1. INTRODUCTION

Drug discovery and development

Drug discovery begins in the laboratory with scientists, including chemists and pharmacologists, who identify cellular and genetic factors that play a role in specific diseases. They search for chemical and biological substances that target these biological markers and are likely to have drug-like effects. Out of every 5,000 new compounds identified during the discovery process, approximately 5 are considered safe for testing in human volunteers after preclinical evaluations. After three to six years of further clinical testing in patients, only one of these compounds is ultimately approved as a marketed drug for treatment. Today there is a considerable search for the discovery of newer drugs with advantages over the existing drugs in the market.

1.1 Diabetes mellitus

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia arising as a consequence of a relative or absolute deficiency of insulin secretion or resistance to insulin action or both [1-4]. The number of people with diabetes are increasing in every country throughout the world. In 2011, 366 million people were suffering from diabetes globally and 4.6 million died. It has been estimated 552 million people are likely to be affected by this metabolic disorder by 2030. Diabetes caused 465 US billion dollars in healthcare expenditure in 2011. As per the study conducted by the Indian Council of Medical Research, 62.4 million people were affected with diabetes and 77.2 million people with prediabetes in 2011 in India [5-9].

The vast majority of diabetes cases fall into two main categories, Insulin dependent diabetes mellitus (Type I) and non-insulin dependent diabetes mellitus (Type II). Type I diabetes is usually due to an immune-mediated destruction of pancreatic islet β-cells with consequent insulin deficiency and the need to replace insulin. Type II diabetes (T2DM) is characterized by tissue resistance to the effect of insulin secreted by pancreatic β-cells. The ability of pancreatic β-cells to continue production of insulin diminishes over time. When insulin production declines in the phase of insulin resistance, glucose disposal from the muscle is diminished and suppression of hepatic glucose
output is decreased leading to diabetes. Insulin resistance is also a contributing factor in atherosclerosis, hypertension, lipid disorders and polycystic ovarian syndrome. Diabetes is associated with several complications of eye, kidney, nervous system, blood vessels, heart and metabolic derangements such as ketoacidosis, hypertriglyceremia, glycosuria, atherosclerosis, retinopathy, nephropathy, neuropathy, skin disease, etc. [10-14].

1.2 Pathogenesis of Type II diabetes mellitus
T2DM is a multifactorial and complex disease resulting from insulin resistance, impairment in insulin secretion and unrestrained hepatic glucose production. It is influenced by both genetic and environmental factors. Multiple genes are involved in the pathogenesis of T2DM and only 5-10% of this disease occurs due to monogenic defects. The mutations in the genes involved in insulin secretion (HNF-1α, HNF-1β, HNF-4α, IPF-1, Glucokinase, Neuro-D1 factor, SUR-1, KCNJ11, etc) and insulin resistance (PPAR-γ, PGC-1α, IRS-1, Calpain-10, ADIPO R2, etc) have been identified to play an important role in the pathogenesis of this disease. Some of the environmental factors which play an important role in the pathogenesis of the disease [15-17] include high calorie diet, obesity, lack of physical activity, smoking, low fibre diet, high intake of saturated fats, etc [18]. The diabetic genotype predisposes an individual for glucose intolerance. Development of T2DM is also influenced by environmental factors.

1.3 Pathophysiology of hyperglycemia
A balanced interplay between insulin action and insulin secretion is required to maintain the normal glycemia. Pancreatic β-cells, by adopting themselves to the changes in insulin sensitivity, play a vital role in the maintenance of normal glycemia (Figure 1).
As a result of this a curvilinear relationship exists between β-cell function and insulin sensitivity. In T2DM patients, a deviation from this normal curvilinear relationship (hyperbola) occurs as a result of inadequate β-cell function, i.e. β-cells can not compensate for the reduced insulin sensitivity resulting in the development of hyperglycemia (Figure 2) [18]. In addition to this deviation, progression along the hyperbola also results in hyperglycemia. In this condition although there is a compensatory increase in the β-cell function to adapt for the reduced insulin sensitivity, a slight hyperglycemia is generally observed at fasting and 2h after glucose load [19]. This increase over a period of time becomes damaging because of glucotoxicity and this in itself is a cause for β-cell dysfunction. Thus, in spite of unlimited β-cell reserves, insulin resistance paves the way for hyperglycemia and T2DM.
The treatment generally prescribed for T2DM has been a combination of diet, exercise, and a hypoglycaemic agent, commonly sulfonylureas and biguanides. Sulfonylureas, which are insulin secretagogues, stimulate insulin secretion from pancreatic β-cells and are often known to induce severe hypoglycemia and weight gain. In addition, both primary and secondary treatment failure rates with sulfonylureas are high, leading to complications. Therefore, drugs that reverse insulin resistance without stimulating insulin release from β-cells fulfill a major medical need in the treatment of T2DM and hence has the potential to reduce long term complications of T2DM. Since the pioneering discovery of the drug, ciglitazone, by a group of scientist at Takeda, which effectively reduces insulin resistance by potentiating insulin action in genetically diabetic and/or obese animals, several new thiazolidine-2,4-diones have been developed.

