Chapter 1

Current Scenario of Drug Development for the treatment strategies of Diabetes

1.1 Introduction

Diabetes mellitus is a major and growing public health problem throughout the world, with an estimated worldwide prevalence in 2000 of 150 million people, expected to increase to 220 million people by 2010. Pronounced changes in human behaviour and lifestyle over the last century have resulted in a dramatic increase in the incidence of diabetes worldwide. Presently, diabetes is the 4th leading cause of death in developed countries and its management exerts a vast economic and social burden.

Many people also have other abnormalities of glucose (sometimes called “prediabetes”) manifest either as impaired fasting glucose (IFG) levels or as impaired glucose tolerance (IGT). The criteria for diagnosis of diabetes and prediabetes are summarized in Table 1. Collectively, diabetes, IFG, and IGT have been dubbed “dysglycemia”. The combination of dysglycemia, obesity, dyslipidemia, and blood pressure elevation is known as the “metabolic syndrome” or the “dysmetabolic syndrome” or “diabesity”. A number of metabolic and clinical abnormalities are included in the dysmetabolic syndrome, as summarized in Table 2.

Table 1. Diagnostic Criteria for Diabetes Mellitus and prediabetes

<table>
<thead>
<tr>
<th></th>
<th>fasting plasma glucose mg/dL (mmol/L)</th>
<th>2 h plasma glucose mg/dL (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal</td>
<td>&lt;100 (5.6)</td>
<td>&lt; 140 (7.8)</td>
</tr>
<tr>
<td>Prediabetes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>impaired fasting glucose</td>
<td>100-125 (5.6-6.9)</td>
<td></td>
</tr>
<tr>
<td>impaired glucose tolerance</td>
<td></td>
<td>140-199 (7.8-11.0)</td>
</tr>
<tr>
<td>diabetes mellitus</td>
<td>≥126 (7.0)</td>
<td>or ≥200 (11.1)</td>
</tr>
</tbody>
</table>

*Diabetes also may be diagnosed with plasma glucose greater than 200 mg/dL (11.1 mmol/L) and unequivocal symptoms (polyuria, polydipsia, unexplained weight loss). Diagnostic criteria for diabetes mellitus includes confirmation on a subsequent day.

Fasting = no caloric intake for at least 8 h. 2 h following a 75g oral glucose load, i.e., oral glucose tolerance test (OGTT).

Table 2. Components of the Dysmetabolic syndrome

- insulin resistances hyperinsulinemia relative to glucose levels
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- acanthosis nigricans
- central obesity
- glucose intolerance or type 2 diabetes
- blood pressure elevation or hypertension
- dyslipidemia/hypertriglyceridemia and decreased HDL cholesterol, small, dense LDL cholesterol particles
- increased plasma uric acid
- Hypercoagulability-increased plasminogen activator inhibitor
- vascular endothelial dysfunction
- coronary artery disease

Diabetes mellitus is a group of metabolic disorders, characterized by hyperglycemia arising as a consequence of a relative or severe deficiency of insulin secretion, or its resistance or both along with the metabolic derangements of carbohydrates, fats and proteins. Diabetes mellitus is commonly classified into two broad types:

1. Insulin Dependent Diabetes Mellitus (IDDM) or type 1 diabetes characterized by severe deficiency of insulin production, usually due to autoimmune destruction of β-cells of pancreas.

2. Non-Insulin Dependent Diabetes Mellitus (NIDDM) or type 2 diabetes, the more common type, is usually due to resistance to insulin action in the setting of inadequate compensatory insulin secretory response.

Type 2 Diabetes Mellitus (T2DM) is the most important type of diabetes because more than 90% of diabetics are of this type. Further, 80 percent of T2DM patients are obese and most of them hyperinsulinaemic and insulin-resistant. T2DM is the result of impaired insulin stimulated glucose uptake and utilization in liver, skeletal muscle, adipose tissue and impaired suppression of hepatic glucose output.

Figure 1. Type 2 diabetes accounts for up to 95% of all cases of diabetes.

Insulin resistance is very common because of obesity, a sedentary life style and aging (Figure 2) resulting in high blood pressure, dyslipidemia and diabetes. Type 2 diabetes does not always occur with insulin resistance but defect in insulin secretion is associated with
microvascular complications viz. blindness, neuropathy and nephropathy and macrovascular complications leading to atherosclerosis, limb amputation. In this situation presence of C-peptide and absence of markers of autoimmunity such as antibodies to glutamic acid decarboxylase may help to diagnose T2D. In view of rapid increase in diabetes cases WHO and American Diabetes Association have reduced the figure of the blood glucose level from 140mg/dl to 126mg/dl for the risk of diabetes. Estimation of glycosylated haemoglobin (HbA$\text{Ic}$), which is a marker of average plasma glucose concentration, is still valid for the diagnosis of T2D.

Figure 2. Causes and consequences of insulin resistance.

Hyperglycemia occurs in the basal or fasting state because of increased hepatic glucose production as a consequence of abnormality in insulin secretion and hepatic insulin resistance. In contrast, hyperglycemia in prandial state arises due to increased absorption of glucose from the gastrointestinal track and its reduced disposal because of deficient insulin secretion to compensate insulin resistance Figure 3.

Figure 3. Regulation of blood glucose (J. Med. Chem. 2004, 47, 4113-4117).

