1 Introduction

1.1 PPARs and Nuclear Hormone Receptors

Nuclear hormone receptors (NR) are ligand activated transcription factors that regulate a wide range of biological processes via gene expression in response to small lipophilic molecules and are thus considered relevant targets in drug discovery. The well-known members of this NR family are androgen receptor (AR), liver X receptor (LXR), estrogen receptor (ER), pregnane X receptor (PXR), retinoic X receptor (RXR), thyroid hormone receptor (TR), farsenoid X receptor (FXR), vitamin D receptor (VDR) and peroxisome proliferator-activated receptors (PPARs).

Increasing attention has been focused on the role of PPARs in the past decades. The PPARs comprise an important subfamily of the NR super family that function as lipid sensors to regulate a broad range of genes[1] in many metabolically active tissues and plays a central role in the modulation of multiple

aspects of lipid and carbohydrate metabolism.[2-4] PPARs play essential roles in the regulation of cellular differentiation, development, and metabolism (carbohydrate, lipid, and protein) of higher organisms.[5,6] Fibrates and glitazones are the well-established synthetic ligands for PPARs. It is very interesting that fibrates which were discovered and approved for the treatment of dyslipidemia and glitazones which were developed subsequently for the treatment of type 2 diabetes were discovered before the discovery of PPARs.

The first fibrate class of compound discovered was ethyl-*α*-4-chlorophenoxyisobutyrate that later on was known as clofibrate.[7] Clinical trials showed that clofibrate decreased lipid levels in hypercholesterolemic patients.[8] However, the mode of action of clofibrate was still unclear. Other fibrates such as gemfibrozil, bezafibrate, fenofibrate, ciprofibrate etc. were discovered subsequently in the period of 1970-80. In an attempt to get more potent fibrates, in 1975 scientists at Takeda laboratories in Japan observed that some of alkanoic acids containing a biphenyl ether moiety showed very good hypoglycemic activity along with hypolipidemic properties.[9] Further extensive studies led to the discovery of the first glitazone class of compound ciglitazone which was found to control hyperglycemia, hyperinsulinemia and hypertriglyceridemia in animal models.[10] Consecutively troglitazone,[11] pioglitazone[12] and rosiglitazone[13] were discovered.

In later years of 1980s it was observed that fibrates increase the transcription of peroxisomal fatty acid *β*-oxidation genes in liver of the rat[14] and also regulate the expression of genes involved in lipoprotein metabolism.[15] In view of understanding the action of fibrates, a novel member of the steroid hormone receptor superfamily of ligand-activated transcription factors was identified which was believed to mediate the peroxisome proliferation response and therefore was called peroxisome proliferator-activated receptor.[15,16] Later on it was identified as PPAR*α.* After a couple of years of the discovery of PPAR*α*, two other members of the same family were cloned and were named PPARγ and

PPARδ. In 1994, PPARγ was identified as a major adipogenic transcription factor[17] and in 1995, PPARγ was identified as the target of the glitazones.[18] Fibrates such as gemfibrozil, fenofibrate, bezafibrate, ciprofibrate and glitazones such as rosiglitazone, pioglitazone are still widely prescribed hypolipidemic drugs and hypoglycemic drugs respectively.

1.2 Protein Structure of PPAR

Like other nuclear receptors, all three PPAR isoforms possess similar structural and functional features. Principally, four functional domains have been identified, called A/B, C, D and E/F.[19]

Figure 1: The functional domains of PPARs

The region A/B consists of N-terminal with ligand-independent activation function 1 (AF-1) and is responsible for phosphorylation of PPAR. The region C is a DNA binding domain (DBD) which promotes the binding of PPAR to the peroxisome proliferator response element (PPRE) in the promoter region of target genes.[20] D is a flexible central docking region for cofactors. The E domain is responsible for ligand specificity and activation of PPAR binding to the PPRE which increases the expression of targeted genes. Recruitment of PPAR co-factors to assist the gene transcription processes is carried out by the ligand-dependent activation function 2 (AF-2), which is located in the E/F domain.[5]

The DBD contains two zinc finger motifs which bind to specific sequences of DNA known as hormone response elements when the receptor is activated. The LBD has an extensive secondary structure consisting of 13 alpha helices and a beta sheet.[21] Natural and synthetic ligands bind to the LBD, either activating or repressing the receptor.

1.3 Nomenclature and tissue distribution

Three types of PPARs have been identified: alpha, gamma, and delta (beta)[5]

- ❖ PPAR*α* : Expressed in liver, kidney, heart, muscle, adipose tissue, and others
- ❖ PPAR*β*/*δ* : Expressed in many tissues but markedly in brain, adipose tissue, and skin
- ❖ PPARγ : Although transcribed by the same gene, this PPAR is further categorized based on their tissue distribution:
    - o γ1: Expressed in virtually all tissues, including heart, muscle, colon, kidney, pancreas, and spleen
    - o γ2 : Expressed mainly in adipose tissue (30 amino acids longer)
    - o γ3 : Expressed in macrophages, large intestine, white adipose tissue.

1.4 Isoforms

1.4.1 PPARα

PPAR*α*, cloned in the early 1990s, plays an important role in the oxidation of fatty acids in the liver.[22] Receptor activation stimulates fatty acid oxidation, a crucial adaptive response to nutritional challenges such as fasting. The receptor regulates genes that control fatty acid uptake, activation of acyl-CoA esters, and degradation by way of peroxisomal and mitochondrial beta-oxidation pathways. PPAR*α* activators induce fatty acid translocase protein mRNA levels in rat liver and intestine, and acyl- CoA synthetase mRNA levels in the liver and kidney.[23] On the whole, these agents affect a variety of steps in fatty acid oxidative

metabolism in different tissues, particularly in the liver where they reduce quantities of available fatty acids for triglyceride-rich VLDL synthesis.

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. Activation of PPARα causes the proliferation of peroxisomes and hepatomegaly in rodents.[16] However this has not been observed in nonrodent species, including human. 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 species. The wild-type PPARα is expressed in rodent liver at 10 times higher levels than in human liver.[24] 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 the rodent and the human.[25] 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.

1.4.2 PPARγ

PPARγ is the most extensively studied of the three PPAR subtypes to date. The human PPARγ protein is homologous to the murine PPARγ protein, with 95% identity at the amino acid level. In fact, PPARγ protein shows a remarkable conservation across all the species in contrast to PPARα and PPARδ. This high level of conservation reflects the pivotal role that PPARγ plays as a regulator of glucose and lipid homeostasis across all the species.

1.4.3 PPARδ

PPARδ which is also known as PPARβ has been cloned from a number of

species and initially given a variety of names. The receptor was first reported in *Xenopus laevis*.[26] Subsequently, the receptor was cloned from human[27] and mice.[28] The human and rodent receptors are about 90% identical in the ligand binding domain (LBD). In humans PPARδ is present in tissues those control lipid metabolism like liver, intestine, kidney, abdominal adipose and skeletal muscle.[29]

Structural similarities of all the three PPAR isoforms were demonstrated by *Henke et al* (Figure 2). The sequence identities 62%, 63% and 70% were found from a structure-based alignment of the ligand binding domains generated with the program MVP.[30]

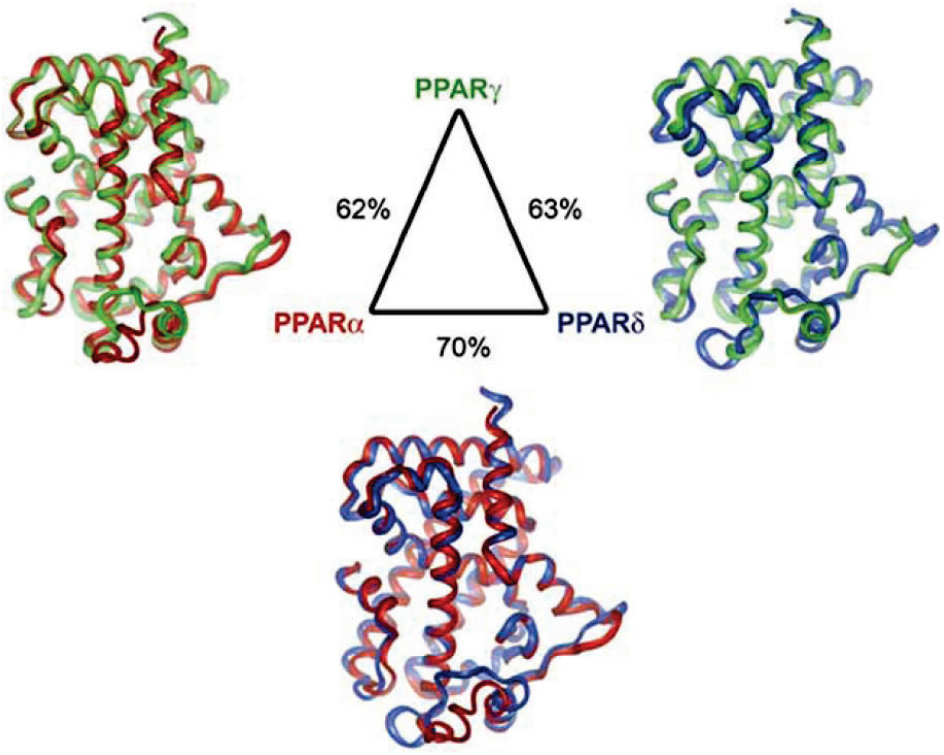


Figure 2: Overlays of PPAR isoforms

PPARα (red), PPARγ (green) and PPARδ (blue) have similar sequences and similar structures. Overlays of PPARα with PPARγ, PPARγ with PPARδ, and PPARα with PPARδ, using x-ray crystal structures of PPARα bound to GW 409544, PPARγ bound to farglitazar and PPARδ bound to GW 501516.