1.4 Pathophysiology of insulin resistance
The metabolic effect of insulin is mediated by a specific plasma membrane receptor with tyrosine kinase activity. Binding of insulin to its receptors activates the intrinsic tyrosine kinase activity of the receptors and results in phosphorylation of tyrosine residues of insulin receptor substrate (IRS) proteins. These serve as binding scaffolds for various adaptor proteins and lead to downstream signaling cascade. This process activates a series of lipid and protein kinase enzymes involved in the translocation of glucose transporters to the cell surface, synthesis of glycogen, protein, mRNAs and nuclear DNA, which affect the cell survival and proliferation (Figure 3) [20]. Any interference with this normal signaling process would result in negative regulation and the development of insulin resistance.

1.5 Pharmacological therapy of T2DM
Pharmacological therapy is warranted in patients who are unable to change their life style through hypocaloric diet, increased physical activity and weight loss, and for those who continue to have glycemia in spite of these changes. Currently, there are 11 classes of FDA approved antihyperglycemic agents available for the management of T2DM (Table 1) [21]. All these agents,
however, suffer with one or more limitations such as limited efficacy, limited tolerability and significant mechanism based side effects.

![Figure 3. Insulin signalling and resistance](image)

**Table 1. FDA approved agents for the treatment of T2DM**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Antidiabetic class</th>
<th>Available Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sulfonyleureas</td>
<td>First generation - Tolbutamide, Chlorpropamide&lt;br&gt;Second generation - Glibenclamide, Glipizide, Gliclazide, Glimepride</td>
</tr>
<tr>
<td>2</td>
<td>Biguanides</td>
<td>Phenformin, metformin</td>
</tr>
<tr>
<td>3</td>
<td>Meglitinide analogues</td>
<td>Repaglinide, Nateglinide</td>
</tr>
<tr>
<td>4</td>
<td>α-Glucosidase inhibitors</td>
<td>Acarbose, Miglitol</td>
</tr>
<tr>
<td>5</td>
<td>Thiazolidinediones</td>
<td>Rosiglitazone, Pioglitazone</td>
</tr>
<tr>
<td>6</td>
<td>Parenteral insulin and its analogs</td>
<td>Regular, Lispro, Aspart, Lente, Insulin glargine</td>
</tr>
<tr>
<td>7</td>
<td>Dipeptidyl peptidase-4 Inhibitors</td>
<td>Sitagliptin, Saxagliptin</td>
</tr>
<tr>
<td>8</td>
<td>Bile acid sequestrant</td>
<td>Colesevelam</td>
</tr>
<tr>
<td>9</td>
<td>Dopamine receptor agonist</td>
<td>Bromocriptine mesylate</td>
</tr>
<tr>
<td>10</td>
<td>Amylinomimetics</td>
<td>Pramlintide acetate</td>
</tr>
<tr>
<td>11</td>
<td>Glucagon like peptide-1 receptor agonists</td>
<td>Exenatide</td>
</tr>
</tbody>
</table>
1.6 Peroxisome proliferated activated receptors

Peroxisome proliferated activated receptors (PPARs), namely PPAR-α, PPAR-β, PPAR-γ and PPAR-δ, (Figure 4) are members of nuclear receptor superfamily of ligand activated transcription factors. PPAR-α was the first one to be identified and they have been cloned from mouse and human [22-26]. PPARs are believed to play a physiological role in the regulation of lipid metabolism. They can be activated by high concentrations of fatty acids (FA) and have been shown to regulate the expression levels of FA binding proteins or enzymes involved in FA oxidation [27-28]. Many of the PPAR receptors such as steroids, thyroxine and retinoids have been studied for their physiological ligands. The natural ligands of these receptors, however, are yet to be identified and hence they are still classified as “orphan” receptors [29].

