PART-I

Pharmacokinetics of Novel Antidiabetic Agents (S002-853, S002-857 & S001-469)
Chapter 1

Review of Literature
Section 1.1

Pharmacokinetics: Role in Drug Discovery & Development
1.1.1 Introduction

Drug research is a unique multi-disciplinary process heading towards the development of therapeutic agents in the area of currently unmet medical needs. The drug research can be divided functionally into discovery and development process [1]. The discovery and development process of therapeutically useful drugs requires an enormous amount of money and time due to high attrition rates of new chemical entities (NCEs)/drug candidate. Lack of efficacy and toxicity are considered to be major reasons for drug failures and pharmacokinetics (PK) governs them to large extent [2]. According to recent literature, it is estimated that only one in ten of the agents that enter clinical development are successful, with an average cost of US $ 500-800 million and a typical time scale of 10-15 years from preclinical discovery research to regulatory approvals [3].

Pharmacokinetics is the study of the time course of absorption, distribution, metabolism and elimination (ADME) of the drugs in biological system and helps to understand the relationship between pharmacological and toxicological effects and concentration of a drug and its metabolites in the body fluid. The systematic application of PK can therefore considerably reduce the cost and time involved in new drug development [4]. Today PK and metabolism are among the most highly interactive disciplines in the pharmaceutical research and development and intimately involve in the design of new chemical entities [5-7]. Fundamentally, ADME/PK information is critical in all phases of a fully integrated drug development program (Table 1.1). The goal of ADME-guided synthesis is to maximize the ability of NCEs to access the therapeutic target. Once a lead compound is identified, it is subjected to preclinical PK/ADME studies. Preclinical pharmacokinetics broadly divided into three areas: (i) PK at the discovery levels that enables selection of lead chemical, (ii) toxicokinetics, or PK during toxicology studies, and (iii) preclinical studies in animals that support the clinical studies and regulatory obligations. Historically, an inappropriate pharmacokinetic property has been a major reason for the failure of compounds in the later stage of development. This is supported by an analysis of cause of failure of drugs selected for development. It has been reported that inappropriate kinetics in humans accounted for 39 % of the failures [8] (Figure 1.1.1).
Table 1.1 Role of pharmacokinetics in the development of new drugs.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Stage</th>
<th>Purpose</th>
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<tbody>
<tr>
<td>Preclinical PK</td>
<td>Discovery</td>
<td>To chose the optimum candidate</td>
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<td>Development</td>
<td>To understand the pharmacology in experimental animals</td>
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<td>Toxicity testing</td>
<td>To determine exposure in two nonhuman species</td>
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<td>Phase I</td>
<td>Dose ranging</td>
<td>Tolerability over range of doses</td>
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<td></td>
<td>Dose linearity</td>
<td>Establishing whether plasma concentration increases in proportion to dose administered in humans</td>
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<td></td>
<td>Definitive kinetics</td>
<td>Measurement of half-life, C&lt;sub&gt;max&lt;/sub&gt; and AUC using single and multiple doses</td>
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<td>Phase II</td>
<td>Sex differences</td>
<td>Assessment of the influence of gender on PK profile</td>
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<td>Food interaction</td>
<td>Assessment of the influence of food on PK profile</td>
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<td>Absolute bioavailability</td>
<td>Quantitative determination of the distribution of a compound in body fluids and tissues</td>
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<td>PK-PD relationships</td>
<td>Establishing clear dose-response relationship, avoids drugs being marketed at excessive doses</td>
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<td>Genetic polymorphisms</td>
<td>Assessment of the influence of genetic differences in drug metabolising enzymes on PK profile</td>
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<td>Phase III</td>
<td>Effect of disease</td>
<td>Determination of the PK profile in the target population</td>
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<td>Subgroup analysis</td>
<td>Determination of the PK profile in subgroup of the target population</td>
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<td>Final dosage form PK</td>
<td>Determination of the PK profile of the final formulation of the drug candidate</td>
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<td></td>
<td>Dose response</td>
<td>Determination of a clear dose-response relationship in the target population. Response can be determined on the basis of a biochemical, imaging or clinical surrogate or the explicit demonstration of therapeutic efficacy</td>
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<td>Phase IV</td>
<td>Dosage form improvements</td>
<td>Determination of the PK profile of new formulations</td>
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<td>Change of formulation</td>
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<td>Line Extensions</td>
<td>Determination of the PK profile of modified drugs designed to extend patent life</td>
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<td>Drug interactions</td>
<td>Determination of the possible influence of the other drugs on the PK profile</td>
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<td>Pharmacovigilence</td>
<td>Continual assessment of competitor and potential competitor compounds</td>
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Figure 1.1.1 Reasons for marketed drugs to be withdrawn.