Current therapies for the management of T2D include suitably balanced diet, exercise, and variety of pharmacological agents including insulin, sulfonylureas, biguanides and
thiazolidinediones (TZDs). These agents act by different mechanisms to normalize blood glucose levels and avoid serious complications that affect the kidney, cardiovascular, ophthalmic and nervous systems.\textsuperscript{19} There are mainly five categories of orally active antidiabetic drugs on the market: sulfonylureas (SU) and non-sulfonylurea (non-SU) insulin secretagogues that stimulate insulin secretion by pancreatic cells; with increasing severity of diabetes, insulin administration is prescribed, metformin, a biguanide for type 2 diabetes, thiazolidinedione class of Peroxisome Proliferator-Activated Receptor Gamma (PPAR-\(\gamma\)) activators such as pioglitazone and rosiglitazone and the \(\alpha\)-glycosidase inhibitors that delay intestinal carbohydrate absorption and blunt postprandial glucose excursions. In 1999, the discovery was announced that a PTP1B knockout mouse displays enhanced insulin sensitivity,\textsuperscript{20} and resistant to weight gain.\textsuperscript{21} These findings generated a vivid interest in PTP1B and other PTPs\textsuperscript{22}•\textsuperscript{23} as drug targets for diabetes and, possibly, obesity. Our objective in this review is to project the current scenario of treatment strategies for T2DM.

\textbf{1.2 Insulin or Insulin Secretagogues}

Oral agents that increase pancreatic insulin secretion are called secretagogues. This class of therapeutics can be divided into sulfonylureas and non-sulfonylureas.

\textbf{1.2.1 Insulin}

Insulin is a peptide hormone secreted from \(\beta\)-cells of pancreas, which mediates glucose transport from blood to the cells (figure 4). For the past several years, insulin is being also used for the treatment of T2D.\textsuperscript{19} It regulates the blood glucose primarily by stimulating translocation of glucose transporter GLUT\(_4\) from intracellular sites to the membrane. It also controls the blood sugar level by inhibiting gluconeogenesis, glycogenolysis\textsuperscript{24} and metabolism of free fatty acid.\textsuperscript{25} Insulin acts through signal transduction mechanism by interacting with insulin receptor present on the cell membrane of all cell types mostly liver and fats. Insulin receptor is tetrameric glycoprotein consisting of 2\(\alpha\) and 2\(\beta\) subunits, linked by disulfide bond and spread across the membrane. The \(\alpha\)- and \(\beta\)-subunits function as allosteric enzymes in which \(\alpha\)-subunit inhibits the tyrosine kinase activity of \(\beta\)-subunit. Binding of insulin to the binding site present in \(\alpha\)-subunit induces aggregation and internalization of receptors along with the bound insulin molecule leading to the stimulation of kinase activity in \(\beta\)-subunit followed by transphosphorylation and a conformational change of receptor that further increases kinase activity of \(\beta\)-subunit\textsuperscript{26} which ultimately leads to the stimulation of insulin metabolizing enzymes. Insulin action is also mediated by certain second messengers like phosphatidylinositol glycan (PIG) and diacylglycerol (DAG) formed by specific phospholipase C.
Very recently, a new chemical substance, hepatic insulin sensitizing substance (HISS) has been discovered which is yet to be identified chemically.\textsuperscript{27} HISS is secreted from liver in response to the injection of insulin and brings about 50-60\% of glucose disposal in a dose dependent manner of insulin for a wide range (5-100 mL/Kg).

Insulin is administered by subcutaneous injection, which is not always convenient mode of drug administration. Moreover, exogenous insulin does not replicate the normal pattern of the nutrient related basal insulin secretion. The difference in the action of injected and endogenously secreted insulin is due to difference in their pharmacokinetic pathways.\textsuperscript{28,29} These shortcomings have been minimized by developing rapid acting insulin analogs, insulin lyspro and insulin aspartate.\textsuperscript{30} Long acting insulin analogs, insulin glargine and insulin detemir\textsuperscript{31,32} are available for the treatment of type 2 diabetes. Another analog of insulin, glulisine, is currently in advance clinical trials.\textsuperscript{33,34}

1.2.2 Sulfonylureas as Oral Antidiabetic Agents

Sulfonylurea receptor represents one of the most important classes of K\textsubscript{ATP} channels, found in \(\beta\)-cells of pancreas. Sulfonylurea insulin secretagogues act through these receptors, which exert their actions in response to the cytosolic concentration of ATP. The increase in ATP concentration on glucose metabolism in normal conditions closes the K\textsubscript{ATP} channels leading to membrane depolarization of the \(\beta\)-cells of pancreas. The depolarized membrane opens intracellular calcium ion channel, resulting Ca\textsuperscript{2+} entry into the cell. The increase in intracellular calcium ion concentration triggers insulin exocytosis.

1.2.2.1 Sulfonylureas as Insulin Secretagogues

Sulfonylurea insulin secretagogues, being the first line of oral antidiabetic agents, have been studied extensively. Carbutamide, the first sulfonylurea was initially discovered serendipitously, followed by other members of this series. These compounds have been classified as first and second-generation of sulfonylureas. The important first generation sulfonylureas are shown in Table 1. Refractory failures, hypoglycemia and weight gain are the major side effects of these agents.\textsuperscript{35}

1.2.3 Non-sulfonylureas as Insulin Secretagogues

These compounds have rapid onset but short duration of action that makes them suitable agents for the treatment of hyperglycemia as well as hyperinsulinemia. Initially, meglitinide the first member of this series, an analog of glibenclamide was studied for its antidiabetic and insulinotropic activity more than two decades ago.\textsuperscript{36} Structural
manipulations of this compound led to the development of other members. Repaglinide (prandin), nateglinide, mitiglinide and an interesting morpholinoguanidine derivative BTS 67582 are new insulinotropic agents. Non-sulfonylurea compounds are superior drugs with respect to first and second generation of sulfonylureas because they do not cause hypoglycemia and weight gain. Repaglinide is more active compared to nateglinide.

