Introduction
1. INTRODUCTION

The discovery of the crucial role of Peroxisome Proliferator Activated Receptors (PPARs) as regulators of lipid and glucose metabolism has raised interest in the development of synthetic ligands as potential tools for therapeutic intervention in Type 2 Diabetes Mellitus (T2DM) and the Metabolic Syndrome. PPAR\(\alpha\) activators Fibrates, primarily improve dyslipidemia, whereas Glitazones are potent PPAR\(\gamma\) activators that improve insulin resistance. Important research programs to develop agonists that combine the therapeutic effects of both PPAR\(\alpha\) and PPAR\(\gamma\) Selective agonists, creating the expectation of greater efficacy and other advantages in the treatment of Type 2 Diabetes and the Metabolic Syndrome, have therefore been undertaken by the pharmaceutical sector. Among these dual PPAR\(\alpha/\gamma\) agonists, compounds that belong to the Glitazar class were in the most advanced stage of development. However, although they demonstrated beneficial impact over selective PPAR agonists by improving lipid and glucose homeostasis, safety has been a critical issue and has led to the discontinuation of their development because of adverse toxicity profiles. However, the target-related mechanism responsible for the identified safety issues and the relevance of rodent toxicities to the human situation are unclear. Therefore, future development of dual PPAR\(\alpha/\gamma\) agonists with selective PPAR\(\gamma\) modulator activity appears appropriate and prompted us to work in this direction.

In the present study we undertook the design, synthesis and computational study of a novel series of PPAR ligands. The PPAR\(\gamma\) full agonist Rosiglitazone and PPAR\(\alpha/\gamma\) dual agonist Tesaglitazar were selected as leads, and accordingly geometrically and conformationally constrained analogous compounds were designed and selected for synthesis and were analysed in term of their binding modes by docking analysis.

1.1. History of Diabetes

Diabetes was one of the first diseases described,\(^1\) with an Egyptian manuscript from 1500 BCE mentioning ‘too great emptying of the urine’.\(^2\) Indian physicians around the same time identified the disease and classified it as madhumeha or "honey urine", noting the urine would attract ants.\(^2\) The word diabetes comes from Latin ‘diabētēs’, which in turn comes from Ancient Greek ‘diabētēs’ which literally means ‘a passer through; a siphon.’\(^3\) The term "diabetes" or "to pass through" was first used in 230 BCE by the Greek
Appollonius of Memphis. Ancient Greek physician Aretaeus of Cappadocia (1st century CE) used that word, with the intended meaning "excessive discharge of urine", as the name for the disease. Ultimately, the word comes from Greek ‘*diabainein*’, meaning "to pass through," which is composed of ‘*dia*’-, meaning "through" and ‘*bainein*’, meaning "to go". The word "diabetes" is first recorded in English, in the form *diabete*, in a medical text written around 1425. The word *mellitus* comes from the classical Latin word *mellītus*, meaning "mellite" (i.e. sweetened with honey; honey-sweet). The Latin word comes from *mell-,* which comes from *mel*, meaning ‘honey’, sweetness; pleasant thing, and the suffix -*ītus*, whose meaning is the same as that of the English suffix "-ite". It was Thomas Willis who in 1675 added "mellitus" to the word ‘diabetes’ as a designation for the disease, when he noticed the urine of a diabetic had a sweet taste (glycosuria). This sweet taste had been noticed in urine by the ancient Greeks, Chinese, Egyptians, Indians, and Persians. The disease was rare during the time of the Roman empire, with Galen commenting he had only seen two cases during his career. Type 1 and type 2 diabetes were identified as separate conditions for the first time by the Indian physicians Sushruta and Charaka in 400-500 AD with type 1 associated with youth and type 2 with being overweight. The term "mellitus" or "from honey" was added by the Briton John Rolle in the late 1700s to separate the condition from diabetes insipidus, which is also associated with frequent urination.

1.2. Definition
Diabetes mellitus (DM), often simply referred to as diabetes, is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). In other words, DM is a set of related diseases in which the body cannot regulate the amount of sugar (specifically, glucose) in the blood. The blood delivers glucose to provide the body with energy to perform all of a person's daily activities.

- The liver converts the food a person eats into glucose. The glucose is then released into the bloodstream.
- In a healthy person, the blood glucose level is regulated by several hormones, primarily insulin. Insulin is produced by the pancreas, a small organ between the
stomach and liver. The pancreas also makes other important enzymes released directly into the gut that helps digest food.

- Insulin allows glucose to move out of the blood into cells throughout the body where it is used for fuel.
- People with diabetes either do not produce enough insulin or cannot use insulin properly, or both (which occurs with several forms of diabetes).
- In diabetes, glucose in the blood cannot move efficiently into cells, so blood glucose levels remain high. This not only starves all the cells that need the glucose for fuel, but also harms certain organs and tissues exposed to the high glucose levels.

1.3. Classification

Diabetes mellitus is classified into four broad categories: type 1, type 2, gestational diabetes and "other specific types". The "other specific types" are a collection of a few dozen individual causes. The term "diabetes", without qualification, usually refers to diabetes mellitus. The rare disease diabetes insipidus has similar symptoms as diabetes mellitus, but without disturbances in the sugar metabolism (insipidus means "without taste" in Latin).

The term "type 1 diabetes" has replaced several former terms, including childhood-onset diabetes, juvenile diabetes, and insulin-dependent diabetes mellitus (IDDM). Likewise, the term "type 2 diabetes" has replaced several former terms, including adult-onset diabetes, obesity-related diabetes, and noninsulin-dependent diabetes mellitus (NIDDM). Beyond these two types, there is no agreed-upon standard nomenclature. Various sources have defined "type 3 diabetes" as: gestational diabetes, insulin-resistant type 1 diabetes (or "double diabetes"), type 2 diabetes which has progressed to require injected insulin, and latent autoimmune diabetes of adults (or LADA or "type 1.5" diabetes).

The three main types of diabetes mellitus (DM) are:

- Type 1 DM results from the body's failure to produce insulin, and presently requires the person to inject insulin. (Also referred to as insulin-dependent diabetes mellitus (IDDM) or "juvenile" diabetes)
- Type 2 DM results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency.
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(Formerly referred to as noninsulin-dependent diabetes mellitus (NIDDM) or "adult-onset" diabetes)

- Gestational diabetes is when pregnant women, who have never had diabetes before, have a high blood glucose level during pregnancy. It may precede development of type 2 DM.

Other forms of diabetes mellitus include congenital diabetes, which is due to genetic defects of insulin secretion, cystic fibrosis-related diabetes, steroid diabetes induced by high doses of glucocorticoids, and several forms of monogenic diabetes.

1.3.1. Type 1 diabetes

Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas, leading to insulin deficiency. The body stops producing insulin or produces too little insulin to regulate blood glucose level. This type can be further classified as immune-mediated or idiopathic. The majority of type 1 diabetes is of the immune-mediated nature, in which beta cell loss is a T-cell-mediated autoimmune attack. There is no known preventive measure against type 1 diabetes, which causes approximately 10% of DM cases in North America and Europe. Most affected people are otherwise healthy and of a healthy weight when onset occurs. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type 1 diabetes can affect children or adults, but was traditionally termed "juvenile diabetes" because a majority of these diabetes cases were in children.

- Type 1 diabetes is typically diagnosed during childhood or adolescence. It used to be referred to as juvenile-onset diabetes or insulin-dependent diabetes mellitus.
- Type 1 diabetes can occur in an older individual due to destruction of the pancreas by alcohol, disease, or removal by surgery. It also results from progressive failure of the pancreatic beta cells, the only cell type that produces significant amounts of insulin.
- People with type 1 diabetes require insulin treatment daily to sustain life.

"Brittle" diabetes, also known as unstable diabetes or labile diabetes is a term that was traditionally used to describe to dramatic and recurrent swings in glucose levels, often occurring for no apparent reason in insulin-dependent diabetes. This term, however, has no biologic basis and should not be used. There are many different reasons for type 1 diabetes to be accompanied by irregular and unpredictable hyperglycemias, frequently with ketosis, and sometimes serious hypoglycemias, including an impaired
counterregulatory response to hypoglycemia, occult infection, gastroparesis (which leads to erratic absorption of dietary carbohydrates), and endocrinopathies (e.g., Addison's disease). These phenomena are believed to occur no more frequently than in 1% to 2% of persons with type 1 diabetes.