The drug candidate succeeds in passing the preclinical drug development stage (drug metabolism, PK, toxicity testing, etc) [9], is submitted as an investigational new drug application (IND) and may enter full development: Phase I (safety and tolerability in healthy volunteers), Phase II (efficacy and dose-effect relationships using a small number of patients), and Phase III (efficacy studies using a large number of patients) [10]. If the IND passes all three clinical phases, it is submitted as a new drug application (NDA) and upon FDA approval, eventually enters for the clinical use/marketplace. Thus PK/ADME play an increasingly important role in drug development starting from drug discovery and lead optimization, pharmacology and safety evaluation continuing into clinical development and finally helping to position the NCEs as the DRUG [11].

1.1.2 Preclinical Strategies

Pharmacokinetics at preclinical level is basically of two types: in-vitro and in-vivo. Studies of drug databases showed that successful drug candidates tend to have 'drug like
properties' [12]. Drug likeness, when viewed at the in-vivo level, is thought of in term of PK and safety. Complex in-vivo properties result from an interaction of physiochemical and structural properties, such as solubility, permeability and stability, which are studied in-vitro. These properties are, in turn, dictated by fundamental molecular properties, such as molecular weight, hydrogen bonding and polarity. As a result of the importance of these properties, a new strategy emerged: testing the 'drug-like' properties of compounds during early discovery using high-throughput property screening methods in-silico, in-vitro and in-vivo (often termed 'pharmaceutical profiling) [13, 14].

1.1.2.1 In-silico Strategies

The high throughput screening (HTS) of large proprietary compound collections and combinatorial libraries has increased the pressure on gathering pharmacokinetic and drug metabolism data as early as possible. Properties related to ADME can be estimated by a range of in-vivo and in-vitro methods [15-18]. In addition, progress has been made in in-silico methods using various quantitative structure-activity relationship (QSAR) and molecular modeling techniques that employ a range of recently introduced descriptors tailored to ADME in-silico (e-ADME) [19]. The computational approach is one of the newest and fastest developing techniques in PK, ADME evaluation, drug discovery and toxicity [20-22]. However, to date, the software packages devoted to ADME prediction, especially of metabolism, have not yet been adequately validated and still require improvements to be effective [23, 24]. Quantitative in-silico predictions are now possible for several PK parameters, particularly absorption and distribution [25, 26]. The emerging consensus is that the predictions are no worse than those made using in-vitro tests, with the decisive advantage that much less investment in technology, resources and time is needed. In addition, and of critical importance, it is possible to screen virtual compounds. Some packages are able to handle thousands of molecules in a few hours. However, common experience shows that, in part at least for essentially irrational reasons, there is currently a lack of confidence in these approaches. An effort is required by the software producers towards more transparency, in order to improve the confidence of their consumers. It seems highly probable that in-silico approaches will evolve rapidly, as did in-vitro methods during the last decade. Past experience should be helpful in
avoiding repetition of similar errors and in taking the necessary steps to ensure effective implementation. A general concern is the lack of access to the large amounts of data on compounds no longer in development, but still kept secret by the pharmaceutical industry. Controlled access to these data could be particularly helpful in validating new *in-silico* approaches. These *in-silico* approaches are promising filters for virtual libraries to aid synthesis as well as the selection of compounds for acquisition and screening in the early stages of drug discovery.