Comparison of all the PPAR subtypes in terms of structure, homology and tissue distribution is presented in Table 1.

Table 1: Summary of structure, homology and tissue distribution of PPARs

	PPAR α	PPAR γ	PPAR δ
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

1.5 PPARs as a Therapeutic Target

1.5.1 PPARs for the treatment of Metabolic Syndrome

1.5.1.1 Metabolic Syndrome

Metabolic syndrome (MS) refers to a cluster of metabolic disorders, such as insulin resistance, hyperinsulinaemia, hyperglycemia, dyslipidemia, high blood pressure and obesity. The first report of the metabolic syndrome was stated in 1923 as the syndrome comprising hypertension, hyperglycemia and hyperuricaemia.[31] Obesity was reported as component of MS in 1947[32] and after

several decades MS was given several names like syndrome X,[33] The Deadly Quartet[34] and the Insulin Resistance Syndrome.[35] Finally the term Metabolic Syndrome (MS) is well accepted all over the globe.

1.5.1.1.1 Management and Treatment of the Metabolic Syndrome

As, the root cause of the metabolic syndrome is unknown, till the date it is not possible to cure it. One can only reduce the severity of the risk factors by life style modification. Loss of weight, increase in physical activity and a healthy diet are proven to be effective in the management of metabolic syndrome. However, when the risk factors are increased, the metabolic disorder could be controlled by combination therapy (summarized in Table 2) in absence of unavailability of mono drug therapy.

Dislipidemia is effectively treated by statins (*e.g*. Atorvastatin, Rosuvastatin, Simvastatin, *etc*.) which lower cholesterol by inhibiting the enzyme HMG-CoA reductase. This class of drugs inhibits cholesterol synthesis as well as increases the clearance of low-density lipoprotein (LDL) form the blood stream by promoting synthesis of LDL receptors. However statins failed in reducing levels of triglycerides and in raising high-density lipoprotein cholesterol (HDLC). PPARα activators, fibrates (*e.g*. Fenofibrate, Bezafibrate *etc*.) remain the choice of treatment for dyslipidaemia as they effectively reduce triglycerides, increase HDL-cholesterol and lower LDL-cholesterol. The only disadvantage of fibrates is their poor efficacy and due to that high dose is required to show the therapeutic effect.

Currently marketed *anti*-obesity drugs are based on suppression of the appetite (*e.g*. Sibutramine) or by increasing body metabolism or by interfering with the body's ability to absorb specific nutrients in food. Because of potential side effects, it is recommended that *anti*-obesity drugs only be prescribed when the probable benefits of the treatment are more than its risks.

In atherosclerosis and cardiovascular segment, Clopidogrel a platelet aggregation inhibitor and Aspirin are proven to reduce the risk of clot formation. Recently Factor Xa inhibitor Rivaroxaban and thrombin inhibitor Dabigataran etaxilate have been approved for the treatment of thrombotic disorders. Sulfonylureas (*e.g*. Glipizide, Glyburide, Glimepiride, and Gliclazide) were the first widely used drugs as oral hypoglycemic drugs. Meglitinides (*e.g*. Repaglinide, Nateglinide) increase insulin production in the pancreas through closing the potassium channels and opening the calcium channels. Metformin that does not cause weight gain is widely used oral drug for the first-line medication prescribed for treatment of type 2 diabetes. PPARγ agonists thiazolidinediones (TZDs) or glitazones (*e.g*. Rosiglitazone and Pioglitazone) are proven to be effective in controlling hyperglycemia by improving insulin sensitization. However edema and weight gain are the major adverse effects caused by glitazones. α-glucosidase inhibitors (*e.g*. Miglitol, Acarbose) work through the decrease of digestion of starch in the small intestine. Dipeptidyl peptidase-4 (DPP-4) inhibitors increase blood concentration of the incretin GLP-1 (glucagon-like peptide-1) by inhibiting its degradation by DPP-4. Vidagliptin and Sitagliptin are the approved drugs of this class.

A variety of drugs are currently in use for the treatment of hypertension. Beta blockers (*e.g*. Atenolol, Metoprolol, Propranolol), alpha blockers (*e.g*. Doxazosin, Prazosin, Terazosin and Tolazoline), mixed alpha + beta blockers (*e.g*. Bucindolol, Carvedilol, Labetalol) are adrenergic receptor antagonists. Calcium channel blockers block the entry of calcium into muscle cells in artery walls (*e.g*. Amlodipine, Nifedipine, and Nimodipine). ACE inhibitors (*e.g*. Captopril, Enalapril, Ramipril and Trandolapril) inhibit the activity of Angiotensin-converting enzyme (ACE), an enzyme responsible for the conversion of angiotensin I into angiotensin II, a potent vasoconstrictor. Angiotensin II receptor antagonists (*e.g*. Losartan, Olmesartan and Telmisartan) antagonise the activation of angiotensin receptors.

Table 2: Available treatment for metabolic syndrome

Metabolic Syndrome				
Dyslipidaemia	**Hyperglycemia & Insulin resistance**	**Elevated blood pressure**	**Cardiovascular diseases**	**Obesity**
• Statins • Niacin • Fibrates	• Sulfonylureas • Meglitinides • α-glucosidase inhibitors • DPP-4 inhibitors • GLP agonists • Glitazones • Biguanides	• ACE-inhibitors • β-blockers • α-blockers • Calcium channel blockers • Angiotensin II receptor antagonists	• Clopidogrel • Aspirin • Rivaroxaban • Dabigataran etaxilate	• Sibutramine • Orlistat

Diabetes and cardiovascular diseases (CVD) are the main risk factors of metabolic syndrome and the PPARs play an important role in maintaining glucose and lipid homeostasis by modulating gene expression. Thus looking for PPAR agonists appears to be promising therapeutic option for the treatment of metabolic syndrome.

1.5.1.2 Mechanism of metabolic action of PPAR's

The gene transcription mechanism is identical in all PPAR subtypes and the process of transcription begins with the binding of a ligand (endogenous or exogenous) to the PPAR receptor. Ligand-bound PPAR heterodimerises with RXR.[36] This heterodimer complex binds to peroxisome proliferator response element (PPRE) located in the regulatory (promoter) region of the target genes. PPRE consists of direct repeat (DR)-1 elements of two hexanucleotides with the AGGTCA sequence separated by a single nucleotide spacer.[37,38]

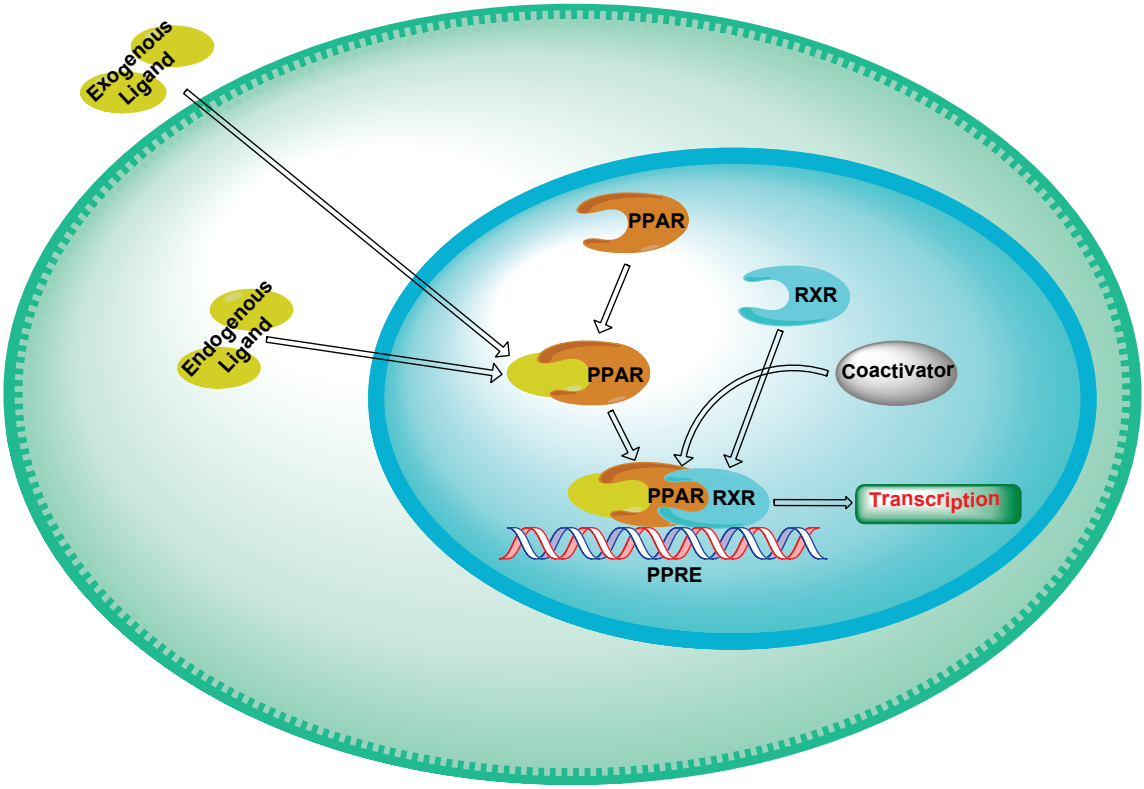


Figure 3: Gene transcription mechanisms of PPAR

In the unliganded state, PPAR interacts with anti-repressor complex; the deacetylated state activity of corepressor inhibits gene transcription. Upon binding of exogenous (drugs) or endogenous (fatty acids, PGs, etc.) ligands, PPAR heterodimerises with RXR and recruits the coactivator containing histone acetylase activity and facilitates transcription of various genes.

