Introduction

"Chance favors the prepared mind."

Louis Pasteur

1. INTRODUCTION

1.1. Metabolic Syndrome

Metabolic syndrome (MS) refers to a cluster of metabolic disturbances, such as insulin resistance, hyperinsulinaemia, hyperglycemia, dyslipidemia, high blood pressure and obesity.

Obesity, a diet-related disease is a major risk factor for developing the cluster of these metabolic diseases while cardiovascular disease and type 2 diabetes are the major manifestations of metabolic syndrome. The incidence of metabolic syndrome has reached global epidemic proportions [**1**]. A variety of names like pluri-metabolic syndrome, insulin resistance syndrome, syndrome X, dysmetabolic syndrome are also coined to describe this cluster of metabolic conditions.

1.1.1. History of metabolic syndrome

The description of metabolic syndrome as a syndrome involving hypertension, hyperglycemia and hyperuricaemia dates back to 1923 [**2**]. Obesity as a component of metabolic syndrome was reported in 1947 [**3**] and subsequently described as a syndrome, which comprised hypertension, hyperglycemia, and obesity in 1965 [**4**]. After a long gap, in 1988 a cluster of risk factors for diabetes and cardiovascular diseases were described and named it Syndrome X [**5**]. The introduction of concept of insulin resistance and its inclusion in the cluster of metabolic syndrome was the most significant move in this area. The syndrome was renamed as 'The Deadly Quartet' [**6**] and The Insulin Resistance Syndrome [**7**] in 1989, and in 1992 respectively. Today the well established term metabolic syndrome remains the most usual description of this cluster of metabolic abnormalities.

1.1.2. Definitions of the metabolic syndrome

A number of expert groups have attempted to develop a unifying definition for the metabolic syndrome. Important definitions have been produced by The

World Health Organization (WHO), The European Group for study of Insulin Resistance (EGIR), and The National Cholesterol Education Program-Third Adult Treatment Panel (NCEP ATP III).

1.1.2.1. The WHO definition [8]

This definition was written in 1999 by diabetologists considering insulin resistance as one of the major underlying contributors to the metabolic syndrome. In addition to insulin resistance or related complications like impaired glucose tolerance or diabetes, presence of any two or more additional risk factors including hypertension, obesity, raised triglycerides (TG), or low levels of high density lipoprotein (HDL) cholesterol.

1.1.2.2. The European Group for study of Insulin Resistance (EGIR) definition [9]

According to WHO definition, diabetes is the primary criteria for metabolic syndrome. Hence the EGIR in 1999 proposed a modified version to be used in nondiabetic subjects only for the reasons, that these patients should already be in the health care system and long standing diabetic patients have lower insulin levels, which do not reflect the level of insulin resistance, a key element of this definition. The group was interested in insulin resistance and proposed the use of fasting insulin levels to estimate insulin resistance and impaired fasting glucose (IFG) as a substitute for impaired glucose tolerance (IGT).

1.1.2.3. The 2001 ATP III definition [10]

This definition was designed by cardiologists and lipidologists in the United States in 2001 to facilitate diagnosis in clinical practice and does not include a measurement of insulin resistance. The ATP III guidelines state that metabolic syndrome may be diagnosed when a patient has three or more of five clinically identifiable risk factors. These include abdominal obesity, high TG level, low HDL cholesterol level, hypertension, and an elevated fasting glucose level.

Though most of the criteria are similar according to the above definitions, the threshold limits of individual components vary (**Table 1**) and this led to confusion in publishing many studies and estimation of the prevalence of the syndrome. To address this issue the International Diabetes Federation (IDF) held a workshop in 2004 and with the consensus of participants defined the general features of metabolic syndrome as abnormal body fat distribution, insulin resistance, atherogenic dyslipidemia, elevated blood pressure, *pro*-inflammatory state and *pro*-thrombotic state.

Table 1

Comparison of the different definitions of metabolic syndrome

WHO definition	EGIR definition	ATP III definition
Diabetes, Impaired glucose tolerance or insulin resistance and two or more of the following complications	Insulin resistance, hyper insulinemia and two or more of the following complications	Three or more of the following complications
Central obesity waist to hip ratio >0.90 for males and >0.85 for females and/or BMI >30 kg/m^2	**Central obesity** waist circumference ≥94 cm for males and ≥80 cm for females.	**Central obesity** Waist circumference >102 cm for males and >88 cm for females.
Plasma TG ≥1.7 mmol/L or 50 mg/dL	**Plasma TG** ≥ 2.0 mmol/L	**Plasma TG** ≥1.7 mmol/L or 150 mg/dL
HDL cholesterol <0.9 mmol/L or 35 mg/dL for males and <1.0 mmol/L or 39 mg/dL for females.	**HDL cholesterol** <1.0 mmol/L	**HDL cholesterol** <1.03 mmol/L or 40 mg/dL for males and <1.29 mmol/L or 50 mg/dL for females.
Microalbuminuria urinary albumin excretion rate ≥ 20 g/min or albumin:creatinine ratio 30 mg/g)	**Fasting glucose** ≥6.1/7.8 mmol/L but <7.0/11.1 mmol/L	**Fasting glucose** ≥110 mg/dL (6.1 mmol/L)
Arterial pressure ≥140/90 mmHg	**Arterial pressure** ≥140/90 mmHg	**Arterial pressure** ≥130/85 mmHg

1.1.3. Prevalence of metabolic syndrome

Prevalence data for the metabolic syndrome in different countries and in different ethnic groups clearly show that the syndrome is a severe problem everywhere in the world and the number of people affected continues to grow.

Table 2

World MS Market by sales in 2003 & 2008

Application	2003 ($)	2008 ($)
Hypertension	21.0	31.0
Dyslipidemia	20.0	44.0
Diabetes	5.0	7.5
Obesity	3.0	6.0
Diabetes diagnostics	4.2	7.2
Lipid diagnostics	0.5	2.0
Total:	**54.3**	**97.7**

Patients with metabolic syndrome are at a high risk of developing cardiovascular disease and/or type 2 diabetes. The market size of drugs and health care products related to metabolic syndrome stands at $97.7 billion (**Table 2**) in 2008 [**11**] which shows the alarming prevalence of the syndrome. Even more worrying factors include the undiagnosed population and its occurrence in younger population.