1.1.2.2 *In-vitro* Strategies

Assessment of ADME properties is now conducted at very early stages of drug discovery for the purpose of accelerating the conversion of Hits and Leads into qualified developmental candidates. To meet this need, high throughput *in-vitro* tests have been developed that can profile NCEs in a model for each of the major barriers to good bioavailability post-oral dose and are often able to screen hundreds of compounds per week with better projections for PK properties of compounds in humans [27-29]. A number of new *in-vitro* techniques are available to screen compounds for key ADME characteristics such as absorption and metabolic stability, which, when applied within a rational strategy, can make a major contribution to the design and selection of successful NCEs [30, 31]. One or all of them will have a major impact on the exposure of individuals to orally administered drugs and to their efficacy. These assay models have been applied not only to screening and ranking of potential drug candidate but also for understanding the mechanisms leading to *in-vivo* pharmacokinetic outcomes. A number of *in-vitro* and cell culture techniques have evolved in recent years that have facilitated the assessment of intestinal permeability. Most notable among them are CaCO-2 cells. Permeability measurements are based on the rate of appearance of test compound in the receiver compartment [32, 33]. The CaCO-2 cells, everted intestinal rings, and *in-situ* perfusions techniques have strong potential for predicting fraction absorbed in humans [34].

As with absorption, valuable metabolic input can be imparted to the drug discovery team based on information gleaned from *in-vitro* metabolic techniques such as hepatic microsomes, S9 fractions, hepatocytes and recombinant CYP-450 enzymes [35]. *In-vitro*
metabolic stability profile is a qualitative as well as quantitative comparison of metabolism of a compound in human and animal models. It helps in identifying the right model for toxicity studies. Extensive metabolism is generally considered a liability as it limits the systemic exposure and shortens the half-life of a compound. Metabolic stability results are usually reported as measures of intrinsic clearance, from which secondary pharmacokinetic parameters such as bioavailability and half-life can be calculated when other data of volume of distribution and fraction absorbed are available. Since these parameters are very important in defining the pharmacological and toxicological profile of drugs as well as patient compliance, the pharmaceutical industry has a particular interest in optimising the metabolic stability during the drug discovery and development process [36]. Several strategies such as reduction of lipophilicity, modification and/or blocking of metabolically soft spots and use of enzyme inhibitors; have been developed to combat metabolism. In spite of several concerns, the fact that active metabolites of several marketed drugs have been developed as drugs with better efficacy, safety and pharmacokinetics profile; cannot be denied [37, 38]. Therefore, instead of considering metabolic instability a liability, it can be exploited as a tool for discovering better drugs. It is equally important to identify the metabolic pathways of the drug candidates by conducting in-vitro CYP450 reaction phenotyping assays. The identification of drug metabolizing enzymes involved in the major metabolic pathways of a compound helps in predicting the probable drug-drug interactions in human [39]. It is known that only unbound drug is pharmacologically active and therefore the assessment of bound fraction by plasma protein binding of a compound is another important parameter to be explored in-vitro.

Available in-vitro metabolism technologies are considerably more efficient than traditional in-vivo methods. With these systems, it is possible to assess the relative rates and routes of biotransformation of a handful of compounds in the time required to comparably characterize one compound using in-vivo approaches. In the past, a relative paucity of tissues was the most notable impediment to conduct large-scale screening efforts with in-vitro tools [40, 41]. However, the continued growths of in-house tissue banks, in concert with the optimization of isolated enzyme expression systems, have made high-throughput metabolism assessments in the discovery arena a reality.
Mass spectrometry (MS) and nuclear magnetic resonance (NMR) have played an invaluable role in the structural characterization and quantification of drug metabolites. Indeed, liquid chromatography (LC) coupled with atmospheric pressure ionization (API) MS has now become the most powerful tool for the rapid detection, structural elucidation, and quantification of drug-derived materials within various biological fluids [42-44]. Often however, MS alone is insufficient to elucidate unambiguously the structure of metabolite in terms of stereochemistry and exact position of functional groups. In such cases, multiple analytical and wet chemistry techniques, such as LC-NMR, enzymatic hydrolysis, chemical derivatization, and hydrogen/deuterium-exchange (H/D-exchange) combined with MS are used to characterize the novel and isomeric metabolites of drug candidates [45].