![Figure 4. Peroxisome proliferated activated receptors](image)

PPAR-β and PPAR-γ have been reported to be involved in embryo implantation, skin proliferation and differentiation [30], preadipocyte proliferation, and the up regulation of lipid metabolism in the muscle. PPAR-γ gene produces two protein isoforms-PPAR-γ1 and PPAR-γ2. PPAR-γ1 is expressed at low levels in many tissues including adipose tissue and muscle, whereas PPAR-γ2 is most abundantly expressed in the adipose tissue. As a transcription factor, PPAR binds to specific DNA response elements after forming heterodimers with retinoid X receptors (RXRs) to regulate transcription of target genes [31]. Growing evidence suggests that PPAR-γ is master transcriptional regulator involved in adipogenesis, energy storage and lipid redistribution [32].
Activation of PPAR-γ by thiazolidine-2,4-diones (TZDs) improves glycaemic control and insulin sensitivity. Hyper insulinemic clamp studies have reported that TZDs can improve whole body insulin sensitivity by increasing glucose and lipid uptake and glucose oxidation in the target tissues [33].

The major task of drug design and development in this connection is, therefore, to aim for selective agonists that bind to PPAR-γ. Several glitazones and non glitazone PPAR-γ agonists are under intensive clinical development for T2DM and other components of the metabolic (insulin resistance) syndrome, including hypertriglyceremia, hypertension and increased cardiovascular risks [34-38].

1.7 PPAR agonists
TZDs, also known as glitazones or thioglitazones, are insulin sensitizing pharmacological agents that reduce insulin resistance and preserve islet β-cell function in T2DM [39-42]. These effects are largely mediated through the ability of these compounds to activate PPAR-γ, the nuclear transcription factor, that controls genetic programs involved in glucose and lipid homeostasis, energy metabolism, adipocyte differentiation and maturation (Figure 5) [43-44]. PPAR-γ, like putative natural ligands, are composed of long chain fatty acid, cyclopentenone, prostaglandins, phenyl acetic acids and tyrosine based compounds [45-47].

![Figure 5. Role of PPARs](image-url)
In the course of investigation on the fibrate class of hypolipidemic agents [48-49], a series of 5-(4-alkoxybenzy1)-2,4-thiazolidinediones were shown to reduce insulin resistance in genetically diabetic and obese animals. With the pioneering discovery of ciglitazone which normalizes the plasma glucose and effectively reduces insulin resistance by potentiating insulin action in genetically diabetic and/or obese animals, several new TZDs have been developed (Figure 6). Ciglitazone, which became the prototype for this class of drug, was found to reduce hyperglycemia, hyperlipidemia in insulin-resistant animal models, but not in normoglycemic animals [50-51]. Ciglitazone was taken into human trials in T2DM subjects, but was withdrawn later because of its low potency and the appearance of cataracts in animals receiving long-term exposure to the drug.

Figure 6. Development of glitazones

In late 1990’s, troglitazone was approved by Food and Drugs Administration (FDA) for the treatment of T2DM but was suspended from the UK market in
December 1997 due to concerns of drug induced hepatotoxicity. In June 1998, the National Institute of Health (NIH) terminated a study investigating troglitazone’s potential for preventing T2DM due to documented cases of fatal hepatotoxicity.

In 1999, two new TZDs, pioglitazone and rosiglitazone, were approved by the United States Food and Drugs Administration (USFDA) for the treatment of T2DM. Later researchers have reported that TZDs should be avoided in patients with ventricular function or chronic renal insufficiency based on patient records [52]. USFDA suggested some additional warnings with respect to cardiovascular risks associated with the use of rosiglitazone (Windia). FDA also stated that development of newer and safer drugs from this class are necessary. Realizing the importance of the development of oral antidiabetic agents in india, companies like Dr. Reddy’s Research Foundation (DRF), Hyderabad, has been working on newer improved thioglitzazone analogs, namely balaglitazone and ragaglitazar (DRF-2189), for the treatment of T2DM. The molecule ragaglitazar did live upto its promise in phase III, but unfortunately the dose at which it reproduced the animal data in humans also caused unacceptable side effects. The development of ragaglitazar was, therefore, suspended in December 2002. However, balaglitazone is undergoing phase III clinical trials [53].

The exact mechanism of action for TZDs is not fully understood. TZDs are known to act on PPAR-γ receptors which regulate the gene expression mainly in the adipose tissues. Thus, PPAR-γ agonists, like thiazolidinediones, may have a direct action on muscle and liver which is independent of their action on fats. Alternatively, improved insulin sensitivity and lowered blood glucose with these agents in the treatment of T2DM may be an indirect effect of their action on adipose tissue. Signaling through a number of adipocyte-derived factors, including TNF-α, leptin, resistin and adiponectin, is altered by PPAR-γ activation in ways that could lead to enhanced insulin sensitivity. In addition, free fatty acids, triglycerides and products of fatty acid metabolism regulate insulin sensitivity. PPAR-γ has a significant role in adipocyte differentiation
and in the regulation of fat storage and utilization. However, TZDs are used clinically as hypoglycemic agents and to improve insulin sensitivity. Glitazones share a common partial chemical structure. TZD is a five-membered heterocyclic ring (Figure 7). It is planar in nature and shows keto-enol tautomerism due to the 1,3-hydrogen shift which leads to the formation of enol isomer of the TZD ring. It has been proposed that formation of the enol isomer is mainly responsible for the rapid racemization of TZD ring. Computational studies indicate that higher acidity at the chiral centre is responsible for the observed racemization and not the rapid keto-enol tautomerization. Glitazones contain a stereogenic centre at the C-5 position of the TZD ring.