1.3.2. Type 2 diabetes
Type 2 diabetes mellitus is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion. Although the pancreas still secretes insulin, the body of someone with type 2 diabetes is partially or completely unable to use this insulin. This is sometimes referred to as insulin resistance. The pancreas tries to overcome this resistance by secreting more and more insulin. The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor. However, the specific defects are not known. Diabetes mellitus cases due to a known defect are classified separately. Type 2 diabetes is the most common type.

In the early stage of type 2, the predominant abnormality is reduced insulin sensitivity. At this stage, hyperglycemia can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce glucose production by the liver.

People with insulin resistance develop type 2 diabetes when they fail to secrete enough insulin to cope with their higher demands.

- At least 90% of adult individuals with diabetes have type 2 diabetes.
- Type 2 diabetes is typically diagnosed in adulthood, usually after age 45 years. It used to be called adult-onset diabetes mellitus, or non-insulin-dependent diabetes mellitus. These names are no longer used because type 2 diabetes does occur in younger people, and some people with type 2 diabetes require insulin therapy.

Type 2 diabetes is usually controlled with diet, weight loss, exercise, and oral medications. However, more than half of all people with type 2 diabetes require insulin to control their blood sugar levels at some point in the course of their illness.

1.3.4. Metabolic syndrome
Metabolic syndrome (also referred to as syndrome X) is a set of abnormalities in which insulin-resistant diabetes (type 2 diabetes) is almost always present along with hypertension (high blood pressure), high fat levels in the blood (increased serum lipids, predominant elevation of LDL cholesterol, decreased HDL cholesterol, and elevated triglycerides), central obesity, and abnormalities in blood clotting (fibrinolysis,
procoagulation) and inflammatory responses. A high rate of cardiovascular disease is associated with metabolic syndrome.

Metabolic syndrome is defined by the National Cholesterol Education Program\textsuperscript{15} as the presence of any three of the following conditions (Table I). \medskip

\textbf{Table I: Limiting conditions of the metabolic syndrome} \medskip

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excess weight around the waist</td>
<td>waist measurement of more than 40 inches for men and more than 35 inches for women</td>
</tr>
<tr>
<td>High levels of triglycerides</td>
<td>150mg/dL or higher</td>
</tr>
<tr>
<td>Low levels of HDL or ‘good’ cholesterol</td>
<td>below 40mg/dL for men and below 50mg/dL for women</td>
</tr>
<tr>
<td>High blood pressure (Hypertension)</td>
<td>130/85 mmHg or higher</td>
</tr>
<tr>
<td>High fasting blood glucose level</td>
<td>110mg/dL or higher</td>
</tr>
</tbody>
</table>

Although the metabolic syndrome is not exclusively associated with T2D and the associated insulin resistance, the increasing prevalence of obesity and associated development of T2D places insulin resistance as a major contributor to the syndrome. The role of adipose tissue stems from the fat that the organism active at secretion of cytokines, termed adipocytokines. These include tumor necrosis factor-\(\alpha\) (TNF\(\alpha\)), interleukin-6 (IL6), leptin, adiponectin and resistin. Leptin has received particular attention of late due to its role in obesity in addition to the fact that recent data indicates that plasma leptin levels are found to be predictive of the potential for cardiovascular pathology.

Many clinicians and researchers believe that insulin resistance underlies the cardiovascular pathogenesis of the metabolic syndrome. One primary reason for this is the role of insulin in fat homeostasis. As discussed above, the major role of insulin is to induce the storage of fuel. This can be as fat (triacylglycerides, TGs) in adipose tissue or as carbohydrate in the form of glycogen in liver and skeletal muscle. The effect of insulin resistance at the level of fat homeostasis is an increase in circulating TGs, referred to as dyslipidemia. Due to insulin resistance there is an increase in the delivery of peripheral fatty acids to the liver which in turn drives hepatic TG synthesis. These TGs are then packaged into lipoprotein particles termed VLDLs (very low density lipoproteins) which are returned to the circulation. Taken together, the insulin resistance and its associated negative effects on metabolism, the increased levels of circulating TGs, the reduced levels of HDLs and
hypertension, all contribute to the progression of atherosclerosis. With associated coagulation and fibrinolysis pathogenesis, the cardiovascular events of the metabolic syndrome can be devastating.

Since many of these pathogeneses can be reversed with proper diet and exercise, it is in a person’s best interest to take responsibility for the role their lifestyle choices play in the development of the metabolic syndrome.¹⁶

1.3.5. Prediabetes

It is a common condition related to diabetes. In people with prediabetes, the blood sugar level is higher than normal but not yet high enough to be considered diagnostic of diabetes.

- Prediabetes increases a person's risk of developing type 2 diabetes, heart disease, or stroke.
- Prediabetes can typically be reversed (without insulin or medication) with lifestyle changes such as losing a modest amount of weight and increasing physical activity levels. Weight loss can prevent, or at least delay, the onset of type 2 diabetes.
- An international expert committee of the American Diabetes Association redefined the criteria for prediabetes, lowering the blood sugar level cut-off point for prediabetes. Approximately 20% more adults are now believed to have this condition and may develop diabetes within 10 years if they do make lifestyle changes such as exercising more and maintaining a healthy weight.

About 17 million Americans (6.2% of adults in North America) are believed to have diabetes. It has been estimated that about one third of adults with diabetes do not know they have diabetes.

- About 1 million new cases of diabetes is diagnosed occur each year, and diabetes is the direct or indirect cause of at least 200,000 deaths each year.
- The incidence of diabetes is increasing rapidly. This increase is due to many factors, but the most significant are the increasing incidence of obesity associated with the prevalence of a sedentary lifestyle.

1.4. Relation among Insulin Resistance, Pre-diabetes and T2DM

If someone has insulin resistance his/her muscle, fat and liver cells do not use insulin properly. The pancreas tries to keep up with the demand for insulin by producing more. Eventually, the pancreas cannot keep up with the body’s need for insulin, and excess
glucose builds up in the bloodstream. Many people with insulin resistance have high level of blood glucose and high levels of insulin circulating in their blood at the same time. People with blood glucose levels that are higher than normal but not yet in the diabetic range have “pre-diabetic”. Doctors sometimes call this condition impaired fasting glucose (IFG) or impaired glucose tolerance (IGT), depending on the test used to diagnose it. If someone has pre-diabetes, he/she has a higher risk of developing type 2 diabetes, formerly called adult-onset diabetes or non insulin dependent diabetes. Studies have shown that that most people with pre-diabetes go on to develop type 2 diabetes within 10 years, unless they loose 5 to 7 percent of their body weight – which is about 10 to 15 pounds for someone who weighs 200 pounds – by making modest changes in their diet and level of physical activity. People with pre-diabetes also have a higher risk of heart disease. Type 2 diabetes is sometimes defined as the form of diabetes that develops when the body does not respond properly to insulin, as opposed to type 1 diabetes, in which the pancreas makes no insulin at all. At first, the pancreas keeps up with the added demand by producing more insulin. In times, however, it loses the ability to secrete enough insulin in response to meals. Insulin resistance can also occur in people who have type 1 diabetes, especially if they are overweight. 

1.5. Complications of diabetes

Both type 1 and type 2 diabetes ultimately lead to high blood sugar levels, a condition called hyperglycemia. Over a long period of time, hyperglycemia damages the retina of the eye, the blood vessels of the kidneys, the nerves, and other blood vessels.

- Damage to the retina from diabetes (diabetic retinopathy) is a leading cause of blindness.
- Damage to the kidneys from diabetes (diabetic nephropathy) is a leading cause of kidney failure.
- Damage to the nerves from diabetes (diabetic neuropathy) is a leading cause of foot wounds and ulcers, which frequently lead to foot and leg amputations.
- Damage to the nerves in the autonomic nervous system can lead to paralysis of the stomach (gastroparesis), chronic diarrhea, and an inability to control heart rate and blood pressure during postural changes.
- Diabetes accelerates atherosclerosis, (the formation of fatty plaques inside the arteries), which can lead to blockages or a clot (thrombus). Such changes can then
lead to heart attack, stroke, and decreased circulation in the arms and legs (peripheral vascular disease).