**Table 1:** First generation of sulfonylureas.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Name of the drug</th>
<th>R₁</th>
<th>R₂</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Carbutamide</td>
<td>NH₂</td>
<td>C₄H₉</td>
</tr>
<tr>
<td>2</td>
<td>Tolbutamide</td>
<td>CH₃</td>
<td>C₄H₉</td>
</tr>
<tr>
<td>3</td>
<td>Chlorpropamide</td>
<td>Cl</td>
<td>C₃H₇</td>
</tr>
<tr>
<td>4</td>
<td>Tolazamide</td>
<td>CH₃</td>
<td>Azepan-1-yl</td>
</tr>
<tr>
<td>5</td>
<td>Acetohexamide</td>
<td>H₃CCO</td>
<td>Cyclohexyl</td>
</tr>
</tbody>
</table>

**Figure 4:** Second generation of sulfonylureas

**Figure 5:** Non-sulfonylureas secretagogues.
1.3 Glucose Mediated Insulin Secretion Enhancers

1.3.1 Glucagon like peptides and Dipeptidylpeptidase-IV

The two major adverse side effects of weight gain and loss of β-cell function associated with current regime of drugs have shifted the focus on glucagon like peptide hormones as new potential drugs for the treatment of type 2 diabetes.\(^{39}\)

It has long been known that oral administration of glucose results in increased insulin secretion relative to intravenous glucose administration. The increased insulin secretion in response to oral nutrients is known as the “incretin” effect. In recent years, the incretin hormone glucagon-like peptide 1 (GLP-1) has been the subject of intense research efforts related to the treatment of type 2 diabetes.\(^{40,41}\) GLP-1 is a 30 amino acid hormone produced by L-cells of the intestinal mucosa that results from tissue-specific processing of the proglucagon gene.\(^{42}\) This is the same gene that is present in a cell of the endocrine pancreas. Posttranslational processing of the proglucagon gene transcript in a cell produces the 29 amino acid hormone glucagon as the primary bioactive product, along with other proglucagon-derived fragments. The primary products of the L-cell, GLP-1 and GLP-2 are \(\sim 50\%\) homologous to glucagon. GLP-2 regulates gastric acid secretion and intestinal motility, possesses intestinal trophic activity.

GLP-1 and GLP-2 induce insulin secretion to transport glucose across plasma membrane in response to food intake. The other major physiological functions of GLPs are inhibition of glucagon secretion, gastric emptying,\(^ {43}\) and reduction of appetite.\(^ {44}\) The insulinogetic function of GLP-1 and GLP-2 are mediated by glucagon like peptide-1 and peptide-2 receptors. The structures of these receptors are quite similar with each other and possess significant homology with other endocrine hormone glucagon, and glucose dependent insulinogetic polypeptide (GIP) receptors. These are G-protein linked receptors localized mainly in β-cells. GLP-1 and GLP-2 activate second messenger cAMP to induce their insulinogetic activity.

It is also predicted that GLP-2 mediates its biological activity via activating \(\text{Ca}^{2+}\) inositol triphosphate pathways\(^ {45}\) The GLP therapy is preferred over sulfonylurea because they affect insulinogetic properties only in presence of glucose and do not cause hypoglycemia, which is generally associated with sulfonylurea. The basic limitation of this therapy is the low bioavailability of these peptides due to short half-life as they are rapidly degraded by enzyme dipeptidylpeptidase IV (DPP IV), a serine protease which cleaves a dipeptide from the N-terminus to give the inactive GLP-1 [9-36] amide.\(^ {46}\) These limitations
have been minimized either by synthesizing inhibitors for dipeptidylpeptidase enzyme or by developing analogs of GLPs which are resistant to DPP-IV.

1.3.2 DPP-IV Characteristics & Mechanism:

X-ray structures of DPP-IV that have been published since 2003 give rather detailed information about the structural characteristics of the binding site. Many structurally diverse DPP-IV inhibitors have been discovered considering the properties of the binding site: 48

1. A deep lipophilic pocket combined with several exposed aromatic side chains for achieving high affinity small molecule binding.

2. A significant solvent access which makes it possible to tune the physico-chemical properties of the inhibitors that leads to better pharmacokinetic behavior.

DPP-IV is a 766 amino acid transmembrane glycoprotein which belongs to the prolyloligopeptidase family. It consists of three parts; a cytoplasmic tail, a transmembrane region and an extracellular part. The extracellular part is divided into a catalytic domain and an eight-bladed \( \beta \)-propeller domain. The latter contributes to the inhibitor binding site. The catalytic domain shows an \( \alpha/\beta \)-hydrolase fold and contains the catalytic triad Ser630 - Asp708 - His740. The S1-pocket is very hydrophobic and is composed of the side chains: Tyr631, Val656, Trp662, Tyr666 and Val711 (figure 6a). Existing X-ray structures show that there is not much difference in size and shape of the pocket that indicates that the S1-pocket has high specificity for proline residues. 49

![Figure 6a.](image)

![Figure 6b.](image)

**Figure 6:** (a) A generic structure of a substrate like inhibitors. (b) The key interactions between the ligand and DPP-IV complex. The ligand's basic amine forms a hydrogen bonding network. The nitrile reacts with the catalytic active serine and forms an imidate adduct.
1.3.3 Distribution and Mechanism:

During a meal the incretins glucagon-like peptide 1 (GLP-1) and glucose-dependent gastric inhibitory polypeptide (GIP) are released from the small intestine into the vasculature. The hormones regulate insulin secretion in a glucose-dependent manner. GLP-1 has many roles in the human body; it stimulates insulin biosynthesis, inhibits glucagon secretion, slows gastric emptying, reduces appetite and stimulates regeneration of islet β-cells. GIP and GLP-1 have extremely short plasma half-lives due to a very rapid inactivation. The enzyme responsible for the metabolism is DPP-IV. Inhibition of DPP-IV leads to potentiation of endogenous GIP and GLP-1 and hence improves treatment of type 2 diabetes (Figure 7a). 50

![Diagram](image)

**Figure 7.** (a) DPP-IV inhibitors inhibit DPP-IV and thus prolong the duration of GLP-1 and GIP activity, resulting in lower blood glucose level. (b) DPP-IV cleaves two amino acids from the N-terminal end of peptides.

