *in vivo* results are further supported by *in vitro* data showing that embryonic stem cells lacking PPARγ fail to differentiate into adipocytes after appropriate treatment, whereas embryonic stem cells expressing PPARγ readily differentiate.[79] In humans, genetic studies have further contributed to clarify the role of PPARγ in fat metabolism. Several mutations in the PPARγ gene have been described. Out of these mutations, loss-of-function and dominant negative ones are observed to be related to lipodystrophic phenotype, while gain-of-function mutations were observed to be related to increased adipogenesis and

weight gain.[80] This mutation, which inhibits phosphorylation at Ser112, resulted in permanently active PPARγ protein and led to increased adipocyte differentiation and obesity. On the other hand, a much more common Pro12Ala substitution in exon B, resulting in a less active PPARγ form was found to be associated with a lower BMI.[81] These findings provide strong evidence for the *in vivo* role of PPARγ in the control of adipogenesis. In addition to the stimulation of adipocyte differentiation, activation of PPARγ also promotes apoptosis in mature lipid-filled adipocytes. This ligand-induced apoptosis in mature cells causes the stimulation of adipogenesis from pre-adipocyte precursors, resulting in an increased number of small, relatively insulin sensitive adipocytes.[82]

1.2.6.1.2 Insulin sensitivity and dyslipidemia

PPARγ exerts antidiabetic and antidyslipidemic effects mainly through improving the insulin sensitivity by modulating the genes involved in lipid and glucose metabolism. In adipocytes, PPARγ regulates the expression of numerous genes involved in lipid metabolism, including fatty acid-binding protein aP2[83], PEPCK [84], acyl-CoA synthase[52], and lipoprotein lipase (LPL).[85] PPARγ has also been shown to control the expression of FATP-1[86] and CD36[87], both involved in lipid uptake into adipocytes. PPARγ also regulates genes those control cellular energy homeostasis. It has been shown to increase expression of the mitochondrial uncoupling proteins, UCP-1, UCP-2, and UCP-3.[88] PPARγ downregulates leptin, an adipokine that inhibits feeding and augments catabolic lipid metabolism[89,90] Activation of PPARγ in adipose tissue may impact whole-body insulin sensitivity by inhibiting the expression and / or secretion of adipocity-derived cytokines such as tumour necrosis factor α (TNFα). TNF-*α*, which blocks the insulin signalling pathway, represses GLUT4 gene expression and increases plasmatic FFA.[91-93] PPAR-*γ* neutralises TNF-*α*-induced insulin resistance by blocking the NF-κB pathway. IL-6 also reduce insulin signaling through down regulation of insulin receptor substrate-1 and AKT, an effect that can be suppressed by activated PPARγ.[94] Plasminogen activator inhibitor 1 (PAI1)

secreted by adipocytes, which promote insulin resistance is down regulated by activated PPARγ.[95] PPARγ agonists stimulate the production of adiponectin, a direct PPARγ target gene in adipocytes[96], which promotes FFA oxidation and insulin sensitivity in muscle and liver through the activation of AMP-activated protein kinase. Adipocyte-related complement protein 30 (Acrp 30) is a secreted adipocyte-specific protein that exerts *in vivo* effects including decreased glucose, triglycerides, and free fatty acids.[97,98] Patients with type 2 diabetes have reduced plasma levels of Acrp30[99] and PPARγ activation increases the plasma Acrp30 levels.[100] Overexpression of 11β-hydroxysteroid dehydrogenase (11β-HSD1), an enzyme that generates the active cortisol from the inactive precursor cortisone in adipocytes causes insulin resistance.[101] PPARγ downregulates 11β-HSD1[102,103] and promotes insulin sensitivity, either by reducing glucocorticoid induced gene expression in the adipocyte or by reducing adipocyte secretion of glucocorticoids.

PPARγ activation increases the expression and translocation (to the cell surface) of the glucose transporters GLUT1 and GLUT4, thus enhancing glucose uptake in adipocytes and muscle cells[104] and lowering plasma glucose levels. PPARγ activation also modulates the insulin signal transduction pathway by increasing the expression of intracellular proteins such as c-Cbl-associated protein (CAP)[105,106] which play a positive role in the insulin signaling pathway.[107] Expression of IRS-2, a protein with a proven role in insulin signal transduction in insulin-sensitive tissue, was also increased in cultured adipocytes and human adipose tissue incubated with PPARγ agonists.[108] PPARγ agonists modulate the endocrine activity of adipose tissue by regulating the synthesis of secreted adipocyte proteins (adipokines) that affect insulin signaling in hepatic and peripheral tissue.[109] PPARγ activation upregulates adiponectin[110] which potentiates insulin sensitivity in liver[97] and skeletal muscle.[111] PPARγ activation increases glucose oxidation by down regulating pyruvate dehydrogenase kinase 4 (PDK-4) in skeletal muscle.[112] The effects of PPARγ activation on muscle and liver involve enhanced insulin-mediated adipose tissue uptake, storage of free fatty acids[113] increased production of adipose-derived factors with potential

insulin-sensitizing activity and suppressed circulating levels of insulin resistance-causing adipose-derived factors such as TNFα or resistin.[114] These findings suggest that PPARγ activation exerts direct actions on adipose cells, with secondary effects in key insulin-responsive tissues such as skeletal muscle and liver.

1.2.6.1.3 Inflammation and atherosclerosis

PPARγ receptors are expressed in monocytes / macrophages, vascular smooth muscle cells, and endothelial cells all of which have a role in the development of atherosclerosis.[115] Activation of PPARγ induces lipid efflux from macrophages and inhibits their transformation into foam cells by upregulating the expression of the ABCA1 cholesterol transporter[116] through a transcriptional cascade that involves the oxysterol receptor, LXR-α.[117] Activation of PPARγ promotes monocyte differentiation and uptake of oxLDL through the expression of the scavenger receptor CD36.[118,119] In addition to lipid uptake through CD36, PPARγ activation down regulates class A scavenger receptor (SR-A) expression[120] and promotes cholesterol efflux from the macrophage foam cell.[117] In addition to their effect on macrophage lipid accumulation, PPARγ activation has been shown to inhibit production of proinflammatory cytokines (such as TNFα, IL-1 and IL-6)[121], the expression of the monocyte chemotactic protein 1 (MCP1/ CCL2) and inflammatory enzymes such as inducible nitric oxide synthase.[120,122] Recently it has been reported by our group that activation of PPARγ by Pioglitazone even at subtherapeutic dose produces anti-inflammatory effects *via* suppression of TNFα and IL-6 in rodents.[123] PPARγ agonists promote plaque stability by reducing the production of metalloproteinases by activated plaque macrophages.[117] Activation of PPARγ in vascular cells activates specific transcription factors that contribute to cell growth and movement[124] by inhibiting nuclear effects of MAPK signaling. PPARγ activation blocks proliferation[125,126] and increases apoptosis of VSMCs[127] and provides vasoprotection. PPARγ activation reduces the expression of adhesion molecule in endothelial cells and

prevents the formation of plaque.[128] PPARγ also exhibit antithrombotic effects by reducing the production of PAI1 (Plasminogen Activator Inhibitor 1) by endothelial cells.[129] PPARγ agonists reduce plasma levels of inflammatory biomarkers that are predictive of cardiovascular disease[130] and these decreases are observed before changes in metabolic parameters.[131] These findings support the direct vascular actions of PPARγ activation.