1.1.2.3 In-vivo Strategies

Unfortunately, there is no substitute for actual in-vivo data in assessing pharmacokinetic profiles of drug candidates. While insight into various aspects of the pharmacokinetic profile (ADME) can be gleaned from in-vitro techniques, there are yet no methods available for accurately predicting what will happen to a drug when it is put into a whole animal. The primary information is needed about drug candidate before it can be taken to clinical studies are: whether it can be given orally, its rates of uptake, distribution and elimination. The first such information is generally generated in rat model, where the rat body is treated as black box: NCEs enters and leaves the body in quantities and at rate that can be precisely quantitated, but what goes on in the in the body can only be inferred through first order kinetics [46].

The preclinical PK work first requires development and validation of an assay for measurement of the drug in rat plasma and serum. While many analytical techniques may use for this purpose, HPLC-UV, LC-MS or LC-MS-MS are most used methods. Often the plasma assay developed at this stage is used to measure drug levels in plasma/serum during clinical/preclinical PK studies. The concentration time data are generally analyzed using compartmental or non-compartmental approach and various PK characteristics such as half-life (t1/2), volume of distribution (Vd), clearance (Cl), area under curve (AUC)
If both oral and intravenous formulations are available, bioavailability (% F) i.e. fraction of an orally administered dose that reaches the bloodstream is also calculated.

The other important information at preclinical stage generated includes tissue distribution and some information about its in-vitro/in-vivo metabolism. Tissue distribution studies reveal the information about where the drug candidate goes in the body. Does it have affinity for a particular tissue and thus accumulates there; does it penetrate the blood brain barrier, how much drug is eliminated in the feces or urine? Plasma, urine and feces sample also provide an opportunity to search for metabolites.

Due to in-vivo constraints, classical PK methods are not high throughput and have often impeded the quick development of new drug candidates. To overcome this problem, significant progress has been made in high-throughput of in-vivo pharmacokinetic studies, with the introduction of cassette, or multiple-in-one, protocols. Cassette dosing has become widely applied to PK screening using LC/MS-MS [47]. This technology has proven to be an effective method to improve the high-throughput of PK screening. Cassette dosing has the advantage of testing more compounds in a shorter time using fewer animals; on the other hand, misleading PK results may be obtained due to problems such as drug-drug interactions. Therefore, the error potentially incurred with cassette dosing for any single compound may be well within the variability encountered in discrete PK studies with relative few animals.
Section 1.2

Type-2 Diabetes Mellitus: Emerging Trends
1.2.1 Epidemiology and Pathophysiology of Type-2 Diabetes

An estimated 285 million people, corresponding to 6.4% of the world's adult population, live with diabetes in 2010. The number is expected to grow to 438 million by 2030, corresponding to 7.8% of the adult population [48]. Diabetes mellitus is one of the most common endocrine metabolic disorders. The prevalence increases with age and varies widely between different populations and ethnic groups. Type-2 diabetes mellitus (noninsulin-dependent diabetes) accounts for approximately 90% of patients with diabetes mellitus, with type-1 (insulin-dependent diabetes) accounting for the remainder [49, 50]. The main force driving this increasing incidence is due to staggering increase in obesity, the single most important contributor to the pathogenesis of diabetes mellitus. Prolonged disease condition leads to chronic macrovascular complications such as retinopathy and nephropathy. The disease is collectively referred, as metabolic syndrome encompasses type-2 diabetes and common constellation of closely linked clinical features. Characteristic factors include insulin resistance, obesity, hypertension and a common form of dyslipidemia and low high-density lipoprotein cholesterol. Metabolic syndrome is associated with marked increased incidence of coronary, cerebral and peripheral artery disease [51].