The DR-1 pattern is specific for PPAR–RXR heterodimer, which distinguishes it from the DR-3, DR-4 patterns of the 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 those mediate the ability of nuclear receptors to initiate or suppress the transcription process. They interact with nuclear receptors in a ligand-dependent manner.[38] 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).[39-41] Alternatively, coactivators such as steroid receptor co-activator (SRC)-1 and the PPAR binding protein (PBP) with histone acetylase activity[42,43] initiate a sequence of events which induce the gene transcription process upon ligand binding (Figure 3). This results in increase in transcription activities of various genes involved in diverse biological processes.

1.5.1.3 PPARα and Metabolic Syndrome

PPAR*α* has been identified as a key regulator of the genes involved in fatty acid oxidation, which occurs in mitochondria, peroxisomes, and microsomes in the liver.[44] Transcription and protein levels of critical enzymes in *β*-oxidation and *ω*-oxidation pathways are direct targets of PPAR*α*, including acyl CoA oxidase, carnitine palmitoyl transferase-I, mitochondrial hydroxymethylglutaryl-CoA synthase, and cytochrome P450 4A enzymes (CYP4A).[45] By increasing the expression of these genes, PPAR*α* ligands significantly activate hepatic fatty acid oxidation, whereas genetic inactivation of the PPAR*α* gene results in massive accumulation of lipids in the liver, severe hypoketonemia, hypoglycemia, hypothermia, and elevated plasma free fatty acid levels.[46]

PPAR*α* also plays a critical role in lipid metabolism. Its known target genes are involved in almost all aspects of lipid metabolism, including uptake, binding, and oxidation of fatty acids; lipoprotein assembly; and lipid transport.[45,47] Synthetic PPAR*α* ligands, such as gemfibrozil, fenofibrate, and clofibrate increase the synthesis of HDL through several PPAR target genes. PPAR*α* activation also increases fatty acid uptake, decreases triglyceride level, and promotes triglyceride-VLDL lipolysis.[47-49] Taken together, it seems that PPAR*α* may be a lipid sensor and can exert beneficial metabolic effects on lipid metabolism. In another aspect, PPAR*α* has also been implicated in the pathogenesis of obesity and insulin resistance.[50] Activation of PPAR*α* reduces weight gain in rodents, and inactivation of PPAR*α* results in a late onset of obese

phenotype.[51] An evidence has recently emerged suggesting that PPAR*α* is an important regulator of insulin sensitivity. Treatment with PPAR*α* activators dramatically improved insulin resistance and glycemic control in type 2 diabetic db/db mice and OLETF rats.[52] Similarly, the PPAR- agonist bezafibrate markedly improved glucose intolerance and insulin resistance in a lipoatrophic diabetic patient.[53] PPAR*α* has also been implicated in BP regulation and vascular inflammation. PPAR*α* in both vascular endothelial cells and smooth muscle cells[54] inhibits vascular inflammation, oxidative stress, and cell growth and migration through blocking NF-κB, TGF-*β*/Smad, and mitogen-activated protein kinase (MAPK) pathways.[55,56] *In vivo* studies further demonstrate regulation of CYP4A by PPAR*α* ligands could play an important role in sodium homeostasis and BP regulation.[57]

Finally, fibrate PPAR*α* activators have been reported to potently reduce atherosclerosis both in apoE -/- mice and in human ApoAI transgenic apoE -/- mice.[58]

1.5.1.4 PPARγ and Metabolic Syndrome

PPARγ has been implicated in almost all aspects of metabolic disorders, including obesity, insulin resistance, dyslipidemia, inflammation and hypertension that are evidenced by the increasing clinical use of synthetic TZD ligands of PPARγ. It may also provide an attractive therapeutic target in the treatment of obesity, hypertension, and atherosclerosis. PPARγ is a key player in adipogenesis, i.e., fat cell differentiation. Many genes involved in fatty acid transport and metabolism are transcriptionally regulated by PPARγ, including adipocyte fatty acid binding protein, phosphoenolpyruvate carboxykinase, acyl-CoA synthase, fatty acid transport protein (FATP), fatty acid translocase (CD36), and lipoprotein lipase.[59] Collectively, these data demonstrate that PPARγ is a key transcription factor in adipogenesis, and overactive PPARγ may contribute to the pathogenesis of obesity.

Importantly, loss-of-function mutation of PPARγ results in severe insulin resistance in humans.[60] It indicates the key role of PPARγ in insulin resistance. The synthetic PPARγ agonists TZD effectively improve insulin sensitivity and lower blood glucose level in patients with type 2 diabetes is consistent with the possibility that PPARγ activation improves insulin resistance. One possible mechanism by which PPARγ increases insulin sensitivity relates to adipocyte-derived signaling molecules or adipocytokines, including TNF-α, IL-6, leptin, resistin, and adiponectin.[61] Increased glucose uptake in adipose tissue, reduction in free fatty acid burden on liver and muscle, a decrease in free fatty acid release from adipocyte, and an increase in energy expenditure also have been suggested to contribute to the beneficial effects of PPARγ agonists on insulin resistance.[62]

As loss-of-function mutation of PPAR- causes elevated triglycerides and low HDL levels in humans,[63] PPARγ has been proved to be pivotal in controlling adipogenesis and fatty acid metabolism. In fact, significant and sustained increases in HDL cholesterol and decreased free fatty acid levels and total cholesterol to HDL cholesterol ratio have been observed in people who were treated with troglitazone and pioglitazone.[64] Furthermore, both TZD rosiglitazone and pioglitazone have been shown to be effective and well tolerated when used in combination with statins,[65] likely adding beneficial effects on dyslipidemia in the patients with the metabolic syndrome.

PPARγ activity has also been suggested to play an important role in BP regulation. TZDs have been found to significantly lower BP in both diabetic animals and humans.[66,67] Studies on hypertensive models not associated with insulin resistance show that TZD treatment significantly lowers BP,[68] arguing for direct vascular action of PPARγ. Although the underlying mechanisms remain unclear, inhibition of vasoconstrictors such as endothelin-1 and stimulation of vasodilators including prostacyclin and nitric oxide have been proposed to play a

role.[69] The long-term consequences of TZD-associated fluid retention on BP remain to be determined.

1.5.1.5 PPARδ and Metabolic Syndrome

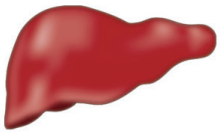
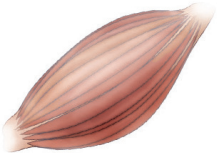
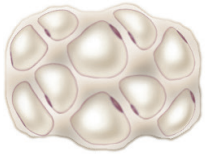
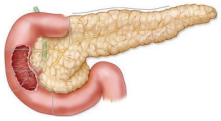
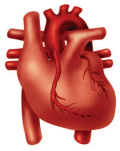
Although the existence of PPAR*δ/β* has been recognized for several years, its biologic role and medical relevance have just recently come under investigation. It has been found that PPARδ action encompasses a panoply of physiologic and pathophysiologic activities, including effects on reproduction, mast cell immunity, bone formation, skin and brain development, wound healing, and tumorigenesis.[45] Similar to the other two isoforms of the PPAR family, emerging evidence suggests that PPARδ may be a pivotal factor in metabolic control.

As PPARδ in NIH 3T3 fibroblast induced PPAR-γ2 expression, PPARδ may be involved in adipogenesis via promoting proliferation of adipocyte precursor cells. On the basis of several studies on PPARδ null mice,[70] PPAR-/ mice[71] and PPARδ transgenic mice,[72] an important role for PPARδ in the pathogenesis of obesity has also been suggested. PPARδ selective agonists play key roles for PPARδ in lipid metabolism. Animal studies on PPAR*δ* specific agonists L-165041 and GW-501516 showed increase in serum HDL cholesterol, reduction in serum LDL and serum triglycerides.[73,74] The results suggest that activation of PPARδ is associated with a less atherogenic lipid profile. PPARδ receptor may also be critically involved in insulin resistance. Studies of PPARδ selective agonists in various animal models proved that a PPARδ selective agonist could effectively lower plasma insulin levels, without adverse effects on glycemic control[74] and markedly improve glucose tolerance and insulin resistance.[75] Although the underlying mechanism is unclear, activation of PPARδ in skeletal muscle, which has a significant role in insulin sensitivity, has been proposed to account for the beneficial metabolic effects of PPARδ agonists on lipid profile and insulin resistance. Taken together, PPARδ is a critical player in

the pathogenesis of the metabolic syndrome, and its ligands may provide useful agents for treating dyslipidemia, obesity, insulin resistance, and atherosclerosis.

Tissue specific roles of all PPAR isoforms for the treatment of metabolic syndrome are summarized in Table 3.