1.2.7 PPARδ

PPARδ has been cloned in the early 1990s from a number of species and initially given a variety of names. Human PPARδ has been mapped to chromosome 6p21.1-p21.2. PPARδ mRNA is ubiquitously expressed in adult rat tissues, but often at lower levels than either PPARα or PPARγ.[132] In humans PPARδ is present in tissues those control lipid metabolism like liver, intestine, kidney, abdominal adipose and skeletal muscle.[43]

1.2.7.1 Therapeutic significance of PPARδ

1.2.7.1.1 Dyslipidemia and diabetes

PPARδ regulates lipid and lipoprotein metabolism and its activation raises HDL-C, reduces LDL-C & VLDL-TG and normalizes insulin levels.[133] The mechanism by which PPARδ activation influences these metabolic parameters are still unknown. However, PPARδ may regulate cholesterol metabolism by promoting reverse cholesterol transport *via* the induction of ABCA1 in peripheral tissues such as skeletal muscle.[134,135] In intestine PPARδ, through inhibition of cholesterol absorption *via* the down-regulation of the cholesterol transporter NPC1-L1, was proposed to participate in the regulation of HDL-C.[136] Activated PPARδ induces the expression of genes involved in FA oxidation and in energy expenditure through the induction of uncoupling proteins (UCPs) in brown adipose tissue and in skeletal muscle.[137-139] PPARδ agonists on metabolic function are associated with an increase in adiponectin and a decrease in resistin secretion by adipose tissue.[140] Furthermore, activation of PPARδ triggers skeletal

muscle remodeling towards fibers with high FA oxidative capabilities.[137,138] In skeletal muscle, PPARδ activation induces a similar gene expression profile to that of fasting and prolonged exercise. FFAs release from adipocytes during fasting and exercise are believed to produce endogenous PPARδ ligands. The physiological role of PPARδ is believed to be in the adaptive response of muscle to switch from glucose to lipid metabolism. PPARδ activation stimulates AMPK and p3-MAPK dependent signaling pathways by which glucose uptake increases.[141] In mouse liver PPARδ was shown to reduce hepatic glucose output by increasing the glucose flux through the pentose phosphate pathway and enhancing FA synthesis. This increased hepatic fatty acid production is believed to be metabolized by the increased β-oxidation rate in muscle.[142]

Table 5. Pharmacological effects of PPARα, γ *and* δ activation.

PPARα activation	**PPARγ activation**	**PPARδ activation**
↓Plasma triglycerides (TG)	↑ Adipogenesis	↓ Plasma triglycerides (TG)
↑ Plasma HDL-C	↑ Insulin sensitivity	↑ Plasma HDL-C, ↓ Plasma LDL-C
↓ Plasma LDL-C	↑ Glucose uptake	↑ Adipogenesis, ↑ Insulin sensitivity
↑ Fatty acid uptake and oxidation in liver and muscle	↓ Plasma glucose	↑Fatty acid oxidation and uncouples energy matabolisim in skeletal muscle
↓ Weight gain	↓Plasma triglycerides (TG)	↓ Weight gain
↓Inflammatory cytokines	↓Inflammatory cytokines	↓Inflammatory cytokines

↓ denotes decrease and ↑ denotes increase

1.2.7.1.2 Inflammation and atherosclerosis

PPARδ also plays an important role in the regulation of macrophage-mediated inflammatory processes and as such exerts an anti-atherogenic function. Experiments with PPARδ knock-out mice have shown that loss of this receptor is correlated to reduced levels of the inflammatory mediators monocyte

chemoattractant protein-1 (MCP-1), IL-1β, and matrix metalloprotein-9 (MMP-9).[143] In addition, it was shown that the inflammatory suppressor protein B cell lymphoma-6 (BCL-6) forms a complex with PPAR*δ* and that it is released from PPAR*δ* upon ligand binding. Thus, activation of PPAR*δ* results in the release and activation of an important anti-inflammatory mediator.[143,144] When synthetic PPAR*δ* ligands are used in experimental animals a significant decrease in the expression of vascular wall inflammatory mediators is observed. These include MCP-1, IL-1β, TNF-*α*, interferon-γ (IFN-γ), and the adhesion molecules vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). These animal studies indicate that there is potential for the use of synthetic PPAR*δ* ligands to reduce the inflammatory events associated with atherosclerosis.

1.2.8 PPAR agonists and their biological significance

In fact the role of PPARα and γ activation in ameliorating hyperglycemia and hyperlipidemia associated with type 2 diabetes originated with two classes of compounds, the fibrates and glitazones which were empirically developed based on rodent pharmacology and eventually characterized as PPARα and γ agonists respectively much later. Thereafter, research on PPARs has unveiled new mechanisms for the regulation of lipid and carbohydrate metabolism and possible molecular determinants of metabolic syndrome. This resulted in the development of several synthetic compounds beyond fibrates and glitazones as agonists of PPARs (**Table 6**).

1.2.8.1 PPARα agonists

1.2.8.1.1 Natural ligands

PPAR*α* activated by wide range of saturated and unsaturated fatty acids, including palmitic acid, linoleic acid, arachidonic acid and oleic acid.[145] Many of the fatty acids bind to PPARα with micromolar affinities.[146] The lipoxygenase metabolite 8(S)-HETE has been identified as a high affinity ligand for PPARα (**Figure 5**).[147,148]