1.1.4. Management and Treatment of the syndrome [12]

As the syndrome does not have a known cause, the cause could not be treated. To reduce the severity of all of the risk factors including dyslipidaemia, diabetes, elevated blood pressure, *pro*-thrombotic and *pro*-inflammatory state, life style modification including loss of weight, increase in physical activity, a healthy diet are proven to be effective in the management of metabolic syndrome. However, if the risk factors are severe one must be subjected to drug

therapy. Since mono therapy is unavailable for the treatment of metabolic syndrome, patient need to be treated for different elements of the syndrome separately with several drug combinations (**Table 3**).

Dyslipidaemia is better treated currently by administering statins (*e.g.* Atorvastatin, Rosuvastatin, Simvastatin, *etc.*). These drugs lower cholesterol by inhibiting the enzyme HMG-CoA reductase, which is the rate-limiting enzyme of the mevalonate pathway of cholesterol synthesis. Inhibition of this enzyme in the liver results in decreased cholesterol synthesis as well as increased synthesis of low-density lipoprotein (LDL) receptors, resulting in an increased clearance of LDL from the bloodstream. However, they are not effective in reducing triglycerides and raising high-density lipoprotein cholesterol (HDLC). Fibrates (peroxisome proliferator activated receptorα (PPARα) activators (*e.g.* Fenofibrate, Bezafibrate *etc.*) remain the choice of treatment for dyslipidaemia and are effective in reducing triglycerides, increasing HDL-cholesterol and lowering LDL-cholesterol. But these are of poor efficacy and thus need high doses to show therapeutic effect. Niacin (vitamin B_3 or nicotinic acid) has been proven to reduce total cholesterol, triglycerides, very-low-density lipoprotein (VLDL), and low-density lipoprotein (LDL) and increase high-density lipoprotein (HDL). Pharmacological doses of Niacin often lead to side-effects that can include dermatological complaints such as skin flushing, itching, dry skin, and skin rashes.

Currently marketed *anti*-obesity drugs operate through 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 (*e.g.*, Orlistat which blocks fat breakdown and thereby prevents fat absorption). Because of potential side effects, it is recommended that *anti*-obesity drugs only be prescribed for obesity where it is hoped that the benefits of the treatment outweigh its risks.

In atherosclerosis and cardiovascular segment, Clopidogrel, a platelet aggregation inhibitor is used in case of cardiovascular events to prevent the progression of arterial clot formation. Aspirin is also proven to reduce the risk of clot formation and its combination with clopidogrel is widely used in patients with

cardiovascular risk. Recently Factor Xa inhibitor Rivaroxaban and thrombin inhibitor Dabigataran etaxilate have been approved for the treatment of thrombotic disorders.

Table 3

Current marketed drugs for the treatment of metabolic syndrome

Indication	Currently available therapy
Dyslipidaemia	Statins
	Niacin
	Fibrates
Hyperglycemia & Insulin resistance	Sulfonylureas
	Meglitinides
	α-glucosidase inhibitors
	Dipeptidyl peptidase-4 (DPP-4) inhibitors
	GLP agonists
	Glitazones
	Biguanides
Elevated blodd pressure	ACE-inhibitors
	β-blockers, α-blockers
	Calcium channel blockers
	Angiotensin II receptor antagonists
CVD (*pro*-thrombotic state)	Clopidogrel
	Aspirin
	Rivaroxaban and Dabigataran etaxilate
Obesity	Sibutramine
	Orlistat

Sulfonylureas (*e.g.* Glipizide, Glyburide, Glimepiride, Gliclazide) were the first widely used oral hypoglycemic medications. They are insulin secretagogues, triggering insulin release by direct action on the K_{ATP} channel of the pancreatic β-cells. They can be safely used with Metformin or glitazones. The primary side effect is hypoglycemia. Meglitinides (*e.g.* Repaglinide, Nateglinide) help the pancreas to produce insulin by closing the potassium channels of the pancreatic β-cells and opening the calcium channels resulting in enhancement of insulin

secretion. They are often called short-acting secretagogues and are taken with or shortly before meals to boost the insulin response to each meal. If a meal is skipped, the medication is also skipped. Adverse reactions include weight gain and hypoglycemia. Biguanides reduce hepatic glucose output and increase uptake of glucose by the periphery, including skeletal muscle. Although it must be used with caution in patients with impaired liver or kidney function, metformin, a biguanide, has become the most commonly used agent for type 2 diabetes in children and teenagers. Amongst common diabetic drugs, Metformin is the only widely used oral drug that does not cause weight gain. Metformin is usually the first-line medication prescribed for treatment of type 2 diabetes. It is generally prescribed at initial diagnosis in conjunction with exercise and weight loss as opposed to in the past, where Metformin was prescribed after diet and exercise had failed. Thiazolidinediones (TZDs) or glitazones (*e.g.* Rosiglitazone and Pioglitazone) are peroxisome proliferator activated receptorγ (PPARγ) agonists and proven to be effective in improving insulin sensitization and exert *anti*-hyperglycemic and hypolipidemic effects. However the treatment with these drugs is associated with adverse effects like edema and weight gain. α-glucosidase inhibitors (*e.g.* Miglitol, Acarbose) do not have a direct effect on insulin secretion or sensitivity. These agents slow the digestion of starch in the small intestine, so that glucose from the starch of a meal enters the bloodstream more slowly, and can be matched more effectively by an impaired insulin response or sensitivity. However, these medications are rarely prescribed because of the severity of their side effects (flatulence and bloating). Dipeptidyl peptidase-4 (DPP-4) inhibitors increase blood concentration of the incretin GLP-1 (glucagon-like peptide-1) by inhibiting its degradation by dipeptidyl peptidase-4 (DPP-4). This class of agents were very recently developed and Vidagliptin and Sitagliptin are approved.

Incretins are insulin secretagogues. The two main candidate molecules that fulfill criteria for being an incretin are Glucagon-like peptide-1 (GLP-1) and Gastric inhibitory peptide (glucose-dependent insulinotropic peptide or GIP). Both GLP-1 and GIP are rapidly inactivated by the enzyme dipeptidyl peptidase-4

(DPP-4). GLP agonists (*e.g.* Exendin) bind to a membrane GLP receptor. As a consequence of this, insulin release from the pancreatic β-cells is increased. These agents may also cause a decrease in gastric motility, responsible for the common side effect of nausea.