Table 3: Therapeutic targets of PPAR in the metabolic syndrome

	PPARα	PPARγ	PPARδ
 **Liver**	↑ Fatty acid uptake ↑ Fatty acid oxidation ↑ HDL apolipoproteins ↓ VLDL production ↓ Acute-phase reactants ↓ Inflammation	↑ Lipogenesis ↑ Insulin sensitivity	↓ Glucose production ↑ Pentose phosphate shunt
 **Skeletal muscle**	PPARα overexpression ↑ Fatty acid uptake ↑ Fatty acid oxidation ↑ Triglyceride lipolysis ↓ Glucose intolerance ↑ Glucose utilization	↑ Insulin sensitivity	↑ Endurance capacity ↑ Fatty acid oxidation and transport ↑ Thermogenesis ↑ Slow-switch fibers
 **Adipose tissue**	↑ Lipolysis during starvation	↑ Adipocyte differentiation ↑ Adipocyte Survival ↑ Lipogenesis ↑ Insulin sensitivity ↑ Adipokine secretion	Prevention obesity ↑ Fatty acid oxidation and transport ↑ Thermogenesis
 **Pancreas**	↓ β-cell lipotoxicity ↑ Glucose stimulated insulin secretion ↑ Fatty acid oxidation		↓ insulin secretion in rodent
 **Heart**	PPARα overexpression ↑ Cardiomyopathy ↑ Fatty acid uptake ↑ Fatty acid oxidation ↓ Glucose oxidation		↑ Fatty acid oxidation and transport ↑ Contractile function
 **Blood vessel**	↓ Atherosclerosis ↑ Reverse cholesterol transport ↓ Inflammatory response		↑ HDL cholesterol

1.5.2 PPARs in inflammation and arthrosclerosis

Inflammation is a multistep process by which the host responds to the perturbation of homeostasis occurred by microbial infections, injuries or altered physiological conditions. Upon elimination of the source of inflammation, a resolution phase proceeds in which the damaged surrounding tissue is repaired and homeostasis is restored. Inflammatory reactions are further classified as acute inflammation and chronic inflammation. Typically, chronic inflammation is developed gradually either by an acute reaction if the host is not able to eliminate the inflammatory inducer[76] or by altered homeostatic conditions, such as obesity and the related type 2 diabetes or atherosclerosis. An inflammatory reaction can be regulated by the detection and interpretation of inflammatory signals, the response of the activated immune cells (such as cytokine release) or the reaction of the surrounding tissue to inflammatory agents.

Interestingly, several regulatory mechanisms of the inflammatory reaction were demonstrated to be influenced by lipid molecules. As PPARs are specialized receptors to detect fatty acid derived signal molecules, they are key candidate for being the receptors that transduce a fraction of the lipid mediated inflammatory signaling events. Certainly, several instances were identified in which certain fatty acid derived molecules were shown to activate PPARs and modulate inflammation.[77,78] Due to their role in the above medical conditions, the metabolic roles of PPARs have been the target of intensive research. As their functions in inflammation slowly emerged, an increasing number of studies were devoted to dissect the role of PPARs in different types of inflammation. The role of PPARs in inflammation is especially relevant in the case of MS and atherosclerosis.

1.5.2.1 Mechanism of the inflammatory actions of PPARs

PPARs up-regulate the target genes involved in lipid and glucose metabolism by transactivation whereas inflammatory processes are down-

regulated by PPARs via mechanisms of transrepression.[79] Cellular and in vivo studies suggest that PPARs can exert their anti-inflammatory effects by several distinct molecular mechanisms (Figure 4).

The anti-inflammatory activities of PPARs are partially effectuated by inhibition of transcription factors responsible for initiating inflammatory responses. Nuclear factor κB (NF-κB) which is one of the transcription factors plays a key role in inflammation pathways.[80] Upon activation by pro-inflammatory cytokines such as TNFα and IL-6, the complex activates the expression of inflammatory genes.[81]

PPARs are able to attenuate NF-κB function in two different ways: by physical interaction with components of the NF-κB complex, PPARs impairs binding of NF-κB to the DNA and subsequent activation of inflammatory genes.[82] (Figure 4A) and secondly, PPARs activation results in increased expression of IκB, the inhibitory protein that prevents the transfer of NF-κB to the nucleus.[83] Apart from interference with NF-κB, additional molecular pathways by which PPARγ inhibits the transcription of pro-inflammatory genes by preventing the release of co-repressor complexes from promoters of pro-inflammatory genes thereby inhibiting their transcription[84] (Figure 4B). PPARδ activation indirectly regulates inflammatory reactions by releasing the inflammatory suppressor protein B cell lymphoma-6 protein (BCL-6).[85] Upon ligand binding, BCL-6, which is known to play a key role in inhibition of inflammatory pathways, is released from PPARδ and is available for the repression of pro-inflammatory genes (Figure 4C).

Alternatively, it is also possible that PPARs modulate inflammatory processes indirectly by altering lipid metabolism. Although the three subtypes of PPAR alter lipid metabolism in three distinctly different ways, they have similar anti-inflammatory roles in the diseases. It suggests that the regulatory circuits of PPARs in metabolism and inflammation are still unidentified.

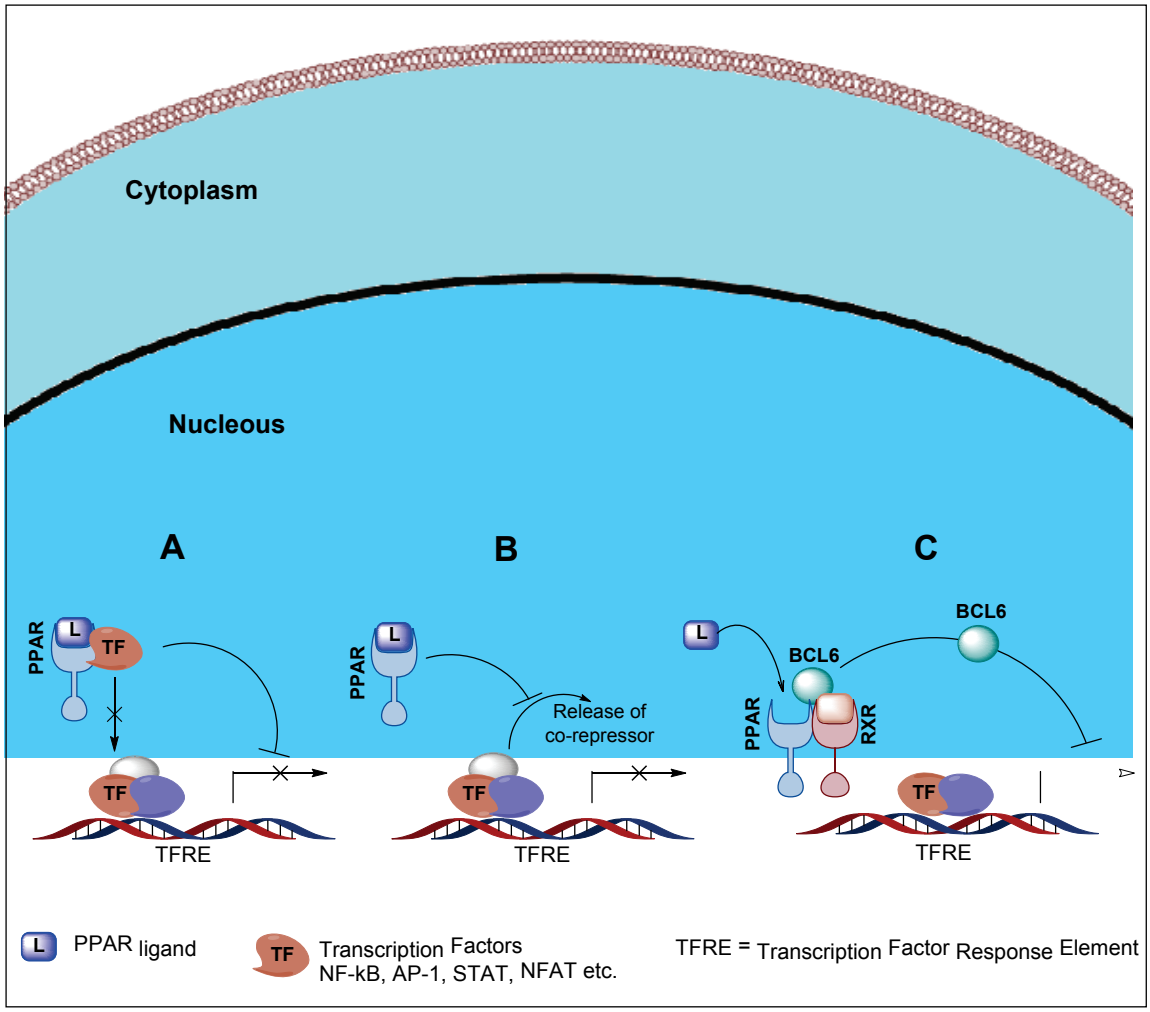


Figure 4: Inflammatory actions of PPARs: Mechanisms of transrepression

1.5.2.2 Role of PPARs in inflammation

PPARs regulate inflammatory processes, mainly by transrepression of pro-inflammatory transcriptional factors such as signal transducer and activator of transcription (STAT), Activator protein–1 (AP-1) and NF-κB.[82,84,86-88] Down-regulation of several inflammatory cytokine (as shown in Table 5) has been observed in many inflammatory animal models when treated with synthetic ligands of PPARs.[89] The role of PPARs in various animal models is extensively studied and reviewed.[89] PPARα has the potential to treat atherosclerosis, colonic inflammation and skin inflammation whereas PPARγ is useful in controlling

inflammatory bowel desiese, CNS inflammation and atherosclerosis. In contrast to the well-established functions of PPARα and PPARγ in controlling inflammation, the role of PPARδ in regulating inflammation and immunity is just emerging. Skin inflammation and wound healing was the first inflammatory model in which the role of PPARβ/δ was investigated.[90] In addition to that the involvement of PPARβ/δ in atherosclerosis is also reported.[85]

Role of all PPAR isoforms for the treatment of inflammation is summarized in Table 4.

Table 4: Role of PPARs in inflammation

PPARα	**PPARγ**	**PPARδ**
Inhibition of Pro-inflammatory Transcription factors		
STAT, AP-1, NF-κB	STAT, AP-1, NF-κB	STAT, AP-1, NF-κB
Down-regulation of Inflammatory cytokines		
IL-6, TNFα, INFγ, IL-1β	MCP-1, VCAM-1, ICAM-1, IFNγ, TNFα	MCP-5, IL-1β, TNFα, IL-6, VCAM-1
Applications in inflammatory diseases		
Atherosclerosis Colonic inflammation Skin inflammation	Inflammatory bowel disease CNS inflammation Atherosclerosis	Skin inflammation wound healing Atherosclerosis

1.6 PPAR Ligands

After identification of relationship between fibrates & glitazones with PPAR and looking at the clinical outcome of these ligands, attention of pharmaceutical researchers diverted towards the development of PPAR agonist. As a result, new mechanisms of PPAR for the regulation of lipid & carbohydrate metabolism were uncovered and several synthetic compounds beyond fibrates and glitazones as agonists of PPARs were discovered. A wide range of saturated and unsaturated fatty acids, polyunsaturated fatty acids and eicosanoids are also identified as endogenous PPAR agonists.