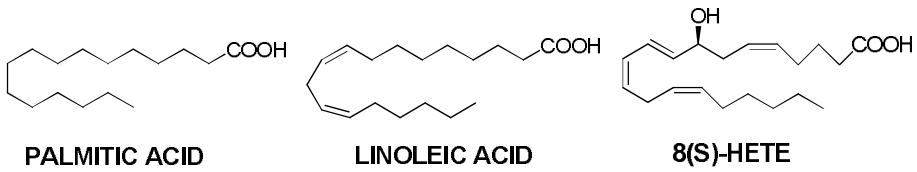


Figure 5: Structures of natural ligands of PPARα

1.2.8.1.2 Synthetic ligands (Figure 6)

Fibrates are a class of drugs which are used for several years for the treatment of dyslipidemia and they continue to remain the treatment for patients with hypertriglyceridemia. Clofibrate and Fenofibrate[45] belong to this class and were developed as hypolipidemic drugs through optimization of their lipid lowering activity in rodents before the discovery of PPARs and later proven to be agonists of PPARα. In fact the biological mechanisms of PPARα are discovered using the fibrates. However these are poor activators of PPARα and need high doses (200 - 1200 mg/day) to exert the clinical effects.[149] Therefore the need exists for the development of more potent and selective PPARα agonists in order to provide superior clinical profile for the treatment of metabolic disorders. Despite remarkable efforts from several research groups of pharmaceutical industry and academia, no potent PPARα agonist has been identified through late 1990s. GlaxoSmithKline identified uredothioisobutyric acid, GW-9578[150] as potent and selective PPARα agonist. This compound, in addition to its lipid lowering activity prevented weight gain and the development of hyperinsulinemia in insulin resistant rats. Scientist at Kyorin identified a series of potent and selective phenyl propionic acid derivatives exemplified by KCL 1998001079 and the *s*- isomer possessing the greatest activity and selectivity profile.[151,152] Merck & Co. developed a conformationally constraint 2,3-dihydrobenzofuran-2-carboxylic acid derivative (Merck compound) that displayed high potency and selectivity over other PPAR subtypes.[153] Subsequently, Lilly identified a compound LY-518674[154] containing triazolone core in the lipophilic tail part and fibric acid as the acidic head. This compound displayed potent hypolipidemic activity and good bioavailability. However, this molecule failed to display the

efficacy in humans and the development was discontinued from phase-II clinical trials.

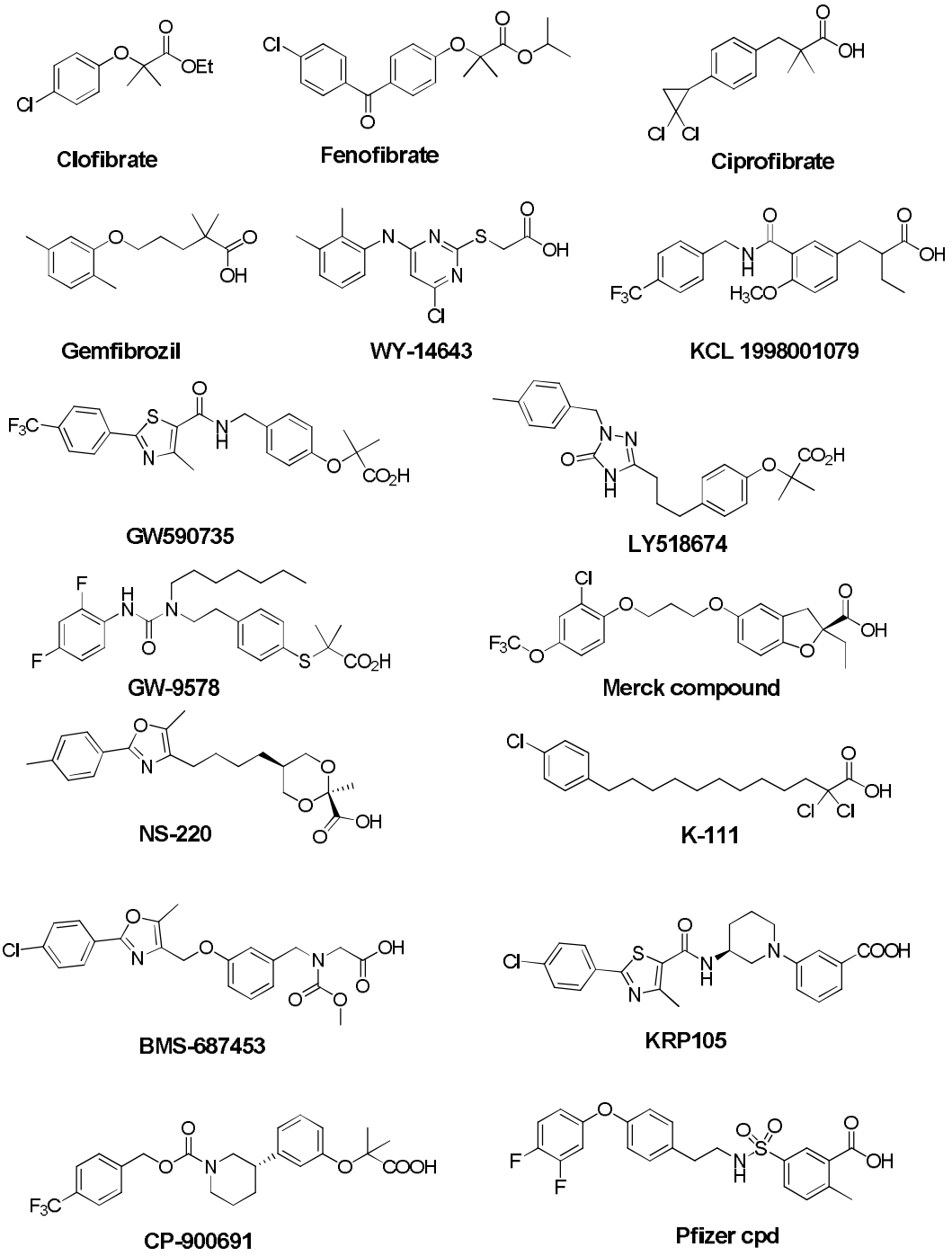


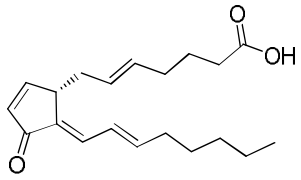
Figure 6: Chemical structures of PPARα agonists

K-111[155] is identified as highly selective PPARα agonist and does not activate γ and δ even at high doses. This compound is also discontinued in

phase-II clinical trials. More recently, Nippon Shinyaku developed a compound NS-220[156] as highly potent and selective PPARα agonist. This compound displayed potent hypolipidemic and antidiabetic effects in animal models. Unfortunately the development of this compound has been terminated from phase-II for unknown reasons. Pfizer reported pharmacokinetic, dipostion and lipid modulating activity of potent and subtype selective PPAR*α* agonist (Pfizer cpd) in preclincal species and human.[157] Scientist at Bristol-Myers Squibb reported, BMS-687453 (preclinical candidate, based on the oxybenzylglycine framework of the PPAR*α*/γ dual agonist (muraglitazar)[158] as a potent, highly selective PPAR*α* agonist with excellent pharmacological and safety profile in preclinical studies and thus was chosen as a development candidate for the treatment of atherosclerosis and dyslipidemia an excellent preclinical safety profile.[159] Further explored the followup SAR of BMS-687453, highly selective PPAR*α* modulators showed excellent anti-dyslipdemic effects *in vivo*.[160] Recently (CP-900691), with a phenylpiperidine carbamate moiety, has been reported to be a highly selective PPAR*α* agonist in T2DM monkeys, with favorable effects upon dyslipidemia as well as upon glycemic control, body weight, and inflammation.[161] More recently scientist at Kyorin Pharmaceutical reported KRP-105 (Backup compound for KRP101, a PPAR*α* selective agonist in Phase II) as a potent and high subtype-selective human PPAR*α* agonist and showed excellent PK profile and effectively lowered triglyceride and total cholesterol in high-fat diet dogs.[162] This compound currently in phase I clinical trial. Several pharmaceutical companies are in search of selective PPAR*α* agonists for the treatment of metabolic disorders, as the medical need for the treatment of these complex diseases remains highly unmet.