A variety of drugs are currently in use for the treatment of hypertension and the choice is made based on the age, compatability of these drugs with other agents when needed to be used in combination. Diuretics (*e.g.* Indapamide, Chlorthalidone, Metolazone, Amiloride) though used as first line therapy can not be classified as *anti*-hypertensive drugs since they help the kidneys to eliminate excess salt and water from the body's tissues and blood. 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) work by antagonizing the activation of angiotensin receptors. To treat the abnormalities of the metabolic syndrome, the first step is lifestyle modification **[13]**. Drug treatment should be used for the specific abnormalities according to current guidelines, and a more aggressive approach may be appropriate when more than one abnormalities are present.

1.2. Peroxisome proliferator activated receptors (PPARs) and metabolic syndrome

Since diabetes and cardiovascular diseases (CVD) are the main risk factors of metabolic syndrome, synchronized therapies, which concurrently control diabetes and inhibit progression of cardiovascular complications will be a fascinating therapeutic option in the treatment of metabolic syndrome. In order to develop such new therapeutic agents for the effective treatment of metabolic

syndrome, identification of molecular mechanisms of the transducer proteins involved in metabolic and anabolic pathways is crucial. Peroxisome proliferator activated receptors (PPARs) are transducer proteins belonging to the nuclear receptor superfamily. These receptors were identified in the 1990s in rodents and named after their property of peroxisome proliferation.

Three distinct receptor subtypes, PPARα, PPARγ and PPARδ or β have been identified 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.

1.2.1. Protein Structure of PPARs

All the three PPAR isoforms possess similar structural and functional features (**Figure 1**). Principally, four functional domains have been identified, called A/B, C, D and E/F (**Figure 1**). The *N*-terminal A/B domain contains a ligand-independent activation function 1 (AF-1) responsible for the phosphorylation of PPAR. 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 [**14**]. The D site is a docking domain for cofactors. The E domain or ligand-binding domain (LBD) is responsible for ligand specificity and activation of PPAR binding to the PPRE, which increases the expression of targeted genes [**15**]. 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 [**15**].

Figure 1. Schematic representation of the functional domains of PPARs.

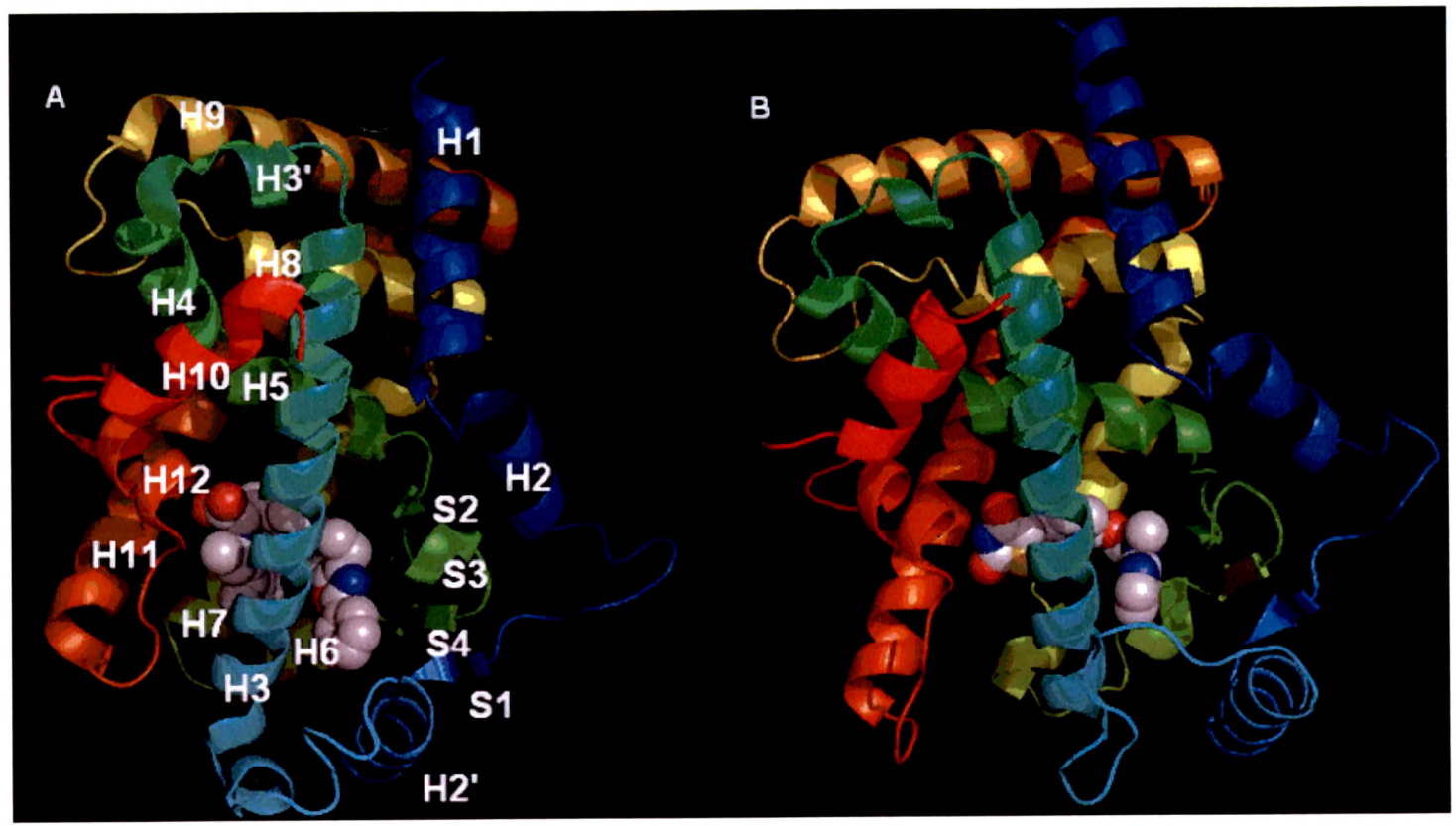


Figure 2. (A). PPARα cocrystalized with GW-409544 (PDB entry no: IK7L); **(B).** PPARγ cocrystalized with Rosiglitazone (PDB entry no: 2PRG)

Figure 2 shows the crystal structures of PPAR isotypes, along with the secondary structure elements; helices H1 to H12 and *ß*-sheets S1 to S4. 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) [**16,17**]. The cavity is Y-shaped and consists 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, and arm II, situated between helix H3 and the *ß*-sheet. Each arm is approximately 12 Å in length. Arm I is mainly hydrophobic, which is not surprising given the hydrophobic nature of the natural ligands. Amino acid residues that are in proximity to binding site form hydrogen-bonding network involving the carboxylate group of natural ligands like fatty acids and Eicosanoic acids [**18,19**] and carboxylic or thiazolidinedione (TZD) of synthetic ligands [**20-22**]. 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 network of protein–ligand interactions, that involves the surrounding amino acid residues helps in holding the AF2-helix in the active conformation, thus promoting the binding of co-activator proteins [**19,21**]. 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 [**19**] or Ragaglitazar [**23**], that the hydrophobic tail is in equilibrium between different positions.