Current research in developing PPAR ligands for the treatment of metabolic syndrome can be bifurcated in two parts. One is the traditional approach that covers continuation of the work which has been done since several decades in developing selective & dual agonists and another is new approach in which focus has been given to the exploratory role of PPAR agonists as a therapeutic target.

1.6.1 Traditional approach in developing PPAR ligands

1.6.1.1 PPARα selective agonists

PPARα agonists are known for the uptake and oxidization of fatty acids and lipoprotein metabolism.[91] The fibrate class of hypolipidemic drugs including Clofibrate, Fenofibrate and Bezafibrate (Figure 5) exert their actions primarily through activation of PPARα. They are used in reducing triglyceride, increasing HDL cholesterol and lowering LDL cholesterol but they need high dose to show significant efficacy because of their poor potency towards PPARα. 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 GW-9578[92] as potent and selective PPARα agonist. Along with its lipid lowering activity, It also prevented weight gain and the development of hyperinsulinemia in insulin resistant rats. Subsequently, Lilly identified a compound LY-518674[93] containing triazolone core in the lipophilic tail part and fibric acid as acidic head. This compound displayed potent hypolipidemic activity and good bioavailability. However, this molecule failed to display the efficacy in the human and the development was discontinued from phase-II clinical trials. Relatively recently NS-220[94] and K-111[95] were identified

as highly potent and selective PPARα agonists that exhibited hypolipidemic and hypoglycemic activities in animal models. K-111 is in phase-II clinical trial but so far no progress has been reported. Whereas Nippon compound NS-220 was unfortunately withdrawn from phase-II study for unknown reason. Since after that several pharmaceutical companies are in search of selective PPARα agonists for the treatment of metabolic disorders but no one has reported any progress in clinical trial.

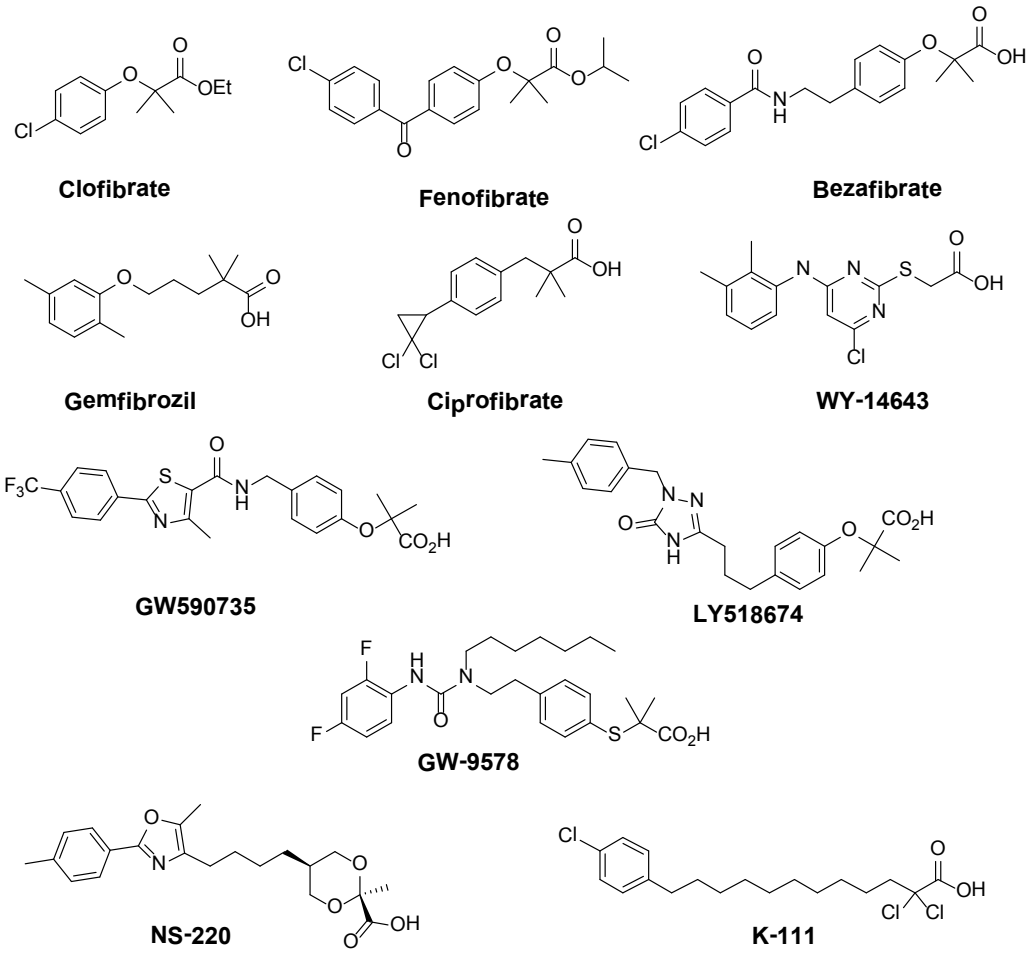


Figure 5: Chemical structures of PPARα agonists

1.6.1.2 PPARγ selective agonists

PPARγ agonists have proven to be efficacious as insulin sensitizer in the treatment of type 2 diebetes.[96,97] PPARγ has been shown to be the molecular target for the thiazolidinedione (TZD) class of antidiabetic agents. The TZDs, commonly referred to as "glitazones", enhance insulin sensitivity in target tissues and lower glucose and fatty acid levels in type 2 diabetic patients. In view of getting more potent fibrate class of compounds, the scientists at Takeda Pharmaceutical Company identified a new compound 2-chloro-3-phenylpropanoic acid ethyl ester, AL-294 which effectively lower plasma glucose as well as triglycerides in 1980.[98] Further modification on the replacement of the acid group of AL-294 with bioisosteric heterocyles resulted in the discovery of the first thiazolidine-2,4-dione (TZD) class of compound AL-321 with enhanced anti-hyperglycemic potency.[99] Subsequent optimization provided a novel, potent glucose-lowering agent Ciglitazone (ADD-3878, U-63287) that effectively improved insulin resistance and glucose disposal[10,99,100] but its clinical development was discontinued mainly due to insufficient efficacy. Further investigation of ciglitazone related TZDs culminated in the discovery of Pioglitazone.[12,101] Moreover, further efforts to the identify more potent TZDs resulted in the discovery of AD-5061,[102] Troglitazone,[103] Rosiglitazone[104] and Englitazone[105] as the first generation insulin sensitizers. Troglitazone (Rezulin) was the first of the TZD class of oral *anti*-diabetic agents to be launched in the United States in 1997 but unfortunately it was subsequently withdrawn from the market due to idiosyncratic hepatotoxicity. Two other TZDs, Pioglitazone (Actos) and Rosiglitazone (Avandia) which have achieved blockbuster status from Takeda Pharmaceutical and GlaxoSmithKline respectively, are currently marketed for the treatment of type 2 diabetes.[106] These two drugs potentiate insulin sensitivity in muscle, liver, and adipose tissue, leading to effective normalization of elevated plasma glucose levels and concomitantly reducing

HbA1c.[107-111] In spite of having good efficacy, these drugs are known to cause side effects such as weight gain and edema.

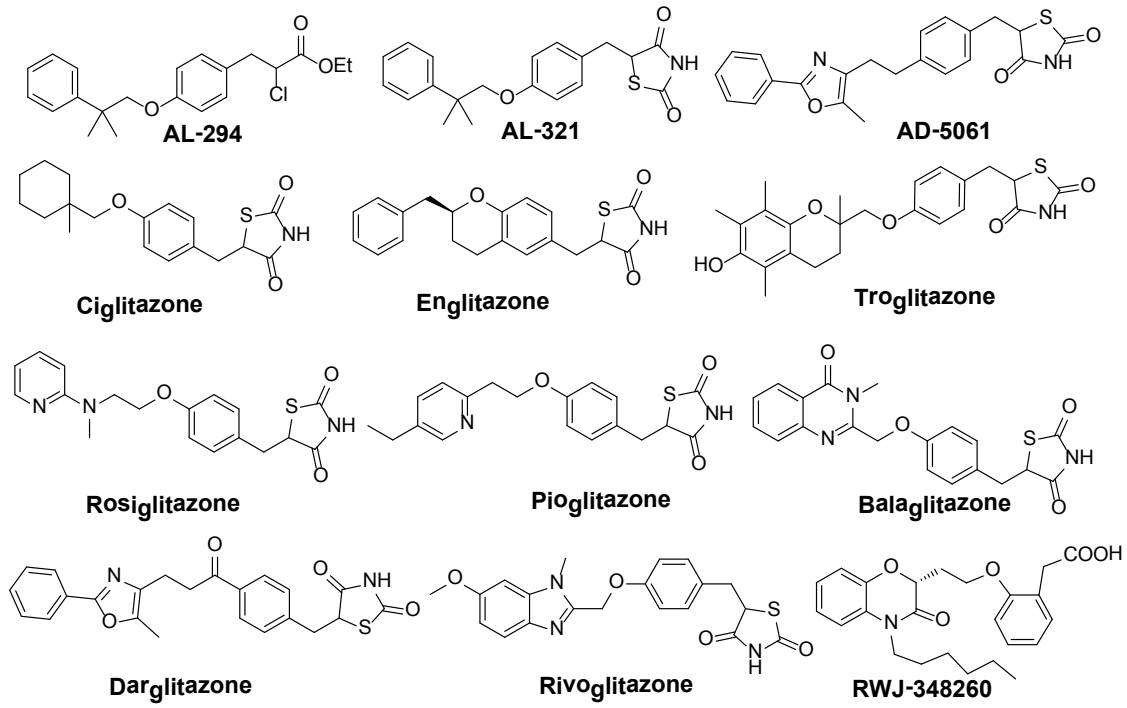


Figure 6: Chemical structures of PPARγ agonists

Since then a lot of afforts have been made by academic institutions along with pharmaceutical and biotechnology companies to get safer and selective PPARγ agonists. Some of them including TZD and non-TZD derived molecules are currently under investigation for the treatment of type 2 diabetes. The TZD based Rivoglitazone (CS-011),[112] 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. The scientists at Dr. Reddy's Research Foundation have discovered Balaglitazone, a novel TZD derivative and a selective PPARγ partial agonist which is currently under Phase-III clinical trials.[113] Further research has been carried out thereafter towards identifying non-TZD PPARγ agonists. RWJ-

348260[114] has been disclosed as a novel non-TZD PPARγ agonist (Figure 6) but no further progress has been reported.