1.2.8.2 PPARγ agonists

1.2.8.2.1 Natural ligands



15-d-PGJ2

Figure 7: Natural ligand for PPARγ

Poly unsaturated fatty acids and eicosanoids are known to activate PPARγ endogenously. 15-Deoxy-$\Delta^{12,14}$-prostaglandin J2 (15-d-PGJ_2) is reported to be the most potent natural ligand for PPARγ.[163] (**Figure 7**) This prostaglandin has become the most widely used naturally occurring PPARγ ligand.

Table 6. Natural and synthetic ligands of PPARα, γ and β/δ.

PPAR ligands		
PPARα	**PPARγ**	**PPARδ**
Natural ligands: Unsaturated fatty acids 8-(S)-HETE **Synthetic ligands:** Fenofibrate Clofibrate Ciprofibrate Gemfibrozil KCL1998001079 WY-14643 GW9578 LY518678 NS-220 K-111 KRP-105 BMS-687453 CP-900691 KRP-105	**Natural ligands:** Poly unsaturated fatty acids Eicosanoids 15-Deoxy-delta 12,14-prostaglandin J2 **Synthetic ligands:** Rosiglitazone Pioglitizone Troglitazone Ciglitazone Englitazone Darglitazone Rivoglitazone	**Natural ligands:** Unsaturated and saturated fatty acids Hydroxyoctadecadienoic acid (HODE) ProstacyclinCarbaprostacyclin **Synthetic ligands:** GW501516 LY-165041 KD3010 MBX-8025

1.2.8.2.2 Synthetic ligands (Figure 8)

More than two decades ago, scientists at Takeda Pharmaceutical Company identified 2-chloro-3-phenylpropanoic acid ethyl ester, AL-294 by serendipity as new lipid-lowering agent. Despite the clofibrate-related structure, the compound had the ability to effectively lower plasma glucose as well as triglycerides.[164] Further efforts focused on the replacement of the acid group of AL-294 with bioisosteric heterocyles led to the discovery of the first and unique thiazolidine-2,4-dione (TZD) AL-321 with enhanced antihyperglycemic potency.[165] Subsequent optimization provided a novel, potent glucose-lowering agent Ciglitazone (ADD-3878, U-63287) that effectively improved insulin resistance and glucose disposal[166-168] but its clinical development was discontinued mainly due to insufficient efficacy. Further investigation of ciglitazone related TZDs culminated in the discovery of Pioglitazone.[169]

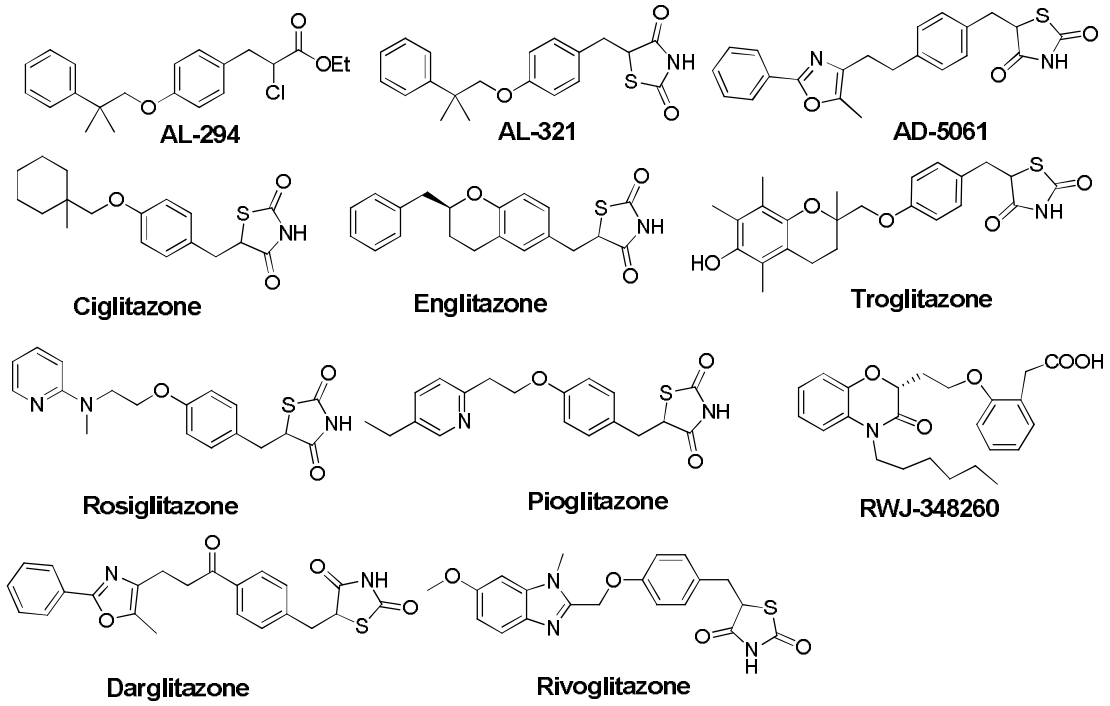


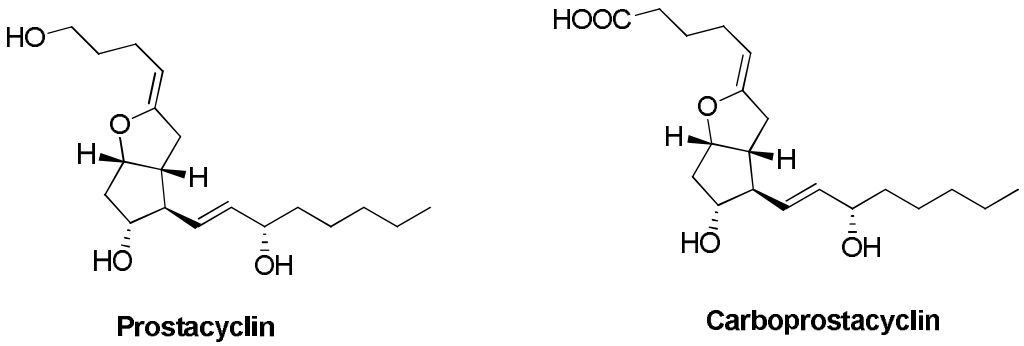
Figure 8: Chemical structures of PPARγ agonists