1.2.2. Mechanism of gene transcription

The gene transcription mechanism is identical in all PPAR subtypes and the process of transcription begins with the binding of ligand (endogenous or exogenous) to the PPAR receptor. Ligand-bound PPAR heterodimerises with RXR [**24**]. This heterodimer complex binds to peroxisome proliferator response

element (PPRE) located in the regulatory (promoter) region of target genes. PPRE consists of direct repeat (DR)-1 elements of two hexanucleotides with the AGGTCA sequence separated by a single nucleotide spacer [**25**, **26**]. 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 those mediate the ability of nuclear receptors to initiate or suppress the transcription process. They interact with nuclear receptors in a ligand-dependent manner [**26**]. 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) [**27**-**29**]. Alternatively, coactivators such as steroid receptor co-activator (SRC)-1 and the PPAR binding protein (PBP) with histone acetylase activity [**30**, **31**] 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.

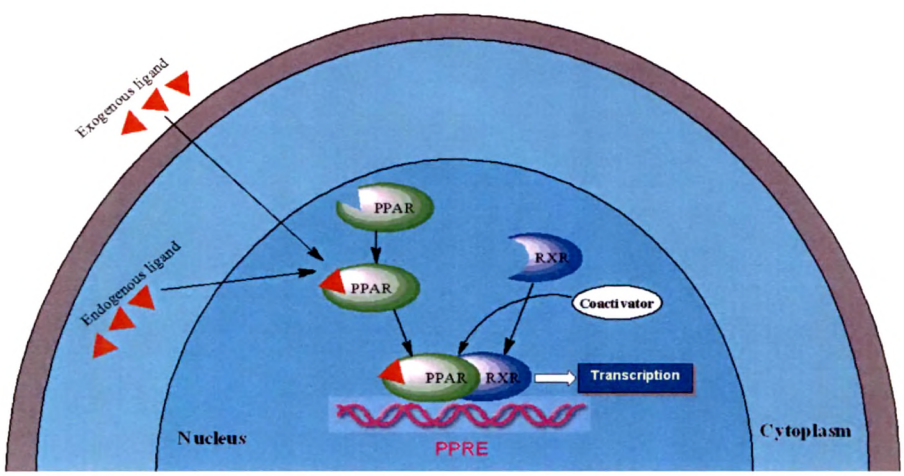


Figure 3. Mechanism of gene transcription

1.2.3. PPARα

PPARα was first cloned from a mouse liver [**32**] and since then it has been cloned from most of rodent and mammalian species [**33-36**] including human [**37**]. The human PPARα gene has been mapped to chromosome 22q12-q13.1 by somatic cell hybridization and linkage analysis [**36**]. Analysis of PPARα tissue distribution in rodents and humans revealed high levels of expression in metabolically active tissues, such as liver, heart, kidney, and muscle [**38,39**]. Although the DNA binding domains (DBDs) are identical across 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 an 85% identity at the nucleotide level and 91% identity at the amino acid level. Activation of PPARα causes the proliferation of peroxisomes and hepatomegaly in rodents [**32**]. 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 [**40**]. 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 [**41**]. 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.

1.2.3.1. Biological Functions of PPARα

1.2.3.1.1. Dyslipidemia

Dyslipidemia in the metabolic syndrome is characterized by elevated triglyceride levels, reduced HDL cholesterol levels, increased LDL cholesterol levels and elevated levels of plasma free fatty acids [**42,43**]. Dyslipidemia leads to obesity, diabetes and CVD [**44-46**]. PPARα plays a crucial role in

dyslipidaemia as it regulates the expression of several genes involved in the lipid and lipoprotein metabolism. PPARα upregulates the genes those 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 [**47-50**]. 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 PPARα activation is mediated through upregulation of lipoprotein lipase [**51**]. 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 [**47**]. All together, PPARα activation contributes to a less atherogenic lipid profile and favors a healthier lipid homeostasis. Thus, drugs with potent activity on human PPARα may be useful adjuncts to current therapies for the treatment of dyslipidemia in patients at risk of cardiovascular disease.

1.2.3.1.2. Obesity/Diabetes

Obesity is a major risk factor for the development of metabolic syndrome. The major evidence showing the role of PPARα in obesity is the observation of increased accumulation of body fat in *PPARα* -/- mice [**52**, **53**]. PPARα activation has been reported to reduce weight gain with no effect on food intake [**54-57**], suggesting the role of PPARα in the regulation of genes coded for uncoupling proteins (UCP) involved in fatty acid catabolism or energy expenditure [**58**]. Uncoupling proteins (UCP) 1-3 are mitochondrial membrane transporters those allow conversion of fuel into heat by uncoupling substrate oxidation from adinosine triphosphate (ATP) synthesis [**59**]. 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 [**60**]. An interesting observation that the mRNA levels of UCP3 are reduced in type 2 diabetics, [**61**] and the upregulation of this UCP3 by the activation of PPARα revels the role of PPARα in diabetes [**62**].

Table 4

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

1.2.3.1.3. Atherosclerosis and inflammation

Inflammation has been shown to be one of the underlying causes of atherosclerosis and insulin resistance [**63**,**64**]. Fat cells secrete a variety of *pro*-inflammatory proteins, such as matrix metalloproteinase-9 (MMP9), tumor necrosis factor-α (TNFα) and interleukin-6 (IL6) [**65**]. These secreted proteins contribute to a state of low-grade systemic inflammation. The increased level of

pro-inflammatory cytokines, together with elevated FFA levels in the plasma of obese subjects stimulate the liver to secrete C-reactive protein (CRP) [**66,67**], an acute phase protein involved in various inflammatory processes. PPARα represses inflammation by a physical interaction and forming inactive complexes with *pro*-inflammatory transcription factors, nuclear factor κB (NFκB) and activating protein 1 (AP1) [**68,69**]. These transcription factors normally induce the transcription of *pro*-inflammatory genes, such as cytokines (IL6, TNFα), chemokines (IL8, MCP1), and cellular adhesion molecules (VCAM1, ICAM1) [**70**]. Another way in which PPAR control inflammation is indirectly by influencing transcription of genes that inhibit NFκB signaling, such as IκBα and IKK.