1.6.1.3 PPARα/γ dual agonists

A majority of type 2 diabetes patients suffer from atherogenic lipid abnormalities in addition to insulin resistance, termed as metabolic syndrome, and given the importance of controlling both glucose and lipid levels in metabolic syndrome. This 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 and led to a hypothesis that probably activation of PPARα may mitigate the weight gain induced by PPARγ activation in humans. This hypothesis that PPARα/γ dual agonism would provide synergistic pharmacological effects has encouraged many research groups to develop these agents.

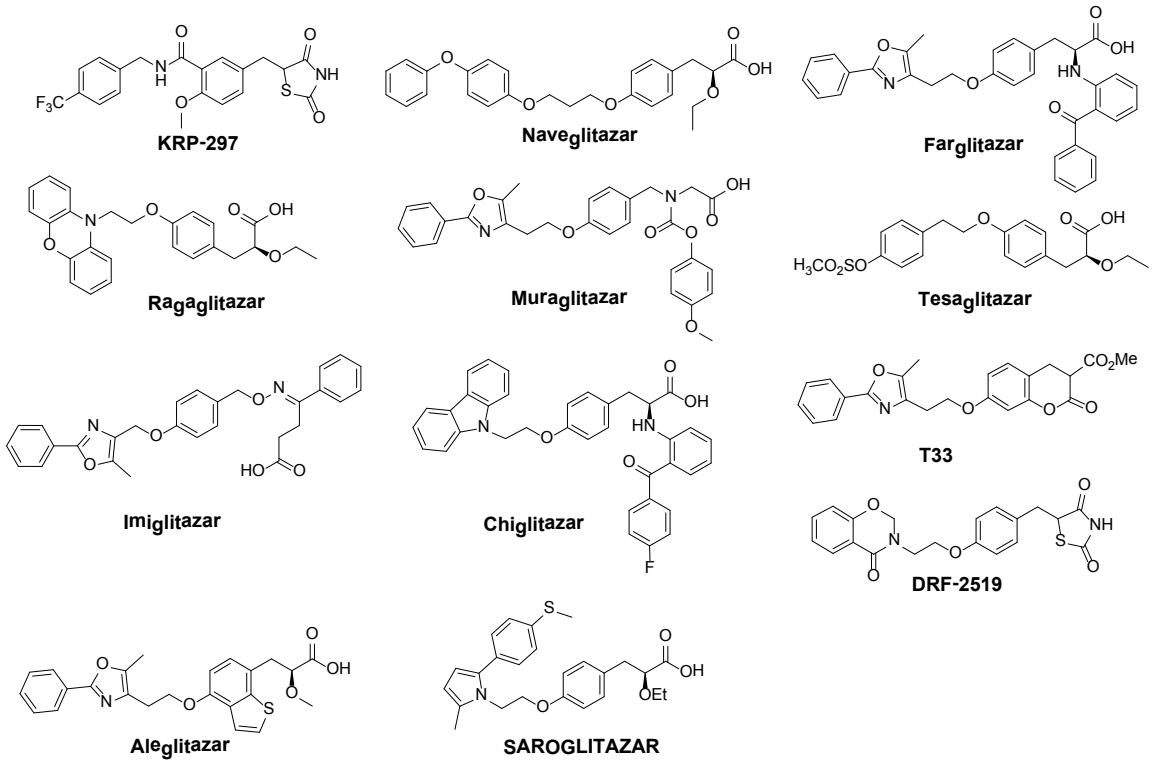


Figure 7: Chemical structures of PPARα/γ dual agonists

A new TZD series of compound KRP-297 was identified as the first PPARα/γ dual agonist which was found effective at normalizing hyperglycemia & hyperlipidemia and reduce FFAs & lipids in healthy subjects in phase-I clinical trial. However, further development was discontinued due to toxicity. The first non-TZD dual agonist Farglitazar[115] (Figure 7) which is a potent PPARγ agonist and moderate PPARα activator was dropped at an advance stage due to the emergence of edema. The only dual agonist that has been advanced to NDA filing, **Muraglitazar**[116] (Figure.7) was dropped due to incidence of edema, heart failure and cardiovascular complication. Like KRP-297, Farglitazar and Muraglitazar, the other PPARα/γ dual agonists including Tesaglitazar,[117] Ragaglitazar,[118] Naveglitazar,[119] and Imiglitazar[120] were dropped in advanced clinical stages due to various toxicological reasons.[121] Few PPARα/γ dual agonists ONO-5129 from Ono Pharmaceuticals, AVE-0897 from Sanofi-Aventis and Chiglitazar from Chipscreen[122] are still left in the development pipeline. A few more compounds DRF-2519[123] and T33 are reported as a novel PPARα/γ dual agonist with insulin sensitizing and hypolipidemic effect.[124] Aleglitazar from Roche which is under phase-III clinical study showed very good efficacy and safety profile.[125] Saroglitazar (Lipaglyn^{TM}) launched by Zydus Cadila in 2014 is the only α/γ dual agonist which is in the market and till date no adverse effect has been observed.[126]

1.6.2 New approaches in developing PPAR ligands

1.6.2.1 PPARδ selective agonists

As discussed above, a journey to accomplish the goal of making safer and efficient PPAR agonist by targeting selective/dual PPARα/γ agonist has met with many safety issues during past few decades. During this period earlier neglected PPARδ has emerged as a potential target for dyslipidemia and inflammation.

Considering these facts, attention has been diverted to the development of PPARδ selective agonists.

The scientists at Merck Research Laboratories have identified a series of compounds such as L-165041,[127] L-783483,[127] L-165461,[128] as PPARδ agonists. The PPARδ selective agonist L-165041 (Figure 8), at doses, which had no effect on plasma glucose and triglyceride levels, raised cholesterol levels in db/db mice. The increased plasma total cholesterol was primarily associated with HDL particles. These compounds have been widely used as a research tool for developing novel compounds. A high affinity, selective PPARδ agonist GW501516 (Figure 8) discovered by GlaxoSmithKline raised HDL-C levels (80%) and decreased triglyceride levels (50%) in obese rhesus monkeys.[74] This compound is in Phase II clinical trial but no progress has been reported since a long time. Subsequently they have reported an aryl sulfonamide derivative (GW-9371) as a partial PPARδ agonist but no efficacy data is reported.[129] Sequentially GlaxoSmithKline has recently reported a series of sulfonamide derivatives (Compound I) as partial PPARδ agonists. Selected compounds from the series showed decrease in body weight and significant improvement in insulin sensitivity in ob/ob mice.[130]

Novartis reported trisubstituted aryls (e.g. LBX001) (Figure 8) as highly potent and selective PPARδ agonists.[131] Simultaneously Novartis has also reported a series of compounds containing substituted isoxazoles linker instead of flexible linker as selective PPARδ agonist.[132] More recently they have also reported biaryl substituted oxazole and thiazole containing PPARδ agonists (Compound III) having improved pharmacokinetic profile. These compounds regulate the genes involved in energy homeostasis in vivo.[133] Bayer is also one of the key players in PPARδ research. It identified a series of compounds with rigid linker (e.g. Compound IV) which possess a high degree of affinity as well as selectivity towards PPARδ.[134] It also reported Indanylacetic acids (e.g.