- Diabetes predisposes people to elevated blood pressure, high levels of cholesterol and triglycerides. These conditions both independently and together with hyperglycemia, increase the risk of heart disease, kidney disease, and other blood vessel complications.

Diabetes can contribute to a number of acute (short-lived) medical problems:

- Many infections are associated with diabetes, and infections are frequently more dangerous in someone with diabetes because the body's normal ability to fight infections is impaired. To compound the problem, infections may worsen glucose control, which further delays recovery from infection.

- Hypoglycemia or low blood sugar, occurs intermittently in most people with diabetes. It can result from taking too much diabetes medication or insulin (sometimes called an insulin reaction), missing a meal, exercising more than usual, drinking too much alcohol, or taking certain medications for other conditions. It is very important to recognize hypoglycemia and be prepared to treat it at all times. Headache, feeling dizzy, poor concentration, tremor of the hands, and sweating are common symptoms of hypoglycemia. A person can faint or have a seizure if blood sugar level becomes too low.

- Diabetic ketoacidosis (DKA) is a serious condition in which uncontrolled hyperglycemia (usually due to complete lack of insulin or a relative deficiency of insulin) over time creates a buildup of ketones (acidic waste products) in the blood. High levels of ketones can be very harmful. This typically happens to people with type 1 diabetes who do not have good blood glucose control. Diabetic ketoacidosis can be precipitated by infection, stress, trauma, missing medications like insulin, or medical emergencies such as a stroke and heart attack.

- Hyperosmolar hyperglycemic nonketotic syndrome is a serious condition in which the blood sugar level gets very high. The body tries to get rid of the excess blood sugar by eliminating it in the urine. This increases the amount of urine significantly, and often leads to dehydration so severe that it can cause seizures, coma, and even death. This syndrome typically occurs in people with type 2 diabetes who are not controlling their blood sugar levels, who have become dehydrated, or who have stress, injury, stroke, or are taking certain medications, like steroids.
1.6. Diabetes Symptoms

Symptoms of type 1 diabetes are often dramatic and come on very suddenly.

- Type 1 diabetes is usually recognized in childhood or early adolescence, often in association with an illness (such as a virus or urinary tract infection) or injury.
- The extra stress can cause diabetic ketoacidosis.
  - Symptoms of ketoacidosis include nausea and vomiting. Dehydration and often-serious disturbances in blood levels of potassium follow.
  - Without treatment, ketoacidosis can lead to coma and death.

Symptoms of type 2 diabetes are often subtle and may be attributed to aging or obesity.

- A person may have type 2 diabetes for many years without knowing it.
- People with type 2 diabetes can develop hyperglycemic hyperosmolar nonketotic syndrome.
- Type 2 diabetes can be precipitated by steroids and stress.
- If not properly treated, type 2 diabetes can lead to complications such as blindness, kidney failure, heart disease, and nerve damage.

1.7. Diagnosis of Diabetes

Physicians prescribe special tests in diagnosing diabetes and also in monitoring blood sugar level control in known diabetics.

A number of laboratory tests are available to confirm the diagnosis of diabetes.

**Finger stick blood glucose:** This is a rapid screening test that may be performed anywhere, including community-based screening programs.

- Although a not as accurate as testing the patient's blood in the hospital laboratory, a fingerstick blood glucose test but is easy to perform, and the result is available right away.
- The test involves sticking the patient's finger for a blood sample, which is then placed on a strip. The strip goes into a machine that reads the blood sugar level. These machines are only accurate to within about 10%-20% of true laboratory values.
- Fingerstick blood glucose values tend to be most inaccurate at very high or very low levels, so this test is only a preliminary screening study. Fingerstick is the way most people with diabetes monitor their blood sugar levels at home.
**Fasting plasma glucose:** The patient will be asked to eat or drink nothing for 8 hours before having blood drawn (usually first thing in the morning). If the blood glucose level is greater than or equal to 126 mg/dL (without eating anything), they probably have diabetes.

- If the result is abnormal, the fasting plasma glucose test may be repeated on a different day to confirm the result, or the patient may undergo an oral glucose tolerance test or a glycosylated hemoglobin test (often called "hemoglobin A1c") as a confirmatory test.
- If fasting plasma glucose level is greater than 100 but less than 126 mg/dL, then the patient has what is called impaired fasting glucose, or IFG. This is considered to be prediabetes. These patients do not have diabetes, but they are at high risk of developing diabetes in the near future.

**Oral glucose tolerance test:** This test involves drawing blood for a fasting plasma glucose test, then drawing blood for a second test at two hours after drinking a very sweet drink containing up to 75 grams of sugar.

- If the blood sugar level after the sugar drink is greater than or equal to 200 mg/dL, the patient has diabetes.
- If the blood glucose level is between 140 and 199, then the patient has impaired glucose tolerance (IGT), which is also a prediabetic condition.

**Glycosylated hemoglobin or hemoglobin A1c:** This test is a measurement of how high the blood sugar levels have been over approximately the last 120 days (the average life-span of the red blood cells on which the test is based). Glycated hemoglobin is better than fasting glucose for determining risks of cardiovascular disease and death from any cause.

- Excess blood glucose hooks itself on to the hemoglobin in red blood cells and stays there for the life of the red blood cell.
- The percentage of hemoglobin that has had excess blood sugar attached to it can be measured in the blood. The test involves having a small amount of blood drawn.
- A hemoglobin A1c test is the best measurement of blood sugar control in people known to have diabetes. A hemoglobin A1c result of 7% or less indicates good glucose control. A result of 8% or greater indicates that blood sugar levels are too high, too much of the time.
The hemoglobin A1c test is the best test for diabetes follow-up care, than to diagnose diabetes. Still, a hemoglobin A1c result greater than 6.1% is highly suggestive of diabetes. Generally, a confirmatory test would be needed before diagnosing diabetes.

The hemoglobin A1c test is generally measured about every 3 to 6 months for people with known diabetes, although it may be done more frequently for people who are having difficulty achieving and maintaining good blood sugar control.

This test is not used for people who do not have diabetes or are not at increased risk of diabetes.

Normal values may vary from laboratory to laboratory, although an effort is under way to standardize how measurements are performed.

Special attention is paid to history including information about the patient's symptoms, risk factors for diabetes, past medical problems, current medications, allergies to medications, family history of diabetes, or other medical problems such as high cholesterol or heart disease, and personal habits and lifestyle.

The limiting values of the parameters of the pathological tests for the diagnosis of DM are listed in table I2.

Table I2: Diagnosis criteria for Diabetes Mellitus

<table>
<thead>
<tr>
<th>Condition</th>
<th>2 hour glucose mmol/l(mg/dl)</th>
<th>Fasting glucose mmol/l(mg/dl)</th>
<th>HbA1c %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt;7.8 (&lt;140)</td>
<td>&lt;6.1 (&lt;110)</td>
<td>&lt;6.0</td>
</tr>
<tr>
<td>Prediabetes Impaired fasting glycaemia</td>
<td>&lt;7.8 (&lt;140)</td>
<td>≥ 6.1(≥110) &amp; &lt;7.0(&lt;126)</td>
<td>6.0-6.4</td>
</tr>
<tr>
<td>Prediabetes Impaired glucose tolerance</td>
<td>≥7.8 (≥140)</td>
<td>&lt;7.0 (&lt;126)</td>
<td>6.0-6.4</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>≥11.1 (≥200)</td>
<td>≥7.0 (≥126)</td>
<td>≥6.5</td>
</tr>
</tbody>
</table>

1.8. Treatment of Diabetes

While many measures were tried, effective treatment was not developed until the early part of the 20th century, when Canadians Frederick Banting and Charles Best developed
insulin in 1921 and 1922. This was followed by the development of the long-acting insulin NPH in the 1940s.

The treatment of diabetes is highly individualized, depending on the type of diabetes, whether the patient has other active medical problems, whether the patient has complications of diabetes, and age and general health of the patient at time of diagnosis.

- A health care professional will set goals for lifestyle changes, blood sugar control, and treatment.
- Together, the patient and the health care professional will formulate a plan to help meet those goals.

Education about diabetes and its treatment is essential in all types of diabetes.