1.2.4. PPARγ

PPARγ is the most extensively studied of the three PPAR subtypes to date. The receptor has been cloned from a number of species, including salmon [**71**], mice [**72**], hamsters [**73**], frogs [**33**], pigs [**74**], rhesus monkeys [**75**] and humans [**76-78**]. The human PPARγprotein 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 reflect the pivotal role that PPARγplays as a 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 [**76,79**].

1. 2. 4.1. Biological Functions of PPARγ

1. 2. 4.1.1. Adipogenesis

PPARγ plays a central role in the process of adipogenesis and is absolutely required for the formation of adipose tissue [**80**]. The *pro*-adipogenic role of PPARγ has been demonstrated by the following observations;

- PPARγ+/– mice are characterized by decreased adipose tissue mass [**81**].

- Injection of PPARγ –/– embryonic mouse cells into wild type blastocytes produces chimeric mice in which adipose tissue is composed exclusively of PPARγ +/+ cells, demonstrating that PPARγ is necessary to ensure development of this tissue [**82**].

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 [**82**].

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 [**83**]. For example, a rare Pro115Gln mutation in the NH_2-terminal ligand-independent activation domain of PPARγ was found in four very obese individuals [**84**]. 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 [**85**]. 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 [**86**].

1. 2. 4.1.2. Insulin Sensitivity and Dyslipidaemia

As described earlier insulin resistance is a central component of metabolic syndrome and has an important role in the pathogenesis of type 2 diabetes mellitus. PPARγ exerts *anti*-diabetic and *anti*-dyslipidemic 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 [**87**], PEPCK [**88**], acyl-CoA synthase [**50**], and lipoprotein lipase (LPL) [**89**]. PPARγ has also been shown to control the expression of FATP-1 [**90**] and CD36 [**91**], 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 [**92**]. PPARγ downregulates leptin, an adipokine that inhibits feeding and augments catabolic lipid metabolism [**93,94**]. Activation of PPARγ in adipose tissue may impact whole-body insulin sensitivity by inhibiting the expression and/or secretion of tumour necrosis factor α (TNFα) and plasminogen activator inhibitor 1 (PAI1), which promote insulin resistance [**95,96**]. PPARγ agonists stimulate the production of adiponectin, a direct PPARγ target gene in adipocytes [**97**], 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 [**98,99**]. Patients with type 2 diabetes have reduced plasma levels of Acrp30 [**100**] and PPARγ activation increases the plasma Acrp30 levels [**101**]. Overexpression of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), an enzyme that generates the active cortisol from the inactive precursor cortisone in adipocytes causes insulin resistance [**102**]. PPARγ downregulates 11β-HSD1 [**103**] and promotes insulin sensitivity, either by reducing glucocorticoid induced gene expression in the adipocyte or by reducing adipocyte secretion of glucocorticoids.

The changes in glucose homeostasis can partly be attributed to a direct action of PPARγ activation on insulin-stimulated glucose disposal. PPARγ activation increases the expression and translocation (to the cell surface) of the glucose transporters GLUT1 and GLUT4, thus enhancing glucose uptake in

adipocytes & 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**] which play a positive role in the insulin signaling. 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 PPARγagonists [**106**].

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 [**107**]. PPARγ activation upregulates adiponectin [**108**] which potentiates insulin sensitivity in liver [**99**] and skeletal muscle [**109**]. PPARγ activation increases glucose oxidation by down regulating pyruvate dehydrogenase kinase 4 (PDK-4) in skeletal muscle [**110**]. The effects of PPARγ activation on muscle and liver involve enhanced insulin-mediated adipose tissue uptake, storage of free fatty acids [**111**] 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 [**112**].

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.

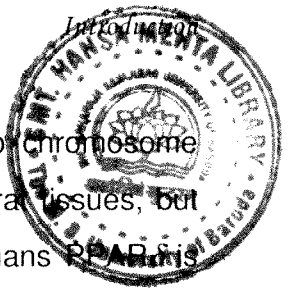
1. 2. 4.1.3. Cardiovascular and Inflammation

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 [**113**]. Activation of PPARγ induces lipid efflux from macrophages and inhibits their transformation into foam cells by upregulating the expression of the ABCA1 cholesterol transporter [**114**] through a transcriptional cascade that involves the oxysterol receptor, LXR-α [**115**]. Activation of PPARγ promotes monocyte differentiation and uptake of oxLDL

through the expression of the scavenger receptor CD36 [**116,117**]. In addition to lipid uptake through CD36, PPARγ activation down regulates class A scavenger receptor (SR-A) expression [**118**] and promotes cholesterol efflux from the macro phage foam cell [**115**]. In addition to their effect on macrophage lipid accumulation, PPARγ activation has been shown to inhibit production of *pro*-inflammatory cytokines (such as TNFα, IL-1 & IL-6) [**119**], the expression of the monocyte chemotactic protein 1 (MCP1/ CCL2) and inflammatory enzymes such as inducible nitric oxide synthase [**118,120**]. Recently it has been reported by our group that activation of PPARPγ by Pioglitazone even at subtherapeutic dose produces *anti*-inflammatory effects *via* suppression of TNFα and IL-6 in rodents [**121**]. PARγ agonists promote plaque stability by reducing the production of metalloproteinases by activated plaque macrophages [**115**]. Activation of PPARγ in vascular cells activate specific transcription factors that contribute to cell growth and movement [**122**] by inhibiting nuclear effects of MAPK signaling. PPARγ activation blocks proliferation [**123**, **124**] and increases apoptosis of VSMCs [**125**] and provides vasoprotection. PPARγ activation reduces the expression of adhesion molecule in endothelial cells and prevents the formation of plaque [**126**]. PPARγ also exhibit *anti*-thrombotic effects by reducing the production of PAI1 (Plasminogen Activator Inhibitor 1) by endothelial cells [**127**]. PPARγ agonists reduce plasma levels of inflammatory biomarkers that are predictive of cardiovascular disease [**128**] and these decreases are observed before changes in metabolic parameters [**129**]. These findings support the direct vascular actions of PPARγ activation.

1.2.5. PPARδ

PPARδ (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* [**33**]. Subsequently, the receptor was cloned from humans [**130**] and mice [**131**]. The human and rodent receptors are about 90% identical in the ligand binding domain (LBD), while the frog receptor shows somewhat lower



sequence identity (72%). 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 [**36**]. In humans PPARδ is present in tissues those control lipid metabolism like liver, intestine, kidney, abdominal adipose and skeletal muscle [**39**].