DPP-IV selectively cleaves two amino acids from peptides, such as GLP-1 and GIP which have proline or alanine in the second position (Figure 7b). At the active site of the protease, there is a characteristic motif of three amino acids, Asp-His-Ser. DPP-IV is the CD26 T-cell activating antigen which is widely distributed in human organs and tissue. Tissues which strongly express DPP-IV include the exocrine pancreas, sweat glands, salivary and mammary glands, thymus, lymph nodes, biliary tract, kidney, liver, placenta, uterus, prostate, skin and the capillary bed of the gut mucosa where most GLP-1 is inactivated locally. DPP-IV is attached to the plasma membrane of the endothelia of almost all organs in the body. It is also present in body fluids, such as blood plasma and cerebrospinal fluid, in a soluble form. DPP-IV inactivates GLP-1 and GIP very rapidly. Regarding GIP and GLP-1, alanine and proline are crucial for biological activity, so elimination of these amino acids leads to formation of metabolites that are inactive. Thus, preventing the degradation of the incretin hormones GIP and GLP-1 by inhibition of DPP-4 is an exciting therapeutic strategy.
1.3.4 Discovery and Development of DPP-IV inhibitors:

It is important to find a fast and accurate system to discover new DPP-IV inhibitors with ideal therapeutic profiles. Three-dimensional models can provide a useful tool for designing novel DPP-IV inhibitors. Pharmacophore models have been made based on key chemical features of compounds with DPP-IV inhibitory activity. The first DPP-IV inhibitors were reversible inhibitors and came with adverse side effects because of low selectivity. Researchers suspected that inhibitors with short half-lives would be preferred in order to minimize possible side effects. However, since clinical trials showed the opposite, the latest DPP-IV inhibitors have a long-lasting effect. One of the first reported DPP-IV inhibitor was P32/98 from Merck. It used thiazolidide as the P1-substitute (figure 6a) and was the first DPP-IV inhibitor that showed effects in both animals and humans but it was not marketed due to side effects. Another old inhibitor is DPP-728 from Novartis, where 2-cyanopyrrolidine is used as the P1-substitute. The addition of the cyano group generally increases the potency.

![Figure 8. Inhibitors of dipeptidylpeptidase-IV enzymes.](image)

A natural peptide agonist, exendin-4 has been isolated from lizard venom with increased half-life in plasma and a prolonged antidiabetic effect. Liraglutide (NN 221), exenatide and ZP10A51 (analog of exendin-4) have been developed as potent DPP-4 resistant. Liraglutide (NN221), as DPP-IV resistant analog of GLP-1 which has the potential for regeneration of beta cells and successfully entered in Phase 2 of clinical trials. Liraglutide plus metformin combination successfully improves glycemic control and lowers body weight subjects with type 2 diabetes.52 Vildagliptin (LAF 237),53 saxagliptin (BMS-477118), ile-
thiazolidine, NVP-DPP728, pyrazolidine derivatives, several nitrile class of compounds like cyclohexyl glycine –(2S)-cyanopyridine and sitagliptin are reported as inhibitors of dipeptidyl peptidase-IV enzyme. Vildagliptin was the first in a new class of oral antidiabetic agents known as DPP-IV inhibitors or “incretin enhancers” and now in advanced-stage of development for the treatment of type 2 diabetes. Sitagliptin (MK-0431-januvia), beta amino acid derived specific DPP-IV inhibitor has recently been developed by MERCK. Sitagliptin plus metformin combination successfully completed clinical trials and finally approved by FDA as a drug. The several DPP-IV inhibitors are shown in figure 7.

1.4 Inhibitors of Hepatic Glucose Production

1.4.1 Glucagon Antagonist

Glucagon a 29 amino acids peptide hormone secreted from α-cells of pancreas catalyzes both gluconeogenesis as well as glycogenolysis. Liver being a metabolic organ maintains normal blood glucose level by regulating production of endogenous glucose through gluconeogenesis and glycogenolysis in diabetics. Thus glucagon antagonists are highly desirable to suppress the formation of glucose by the liver. Hepatic glucose production occurs due to insufficient insulin secretion or development of insulin resistance in the liver. Several enzymes that regulate rate-controlling steps in gluconeogenesis and glycogenolysis are obvious targets to inhibit hepatic glucose output. Glucagon is a seven transmembrane G-protein coupled receptor, which mediates its effect via stimulation of cAMP. Numerous glucagon antagonists have been reported from natural and synthetic sources. Styrylquinazoline CP-99711 (I) has been reported for glucagon antagonistic activity in rats. Pyrrolo[1, 2-a]quinoxaline (II), thiophene derivative (III) showed high affinity for glucagon receptor. The representative examples of each of these classes are shown in Figure 8.
Mercaptobenzimidazole NNC-92-1687 (IV) the first non-peptide competitive human glucagon receptor antagonist has been reported by Novo-Nordisk. Other human glucagon receptor antagonists include alkylidine hydrazide (V), biaryl amides, triaryl imidazoles (VI) and triarylpurroles (VII).

Recently, an entirely new class of compounds, substituted biaryls has been identified through high-throughput screening as glucagon receptor antagonist. These compounds act by inhibiting cAMP, stimulated by glucagon. Out of many derivatives the compounds (VIII) and (IX) were selected for clinical trial but due to undesirable side effects and low bioavailability (40%), further development of these compounds has been discontinued. Glucagon receptor antagonists have been extensively reviewed recently.