- When the patient is first diagnosed with diabetes, the diabetes care team will spend a lot of time with the patient, teaching them about their condition, treatment, and everything they need to know to care for themselves on a daily basis.
- The diabetes care team includes the health care professional and his or her staff. It may include specialists in foot care, neurology, kidney diseases, and eye diseases. A professional dietitian and a diabetes educator also may be part of the team.

1.8.1. Type 1 diabetes

Treatment of diabetes almost always involves the daily injection of insulin, usually a combination of short-acting insulin (for example, lispro [Humalog] or aspart [NovoLog]) and longer acting insulin (for example, NPH, Lente, glargine [Lantus], detemir [Levemir]).

- Insulin must be given as an injection just under the skin. If taken by mouth, insulin would be destroyed in the stomach before it could get into the blood where it is needed.
- Most people with type 1 diabetes give these injections to themselves. Even if someone else usually gives the patient injections, it is important that the patient knows how to do it in case the other person is unavailable.
- The patient should be trained to store and inject the insulin. Insulin is usually given in two or three injections per day, generally around mealtimes. Dosage is individualized and is tailored to the patient's specific needs by the health care
professional. Longer acting insulins are typically administered one or two times per day.

- Some people have their insulin administered by continuous infusion pumps to provide adequate blood glucose control. Supplemental mealtime insulin is programmed into the pump by the individual as recommended by his or her health care professionals.
- It is very important to eat after the taking insulin, as the insulin will lower blood sugar regardless of whether the person has eaten. If insulin is taken without eating, the result may be hypoglycemia. This is called an insulin reaction.
- There is an adjustment period while the patient learns how insulin affects them, and how to time meals and exercise with insulin injections to keep blood sugar levels as even as possible.
- Keeping accurate records of blood sugar levels and insulin dosages is crucial for the patient's diabetes management.
- Eating a consistent, healthy diet appropriate for the patient's size and weight is essential in controlling blood sugar level.

1.8.2. Type 2 diabetes

Depending on how elevated the patient's blood sugar and glycosylated hemoglobin (HbA1c) are at the time of diagnosis, they may be given a chance to lower blood sugar levels through lifestyle changes, without medication.

- The best way to do this is to lose weight if the patient is obese, and begin an exercise program.
- This will generally be tried for 3 to 6 months, then blood sugar and glycosylated hemoglobin will be rechecked. If they remain high, the patient will be started on an oral medication, usually a sulfonylurea or biguanide (metformin [Glucophage]), to help control blood sugar levels.
- Even if the patient is on medication, it is still important to eat a healthy diet, lose weight if they are overweight, and engage in moderate physical activity as often as possible.
- The health care professional will initially monitor the patient's progress on medication very carefully. It is important to receive just the right dose of the right medication, to regulate blood sugar levels in the recommended range with the fewest side effects.
• The doctor may decide to combine two types of medications to achieve blood sugar levels control.
• Gradually, even people with type 2 diabetes may require insulin injections to control their blood sugar levels.
• It is becoming more common for people with type 2 diabetes to take a combination of oral medication and insulin injections to control blood sugar levels.

1.9. Diabetic Medications (Therapeutic Intervention for Hyperglycemia)

Many, if not all, of the vascular consequences of insulin resistance are due to the persistent hyperglycemia seen in T2D. For these reason a major goal of therapeutic intervention in T2D is to reduce circulating glucose levels. Many different types of medications are available to help lower blood sugar levels in people with type 2 diabetes. Each type works in a different way. There are many pharmacologic strategies to accomplish these goals. It is very common to combine two or more types to get the best effect with fewest side effects.

• Sulfonylureas: They are oral hypoglycemic drugs and are referred to as endogenous insulin secretagogues because they induce pancreatic release of endogenous insulin. The first generation sulfonylureas (tolbutamide, acetohexamide, chlorpropamide and tolazamide) are not routinely prescribed in the US. The second generation sulfonylureas include glipizide, gliptin and glyburide. Because all of these drugs can induce pronounced hypoglycemia, treatment is initiated with the lowest possible dose and carefully monitored until the dose is found that results in a fasting plasma glucose (FPG) of 110-140 mg/dL. Sulfonylureas function by binding to and inhibiting the pancreatic ATP-dependent potassium channel that is normally involved in glucose mediated insulin secretion. They have no significant effects on circulating triglycerides lipoproteins or cholesterol.

• Biguanides: They are a class of oral hypoglycemic drugs that function to lower serum glucose levels by enhancing insulin-mediated suppression of hepatic glucose production and enhancing insulin-stimulated glucose uptake by skeletal muscle. Metformin (Glucophage) is a member of this class and is currently the most widely
prescribed insulin-sensitizing drug in current clinical use. Metformin administration does not lead to increased insulin release from the pancreas and as such the risk of hypoglycemia is minimal. Because the major site of action for metformin is the liver its use can be contraindicated in patients with liver dysfunction. The drug is ideal for obese patients and for younger T2 diabetics. Evidence on the mode of action (MOA) of metformin shows that it improves insulin sensitivity by increasing insulin receptor tyrosine kinase activity, enhancing glycogen synthesis and increasing recruitment and transport of GLUT4 transporters to the plasma membrane. Additionally, it has been shown that metformin affects mitochondrial activities dependent upon the model system studies. Metformin has a mild inhibitory effect on complex I of oxidative phosphorylation, has antioxidant properties, and activates both glucose 6-phosphatde dehydrogenase (G6PDH) and AMP-activated protein kinase (AMPK). The importance of AMPK metformin action stems from the role of AMPK in the regulation of both lipid and carbohydrate metabolism. In adipose tissue, metformin inhibits lipolysis while enhancing re-esterification of fatty acids.

In adolescent females with T2D, the use if metformin is highly recommended to reduce the incidence of as well as the potential for polycystic ovarian syndrome, (PCOS). PCOS is brought on by the hyperinsulinemia if T2D. insulin effects on the ovary drive conversion of progesterone to testosterone and a reduction in serum hormone globulin (SHBG). Taken together, the effects of hyperinsulinemia leads to a hyperandrogenic state in the ovary resulting in follicular atresis and ovulatory dysfunction.

- **Alpha-glucosidase inhibitors:** These oral hypoglycemic agents slow absorption of the starches a person eats. This slows down glucose production. Alpha-glucosidase inhibitors like acarbose (Precose) and miglitol (Glyset) function by interfering with the action of the α-glucosidases present in the small intestinal brush border. The consequence of this inhibition is a reduction in digestion and the consequent absorption of glucose into the systemic circulation. The reduction in glucose uptake allows the pancreatic β-cells to more effectively regulate insulin secretion. The advantage to the use of the α-glucosidase inhibitors is that they function locally in the intestine and have no major systemic action. Hypoglycemia does not usually occur
with the use of $\alpha$-glucosidase inhibitors but they are effective at reducing fasting plasma glucose (FPG) levels and levels of glycosylated haemoglobin (HbA$_{1c}$). the adverse side effects of these inhibitors are abdominal bloating and discomfort, diarrhea and flatulence.$^{16}$

- **Thiazolidinediones (TZDs):** These oral hypoglycemic agents increase sensitivity to insulin. The TZDs such as troglitazone (Rezulin: Warner Lambert Co. but this drug was voluntarily removed from the market due to liver damage risk$^{18}$), rosiglitazone (Avandia: Glaxo Smithkline) and pioglitazone (Actos: Eli Lilly and Co.) have been proven useful in treating the hyperglycemia associated with insulin-resistance in both T2D and nondiabetic conditions (though these have also been withdrawn in many countries owing to their cardiovascular adverse effects$^{19}$). The TZDs function as agonist for the nuclear receptor peroxisome proliferator activated receptor-$\gamma$ (PPAR$\gamma$). The net effect of the TZDs is a potentiation of the actions of insulin in liver, adipose tissue and skeletal muscle, increased peripheral glucose disposal and a decrease in glucose output by the liver.