This heterogeneous disorder is characterised by alterations in insulin sensitivity, comprising insulin resistance in peripheral tissues and impaired hepatic insulin sensitivity (alterations in hepatic glucose production), and by impaired insulin secretion by pancreatic β cells [52-54]. Although the primary cause of type-2 diabetes remains unclear, the inability of β cells to compensate for reduced insulin sensitivity in peripheral tissues eventually leads to β-cell failure and deterioration in glucose homeostasis, ultimately leading to overt type-2 diabetes mellitus. Type-2 diabetes mellitus is a complex disease and both genetic and environmental factors appear to contribute to its development [52, 55]. Insulin resistance can be due to multiple defects in signal transduction such as impaired activation of insulin receptor-tyrosine kinase and reduced activation of insulin-stimulated phosphatidylinositol-3-hydroxy kinase. The resistance of insulin due to diet-induced obesity has given the critical role of obesity in the development of insulin resistance and other features of the metabolic syndrome [56].
Abnormalities of fatty acid metabolism are increasingly recognized as key components of the pathogenesis of the metabolic syndrome and type-2 diabetes [57]. Excess levels of oxygen in the living body can also pose a serious health threat; the so-called oxygen toxicity is brought about by oxygen species such as hydrogen peroxide and oxy radicals and damage living tissue. The active oxygen species are also associated with diabetes mellitus and are destructive towards various tissues as occurring in diabetes mellitus. There have been many reports discussing relationships between peroxidation and diseases such as diabetes mellitus, atherosclerosis and myocardial ischemia in terms of radical oxidation [58].

Type-2 diabetes mellitus is a progressive disease which may remain undiagnosed for several years, thus pre-existing diabetic complications are often present at the time of diagnosis. These complications fall into 2 main categories: macrovascular complications (including coronary heart disease and peripheral vascular disease), and microvascular complications (including retinopathy, nephropathy and neuropathy). Recent evidence has established that tight glycaemic control is a major factor in the prevention of these complications in both type-1 and type-2 diabetes mellitus [59-61]. Furthermore, recent epidemiological studies implicate postprandial hyperglycemia, but not fasting plasma glucose levels, as an important factor associated with the development of vascular complications [61-64].

The increasing prevalence of obesity and its associated comorbidities including type-2 diabetes and related cardiovascular disorders has stimulated efforts to develop effective new approaches in the treatment of this condition. While most therapeutic approaches involve altering the balance of metabolic energy by reducing energy intake, an alternative approach for the management of obesity is to affect an increase in the rate of energy expenditure. In 1984, compounds of the phenethanolamine class having thermogenic properties in rodents were first disclosed. Despite their structural similarity to known \( \beta_1 \) and \( \beta_2 \) adrenoceptor ligands, pharmacological studies indicated that these compounds stimulated a third or 'atypical' \( \beta \)-adrenergic receptor (\( \beta \)-AR) that is now described as \( \beta_3 \)-AR. \( \beta_3 \) agonist also increased insulin sensitivity and glucose utilization. Later studies suggested that Tyr 64 Arg \( \beta_3 \)-AR mutation in the human population plays a role in the
development of diabetes mellitus and/or obesity in some individuals possessing this genetic variant [65].

1.2.2 Emerging Drugs for Type-2 Diabetes mellitus

At present, therapy for type-2 diabetes relies mainly on several approaches intended to reduce the hyperglycemia. The natural progression of type-2 diabetes mellitus (T2DM) requires continuing medical care, early insulin intensification and patient self-management education to reduce the risk of long-term complications, including microvascular and macrovascular complications [66]. Several new pharmacological agents have recently been developed to optimise the management of type 2 (non-insulin-dependent) diabetes mellitus.

1.2.2.1 The α-Glucosidase Inhibitors

Alpha-glucosidase inhibitors such as acarbose (Precose) and miglitol (Glyset) function by interfering with the action of α-Glucosidase present in the small intestinal brush border epithelium [67]. 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 α-glucosidase inhibitors but they are effective at reducing fasting plasma glucose levels and levels of glycosylated hemoglobin (HbA1c). The common adverse side effects of these inhibitors are abdominal bloating and discomfort, diarrhea and flatulence.

1.2.2.2 Sulfonylureas

The sulfonylurea and meglitinide classes of oral hypoglycemic drugs are referred to as endogenous insulin secretagogues because they induce the pancreatic release of endogenous insulin [68]. The sulfonylureas have been used in the US for nearly 50 years. The first generation sulfonylureas (tolbutamide, acetohexamide, chlorpropamide and tolazamide) are not routinely prescribed any longer in the US. The second generation
sulfonylureas include glipizide (Glucotrol), glimepiride (Amaryl) 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 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.