1.2.5.1. Biological mechanisms of PPARδ

PPARδ regulates lipid and lipoprotein metabolism and its activation raises HDL-C, reduces LDL-C & VLDL-TG and normalizes insulin levels [**132**]. The mechanism by which PPARδ activation influences these metabolic parameters is 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 [**133**,**134**]. 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 [**135**].

In addition to benefits on lipid and lipoprotein metabolism, PPARδ activation reduces the inflammatory response in macrophages and endothelial cells [**136-139**]. 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 [**140-142**]. It is also believed that PPARδ induces fat burning in muscle, which together with an overall improvement in systemic lipid metabolism is responsible for lowering fat overload in insulin-sensitive tissues, thereby reducing insulin resistance. Also, effects of PPARδ agonists on metabolic function are associated with an increase in adiponectin and a decrease in resistin secretion by adipose tissue [**66**]. Furthermore, activation of PPARδ triggers skeletal muscle remodeling towards fibers with high FA oxidative capabilities [**141**,**142**]. 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 [**143**]. 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 [**144**].

Table 5

Pharmacological effects of PPARα and γ activation[a]

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

[a]↓ denotes decrease and ↑ denotes increase

1.2.6. PPAR agonists

In fact the role of PPARα and γ activation in ameliorating hyperglycemia and hyperlipidemia associated with metabolic syndrome 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. There after, 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.

1.2.6.1. PPARα agonists

1.2.6.1.1. Natural ligands

A wide range of saturated and unsaturated fatty acids are shown to bind to PPARα while palmitic acid, linoleic acid and arachidonic acid (**Figure 4**) are among the prominent ones. Many of the fatty acids bind to PPARα with micromolar affinities.

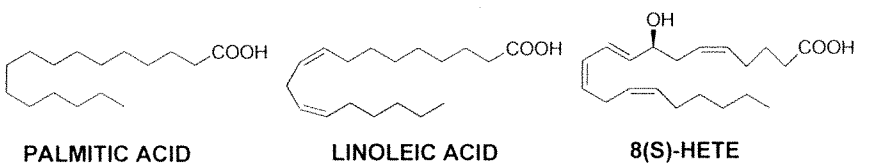


Figure 4. Structures of natural ligands of PPARα

1.2.6.1.2. Synthetic ligands (Figure 5)

Fibrates form a class of drugs which have been used for several years for the treatment of dyslipidemia and they continue to remain the treatment for patients with hypertriglyceridemia. Clofibrate, Fenofibrate and Bezafibrate [**145**] 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 to exert the clinical effects. 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 [**146**] 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.

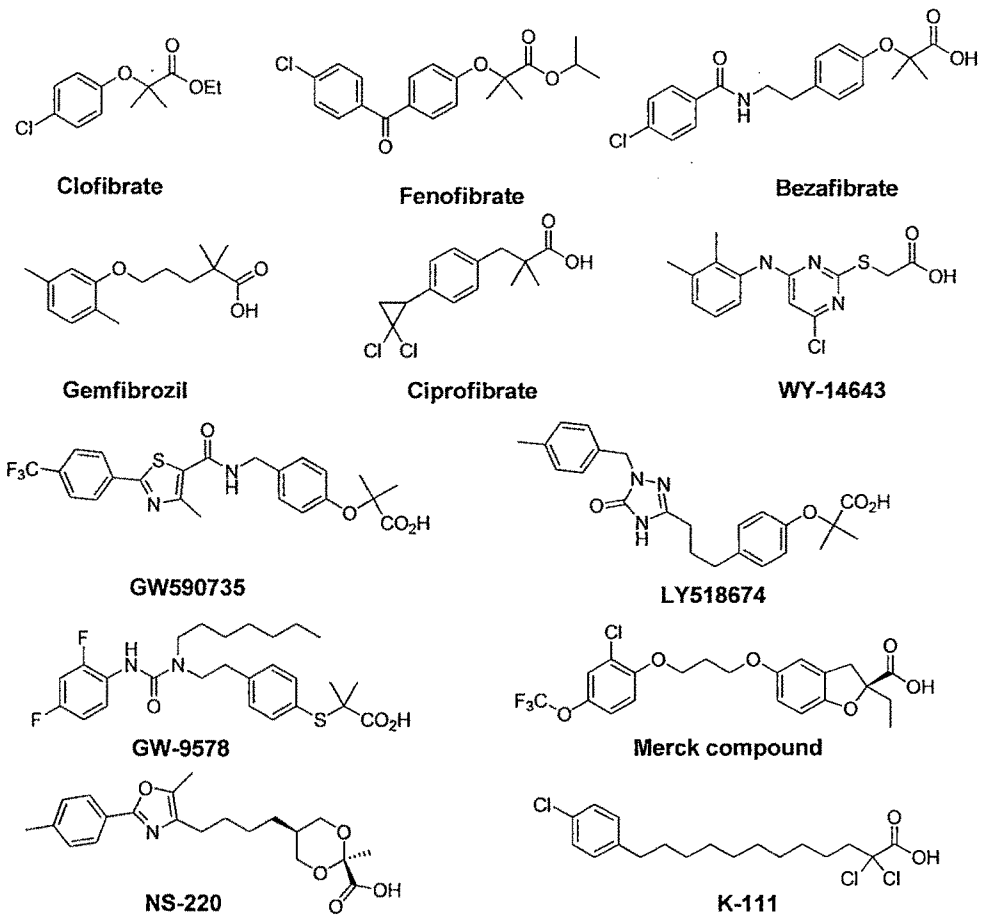


Figure 5. Chemical structures of PPARα agonists

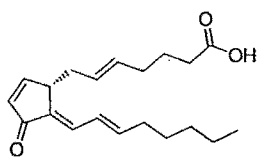
Merck & Co. developed a conformationally constraint 2,3-dihydrobenzofuran-2-carboxylic acid derivative (Merck compound) that displayed high potency and selectivity over other PPAR subtypes [**147**]. Subsequently, Lilly identified a compound LY-518674 [**148**] containing triazolone core in the lipophilic 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 humans and the development was discontinued from phase-II clinical trials. Recently K-111 [**149**] is identified as highly selective PPARα agonist and does not activate γ and δ even at high doses. This compound is currently in phase-II clinical trials. More recently, Nippon developed a compound NS-220 [**150**] as highly potent and selective PPARα agonist. This compound displayed

potent hypolipidemic and *anti*-diabetic effects in animal models. Unfortunately the development of this compound has been terminated from phase-II for unknown reasons. Several pharmaceutical companies are in search of selective PPARα agonists for the treatment of metabolic disorders, as the medical need for the treatment of this complex disease remain highly unmet.