Moreover, further medicinal chemistry efforts led to the identification of extremely potent oxazole-containing TZDs such as AD-5061.[170] These findings

inspired the pharmaceutical industry to pursue follow-on TZDs, and Troglitazone,[171,172] Rosiglitazone[172,173] and Englitazone[174] which constitute first generation insulin sensitizers, were successfully identified by Daiichi-Sankyo (formerly Sankyo), GlaxoSmithKline and Pfizer respectively. Troglitazone (Rezulin) was the first of the TZD class of oral anti-diabetic agents to be launched in the United States in 1997; however the agent was subsequently withdrawn from the market due to idiosyncratic hepatotoxicity. Two other TZDs, Pioglitazone (Actos) and Rosiglitazone (Avandia), which have achieved blockbuster status, are currently marketed for the treatment of type 2 diabetes.[175] The two drugs potentiate insulin sensitivity in muscle, liver, and adipose tissue, leading to effective normalization of elevated plasma glucose levels and concomitantly reducing HbA1c.[176-179] However these drugs were not devoid of side effects. Treatment with these drugs causes weight gain and edema. In Sept 2010, rosiglitazone suspeneded from European market due to potential cardiovascular risks. However patients, currently taking rosiglitazone are advised to take alternative options. Several selective PPARγ agonists including TZD and non-TZD derived molecules are currently under investigation for the treatment of type 2 diabetes. The TZD based insulin sensitizer, Rivoglitazone (CS-011)[180], which advanced to Phase III clinical trials, has proven to be more potent and efficacious than Rosiglitazone in several aspects but administration of the agent at a therapeutic dose led to an increase in body weight. Medicinal chemistry efforts have been made thereafter towards identifying non-TZD PPARγ agonists. RWJ-348260[181] has been disclosed as a novel non-TZD PPARγ agonist (**Figure 8**). Recently Takeda Scientist described novel benzylpyrazole acylsulfonamides as non-TZD, non-carboxylic-acid-based PPARγ agonists. Potent PPARγ agonists showed significant antidiabetic activity with high metabolic stabilty.[182]

1.2.8.3 PPARβ/δ agonists

1.2.8.3.1 Natural ligand



Prostacyclin **Carboprostacyclin**

Figure 9: Natural PPAR*β*/*δ* ligand

PPAR*β*/*δ* is also activated by unsaturated and saturated long-chain fatty acids (LCFA), EPA, arachidonic acid, and a number of eicosanoids, HODE (hydroxyoctadecadienoic acid), and prostacyclin (PGI2). The semisynthetic prostacyclin, carbaprostacyclin was reported to be the most potent PPAR*β*/*δ* natural ligand (**Figure 9**).[183]

1.2.8.3.2 Synthetic ligand

Many research groups tried to prepare selective ligands to study biological functions and possible applications to the human therapy and several pharmacological agonist of PPAR*β*/*δ* identified. But there are no marketed PPARδ agonists mainly due to the lack of selective ligands to aid in study of biological mechanisms. Phenylacetic acid analog L-165041 was reported to be the most potent selective PPARδ ligand in a series identified by researcher at Merck, with high affinity for human PPARδ and 10 fold selectivity.[184] The thio substituted phenoxyacetic acid GW-501516[133,185] is the first PPARδ agonists which exhibited nanomolar potency and 1,000 fold selectivity over other subtypes. GW-501516 (**Figure 10**) advanced to phase II clinical trials but the further development of this compound has been differred for unknown reasons. Bayer identified compound (Bayer compound) with rigid linker who possesses a high degree of affinity as well as selectivity towards PPARδ.[186]

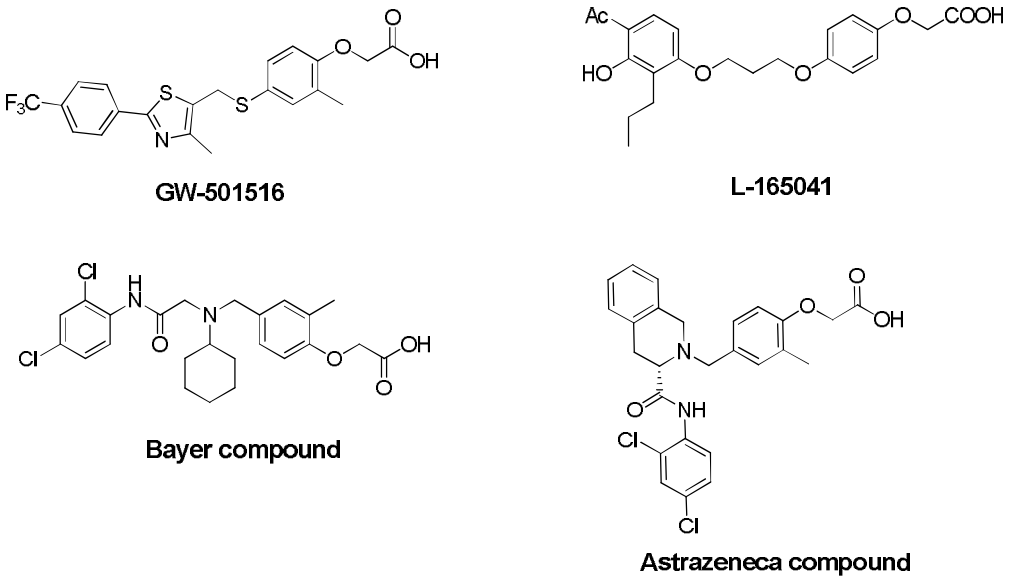


Figure 10: PPARδ agonist

Scientist at Kalypsis identified KD3010 is a potent and selective PPARδ agonist that demonstrates activity in rodent and nonhuman primate pharmacology models of dyslipidemia, diabetes, and obesity**.** This is currently in phase II clinical trial.[187] Metabolex developing the PPAR*δ* agonist MBX-8025 (RWJ800025, currently in Phase II) for the treatement of cardiovascular risk factors associated with mixed dyslipdemia and also improve the insulin resistance that is linked to obesity and other metabolic disorder.[188] Recently Astrazeneca describes the discovery of isoindoline and tetrahydroisoquinoline derivatives as hPPAR*δ* agonists that displayed excellent selectivity over the PPAR*α* and PPARγ subtypes. Astrazeneca compound demonstrated efficacy in upregulation of PDK4 in human primary myotubes, a biomarker for increased fatty acid oxidation.[189] Thereafter many research groups around the globe have been involved in developing selective PPARδ agonists.

1.2.9 Novel therapeutic approaches

PPAR agonist improves T2DM dyslipdemia and atherosclerosis, but their side effects remain important. Combination therapy with agonist with properties of the different PPAR isotypes may increase their beneficial effect compared to single use for both diseases. Several pharmaceutical companies developed

PPARs dual and pan agonists, which are able to activate two or all PPARs (**Table7**). The development of partial agonists should lead to selective regulation of genes, which have beneficial effects on T2DM or atherosclerosis, avoiding the activation of those genes provoking side effects.