- **Meglitinides:** These oral hypoglycemic agents stimulate the pancreas to make more insulin. The meglitinides repaglinide (Prandin) and nateglinide (Starlix) are non sulfonylurea insulin secretagogues that are both fast acting and of short duration. Like the sulfonylureas meglitinide therapy results in significant reduction in FPG as well as HbA$_{1c}$. The MOA of meglitinide is initiated by binding to a receptor on the pancreatic $\beta$-cell that is distinct from the receptors for the sulfonylureas. However, meglitinides do exert effects on potassium conductance like the sulfonylureas, the meglitinides have no direct effects on the circulating levels of plasma lipids.
Introduction

Diagnosis of type 2 DM: use one of three tests (results should be confirmed on a subsequent day)
- FPG ≥ 200 mg per dL (11.1 mmol per L) + symptoms
- FPG ≥ 126 mg per dL (7.0 mmol per L)
- OGGT (75 g) with 2 hr PG ≥ 200 mg per dL (11.1 mmol per L)

Patient education/diet and exercise/HbA1c
Goals: FPG < 126 mg per dL (7.0 mmol per L), 
HbA1c < 7 percent; evaluate in three months

Initiate **monotherapy** if diet and exercise alone are inadequate.

### Options for monotherapy

- **Sulfonylureas**
  - Target population: Recent type 2 DM diagnosis or type 2 DM < 5 years duration
  - Advantages: Rapid FPG reduction, Low cost
  - Disadvantages: Weight gain, Risk of hypoglycemia

- **Meglitinides**
  - Target population: Recent type 2 DM diagnosis or Elevated FPG
  - Advantages: ↓ Risk of hypoglycemia, Short-acting
  - Disadvantages: High cost, Risk of hypoglycemia

- **Biguanides**
  - Target population: Overweight/obese
  - Advantages: No weight gain, ↓ Risk of hypoglycemia
  - Disadvantages: GI side effects, High cost, Rare lactic acidosis

- **Thiazolidinediones**
  - Target population: Insulin resistant, Overweight/obese
  - Advantages: ↓ Amount of insulin, ↓ Risk of hypoglycemia
  - Disadvantages: High cost, Weight gain, Slow onset of action, Issue of liver toxicity

- **Alpha-glucosidase inhibitors**
  - Target population: Elevated FPG
  - Advantages: ↓ Risk of hypoglycemia
  - Disadvantages: High cost, GI side effects

Initiate **combination therapy** if a single agent is inadequate.

### Options for combination therapy

- **Sulfonylurea** + biguanide or thiazolidinediones or alpha-glucosidase inhibitor
- **Biguanide + meglitinide**
- **Biguanide + thiazolidinedione**
- **Biguanide + alpha-glucosidase inhibitor**
- **Triple combination therapy**
  - Sulfonylurea + biguanide + thiazolidinedione or Sulfonylurea + biguanide + alpha-glucosidase inhibitor

If therapeutic goals are not met using the above combinations, switch to insulin +/- oral agent.

**Figure 11:** Flowchart for stepwise therapy of T2DM

- **DPP IV Inhibitors:** DPP-IV inhibitors are compounds that increase the concentration of endogenous incretins, including glucagon like peptide-1 (GLP-1), by limiting the proteolytic cleavage by dipeptidyl peptidase-IV (DPP-IV). The clinical effect is to stimulate insulin secretion in a glucose-specific manner and suppress glucagon secretion. DPP4 inhibitors such as sitagliptin and vildagliptin are novel agents for treatment of type 2 diabetes. They target both prandial and fasting glucose
concentrations, and work by improving β-cell sensitivity to glucose, whereby it increases glucose-dependent insulin secretion. Glitits can be used as monotherapy or combined with metformin or SUs. Glitits are largely weight neutral. No serious adverse events were noted during the clinical trials. Vildagliptin is not recommended in patients with hepatic impairment. Long-term safety regarding cardiovascular outcomes needs to be assessed.\textsuperscript{20}

A flowchart of the currently available stepwise clinical therapy for T2DM is given in figure I.\textsuperscript{21}

1.10. Peroxisome Proliferator Activated Receptors and T2DM

Type 2 diabetes is characterized by hyperglycemia, insulin resistance, and defects in insulin secretion and is usually associated with dyslipidemia, hypertension, and obesity. Although the detailed pathophysiology of this disease remains incompletely understood, metabolic defects in the liver, pancreatic β-cells, adipose tissue, and skeletal muscle all contribute to the development of type 2 diabetes. Though long thought to be mainly a disorder of carbohydrate metabolism, today a great deal of evidence suggests that abnormalities in fat metabolism play a central role in the pathogenesis of this disease.\textsuperscript{22-24}

Peroxisome proliferator-activated receptors (PPARs) are orphan receptors belonging to the steroid/thyroid/ retinoid receptor superfamily of ligand-activated transcription factors. Although cloned only a few decade ago,\textsuperscript{25,26} the rapid progress in functional analysis of these receptors has established that the PPARs play a central role in regulating the storage and catabolism of lipids in both animals and humans (Figure I9). Therefore, much attention has been paid in the research of structural determination and ligand activation of these receptors. There are three PPAR subtypes, which are the products of distinct genes and are commonly designated PPAR\textsubscript{α} [NR1C1], PPAR\textsubscript{γ} [NR1C3], and PPAR\textsubscript{δ} [NR1C2].\textsuperscript{27} The PPARs have a protein domain structure (Figure I2) common to other members of the nuclear receptor gene family. This consists of a variable N-terminal region that contains the transcriptional activation function 1 domain (AF-1), a highly conserved DNA-binding domain (DBD), and a ligand-binding domain (LBD) within which lies a C-terminal region that contains the transcriptional activation function 2 domain (AF-2). The LBD contains certain conserved amino acids that have been mapped to critical receptor functions involved in signal transduction. However, there is significant...
sequence variation in the residues that line the ligand-binding pocket, which is reflected in the fact that each receptor subtype is pharmacologically distinct. The PPARs form functionally active heterodimers with another nuclear receptor, the 9-cis-retinoic acid receptor (RXR). These heterodimers regulate expression of target genes by binding to DNA sequence elements, termed PPAR response elements (PPREs, Figure I3). PPREs have been identified in the regulatory regions of a large number of genes, including many that encode proteins involved in lipid metabolism and energy balance, such as aP2, phosphoenolpyruvate carboxykinase (PEPCK), acyl-CoA synthetase, and lipoprotein lipase (LPL).

**Figure I2:** PPAR family of nuclear receptors. Top: comparison of human PPARs is shown. Numbers represent percent homology with PPARα: AF-1= activation function 1; DBD= DNA binding domain; LBD= ligand-binding domain; AF-2= activation function 2. Bottom: the PPARs bind to DNA response elements in the regulatory regions of target genes as heterodimers with RXR. When an agonist binds to the PPAR receptor, recruitment of coactivator proteins (not shown) leads to transcriptional modulation: PPRE= PPAR response element.

The various beneficial effects of PPAR ligands are depicted pictorially in figure I3.
Figure 13: The beneficial metabolic effects of PPAR ligands. PPARγ agonists are effective in treating T2DM. They modulate the expression of numerous genes in adipocytes, which results in improved insulin sensitivity, increased fatty acid uptake and decreased lipolysis. As a result, circulating FFA levels are diminished. Activation of PPARγ also results in changes in adipokine production, remodeling of adipose tissue, and the concurrent repartitioning of lipids from lipolytic visceral fat into subcutaneous fat that contains newly generated, small insulin-sensitive adipocytes. PPARγ agonists also decrease the inflammation of adipose tissue that is induced by obesity and contributes to increased insulin resistance. As a result of these multiple adipocentric actions (pale orange), PPARγ activation improves insulin sensitivity in skeletal muscle and liver, and reduces hyperglycemia. In dyslipidemic subjects, PPARα agonists induce lipid uptake and catabolism and the production of apolipoproteins A-I and A-II, thereby diminishing circulating TG and increasing HDL-C levels (cream). In addition to their anti-dyslipidemic activities, recent in vitro and preclinical data indicate that PPARα agonists also have direct vasoprotective effects (brown). Activation of PPAR-d increases fatty acid oxidation and uncouples energy metabolism in skeletal muscle. Thus, PPARδ agonists lower triglycerides, increase HDL-C and protect against obesity in preclinical species (dark red). Dotted lines represent activities observed only in preclinical experiments and in vitro.
1.10.1. PPARα

PPARα plays a pivotal role in the uptake and oxidation of fatty acids and also in lipoprotein metabolism. PPARα is expressed highly in liver, heart and skeletal muscle, tissues that extract a high level of their energy requirements from lipids. During prolonged fasting that results in hypoglycemia, fatty acids are released from fat depots and travel to the liver where they are taken up, oxidized and metabolized into ketone bodies to provide fuel for peripheral tissues. The crucial role of PPARα in mediating these metabolic processes and, ultimately, energy homeostasis is demonstrated by the phenotype of PPARα null mice, which, on fasting, are characterized by hypoglycemia, hypoketonemia, hyperlipidemia and hepatic steatosis. Dyslipidemia, characterized by elevated circulating levels of triglycerides (TGs) in combination with decreased levels of high-density lipoprotein cholesterol (HDL-C), is often a forerunner of cardiovascular disease. PPARα agonists decrease plasma TG levels and increase HDL-C levels. The former action is mediated by increasing lipid uptake, activation and catabolism through the transcriptional modulation of numerous genes that control these processes. The latter is mediated, in part, by augmenting hepatic production of apolipoprotein A-I (apoA-I) and apoA-II, which are major proteinaceous components of HDL-C. Additionally, PPARα agonists cause favorable changes in the particle size and subclass distribution of lipoproteins.