1.4.2 Biguanides

Biguanides are basically the guanidine derivatives constitute a well-known class of antimalarials. The antidiabetic activity of guanidine derivatives was initially discovered as a side effect of proguanil, an antimalarial drug that led to the synthesis of potent antidiabetic drugs like metformin, phenformin and buformin. Biguanides, including phenformin and metformin, were introduced in 1957 as oral antidiabetic drugs. Phenformin was later withdrawn in many countries due to its side effect of lactic acidosis. Metformin is now a widely used biguanide for the therapy of type 2 diabetes. Metformin lowers blood glucose level without causing overt hypoglycemia or stimulating insulin secretion.
The molecular targets of these agents have not yet been identified. Their primary mode of action seems to be inhibition of hepatic gluconeogenesis. The main side effect of metformin monotherapy is gastrointestinal (GI) symptoms. At 2550 mg daily dosage, patients experienced abdominal discomfort, bloating, and metallic taste. Metformin monotherapy is considered to be the first line treatment for patients prone to weight gain and/or dyslipidemic who have failed to achieve adequate glycemic control on dietary management. Metformin is also used in combination with other antihyperglycemic agents and insulin. Metformin at higher doses of 0.5g to 1.50g has an absolute oral bioavailability of 50-60%. The bioavailability of the drug declines at higher oral doses.

1.5 Insulin Sensitizers

Compounds which decrease insulin resistance are called insulin sensitizers or enhancer of insulin action. Thiazolidinediones (TZDs) are chemical compounds that reduce insulin resistance, increase insulin-stimulated glucose disposal and improve glycemic control by improving peripheral consumption and suppressing hepatic glucose production. TZDs act by binding to peroxisome proliferator activated receptor-γ (PPARγ) which regulate the transcription of a number of insulin responsive genes intimately involved in the control of glucose and lipid metabolism. PPARγ is a nuclear receptor expressed in adipocyte and interacts with retinoid X receptor and form heterodimer RXR. Interaction of TZDs with PPARγ brings about conformational change in PPARγ-RXR complex, which induce expression of various genes, involved in fatty acid uptake in adipose tissues. Ciglitazone, the first member of this class of compounds was discovered serendipitously. Troglitazone (Rezuli), pioglitazone (Actos), and rosiglitazone (Avandia) are the other important agents of this series. After the approval of troglitazone in 1997, it was withdrawn due to a rare but clinically serious incidence of hepatotoxicity. The mechanism of troglitazone-induced hepatotoxicity is not clear but it is likely related to inflammation via an immunological mechanism. The toxicity does not appear to be associated with the other TZDs approved later, pioglitazone and rosiglitazone, both of which proved to be efficacious in clinical trials in lowering fasting plasma glucose, HbA1c, and triglycerides and increasing HDL.

The major side effects of this class of compounds are edema and weight gain, possibly due to their ability to stimulate adipocyte differentiations. The mechanism of development of edema is still unclear. Current status of therapeutic importance of peroxisome proliferator activated receptor in diabetes has been recently reviewed extensively.
1.6 α-glucosidase inhibitors

α-glucosidase inhibitors are oral anti-diabetic drugs used for T2DM that work by preventing the digestion of carbohydrates (such as starch and table sugar). Carbohydrates are normally converted into simple sugars (monosaccharides), which can be absorbed through the intestine. Hence, α-glucosidase inhibitors reduce the impact of carbohydrates on blood sugar, therefore they are generally used to establish greater glycemic control over hyperglycemia in T2DM, particularly with regard to postprandial hyperglycemia. They may be used as monotherapy in conjunction with an appropriate diabetic diet and exercise, or they may be used in conjunction with other anti-diabetic drugs.

The α-glucosidase inhibitors (AGIs) are a class of nonsystemic drugs that do not target specific pathophysiological defects in type 2 diabetes. The enzyme is located in the brush border of the small intestine and is required for the final step in the breakdown of carbohydrates such as starch, dextrins, and maltose to absorbable monosaccharides.\textsuperscript{81} The important members of this class of compounds include Acarbose (Precose),\textsuperscript{82} miglitol (Glyset),\textsuperscript{83} vogalibose.\textsuperscript{84} The α-glucosidase inhibitors act as competitive inhibitors of α-glucosidase enzyme, which retards the process of carbohydrate absorption and delays meal derived glucose in diabetic as well as in normal subjects.\textsuperscript{81} These drugs are taken before the meal. The striking side effects of these agents are gastrointestinal, abdominal discomfort, and diarrhoea.\textsuperscript{85}
1.7 Targetting Insulin Signaling Pathways

1.7.1 Protein Tyrosine Phosphatase 1B Inhibitors

Protein tyrosine phosphatase-1B (PTP1B) emerged only 9 years ago as a new drug target for the treatment of diabetes and obesity. The enzyme belongs to the family of tyrosine phosphatases, which consists of around 90 members.\(^{86,87}\) Interestingly, this target had a very long gestation period, starting with 19th century observations that vanadium salts are of therapeutic utility in diabetes, followed by the biochemical discovery that vanadate is a potent, nonselective inhibitor of phosphatases. By the mid-1980s, it was understood that blocking one or more phosphatases could enhance the phosphorylation state of the insulin receptor kinase \(\beta\) subunit and/or its downstream signaling partners and revert insulin resistance, which is a characteristic of type 2 diabetes. Experiments based on PTP1B knockout mice showed enhanced IR and IRS-1 phosphorylation in the liver and skeletal muscle and also mice were found healthy, lean and obesity resistant with low level of plasma glucose and insulin.\(^{88,89}\) These findings generated a vivid interest in PTP1B and other PTPs as drug targets for diabetes and obesity.