Compounds V) which were found to be very good insulin sensitizers in vivo. Selected compounds also reduced plasma triglyceride and elevated plasma HDL in hApoA1 mouse model.[135]

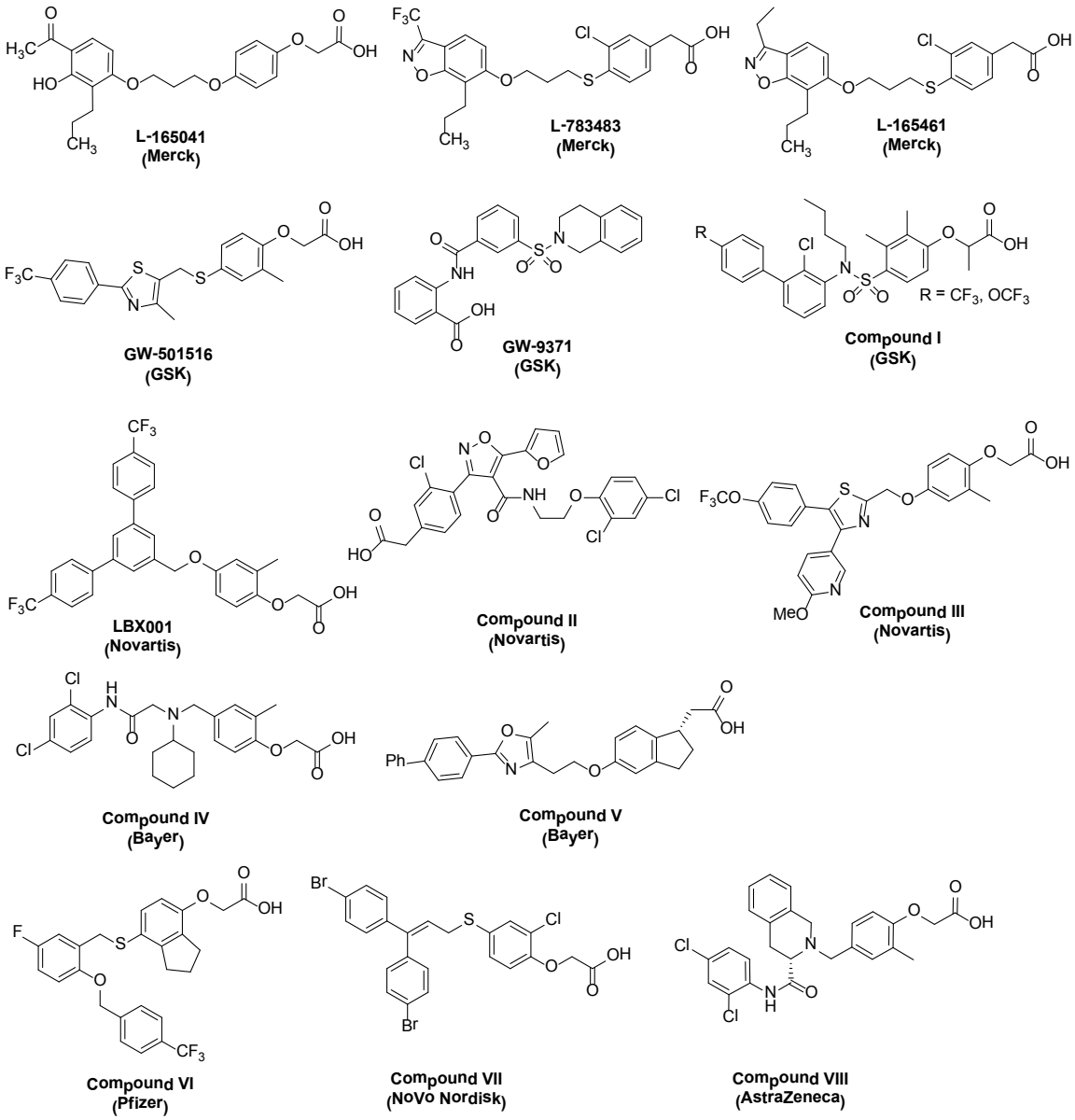


Figure 8: Chemical structures of PPARδ agonists

Simultaneously Pfizer reported the compounds with similar type of scaffold (e.g. Compound VI) possessing 1000 fold PPARδ selectivity over PPARα.[136] At

the same time scientists at NoVo Nordisk identified novel PPARδ partial agonist with full efficacy on lipid metabolism.[137] Chronic treatment of high fat fed ApoB100/CETP-Tgn mice with this compound corrected the plasma lipid parameters and improved insulin sensitivity. More recently AstraZeneca has also reported isoindoline and tetrahydroisoquinoline derivatives as potent and selective PPARδ agonists.[138] MBX-8025 (Metabolex) is the most recent compound which is under Phase II clinical evaluation. Recently it has been reported that MBX-8025 is very effective in atherogenic dyslipidemia.[139]

1.6.2.2 PPARγ/δ dual agonists

Based upon the central roles that both PPARγ and PPARδ play in lipid metabolism, PPARγ/δ dual agonists are assumed as a beneficial combination therapy for type 2 diabetes. Such a combination is predicted to effectively lower glucose and improve insulin sensitivity while simultaneously improving the dyslipidemia common in diabetic patients.[140] The beneficial effects on lipid homeostasis and ability to stimulate reverse cholesterol transport are anticipated to significantly impede the progression of atherosclerosis which should contribute to lowering the mortality rates of type 2 diabetic patients. Furthermore, the propensity of PPARδ activation to improve insulin sensitivity and increase fatty acid oxidation suggests that a dual PPARγ/δ agonist could attenuate the undesired weight gain realized with the selective PPARγ agonists.

Reports of PPARγ/δ dual agonists have been limited. The only PPARγ/δ dual agonist in phase-I clinical trial is DB959 which is developed by Dara Biosciences for the treatment of type 2 diabetes but no activity data has been reported. GlaxoSmithKline has reported a compound as a PPARγ/δ dual agonist with potent anti-hiperglycemic as well as anti-hyperlipidemic effects in animal models.[141] More recently Eli Lilly and Co. has described a dual PPAR$\gamma/\beta(\delta)$ agonist with approximately 17-fold greater functional PPAR$\beta(\delta)$ potency in cell based transactivation assays compared to PPARγ.[142] This dual agonist improves

insulin sensitivity and reverses hyperglycemia with minimized weight gain in preclinical models of type 2 diabetes.

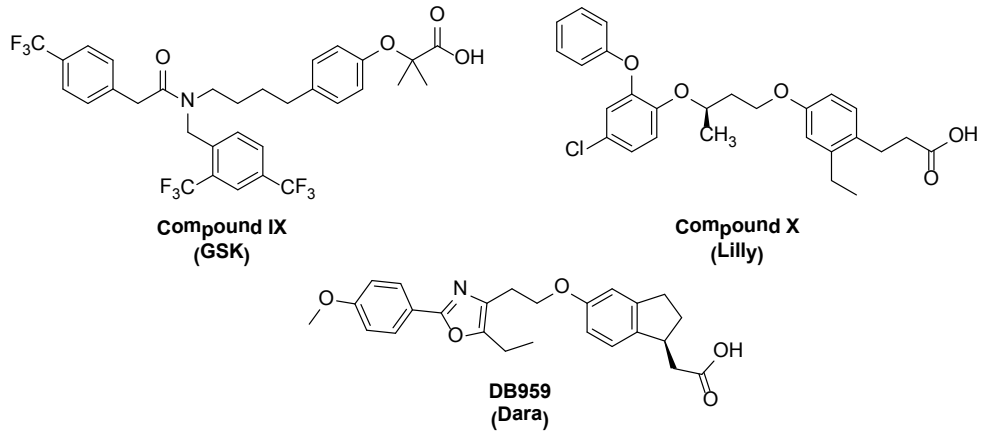


Figure 9: PPARγ/δ dual agonists

1.6.2.3 PPARα/γ/δ Pan agonists

The first PPARα/γ/δ pan agonists from Merck[128,143] and GlaxoSmith Kline[141] (Figure 10) have now been reported. The Merck compound at a 30 mg/kg/day dose for 11 days showed glycemic and triglyceride lowering efficacy in *db/db* mice equal to rosiglitazone, but showed less efficacy in the Zucker Diabetic Fatty rat. Similarly treatment of male *db/db* mice with the Novo-Nordisk compound (Figure 10) (dosed at 1 mg/kg/day) for 7 days resulted in significant reductions in blood glucose (47%) and plasma insulin (71%). In comparison, treatment of *db/db* mice with the PPARα/γ dual agonist ragaglitazar under the same conditions (but at a 3 mg/kg/day dose) resulted in a 51% decrease in blood glucose and a 79% decrease in plasma insulin. Further studies are needed to determine any added advantage to the use of PPARα/γ/δ pan agonists versus PPARα/γ dual agonists for the treatment of various metabolic diseases. Later on GlaxoSmithKline reported Sodelglitazar[144] a potent PPAR-pan agonist with high efficacy. Unfortunately this compound was terminated in Phase II clinical studies due to some safety related issues. More recently TIPP-703[145] (Figure 10) was

developed by the scientists from the University of Tokyo but further development is not reported. Indelglitazar[146] the most recent compound of this class which reached in advanced clinical trial was reported by Plexxikon but recent development is undisclosed.