The effects of PPARα agonists (Figure I4) on circulating lipid parameters and, perhaps, vascular cells are beneficial because these ligands reduce the progression of atherosclerosis and the incidence of coronary events in major clinical studies, including the Helsinki Heart Study and the Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial (VA-HIT). The antiatherosclerotic efficacy of PPARα agonists is particularly pronounced in diabetic patients, as demonstrated in the VA-HIT and the Diabetes Atherosclerosis Intervention Study. Such results are noteworthy because cardiovascular disease is the major cause of mortality in T2DM patients, a cohort in which the prevalence of dyslipidemia is 2–3 times higher than in the general population.
1.10.2. PPARγ

PPARγ is present in high concentrations in adipocytes. Seminal studies in vitro have demonstrated that this receptor is both necessary and sufficient for adipocyte differentiation, and that it promotes lipid accumulation by adipocytes. The importance of PPARγ in adipocyte biology is underscored further by studies in vivo in which adipose-specific ablation of PPARγ expression in mice results in adipocyte hypocellularity, and heterozygous PPARγ knockout mice have reduced adiposity.

Consonant with the idea that PPARγ ligands (Figure I5) mediate their effects primarily through adipose tissue, it has been demonstrated that they alter the expression of genes that are involved in lipid uptake, lipid metabolism and insulin action in adipocytes. As a result, they enhance adipocyte insulin signaling, lipid uptake and anabolic lipid metabolism, and attenuate lipolysis and free fatty acid (FFA) release. Consequently, lipid levels in adipose tissue rise whereas circulating FFAs diminish. It has been proposed that by repartitioning lipids away from liver and muscle, the two primary tissues that are responsible for insulin-mediated glucose disposal and metabolism, PPARγ agonists ameliorate hyperglycemia by reversing lipotoxicity-induced insulin resistance. Thus far, TZD treatment has been shown to diminish the lipid content of liver but not skeletal muscle. Data from patients with type 2 diabetes mellitus (T2DM) and preclinical species also demonstrate that PPARγ agonists function as ‘adipose remodeling factors’ that redistribute lipids from insulin-resistant, lipolytic visceral-fat depots into subcutaneous fat that contains small, newly differentiated, insulin-responsive adipocytes. Because fatty acids that are released from visceral adipose tissue drain into the portal vein and can serve as gluconeogenic substrates in the liver, such anatomical changes are thought to decrease their availability, thereby reducing the hepatic production of glucose and further improving glucose homeostasis. In support of this hypothesis, human probands with inhibitory PPARγ mutations suffer from partial
lipodystrophy, which is characterized by decreased subcutaneous fat, increased visceral fat, hyperglycemia and insulin resistance.\textsuperscript{59,60} In addition to altering fat deposition, PPAR\(\gamma\) 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.\textsuperscript{61} For example, adiponectin, which potentiates insulin sensitivity in liver\textsuperscript{62} and skeletal muscle,\textsuperscript{63} is upregulated in response to PPAR\(\gamma\) activation.\textsuperscript{64,65} Other studies reveal that PPAR\(\gamma\) agonists reduce elevated levels of pro-inflammatory cytokines and chemokines that result from the excessive accumulation of macrophages in adipose tissue of obese, insulin-resistant rodents.\textsuperscript{66} Because pro-inflammatory cytokines derived from adipocytes and macrophages can inhibit insulin stimulated signal transduction and, thereby, induce insulin resistance,\textsuperscript{67,68} PPAR\(\gamma\) agonists might also improve insulin sensitivity through this immunosuppressant mechanism.

\textbf{Figure I5:} Two well known and popular PPAR\(\gamma\) agonists

\begin{center}
\begin{tabular}{ll}
Pioglitazone (4) & Rocsiglitazone (5) \\
\end{tabular}
\end{center}

1.10.3. \textit{PPAR}\(\delta\)

Because of its ubiquitous expression and the paucity of selective ligands, PPAR\(\delta\) is the least understood PPAR subtype. Nevertheless, early PPAR\(\delta\) selective agonists were found to elevate HDL-C levels in diabetic mice,\textsuperscript{69} a seminal observation that indicated that PPAR\(\delta\) ligands might have beneficial effects on dyslipidemia. Subsequently, the potent PPAR\(\delta\) agonist GW501516 [\{2-methyl-4-\{\{4-methyl-2-\{4-(trifluoromethyl)phenyl\}1,3-thiazol-5-yl\}methyl\}thio\}phenoxy \} acetic acid] was shown to increase HDL-C while decreasing elevated TG and insulin levels in obese rhesus monkeys.\textsuperscript{70} GW501516 also attenuates weight gain and insulin resistance in mice fed high-fat diets by increasing the expression in skeletal muscle of genes that promote lipid catabolism and mitochondrial uncoupling, thereby increasing \(\beta\)-oxidation of fatty acids in skeletal muscle.\textsuperscript{71}

The \(\alpha\)-helices of PPAR\(\gamma\) and PPAR\(\delta\) LBDs, represented by tubes are shown in \textbf{figure I6}.
Introduction

Figure I6: Crystal structures of the apo-PPAR LBDs. The α-helices are represented by tubes. Helix 2', helix 3, and the AF-2 helix are designated as H2', H3, and AF-2, respectively. The solvent-accessible ligand-binding site is indicated by the white shaded surface: (A) PPARγ LBD; (B) PPARδ LBD.

1.11. Role of PPAR Agonists in the therapy of Type 2 Diabetes

1.11.1. PPARα and PPARγ Agonists
The role of PPARα and PPARγ activation in ameliorating the hyperglycemia and hyperlipidemia associated with type 2 diabetes originates with two classes of compounds, the fibrates and the glitazones or thiazolidinediones (TZDs), which were empirically discovered via rodent pharmacology to have antihyperlipidemic and antihyperglycemic activity, respectively. The fibrates [e.g., clofibrate (1), fenofibrate (2), and bezafibrate (3); Figure I4] are drugs that have long been shown to effectively reduce triglycerides (TG) and free fatty acids (FFA) and increase high-density lipoprotein cholesterol (HDL) in both rodents and man. The discovery that these compounds as weak activators of PPARα suggested that this receptor may be the primary molecular target of this class of drugs. This hypothesis has been reinforced by the discovery of more potent and selective ligands for PPARs that display improved lipid lowering activity compared to the fibrates. In addition, fibrates have also been shown to improve glucose tolerance in type
2 diabetic patients, although this activity may not be attributable to activation of PPARα because some of these compounds also have appreciable PPARγ activity. Fibrates are generally well-tolerated drugs; however, they are associated with a number of side effects, the most common of which are gastrointestinal side effects such as nausea and diarrhoea, and elevations in liver enzymes. Skeletal myopathy and acute rhabdomyolysis have also been reported during treatment with all the currently marketed fibrates. It is not clear whether the effects on muscle are mediated by PPARα, but it will be important to carefully monitor these side effects with the more potent, selective PPARα agonists currently in clinical development. In addition, fibrates are excreted via the kidneys and thus should be avoided in patients with renal failure. Finally, fibrates have a propensity to cause drug-drug interactions because of their inhibition of cytochrome P450 enzymes and thus must be used with great caution in combination with other lipid-lowering drugs, particularly statins. While the combination of stains and fibrates has shown improved control of lipoprotein risk factors relative to either agent alone, this combination has shown an increase in renal failure, myopathy, and severe rhabdomyolysis.