1.2.6.2. PPARγ agonists

1.2.6.2.1. Natural ligands

Polyunsaturated 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(**Figure 6**) [**151**].



15-d-PGJ2

Figure 6. Natural ligand for PPARγ

1.2.6.2.2. Synthetic ligands

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 [**152**]. 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 *anti*-hyperglycemic potency [**153**]. Subsequent optimization provided a novel, potent glucose-lowering agent Ciglitazone (ADD-3878, U-63287) that effectively improved insulin resistance and glucose disposal [**154-156**] but its clinical development was discontinued mainly due to insufficient efficacy. Further investigation of ciglitazone related TZDs culminated in the discovery of Pioglitazone [**157,158**].

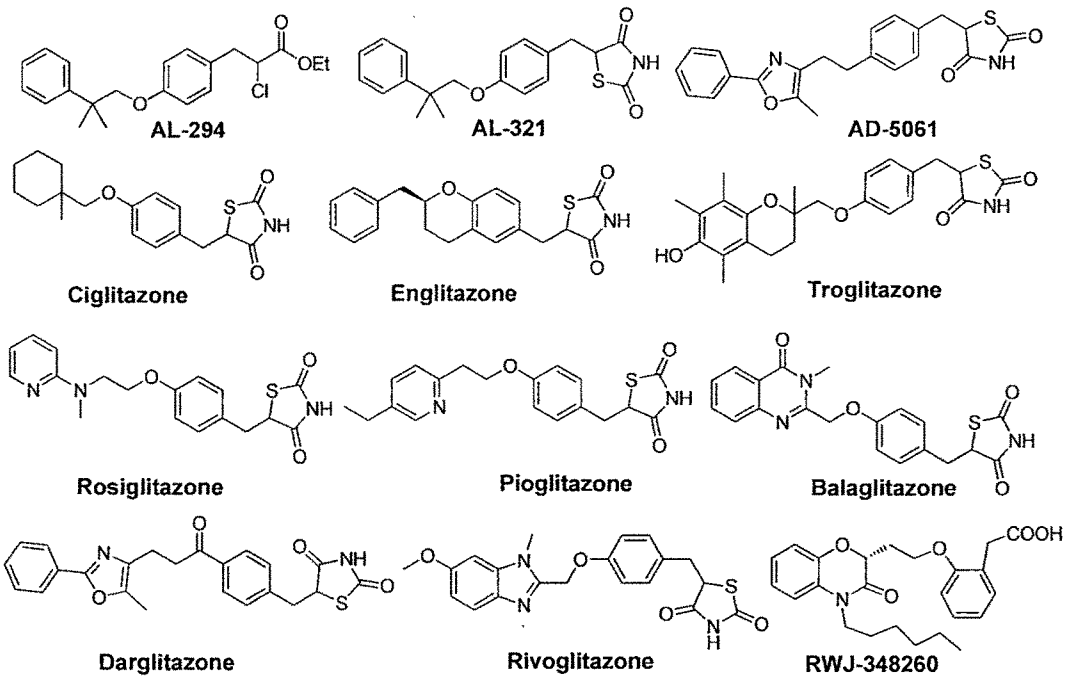


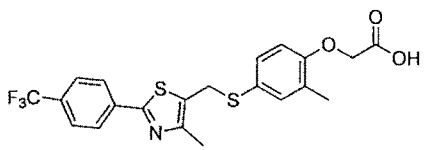
Figure 7. Chemical structures of PPARγ agonists

Moreover, further medicinal chemistry efforts led to the identification of extremely potent oxazole-containing TZDs such as AD-5061 [**159**]. These findings inspired the pharmaceutical industry to pursue follow-on TZDs, and Troglitazone [**160,161**], Rosiglitazone [**161**], and Englitazone [**163**], 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 [**164**]. 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 [**165-169**]. However these drugs were not devoid of side effects. Treatment with these drugs causes weight gain and edema. Huge investments have been made in the last decade and numerous

laboratories, including those in academic institutions along with pharmaceutical and biotechnology companies have been involved in developing novel classes of therapeutic agents interacting with PPAR receptors for the management of type 2 diabetes and the associated metabolic abnormalities. 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) [**170**], 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. Scientists at Dr. Reddy's research foundation have discovered Balaglitazone, a novel TZD derivative and found to be a selective PPARγ partial agonist. 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 [**171**]. Medicinal chemistry efforts have been made thereafter towards identifying non-TZD PPARγ agonists. RWJ-348260 [**172**] has been disclosed as a novel non-TZD PPARγ agonist (**Figure 7**).

1.2.6.3. PPARδ agonists

Most of the saturated and unsaturated fatty acids those activate PPARα and γ are shown to bind to PPARδ also but with lower affinity. There are no marketed PPARδ agonists mainly due to the lack of selective ligands to aid in study of biological mechanisms. GW-501516 [**173**] is the first PPARδ agonists which exhibited nanomolar potency and 1,000 fold selectivity over other subtypes. GW-501516 (**Figure 8**) is the only PPARδ agonists advanced to clinical trials but the further development of this compound has been differred for unknown reasons. There after many research groups around the globe have been involved in developing selective PPARδ agonists.