1.7.2 Validation of PTP1B as a drug target for diabetes and obesity

PTP1B is localized to the cytoplasmic face of the endoplasmic reticulum and is expressed ubiquitously, including in the classical insulin-targeted tissues such as liver, muscle and fat.\(^{90}\) Mounting evidence from biochemical, genetic and pharmacological studies support a role for PTP1B as a negative regulator in both insulin and leptin signaling, which can associate with and dephosphorylate activated insulin receptor (IR) or insulin receptor substrates (IRS) (Figure 12).\(^{91,92}\) Overexpression of PTP1B in cell cultures decreases insulin-stimulated phosphorylation of IR and/or IRS-1, whereas reduction in the level of PTP1B, by
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antisense oligonucleotides or neutralizing antibodies, augments insulin initiated signaling.\textsuperscript{93,94} Analyses of quantitative trait loci and mutations in the gene encoding PTP1B in humans support the notion that aberrant expression of PTP1B can contribute to diabetes and obesity.\textsuperscript{95} Mice that lack PTP1B display enhanced sensitivity to insulin, with increased or prolonged tyrosine phosphorylation of IR in muscle and liver.\textsuperscript{96} Interestingly, PTP1B\textsuperscript{−/−} mice are protected against weight gain and have significantly lower triglyceride levels when placed on a high-fat diet. This is unexpected because insulin is also an anabolic factor, and increased insulin sensitivity can result in increased weight gain. PTP1B was subsequently shown to bind and dephosphorylate JAK2, which is downstream of leptin receptor.\textsuperscript{97} Thus, the resistance to diet-induced obesity observed in PTP1B\textsuperscript{−/−} mice is likely to be associated with increased energy expenditure owing to enhanced leptin sensitivity. Recent tissue-specific knockout results indicate that body weight, adiposity and leptin action can be regulated by neuronal PTP1B.\textsuperscript{98} Inhibiting neuronal PTP1B would require drugs that penetrate the blood–brain barrier. Consistent with the above results, antisense-based oligonucleotides that target PTP1B have shown efficacy in type 2 diabetes and have entered phase 2 clinical trials.\textsuperscript{99} In addition, small-molecule inhibitors of PTP1B can work synergistically with insulin to increase insulin signalling and augment insulin-stimulated glucose uptake.\textsuperscript{100} Moreover, pretreatment of leptin-resistant rats with a potent and selective PTP1B inhibitor results in a marked improvement in leptin-dependent suppression of food intake.\textsuperscript{101} Collectively, these biochemical, genetic and pharmacological studies provide strong proof-of-concept, validating the notion that inhibition of PTP1B could address both diabetes and obesity and making PTP1B an exciting target for drug development.

\textbf{Figure 11. Proposed mode of action of PTP1B:} IR, insulin receptor α subunits; IRS-1, insulin receptor substrate-1; \textit{le}(R), leptin receptor; Jak, Janus kinase; STAT signal transducer and activator of transcription; PI3K, phosphatidylinositol-3-kinase; Akt, protein kinase B. (Drug Discovery Today 2007, 12, 373–381)
1.7.3 Challenges in developing PTP1B-based small molecule therapeutics:

Selectivity is one of the major issues in the development of PTP1B inhibitors as drugs. Because all PTPs share a high degree of structural conservation in the active site, the pTyr (phosphotyrosine)-binding pocket, designing inhibitors with both high affinity and selectivity for PTP1B poses a challenge. Fortunately, PTP substrate specificity studies have shown that pTyr alone is not sufficient for high-affinity binding and residues flanking the pTyr are important for PTP substrate recognition. 102

Bioavailability is another important issue in the development of PTP1B-based small-molecule therapeutics. The active sites of PTPs have evolved to accommodate pTyr, which contains two negative charges at physiological pH. Consequently, most active-site-directed PTP inhibitors (non-hydrolyzable pTyr mimetics) reported to date possess a high charge density to serve as competitive inhibitors. Such molecules are generally not drug-like, with limited cell membrane permeability. Several strategies have been applied to improve the cell permeability and/or bioavailability of PTP1B inhibitors.

1.7.4 Development of Potent and selective PTP1B inhibitors

With the advent of PTP1B as a new target for T2DM various novel and selective PTP1B inhibitors have been developed. Numerous literatures are available covering several classes of molecules as potent PTP1B inhibitors. 103 The development of various potent molecules for PTP1B inhibitory activity can be categories on the basis of following four approaches:

(a) The Library Approach (X & XI). 104
(b) The ‘Linked-fragment’ Approach (XII-XIV). 105
1.7.4.1 The Library Approach

A focused library approach was used to identify highly potent and selective PTP1B inhibitors that are capable of bridging and simultaneously associating with both the active site and an adjacent peripheral site. The library contains (i) a biasing pTyr to ensure association with the active site and (ii) a structurally diverse set of 23 linkers that tether the pTyr moiety to (iii) a structurally diverse set of eight aryl acids, which were designed to associate with the peripheral subsite, positioned near the active site.

1.7.4.2 The 'Linked-fragment' Approach

In addition to the proximal non-catalytic site defined by Lys41, Arg47 and Asp48, a second aryl phosphate-binding site, adjacent to the PTP1B active site, was identified from crystal structures of the protein in complex with pTyr and a small aryl phosphate. This second aryl phosphate-binding site lies within a region (Arg24 and Arg254) that is not conserved among the PTPs. A ‘linked fragment’ approach was employed to develop potent and selective PTP1B inhibitors that can engage both the active site and the second aryl phosphate-binding site.

1.7.4.3 The conformation assisted Approach

Structure-based modelling has been used to target unique PTP1B conformations for inhibitor development with both high affinity and selectivity. A series of benzotriazole phenyl difluoromethyl phosphonic acids were synthesized as non-peptidic PTP1B inhibitors. Many of these compounds showed good inhibitory activity, at the sub-mM level, for PTP1B but none of them had selectivity compared with TC-PTP.