1.2.2.3 Meglitinides

Meglitinides help the pancreas to produce insulin and are often called "short-acting secretagogues." They act on the same potassium channels as sulfonylureas, but at a different binding site [69]. By closing the potassium channels of the pancreatic beta cells, they open the calcium channels, hence enhancing insulin secretion. They are taken with or shortly before meals to boost the insulin response to each meal. If a meal is skipped, the medication is also skipped. The meglitinides used are repaglinide (Prandin), nateglinide (Starlix). Adverse reactions include weight gain and hypoglycemia.

1.2.2.4 Biguanides

Biguanides reduce hepatic glucose output and increase uptake of glucose by the periphery, including skeletal muscles. Metformin administration does not lead to increased insulin release from the pancreas and as such the risk of hypoglycemia is minimal [70]. Although it must be used with caution in patients with impaired liver or kidney function. Amongst common diabetic drugs, metformin is the only widely used oral drug that does not cause weight gain. The metformin (Glucophase), a biguanide, has become the most commonly used agent for type-2 diabetes in children and teenagers. Metformin may be the best choice for patients who also have heart failure [71]. The biguanides phenformin and buformin are withdrawn due to lactic acidosis risk [72, 73]. Metformin is usually the first-line medication which is used for treatment of type-2 diabetes. It is generally prescribed at initial diagnosis in conjunction with exercise and weight loss as opposed to in the past, where Metformin was prescribed after diet and exercise had failed. In adolescent females with type-2 diabetes, the use of metformin is
highly recommended to reduce the incidence as well as the potential for polycystic ovarian syndrome.

1.2.2.5 Thiazolidinediones

Thiazolidinediones (TZDs), also known as "glitazones," bind to PPARγ, a type of nuclear regulatory protein involved in transcription of genes regulating glucose and fat metabolism. These PPARs act on Peroxisome Proliferator Responsive Elements (PPRE). The PPREs influence insulin sensitive genes, which enhance production of mRNAs of insulin dependent enzymes. The final result is better use of glucose by the cells. Main thiazolidinediones used are rosiglitazone (Avandia) and pioglitazone (Actos). The troglitazone (Rezulin) which used in 1990s, withdrawn due to hepatitis and liver damage risk. As a result of multiple retrospective studies, there is a concern about rosiglitazone's safety, although it is established that the group, as a whole, has beneficial effects on diabetes. The greatest concern is an increase in the number of severe cardiac events in patients taking it. The pioglitazone may decrease the overall incidence of cardiac events in people with type II diabetes who have already had a heart attack [74].

1.2.2.6 Emerging Therapies

Several new therapies including new insulin platforms and new classes of pharmaceutical agents with unique modes of action have recently been introduced or are in clinical development for use in patients with type-2 diabetes. These include amylinomimetics, incretinmimetics, DPP-IV inhibitors, and glucagon antagonists [75]. These new agents improve glycemia and in some instances can reduce body weight. Glucagon-like peptide (GLP) analogs and agonists bind to a membrane GLP receptor. As a consequence of this, insulin release from the pancreatic beta cells is increased. Exenatide (also Exendin-4, marketed as Byetta) is the first GLP-1 agonist approved for the treatment of type-2 diabetes [76]. Liraglutide, a once daily human analogue (97% homology), is being developed by Novo Nordisk under the brand name Victoza. The product was approved by the U.S. Food and Drug Administration (FDA) on January 25, 2010. These agents may
also cause a decrease in gastric motility, responsible for the common side effect of nausea, and is probably the mechanism by which weight loss occurs.

Medical plants play an important role in the management of diabetes mellitus especially in developing countries where resources are meager. Plant materials which are being used as traditional medicine for the treatment of diabetes are considered one of the good sources for a new drug or a lead to make a new drug. Now a days more than 400 plants are being used in different forms for hypoglycaemic effects, all the claims practitioners or users are neither baseless nor absolutely. Therefore, a proper scientific evaluation a screening of plant by pharmacological tests followed by chemical investigations is necessary [77].