GW-501516

Figure 8. PPARδgonist

1.2.6.4. PPARα/γdual agonists

The concept of PPARα/γdual 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 [**174**]. The 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. This finding led to a hypothesis that probably activation of PPARα may mitigate the weight gain induced by PPARγ activation. The hypothesis that PPARα/γ dual agonism would provide synergistic pharmacological effects has encouraged many research groups to develop these agents. To date, a large number of structurally diverse PPARα/γdual agonists have been disclosed in the literature and in patent applications. Many of these compounds have been evaluated in clinical trials and some of them have progressed into late-stage development. The first PPARα/γdual agonist to be reported was KRP-297 (MK-0767) [**175**]. Results from a Phase I clinical trial showed that the new TZD derivative was well tolerated and effective at normalizing hyperglycemia & hyperlipidemia and reduce FFAs & lipids in healthy subjects. However, further development was discontinued due to toxicity. Muraglitazar (BMS-298585) is the first PPARα/γdual 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 [**176**]. 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 [**177**], 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) [**178**], Ragaglitazar (DRF-2725) [**179**], Naveglitazar [**180**], Farglitazar (GI262570) [**181**], and Imiglitazar (TAK-559) [**182**], due to various toxicological reasons or a risk-benefit assessment has been disappointing. There are only a few PPARα/γ dual agonists left in the development pipelines. ONO-5129 [**183**], is currently being investigated in Phase II clinical trials for the treatment of type 2 diabetes. AVE-0897 [**184**], a balanced PPARα/γ dual agonist by Sanofi-Aventis, is presently undergoing Phase I clinical trials. Shenzhen chipscreen biosciences is investigating a tyrosine-based PPARα/γ dual agonist Chiglitazar (CS038) [**185**], 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 [**186**]. α-Aryloxyphenylacetic acids have also been reported to be PPARα/γ dual agonists, but preferentially activate PPARα with weak or partial PPARγ activating potency [**187**]. 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 [**188**]. Recently T33 is reported as a novel PPARα/γ dual agonist with insulin sensitizing and hypolipidemic effectc [**189**] (**Figure 9**).

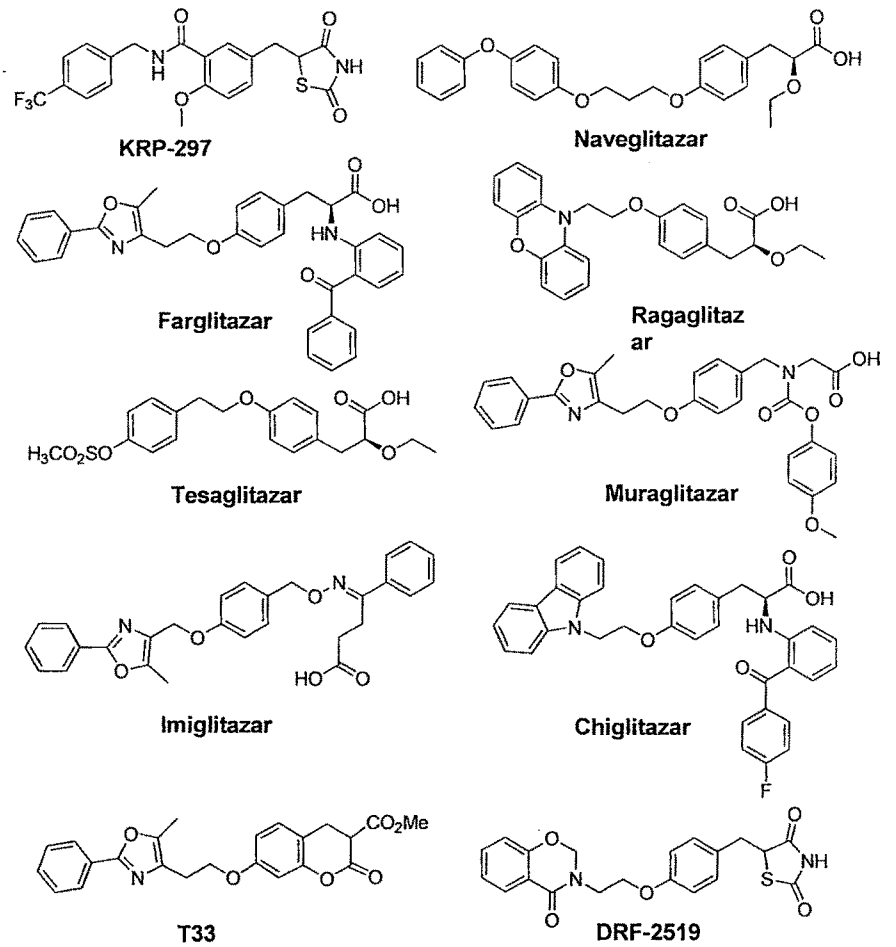


Figure 9. Chemical structures of PPARα/γ dual agonists

More recently ZYH1 (structure undisclosed), 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 [**190**].

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 [**191**-

193]. 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 [**194**].

The *in vitro* activities of few PPAR agonists were presented in **Table 6** below.

Table 6

PPAR transactivation activities of agonists reported in literature[a]

Compound	Originator	hPPAR transactivation EC_{50} (μM)			Ref
		α	γ	δ	
Clofibrate	Astra Zeneca, Wyeth	55	500	IA	[144]
Fenofibrate	Abbott Fournier	30	300	IA	[144]
Bezafibrate	Norwich Eaton, Roche	50	60	20	[144]
WY-14643	Wyeth	5.0	60	35	[144]
GW-590735	Glaxo Smithkline	0.004	10	2.83	[195]
LY-518674	Eli Lilly	0.042	IA	IA	[147]
GW-9578	Glaxo Smithkline	0.05	1.0	1.4	[144]
Merck compound	Merck	0.002	>3	>15 (IC_{50})	[146]
NS-220	Nippon Shinyaku	0.019	9.6	>100	[149]
Troglitazone	Sankyo	IA	0.55	IA	[144]
Rivoglitazone	Sankyo	IA	0.005	IA	[196]
RWJ-348260	Jhonson & Jhonson	0.86	0.19	0.94	[170]
Rosiglitazone	Glaxo Smithkline	IA	0.043	1A	[144]
Pioglitazone	Takeda	IA	0.58	IA	[144]
Balaglitazone	Dr. Reddy's	IA	1.351	IA	[170]
KRP-297	Kyorin	0.85	0.083	9.1	[144]
Naveglitazar	Eli Lilly	0.36	2.81	NR	[179]
Farglitazar	Glaxo Smithkline	0.45	0.00034	IA	[180]
Ragaglitazar	Dr. Reddy's	3.2	0.57	NR	[178]
Tesaglitazar	AstraZeneca	1.7	0.25	NR	[197]
Muraglitazar	Bristol-Myers Squibb	0.32	0.11	NR	[175]
Imiglitazar	Takeda	0.067	0.031	NR	[181]
Chiglitazar	Chipscreen	1.2	0.08	NR	[184]

Compound	Originator	hPPAR transactivation EC_{50} (μM)			Ref
		α	γ	δ	
T33	Shanghai Institute for Biological Sciences	0.148	0.01	NR	[189]
GW-501516	Glaxo Smithkline	1.0	0.80	0.0012	[171]

[a] IA denotes inactive and NR denotes not reported.

These facts made the development of PPARα/γ dual agonists and selective PPARα agonists with distinct biological and safety profiles a challenge among the drug discovery groups around the world as the medical need for metabolic disorders has largely remained unmet.