1.7.4.4 Targeting allosteric sites for improved selectivity and bioavailability

A secondary allosteric site has recently been described for PTP1B, and several small-molecule inhibitors that occupy this site stabilize an inactive conformation of PTP1B. Unlike the pTyr binding active site, the allosteric site is not well conserved and possesses is substantially less polar. Thus, targeting the allosteric site might present an alternative strategy for developing selective inhibitors with acceptable pharmacological properties.

Figure 14a showing the potent and selective PTP1B inhibitors based on the approaches. One of the earliest strategies for designing PTP1B inhibitors has focused on the incorporation of tyrosine phosphate mimetics into peptidomimetic backbones. The
phosphotyrosine moiety which penetrates the catalytic 9Å-deep cleft has been successfully mimicked by three different negatively charged chemical groups: difluorophosphonomethyl aryl, oxalylaminobenzoic acid and carbomethoxybenzoic acid. Among these, difluoromethylene phosphonates have been shown to yield potent phosphatase inhibitors. The bis-difluorophosphonate-phenylanaline (X), one of the most potent inhibitor series of the target to date (Ki) 2.4 nM, has good selectivity over most phosphatases, including T-cell PTP (TC-PTP). Another phosphatase, PTEN/NMAC (phosphatase and tensin homolog) is part of this pathway as phospholipid phosphatase that indirectly stimulates phospho-inositol 3-kinase (PI3K).\textsuperscript{109} PTEN has been recently identified as a significant antidiabetic drug target.\textsuperscript{110} Recently ottaná et al. reported 5-Arylidene-2-phenylimino-4-thiazolidinones (XX) as potent PTP1B and LMW-PTP inhibitors.\textsuperscript{111}

![Figure 14a. Potent PTP1B inhibitors](image)
Pei et al.\textsuperscript{112} have reported several alpha-haloacetophenone derivatives (XXI) as potent neutral protein tyrosine phosphatase inhibitors, which covalently alkylate the conserved catalytic cysteine residue in the PTP active site. Malamas et al.\textsuperscript{113} reported two novel series of benzofuran/benzothiophene- biphenyl oxo-acetic acids and sulfonylsalicylic acid as potent inhibitors of PTP-1B with good antihyperglycemic activity. They found that compound XXII normalizes plasma glucose level at the 25 mg/kg dose (po) and 1 mg/kg dose (ip). Wrobel and co-workers\textsuperscript{114} reported a series of 11-arylbenzo[b]naphtho[2,3-d]furan/thiophene derivatives XXIII, which selectively inhibit PTP-1B over other PTPs and lowered the blood glucose level in ob/ob mice. Hu et al.\textsuperscript{115} reported several flavonoids including a flavonol XXIV, which showed potent inhibitory activity against PTP-1B enzyme. Won et al. isolated 12 new flavonones (XXVa & XXVb) from the stem bark of \textit{Erythrina abyssinica} as potent PTP1B inhibitors. Recently, Goel et al. reported\textsuperscript{116,117} several functionalized acetophenones (XXVI), benzofurans (XXVII), naphthofurans (XXVIII), and dibenzofurans (XXIX) as novel protein tyrosine phosphatase-1B inhibitors and various nature mimicking furanyl-2-pyranones\textsuperscript{118} (XXX) and 1,3-teraryl\textsuperscript{119} (XXXI) as potent antihyperglycemic agents.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure14b.png}
\caption{Natural and synthetic PTP1B inhibitors.}
\end{figure}
Several PTP1B inhibitors are in clinical development. Recently, diaryloxamic acid (XXXII) has been identified as selective and potent PTP1B inhibitor for using a linked-fragment strategy. Recently a newer approach to inhibit PTP1B through peroxides have been reported by several workers. Davis et al. reported the inactivation of PTP1B by amino acid hydroperoxides. Gates et al. reported that organic peroxides have the ability to cause the thio-reversible, inactivation of PTP1B (figure 14 shows the variable organic peroxides).

![Figure 15. Showing variable organic peroxides](image)

1.8 PPARα/γ dual and Pan-agonist

The nuclear receptor PPARα is the legitimate target for fibrate class of lipid lowering agents and PPARγ is the validated target for glitazone class of insulin sensitizers. Thus there is a resurgence of interest in the development of new anti-diabetic drugs that combine the insulin-sensitizing effects of PPARγ together with the additional lipid lowering activity of PPARα. A pan-agonist, capable of stimulating all three peroxisome proliferater-activated receptors (PPARs) as a group, expected to be useful for treatment of T2DM. Clinical evidences obtained from benzafibrate-based trials are in support of the pan-PPAR agonist concept as therapeutic approach. Numerous chemicals have been employed to design new class of dual agonists. One strategy was to cyclize the fibrate structure mimick in order to fibrate and glitazone structures in one chemical entity. The glitazars emerged as first example of dual PPARα/γ class. Muraglitazar was the first dual-PPARα/γ agonist approved by US Food and Drug Administration (FDA). In obese diabetic db/db mice, muraglitazar has efficacious antidiabetic effects and improves metabolic abnormalities. Recently, Artis et al. reported a novel pan-agonist, indeglitazar, which gives partial response against PPARγ and full response against PPARδ, is now progressed to phase II clinical trials. Recently discovered some dual PPARα/γ activators have been depicted in Figure 16. Using carbazol-derived dual PPARα/γ agonist as a structural template, a novel pan-agonist has been designed, and benzofibrate has been approved as belongs to this class. New
Compounds with improved pan-agonist activity compared to benzofibrate have been also developed, such as LY-465608, DRF-11605 and CS-204.\textsuperscript{127} Among these, indole-based compounds such as BPR1H036 display insulin sensitizing effects and in-vitro glucose uptake improvement.\textsuperscript{128} Moreover, a number of pan-agonists, with insulin sensitizing and antihyperglycemic actions, are currently tested in clinical studies. PLX-204 is in pre-clinical phase whereas netoglitazone in phase II clinical trial.