The TZDs have been shown to enhance the sensitivity of target tissues to insulin and to reduce plasma glucose, lipid, and insulin levels in animal models of type 2 diabetes and in humans. The TZDs pioglitazone [Actos (4)] and rosiglitazone [Avandia (5)] (Figure I5) are currently marketed for the treatment of type 2 diabetes and represent important agents in the treatment of this disease both as monotherapy or in combination with existing therapies. These drugs display significant glucose-lowering efficacy, generally achieving mean decreases in haemoglobin A1C (HbA1C) of approximately 1-1.5% and mean decreases in fasting glucose of 60-80 mg/dL in type 2 diabetic patients. These drugs also display modest beneficial effects on TGs, FFAs, and HDL cholesterol. As was the case with fibrates and PPARR, the discovery that the TZDs are potent, selective agonists of PPARγ provided a key link to understanding the molecular mechanism of these drugs. These discoveries have alsoin the effort to optimize selective PPARγ agonists as effective antidiabetic agents and selective PPARα agonists as antihyperlipidemic agents.

While rosiglitazone and pioglitazone have many beneficial effects in type 2 diabetics, they also have some undesirable effects. A gain in weight of 3-5 kg is seen in most patients, with a minority gaining an inordinate amount of weight. This weight gain is accompanied by an increase in subcutaneous fat mass. The clinical significance of this
weight gain requires further evaluation, but in a treatment population that generally is already overweight this can minimally lead to negative psychological effects. In some patients this weight gain is accompanied by an increase in plasma volume leading to edema that is often resistant to diuretic therapy. This plasma volume expansion may precipitate or exacerbate congestive heart failure, and hence the currently marketed TZDs are not recommended in diabetic patients with NYHA class III and class IV cardiac status and should be used with caution in class I and class II patients. While the mechanism of the fluid retention seen with the marketed TZDs has not been elucidated, it is likely to be a PPARγ mediated effect because structurally unrelated selective PPARγ agonists also promote fluid retention.

Given the importance of controlling both glucose and lipid levels in type 2 diabetes, the concept of identifying ligands that bind and activate both PPARα and PPARγ represents a logical continuation in the field of PPAR research. In addition to their benefit on lipids, reports in the literature that fibrates reduce body weight gain in rodents without affecting food intake\textsuperscript{83,84} offer hope that activation of PPARα may mitigate the weight gain induced by PPARγ activation seen in humans. The hypothesis that PPARα/γ dual agonism should provide additive, and possibly synergistic, pharmacology has resulted in an intensive effort within the pharmaceutical industry to develop and evaluate these agents (Figure I7).\textsuperscript{85}

1.11.2. The PPAR dual agonists

PPARα/γ dual agonists

PPARα/γ dual agonists have been postulated as a combination strategy to achieve a broad spectrum of metabolic effects and reduce mortality rates associated with type 2 diabetes by improving insulin resistance, hyperglycemia and alleviating atherogenic dyslipidemia. In addition, PPARα agonists stimulate lipid oxidation and decrease adiposity in rodent obesity models. Thus, the propensity of PPARγ activation to induce weight gain through its adipogenic affects may be offset by the ability of PPARα activation to stimulate lipid catabolism thereby providing a compound with reduced propensity for inducing undesired weight gain.\textsuperscript{86,87} In recent years, a number of structurally diverse PPARα/γ dual agonists have been reported. Many of these have been evaluated in the clinic and some have advanced into late stage development. The clinical efficacy of the initially discovered
dual PPARα/γ agonists has been encouraging. However, the discontinuation of the development of the most promising PPARα/γ dual agonists, including muraglitazar, tesaglitazar, ragaglitazar, TAK559 and KRP297\textsuperscript{88-91} (Figure 17) due to various safety liability issues has been disconcerting. Other dual agonists are advancing through different stages of development. However, growing concerns regarding toxicities, as well as the recent requirement by the FDA to include 2-year rodent carcinogenicity studies prior to advancing any PPAR compound into any clinical study of greater than 6 months in duration, has tempered enthusiasm.

**Figure 17:** PPARα/γ dual agonists
The activities of the discussed PPARα agonists, PPARγ agonists and PPARα/γ dual agonists in terms of binding and cell-based functional assays are listed in table I3.

Table I3: Activity of PPARα/γ Agonists in Binding and Cell-Based Functional Assays

<table>
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<tr>
<th>compd</th>
<th>binding (μM)a</th>
<th>cell-based EC50 (μM)b</th>
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<tr>
<td></td>
<td>PPARα</td>
<td>PPARγ</td>
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<tr>
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</table>

*aAll data were generated using hPPAR LBD.85 Data reported are either Ki or IC50 values in which Ki = IC50/ (1 + [L]/Kd) (where IC50 is the concentration of test compound required to inhibit 50% of the specific binding of the radioligand, [L] is the concentration of the radioligand used, and Kd is the dissociation constant for the radioligand at the receptor). Refer to the original reference to determine whether reported values is Ki or IC50; nr = not reported. bAll data were generated using the hPPAR-GAL4 transactivation assay110; ia = inactive at 10 μM or the highest concentration tested; nr ) not reported. cData are for active metabolites. dData are for murine receptors.

1.12. Partial Agonism or Selective Modulation to the Rescue

Disappointingly, Full PPAR Agonists are plagued by certain adverse side effects. On the up side, Partial PPAR Agonists (selective modulators) have the potential to retain the desired efficacy and beneficial effects of full PPAR agonists while diminishing the unwanted effects. Especially in case of PPARγ; partial agonists are being developed with the goal of retaining the beneficial effects of this class of agents, while diminishing their adverse effects (e.g. metaglidasen).92 Studies with partial agonists of PPARγ suggests that a focus on partial PPAR agonists may be a way of developing agents that have the
desired efficacy of PPAR agonists and are devoid of, at least, some of their potential adverse effects.  

The hypothesis “Partial Agonism at both PPARα and PPARγ receptors by dual partial activators or of PPARγ by partial activators or selective modulators may provide a solution - resulting in the desirable responses and reducing the adverse effects” caused by the individual agonists for the treatment of T2DM attracted our attention and prompted us to develop a research protocol to design, select and synthesize and to carry out the docking analysis of such new chemical entities for the measurement of their binding affinities in the active site of the proteins concerned. Development of such agents being a very challenging endeavor, as the molecules should have individual/simultaneous affinity for all the aimed macromolecular targets with similar binding pockets and also the fine tuning of the conformational and geometrical constrains thus required to obtain the desired binding, which may finally result in desired activity profile and lesser or no adverse effects as compared to the full agonists.

1.13. Computational methods in the design of multiple receptor activating ligands

Computational methods, which has emerged as very useful and money saving tools in drug design, can be effectively employed in designing multiple receptor activating ligands. The design of multiple receptor activating ligands is very challenging as it requires structural fine tuning so that the designed molecule should bind to and activate the targeted receptors of structural similarity. The x-ray crystal structural information of the 3D-geometry of targeted receptors is advantageous for this special effort. Docking of designed molecules into the x-ray crystal structure of the active site of the target protein may reveal valuable approximation regarding binding of the molecules to the protein in biological system. Finally the analysis of the results obtained from Docking Experiments enable to classify among the targeted molecules to be taken forward for synthesis and characterization.

AIM AND OBJECTIVES

We aim to work on the hypothesis that Selective PPARγ Modulators (SPPARγMs) retain the desirable efficacy for Type 2 Diabetes Mellitus, but have diminished potential for, or
ideally lack all together, the undesirable PPARγ mechanism based side effects and laid
down certain objectives to meet and fulfill the latest concept that (SPPARγMs) could bind
to the receptor in a distinct mode relative to Full agonists, thereby providing a physical
basis for different biological effects because of which, such ligands act as Partial
Agonists.

To carry out Theoretical Structure Activity Relationships Studies on some of the
Selected Standard Molecules (PPARγ full and partial agonists, PPARα/γ dual full and
partial agonists and SPPARγMs) available commercially (withdrawn or under
clinical trials).

To modify the Selected Standard Molecules to Design a Virtual Library of NCEs
having almost all the structural feature required for the expected Selective
Peroxisome Proliferator Activated Receptor γ Modulators (SPPARγMs).