1.2.9.1 PPARα/γ dual agonists (Figure 11).

The concept of PPARα/γ dual and pan (PPAR$\alpha/\gamma/\delta$) agonists stems from the fact that activation of different PPAR subtypes leads to a broad spectrum of metabolic effects that may be complementary. Moreover, a recent study has demonstrated that combination therapy of the PPARα and PPARγ agonists, Rosiglitazone and Fenofibrate, results in normalization of triglyceride and total cholesterol levels without increasing body mass index in type 2 diabetic patients.[190] An importance of controlling both glucose and lipid levels in metabolic syndrome gave rise to the concept of identifying dual agonists, which can activate both PPARα and PPARγ. In addition to their hypolipidemic effects, fibrates reduce body weight gain in rodents without affecting food intake. To date, a large number of structurally diverse PPARα/γ dual agonists have been disclosed in the literature and in patent applications.

The first PPARα/γ dual agonist to be reported was KRP-297 (MK-0767).[191] Results from a Phase I clinical trial showed that the new TZD derivative was well tolerated and effective at normalizing hyperglycemia and hyperlipidemia and reduce FFAs and lipids in healthy subjects. However, further development was discontinued due to toxicity. Muraglitazar (BMS-298585) is the first PPARα/γ dual agonist reviewed by the FDA advisory committee. This non-TZD oxybenzylglycine analogue is reported to exhibit potent *in vitro* activities against both PPARα and PPARγ subtypes and exert excellent glucose and lipid lowering effects in rodent models.[158] A longer study of 24-104 weeks resulted in a significantly increased risk in the composite end point of death, myocardial infarction, stroke, transient ischemic attack, and congestive heart failure[192], leading to termination of further development of Muraglitazar. Like KRP-297 and Muraglitazar, the discontinuation of the clinical development of other PPARα/γ

dual agonists, including Tesaglitazar (AZ- 242)[193], Ragaglitazar (DRF-2725)[194] Naveglitazar[195], Farglitazar (GI262570)[196], Imiglitazar (TAK-559)[197] and Naveglitazar (LY-818)[195] due to various toxicological reasons or a risk-benefit assessment has been disappointing.