![Figure 16. PPARα/γ dual and Pan-agonist.](image)

1.9 **Antiobesity Agents**

Obesity is a multigenic disease associated with several metabolic abnormalities such as insulin resistance, cardiovascular morbidity and cancer.\textsuperscript{129} This has established obesity as therapeutic targets for the management of type 2 diabetes. Basically, following three therapeutic approaches are currently being pursued.

1. Agents reducing energy intake or appetite suppressants
2. Agents affecting energy expenditure.
3. Agents affecting dietary absorption of fats.
1.9.1 Agents Reducing Energy Intake or Appetite Suppressants

Agents, which reduce energy, are called appetite suppressants. These compounds affect secretion of various biogenic amines. Based on the nature of the amines (neurotransmitters) secreted, these agents have been classified into following two categories.

1.9.1.1 Noradrenergic Agents

These agents induce release of noradrenalin from nerves endings in hypothalamus. Noradrenalin release activates postsynaptic α1- and β1-adrenoceptor that suppress appetite. Phentermine, phenylpropanolamine, diethylpropion and mazindol are the drugs of this class for the treatment of obesity. Mazindol is the only drug approved for clinical use.

![Figure 17: Noradrenergic agonists.](image)

1.9.1.2 Serotonergic Agents

This group of appetite suppressants acts by inhibiting reuptake of serotonin (5-hydroxytryptamine). Dexfenfluramine, sertraline and fluoxetine are drugs of this class but they are no longer in clinical use for the treatment of obesity due to intolerable side effects and have been reviewed extensively.

1.10 β3-Adrenoceptor Agonists

β3-Adrenoceptor is a member of G-protein coupled adrenoreceptor family. The agonists of this receptor are very effective for thermogenic antiobesity, lipolysis and insulin sensitizing activity in rodents. Their main sites of action are white and brown adipose tissue and muscle. The β3-adrenoceptor mRNA levels are lower in human than in rodent adipose tissue.

![Figure 18: β3-Adrenoceptor Agonists as antidiabetic agents.](image)
β3-Adrenoreceptor agonists fall into three main chemical classes: arylethanolamines (BRL 37344, BRL 35135), aryloxypropanolamines (CGP 12177), and trimetoquinols (CL-316243). The above compounds are in advanced clinical trials. Oral bioavailability and tissue selectivity are striking problems of this compounds.

1.11 Miscellaneous

1.11.1 Tumor Necrosis Factor Alpha (TNF-α)

The mechanisms of insulin resistance caused by obesity are not so far clearly understood. According to first hypothesis it is due to increased flux of substrates into the adipose tissues resulting in elevated levels of secreted factors responsible for insulin resistance but according to second hypothesis the levels of TNF-α mRNA protein is increased. It has been observed that TNF-α inhibits signaling events mediated by insulin resistance. TNF-α is a proinflammatory cytokines responsible for pathogenesis of a spectrum of diseases. The overproduction of these cytokines (TNF-α) also leads to insulin resistance that has been established by null mutation in obese. This notion was further supported by significant increase in peripheral insulin stimulated glucose uptake on neutralization of TNF-α in obese fa/fa rats improving insulin sensitivity in peripheral tissues.

1.11.2 Preparation of P38 MAP Kinase Inhibitors

P38-Mitogen-activated kinase plays a central role in the synthesis of TNF-α. This protein belongs to a group of serine/threonine kinases that include c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK). The compounds RWJ67657 and VX-702 are potent inhibitors of P38 MAP Kinase and are in advanced clinical stage. Another compound pyrazole derivative and BIRB 796 a potent selective inhibitor of P38α is in clinical trial for treatment of autoimmune diseases.
1.11.3 Glucokinase Activators

The pathogenesis of type 2 diabetes is not only obesity or insulin resistance but also mutations in the genes of regulatory enzymes of glucose. This was established with the discovery of a mutated gene encoding glucokinase (GK) in maturity onset diabetes of the young type 2 (MODY2). GK is an enzyme responsible for the phosphorylation of glucose in pancreatic β-cell and hepatocyte leading to glycogen synthesis and decrease in hepatic glucose production. In MODY2 patients, there is a net reduction in the formation of glycogen and increase in the production of the glucose in liver. Based on these observations numerous compounds have been synthesized to obtain potent GK activators.

Screening of 120,000 synthetic organic compounds of diverse chemical structures led to the discovery of a compound RO0281675. It has been found to activate recombinant human glucokinase potentially in a dose dependent manner.

![Figure 20. Glucokinase activators.](image)

1.12 Summary

The current clinical scenario on the management of diabetes and associated complications have highlighted the importance of antihyperglycemic agents for the management of diabetes mellitus and associated microvascular /macrovascular complications. Majority of the earlier therapeutic agents for the management of diabetes had been developed without defined molecular targets or on the basis of understanding of disease pathogenesis. Advances in molecular pharmacology and medicinal chemistry have given a new insight of molecular pathways of diabetes and its complications. These targets have been identified on the basis of predicted roles in modulating one or more aspects of pathogenesis of diabetes. Important strategies for management of diabetes are: 1) augmenting glucose mediated insulin secretion and insulin sensitivity, 2) inhibiting hepatic glucose production and its absorption by the cells, 3) targeting specific molecular targets in insulin signaling pathways, 4) control of obesity and altered lipid metabolism to improve insulin sensitivity.
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