To carry out *Docking and Scoring Studies, of the theoretically selected among the
Designed NCEs and Selected Standard Molecules, in the active site of the proteins
concerned (PPARγ: 1i7i and PPARα: 1i7g) with Tesaglitazar (PARα/γ Dual
Agonist) as the template while using the Surf lexDock and CScore module of
SYBYL (A TRIPOS SOFTWARE) available in our in silico Drug Design Laboratory.

*A preliminary docking study of the N-methylbenzimidazol-2-yl linked meta-substituted
benzylidene/benzyl based NCEs (1-18) containing TZD, DEM and MAA as hydrogen bonding parts
have been carried out into the active site of (PPARγ: 2prg and PPARα: 1k7l) protein with
Rosiglitazone (PPARγ Full Agonist) as the template while using the Surf lexDock and CScore module of
SYBYL.92a.

Additionally a similar preliminary docking study of the N-methylindole-2-y/3-yl linked meta-
substituted benzylidene based NCEs (37-39 and 79-81) containing TZD, DEM and MAA as
hydrogen bonding parts have been carried out into the active site of (PPARγ: 1i7i and PPARα: 1i7g)
protein with Tesaglitazar (PARα/γ Dual Agonist) as the template92b.

To tabulate data obtained as a result of Docking and Scoring Studies of the targeted
among the theoretically selected NCEs and Selected Standard Molecules: Total
Score, D Score, PMF Score, G Score, CHEM Score and C Score in general and G
Score in particular.

To take snap shots, of Binding Disposition in the cavity of the protein concerned
and of Hydrogen Bonding Interactions of the NCEs with the AA residues of the
active site of the protein concerned along with that of Tesaglitazar extracted from
the PPARγ Protein - 1i7i and to tabulate the data in terms of Number and Distances
of the interacting AA residues.
To carry out comparison of the results of the binding modes and also the hydrogen bond interactions, between all the selected/targeted among the designed molecules and the AA residues in the active sites of PPARα and PPARγ proteins, to that of Tesaglitazar and subsequent support from the comparison of predicted binding affinities in terms of G Score values to classify and validate the selected among the designed NCEs into various categories as novel PPAR ligands.

Devising New Synthetic Strategies, which meet the requirement of “Ideal Synthesis” and also take into consideration the concept of ‘Connectivity Analysis’, thereby, providing new insights towards the skeletal and functionality requirement of these novel ligands.

Synthesis and Structural Characterization of the Targeted NCEs.

Keeping in view the importance of above cited facts coupled with the proposed hypothesis, we, in this research work have theoretically designed, selected and computationally docked and then synthesized novel heterocyclyl linked meta-substituted phenyl containing thiazolidinediones and acyclic analogs of isoxazolidinediones - a cyclic analog of thiazolidinedione, while introducing conformational and geometric constrains by decreasing the length of the linker and introducing unsaturation to attach the hydrogen bonding parts with the phenyl moiety, as potential Selective Peroxisome Proliferator Activated Receptorγ partial agonists as potential Selective Peroxisome Proliferator Activated Receptorγ Modulators (SPPARγMs) as partial agonists for the management of Type 2 Diabetes and Metabolic Syndrome.

Docking and Scoring studies of the some selected sets of theoretically designed New Chemical Entities, in the active site of the proteins concerned, have been carried out using the SurflexDock and CScore module of SYBYL (A TRIPOS SOFTWARE) available in our in silico Drug Design Laboratory, with the aim to classify, select for synthesis and validate computationally - the designed NCEs in comparison to some selected standard molecules withdrawn/suspended or are under clinical trials.

The New Chemical Entities were theoretically designed by the modification of some selected standard molecules (Charts I1-I2) acting as full or partial or dual or selective agonists for the PPARs so as to incorporate all The Pharmacophoric (Figures I8) for the molecule to act as PPARγ partial agonists as Selective Peroxisome Proliferator Activated Receptorγ Modulators (SPPARγMs).
Introduction

CHART 1: LITERATURE MOLECULES (PPAR LIGANDS) ATTRACTED OUR ATTENTION

PAT5A

OXADIAZOLE

KRP-297

CHART 1: LITERATURE MOLECULES (PPAR LIGANDS) ATTRACTED OUR ATTENTION

Rosiglitazone

Tesaglitazar

N-Acy1 indole

Oxime ether

Isoxazoline

MK-0533
**FIGURE I₈: GENERATION OF PHARMACOPHORIC FRAGMENTS**

**DESIGN OF NCEs**

A set of 108 NCEs (Table I₄ and Figure I₈) incorporating N-methyl/benzyl substituted benzimidazol-2-yl and indol-2-yl and also indol-3-yl linked at the *meta* position of the central phenyl ring of the benylidene/benzyl moiety through a methyleneoxy (2 atom)/oxy(1 atom)/direct(0 atom) linker was designed. The benylidene/benzyl moiety in turn holds the required hydrogen bonding parts, which as per present design are the Thiazolidine-2,4-dione (TZD), Diethyl malonate (DEM) and Methyl acetoacetate (MAA).

The criterion set for selection of the Targeted NCEs (*conformationally and geometrically constrained*) for synthesis, among the Designed NCEs was both theoretical as well as computational. The General (*Double Bond/ One Atom or Direct Linked/ Indole-3-yl and N-Benzyl etc.*) and Docking and Scoring (Table I₅) and the Binding Dispositions and Hydrogen Bond Interaction in the active site of the proteins concerned and the data thus generated helped us out to validate and compare and classify and select 28 NCEs for final synthesis, with respect to some standard selected molecules, in the present research work (Chart I₃).
TABLE I: DESIGNED SET OF 108 NCEs: SELECTION CRITERION - THEORETICAL AND COMPUTATIONAL

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</table>

DESIGNED: 108 THEORETICALLY DELETED: 36 COMPUTATIONALLY DELETED: 47 AND OTHERWISE ADDED: 3 = 2892a-b
CHART I: THE TARGETED 28 NCEs

Targeted NCEs: 25: Green and Additionally Targeted NCEs: 3 Green*
We accordingly planned to synthesise almost all the Targeted NCEs (conformationally and geometrically constrained) through ‘Knoevenagel Condensation’ of the thiazolidinediones (T: TZD) or diethyl malonate (D: DEM) or methyl acetoacetate (M: MAA) based hydrogen bonding parts with the appropriate heterocyclic linked benzaldehydes, while using piperidinium acetate and toluene under reflux, for example (Schemes 4A).

‘Knoevenagel Condensation’ being unsuccessful in case of heterocyclyl linked benzaldehydes, having methyleneoxy (2 atoms) and oxyp (1 atom) linker, while using methyl acetoacetate based hydrogen bonding part leading to corresponding benzylidenes/benzyl containing Targeted NCEs - prompted us to follow ‘Halide Displacement and Mitsunobu Reaction’ methodology while using appropriately substituted methyl acetoacetate linked benzylidene/benzyl based phenolic derivatives for example (Scheme 4A1 and 6A1).

**SCHEME 4A: METHYL BENZIMIDAZOLYL-2 LINKED NCEs (MBD2T and MBS2D)**

(5Z)-5-{3-[(1-methyl-1H-benzimidazol-2-yl)methoxy]benzylidene}-1,3-thiazolidine-2,4-dione diethyl 2-{3-[(1-methyl-1H-benzimidazol-2-yl)methoxy]benzylidene}propanedioate

- **MBD2T**
- **MBD2D**

a: Piperidinium acetate, Toluene
SCHEME 4\textsubscript{A1}: METHYL BENZIMIDAZOLYL-2 LINKED NCEs
(MBD2M and MBS2M)

methyl (2E)-2-{3-[(1-methyl-1\textsubscript{H}-benzimidazol-2-yl)methoxy]benzylidene}-3-oxobutanoate
methyl 2-{3-[(1-methyl-1\textsubscript{H}-benzimidazol-2-yl)methoxy]benzyl}-3-oxobutanoate

SCHEME 6\textsubscript{A1}: METHYL INDOLYL-2 LINKED NCEs
(MI2D2M)

methyl (2E)-2-{3-[(1-methyl-1\textsubscript{H}-indol-2-yl)methoxy]benzylidene}-3-oxobutanoate

The structure of all the intermediates and Targeted NCEs were confirmed through spectral studies.