![Figure 7. General structure of thiazolidine-2,4-dione](image)

### 1.8 Structure-activity relationships (SAR)

The relationship of structure to hypoglycemic activity and activation of the various PPARs with substituted TZDs and related compounds have been the subject of intensive investigations [48, 49, 55-65]. Some general structure-activity relationships are apparent from a comparison of the common features of the more potent compounds identified in these studies. TZD hypoglycemic agents can be viewed as being composed of an acidic head group connected to a lipophilic tail by a phenoxyalkyl linker (Figure 8). The pKa value for TZDs is about 6.8 and these compounds are thus partially ionized at physiological pH. Other acidic moieties, heterocyclic groups like oxazolidinediones, particularly a-substituted carboxylic acids and rhodanine, can also replace the TZD ring. In the lipophilic tail, incorporation of a wide variety of mostly aromatic and heteroaromatic groups has produced active agents.
Compound 1 (Figure 9) has similar potency [64] in functional transactivation assays for both PPAR-α and PPAR-γ (PPAR-α 13nM, PPAR-γ 4nM), whereas farglitazar 2 is highly selective [60] for PPAR-γ (PPAR-α 450nM, PPAR-γ 0.35nM). Neither 1 nor 2 has any significant PPAR-δ activity. There is a chiral center at the 5 position of the TZD ring, but this is not configurationally stable under physiological conditions. For substituted carboxylic acids, the PPAR-γ activity resides in the S-enantiomer. These compounds, including the TZDs, can be viewed as derivatives of phenylpropionic acid by combining the acidic head group with the phenyl group of the linker. Some arylacetic acids, (3 and 4), are also fairly potent PPAR-γ agonists, although the SAR is not thoroughly explored. Compound 3 is highly selective for PPAR-γ, whereas compound 4 activates both PPAR-δ and PPAR-γ.

A phenoxyethyl group (1, n = 2) as the central phenoxyalkyl linker is commonly found to yield highly active compounds in SAR studies of hypoglycaemic TZDs. Often shorter chain lengths (1, n = 1) or inclusion of the phenoxyethyl group into a heterocyclic ring also leads to active compounds. In the lipophilic tail, incorporation of a wide variety of mostly aromatic and heteroaromatic groups
has produced active agents. In a very limited study [57], the hypoglycemic potency in a series of oxazolidinediones with variations in the lipophilic tail was found to increase with increasing log P. A small number of 3D-QSAR studies on the TZDs have been reported, but these agents and the PPAR receptors are not particularly well suited for this type of analysis. 3D-QSAR studies are highly dependent on alignment, which can be difficult with very flexible molecules that bind to a receptor principally by large regional diffuse hydrophobic interactions.

Due to their long-term side effects, TZDs are less well known compared to other classes of hypoglycaemic agents. Both rosiglitazone and pioglitazone are generally well tolerated. Weight gain and minor oedema are associated with TZD therapy, and their use in patients with moderate to severe chronic heart failure has not been advised. Decreases in hemoglobin levels and hematocrit have also been found. The first marketed TZD, troglitazone, was withdrawn because of increased risk of hepatotoxicity. Approximately 1.9% of patients, treated with troglitazone in preapproval controlled clinical trials showed an increase in plasma of the liver enzyme, alanine aminotransferase (ALT) greater than three times the upper limit of the normal range. In trials with rosiglitizone or pioglitizone, the incidence of significantly elevated ALT was low and similar to that of placebo. However, regular monitoring of liver enzymes is recommended when these drugs are used [66, 67].

The Endocrinologic and Metabolic drugs Advisory Committee and the Drugs Safety and Risk Management Advisory Committee of USFDA have concluded that the use of rosiglitazone for the treatment of T2DM is associated with a greater risk of myocardial ischemic events than placebo, biguanides or sulphonylurea. It based its conclusion on three independently conducted meta-analysis of 42 randomized trials demonstrating an increase in the relative risk of myocardial infarction, angina or sudden death among patients taking rosiglitazone resulting in its withdrawal from the market worldwide.

In the light of above the present research was, therefore undertaken. It was proposed to design, synthesize and evaluate some novel glitazones as possible insulin sensitizers for the treatment of T2DM.