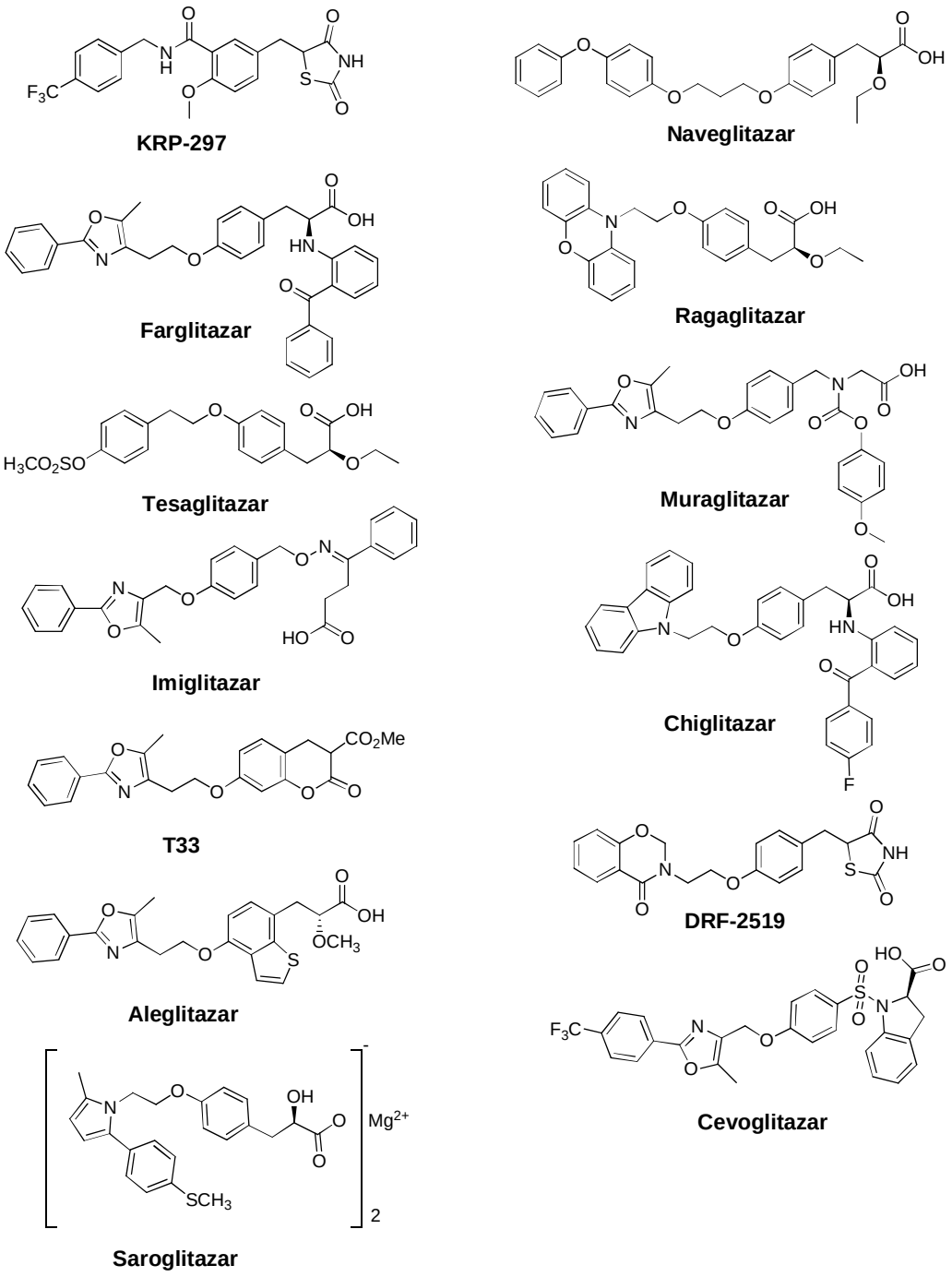


Figure 11: Chemical structures of PPARα/γ dual agonists

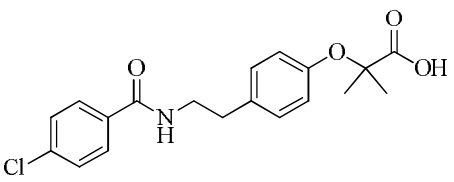
There are only a few PPARα/γ dual agonists left in the development pipelines. Shenzhen chipscreen biosciences is investigating a tyrosine-based PPARα/γ dual agonist Chiglitazar (CS038)[198], which is undergoing Phase II clinical trials. Based on the U-shaped pharmacophore model designed from the binding mode of PPARα and PPARγ to the dual agonist Tesaglitazar, a series of 2-alkoxydihydrocinnamates were synthesized as potent PPARα/γ dual agonists.[199] α-Aryloxyphenylacetic acids have also been reported to be PPARα/γ dual agonists, but preferentially activate PPARα with weak or partial PPARγ activating potency. Cevoglitazar (LBM-642)[200,201], a novel dual agonist identified by Novartis, has been reported to provide improvement in glucose tolerance similar to Pioglitazone and demonstrate superior reductions in total hepatic lipids. Cevoglitazar has discontinued in phase IIa and the reason for this decision was not announced.[202] Dr. Reddy's Laboratories has been investigating a benzoxazinone-based TZD, DRF-2519 that is classified as a PPARα/γ dual agonist for the management of metabolic disorders. Exposure of Zucker fatty rats to DRF-2519 resulted in more effective reduction in plasma insulin, triglycerides, and FFAs than Rosigllitazone.[203] Recently T33 is reported as a novel PPARα/γ dual agonist with insulin sensitizing and hypolipidemic effect.[204] Saroglitazar, a dual PPARα/γ agonist developed by Zydus Cadila has been shown to exert anti-dyslipidemic and insulin sensitization effects in animal models and favorable safety and pharmacokinetic profile in humans[205] Simillarly, Roche is developing Aleglitazar, R1439 (Phase-III), as a PPARα/γ dual agonist and it is very effective to improve peripheral insulin sensitivity and glycemic control and also improved management of dyslipdemia.[206,207] But recently Roche has terminated development of Aleglitazar due to safety concerns and lack of efficacy in phase III clinical trial. Very few PPARα/γ agonists are presently advancing through different stages of clinical studies. Most of the terminated PPAR ligands shared some undesirable adverse events but the reason for discontinuation of development was, in many cases, claimed to be compound specific. Consequently, it is difficult to ascertain whether the toxicological side effects

which motivated their discontinuation was due to the activation of PPARα, PPARγ, or both (class effect), or due to a PPAR unrelated or chemical structure specific effect.[208-210] Since the basis for the observed safety liabilities and the relevance of rodent toxicities to the human situation are still unknown, there seems to be significant opportunities for successful development of this class.[211]

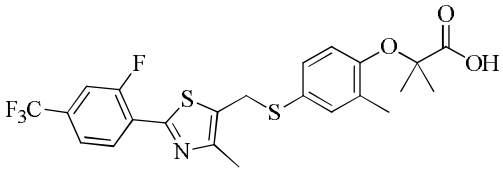
1.2.9.2 PPARα/γ/δ agonists (Pan Agonist)

The PPAR pan agonists can activate all three PPAR subtypes, and they can potentially exert various effects on metabolic disorders such as insulin resistance, obesity, dyslipidemia and hypertension. They are believed to be more effective than dual PPAR agonists.

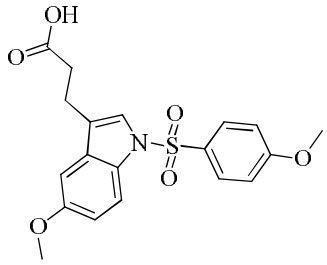
Bezafibrate (**Figure 12**) is the first and still the only pan-PPAR agonist used therapeutically. It activates all three PPARs with the same effectiveness. Bezafibrate raises HDL-C and reduces TGs, improves insulin sensitivity and reduces blood glucose.[212] Bezafibrate thus validates the proof of concept of a therapeutic approach via pan-PPAR activation in the treatment of metabolic disturbances associated with T2DM. Besides all these beneficial points, bezafibrate's weakness lies in its low potency in activating PPARs. The development of more powerful molecules is thus needed, but whether this is possible without increasing side effects remains unknown and further investigations are necessary. Sodelglitazar (GW677954), a novel PPAR pan agonist, is being investigated in Phase II trials for the treatment of metabolic disorders.[213] Indeglitazar (PLX-204), from plexxikon in phase- II and GW-625019 from Glaxosmithkline is also progressing in Phase I trials for the treatment of metabolic disorders. In addition, LY-465608, DRF-11605, CS-204, and DRL-11605 are under investigation, and may be potent therapeutic agents for the treatment of metabolic disorders.[208]



Bezafibrate



Sodelglitazar (GW677954)



Indeglitazar (PLX-204)

Figure 12: pan PPAR agonists

1.2.9.3 Partial agonist

To avoid adverse effects, new chemical class of partial agonist has been designed displaying different binding properties to the PPARs compared with full agonist. Partial agonists are defined as weak activators of PPARs that elicit the same activation pattern and show linked dose−response curves but with lower maximal activity compared to full agonists. They might induce alternative receptor conformation and thus recruit different coactivators, resulting in distinct transcriptional effects compared to full agonists

1.2.9.3.1 PPARγ partial agonist

Scientists at Dr. Reddy's research foundation have discovered Balaglitazone (DRF2593), a novel TZD derivative and found to be a selective PPARγ partial agonist. (**Figure 13**) Studies have shown that treatment with Balaglitazone leads to significant improvement in glycemic control and the HDL-C level, with minimum side effects. Balaglitazone impose less volume expansion and compensatory cardiac hypertrophy when compared to Rosiglitazone in chronic studies. Further lesser haemodilution with equi-efficacious doses of Balaglitazone when compared to both Pioglitazone and Rosiglitazone has been observed in Phase-III clinical trials.[214] Another interesting compound in this

category is PAT5A, selectively modulates PPARγ activity. PAT5A inhibits cholesterol and fatty acid biosynthesis, suggesting that it possesses a unique receptor independent non-PPAR related property. As expected, administration of PAT5A in a rodent model of type 2 diabetes (*db*/*db* mice) resulted in a dose-dependent reduction in plasma glucose with lower drug-induced weight gain.[215] Netoglitazone[216] and NC-2100[217] these structurally related glitazones appeared to be weakly binding full agonists in cell-based reporter assays. They possess *in vivo* activities in obese insulin-resistant mice comparable to rosiglitazone despite their weak agonistic profiles. Furthermore, in mice, Netoglitazone produces less weight gain than other glitazones with comparable levels of glycemic control. Interestingly, Netoglitazone appears to function as a full or partial PPARγ agonist depending on the cell type and the response element used in the transactivation assay.