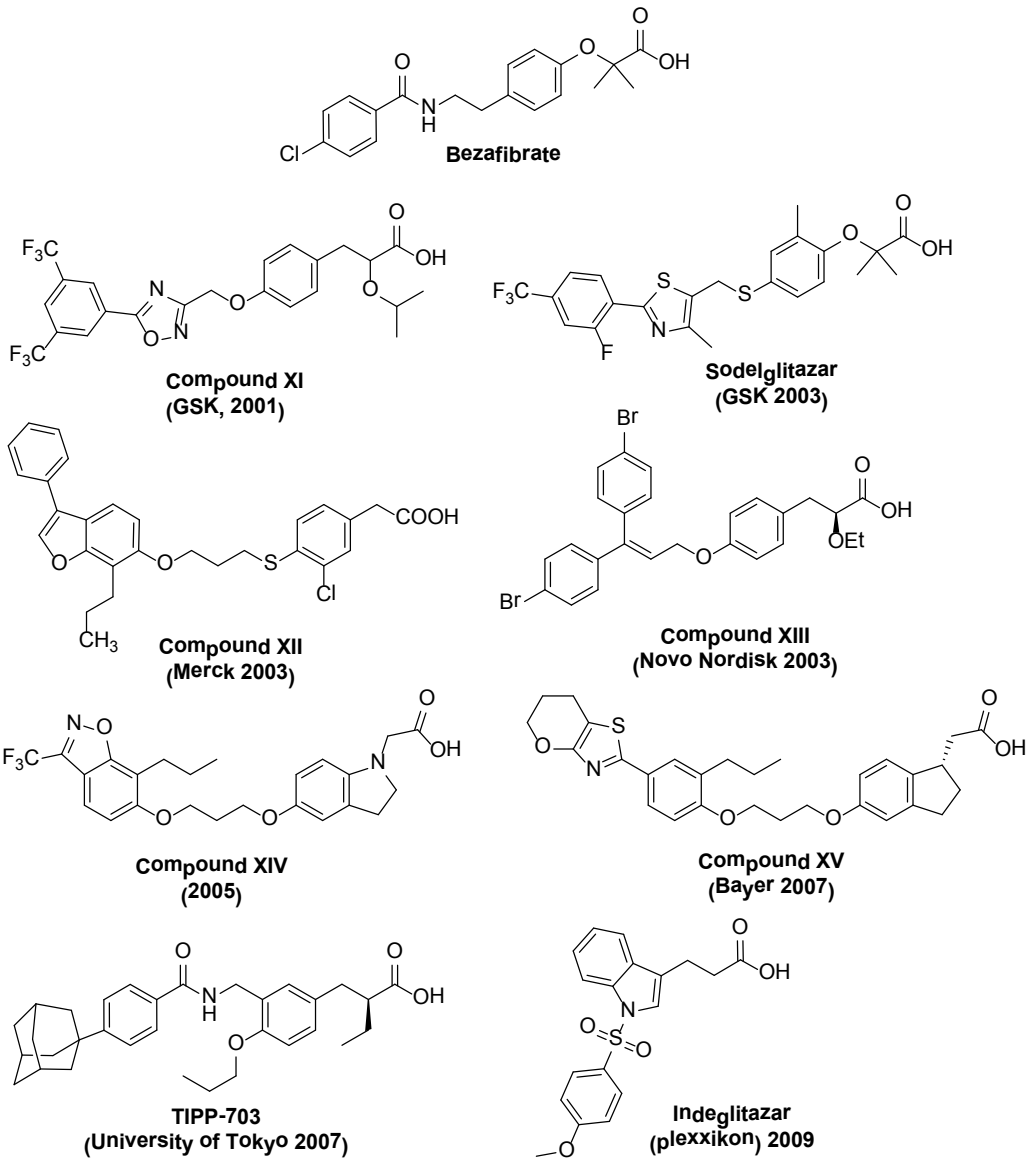


Figure 10: PPAR-pan agonists

1.6.2.4 Selective PPAR modulators (sPPARMs)

Although showing very good clinical and preclinical efficacy, most of the potent PPAR selective agonists and dual agonists were dicontinued due to adverse effects observed at clinical and preclinical stages. Currently the research toward the development of safer PPAR agonist is focused on sPPARM. The idea behind the concept of sPPARM is that a ligand binds to the nuclear receptor and changes a conformation of the receptor in such a way that the heterodimer allow only the selected co-activator to bind with it and the gene transcription pursue in a selective manner. It is believed that unlike full agonists these kind of modulators will minimize the unwanted effects and show only desired efficacy.

Compared to other subtypes selective modulation of PPARγ are studied more extensively to overcome safety issues shown by PPARγ full agonists. GW0072 (Figure 11) was the first compound reported as selective PPARγ modulator (sPPARγM)[147] and after that so many compounds are reported as a sPPARγM till date. FK-614 (Figure 11) was another compound which was reported as sPPARγM. This compound is a moderate (EC_{50} = 200 nM) activator of PPARγ. FK-614 demonstrates the antidiabetic activity in *db/*db mice and *ob/*ob mice similar to full agonist.[148] Further development of this molecule was discontinued after completion of phase-II clinical trial due to lake of sufficient differentiation of safety and efficacy profile from existing PPARγ agonists. Then after the scientists form Roche and Dr. Reddy's Laboratories reported compounds PA-082[149] and PAT5A[150] respectively as sPPARγM. The clinical development of these compounds are undisclosed. Simultaneously Merck also reported indole based compound nTZDpa (Figure 11) as a sPPARγM.[151] This compound was found effective in reducing hyperglycemia as well as hyperinsulinemia in fat-fed C57BL/6J mice. During the animal experiments nTZDpa did not show body weight gain and adipose depot size as shown by full agonists. Due to the poor pharmacokinetic profile of nTZDpa Merck then

alternatively developed compound MK-0533[152] (Figure 3) with improved pharmacokinetic profile but unfortunately this compound was terminated from the phase II clinical study because of lake of superiority. Recently the scientists from Amgen reported PPARγM INT-131 which enhances insulin sensitivity.[153] Further development of this compound is undisclosed.

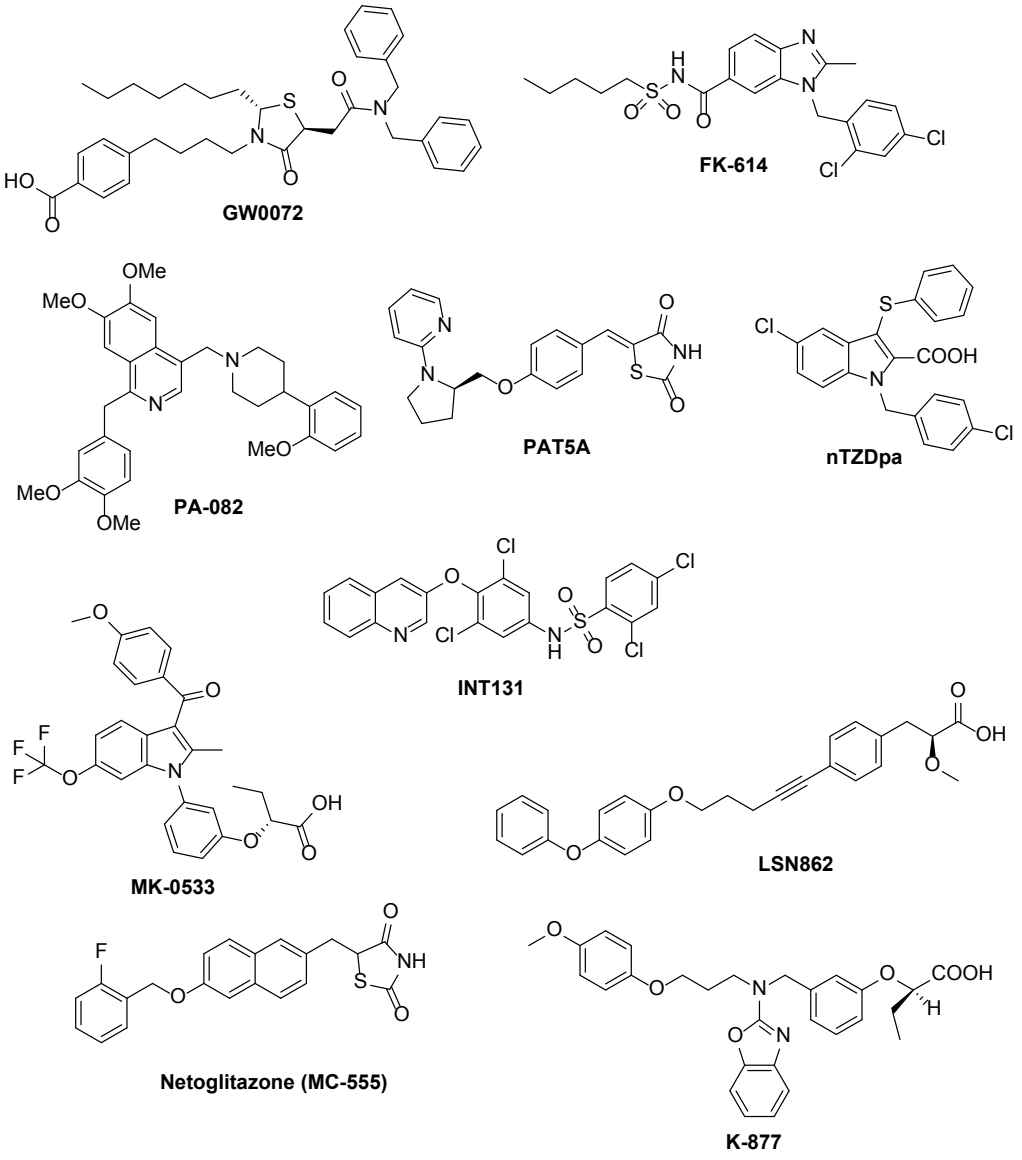


Figure 11: Selective PPAR modulators

A part from PPARγM several compounds are reported as PPARα/γ dual modulators. Netoglitazone and LSN862 (Figure 11) are among this class of compounds. Netoglitazone is a potent PPARα agonist with PPARγM properties whereas LSN862 is a partial agonist of both PPARα and PPARγ. Interestingly more reports are not found for selective PPARα modulators (PPARαM). K-877[154] is the first reported PPARαM with high potency and selectivity. This compound is under Phase III clinical studies for the treatment of atherogenic dyslipidemia.[155]

Considering the distinct therapeutic effects and safety issues associated with PPAR selective and dual agonists, the development of a PPAR agonist with better biological profile and safety window is a challenge among the drug discovery scientists around the world. The ability of each PPAR subtype to regulate distinct metabolic pathways has led to a new approach of activating all the tree PPAR subtypes with a single molecule (PPAR-pan agonism). The ultimate goal of the combination agonist strategy is to provide maximal efficacy while minimizing undesired adverse side effects. Targeting PPARδ for the treatment of metabolic syndrome is an emerging concept. Recently it has been identified that PPARδ is involved in various aspects of lipid metabolism, cell differentiation and inflammation.

These facts led us to target for an equipotent PPAR-pan agonist with moderate potency and a selective PPARδ agonist as the medical need for metabolic disorders has still remained unmet.