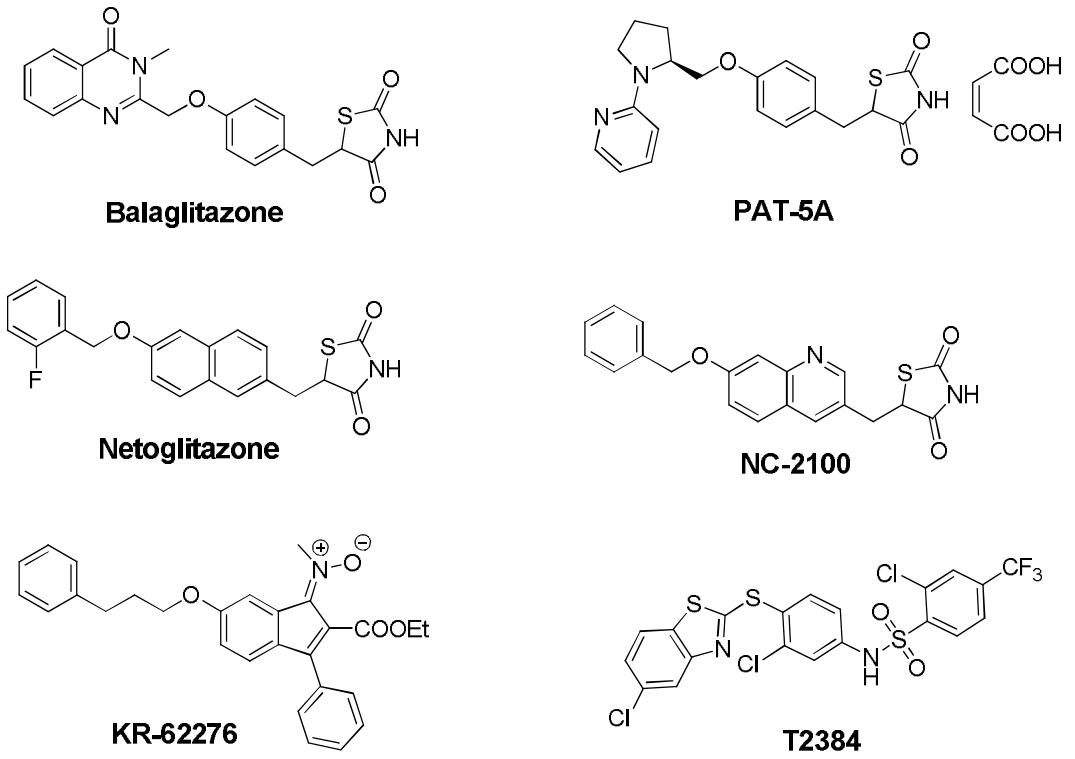


Figure 13: PPARγ partial agonist

More recently, KR- 62776 and KR-62980 indenone derivatives have been developed as PPARγ-specific full agonists and display PPARγ partial agonist

activities. These indenone compounds have been shown to inhibit adipocyte differentiation via activation of ERK; consequently, they do not induce weight gain in animal tests.[218] Another benzenesulfonamide derivative T2384 is a conformationally flexible PPAR*γ* partial ligand that lowers plasma glucose and insulin levels *in vivo*, as seen with Rosiglitazone.[219] However, T2384 treatment does not increase body weight and has been shown to induce weight loss at higher tested doses.

Table 7. Dual, Pan and Partial PPAR agonist

PPAR Agonist		
Dual agonists	**Pan agonists**	**Partial agonists**
KRP-297	Bezafibrate	**PPARγ**
Muraglitazar	Sodelglitazar	Balaglitazone
Farglitazar	Indeglitazar (PLX204)	PAT-5A
Ragaglitazar		Netoglitazone
Tesaglitazar		NC-2100
Imiglitazar		KR62276
Chiglitazar		T2384
Aleglitazar		**PPARδ**
DRF2519		GW9371
Cevoglitazar		GSK7227
Saroglitazar		GSK1115

1.2.9.3.2 PPAR*β*/*δ* partial agonist

The 3,3-bis-(4-bromophenyl) allylsulfanyl derivative (Novo nordisk compound) was recently reported to be a selective partial PPAR*β*/*δ* agonist but with full agonist activity in terms of FFA oxidation in muscle cells both *in vitro* and *in vivo*.[220] (**Figure 14**) In addition, compound displays satisfactory oral pharmacokinetic properties in rats and corrects plasma lipid parameters and improved insulin sensitivity. GW9371 was identified as an another novel class of PPAR*δ* partial agonist through high throughput screening.[221] The design and synthesis of SAR analogues led to disubstituted derivatives GSK7227 and GSK1115, which represent the most potent and selective PPAR*β*/*δ* compounds in this series. These compounds induce expression, but with reduced efficacy compared to the full agonist, of two important PPAR*δ*-regulated genes in human

skeletal muscle cells: CPT1 and PDK4, which play key role in skeletal muscle metabolisim by contributing to regulation of glucose metabolism.[221]

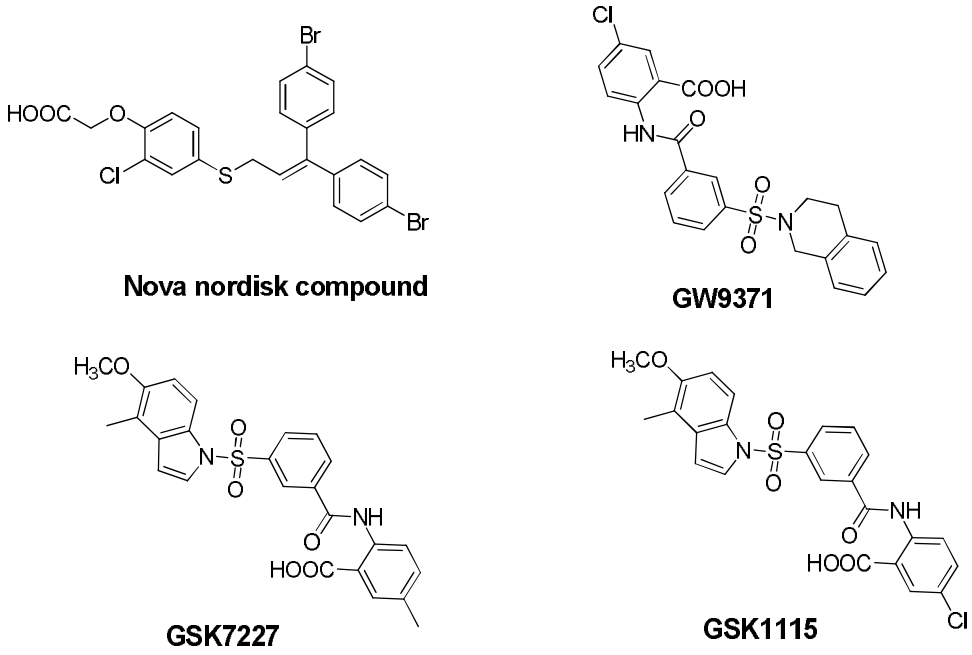


Figure 14: PPAR*δ*/*β* partial agonist

Lipid abnormolities associated with T2DMs are the major metabolic risk factors for cardivascular disease. PPARs and their modulators have emerged as promising group of agents for the treatment of metabolic disorders such as hyperglycemia, hyperlipidemia and atherosclerosis. Selective PPAR modulators, dual and pan agonist seem to provide resonable promise to achive above said objectives. For the prevention and treatment of both lipid and glucose profile disorders, one should consider the potency and affinity of selective PPARs along with their potential side effects. These facts made the development of selective PPARα, and PPARα/γ dual agonists with distinct biological and safety profiles a challenge among the drug discovery groups around the world as the medical need for metabolic disorders is largely remained unmet.