In the quest to develop compound with efficacy, low toxicity, and more ever affordable for treatment of diabetes and diabetes related complications, Central Drug Research Institute (CDRI), Lucknow, India, initiated steps to develop antidiabetic molecules. Flavone and chalcone as a basic skeleton was selected to optimize pharmacophoric properties in order to get desired anti-diabetic activity and property profile. These compounds (S002-853, S002-857 and S001-469) are covered under US patent number US 2006/0142302 A1 and 7635779.
Section 1.3
Research Envisaged
1.3.1 Research Envisaged

Troglitazone effectively reduces hyperglycemia, hyperinsulinaemia and hypertriglyceridemia in patients with type-2 diabetes. The mechanism of pharmacological effects has been shown to involve increased insulin sensitivity effects in skeletal muscle, liver and adipose tissue via the activation of PPAR-γ [78]. As vitamin-E analogue, troglitazone has been demonstrated to be an effective antioxidant; oxidative ring opening and subsequent quinone metabolite formation is believed to be the cause of hepatotoxicity and withdrawal of the drug [79]. This has led to the modification and resulted in several new molecules. Thinking on mechanism of action of troglitazone, Central Drug Research Institute (CDRI), Lucknow, India, initiated steps to develop antidiabetic molecules.

Flavonoids and chalcones are among the most ubiquitous groups of polyphenolic compounds in foods of plant origin. As integral constituents of the diet, they may exert a wide range of beneficial effects on human health. Flavonoid-rich extract of plant is long known for its antidiabetic activities in traditional medicines [80-82]. Flavonoids and chalcones produce such biological effects through their free radical scavenging antioxidant activities and metal ion chelating abilities [83, 84]. On the basis of these properties, flavone and chalcone as a basic skeleton was selected by CDRI to optimize pharmacophoric properties in order to get desired activity and property profile. S002-853, S002-857 are flavones derivatives and S001-469 is a chalcones derivative which were synthesized by Medicinal and process Chemistry Division of Central Drug Research Institute (CDRI), CSIR, Lucknow, India, which has an enormous potential to antidiabetic along with significant antidyslipidemic activity (Figure 1.3.1) [85-88].

The biological screening of the synthesized compounds for antihyperglycemic and antidyslipidemic activities were carried out in Biochemistry Division of the Institute. Sucrose loaded rat model was used for primary screening followed by streptozotocin induced beta cell damaged diabetic model of Sprague Dawley rat model. However, no information of these compounds about their pharmacokinetic profile and metabolism in animal model is available.
The pharmacokinetics and metabolism of flavonoids has been an area of active research in the last decade. The profiles indicate considerable differences among the different types of dietary flavonoids so that the most abundant flavonoids in the diet do not necessarily produce the highest concentration of flavonoids or their metabolites \textit{in-vivo} \cite{89}.

Figure 1.3.1 Chemical structure of [A] S002-853, [B] S002-857 and [C] S001-469.
Characterization of the preclinical pharmacokinetic studies in animal models is required for toxicological and preclinical studies and also for extrapolation of the pharmacokinetics and pharmacodynamics in human [90]. It was essential to characterize S002-853, S002-857 and S001-469 in terms of its pharmacokinetic profile and oral bioavailability.

One of the prerequisites for successfully carrying out preclinical as well as clinical PK studies is the availability of a reliable, reproducible, accurate and precise bio-analytical method for the compounds under consideration. An HPLC-UV method for determination of S002-853, S002-857 and S001-469 was reported in rat plasma, the lowest limit of quantitation (LLOQ) was 15.6 ng/ml (unpublished data). The sensitivity of this assay was found to be inadequate for PK profiling of S002-853, S002-857 and S001-469 by conventional routes of administration. Lengthy process of extraction of S002-857, moreover the method was not successful in estimating major metabolites of S001-469 and S002-853. Therefore, it was deemed necessary to develop a more sensitive and selective assay method for quantitative estimation of S002-853, S002-857 and S001-469 in biological fluids for meaningful preclinical pharmacokinetic evaluation to support the development of candidate drug. The advent of new technology LC-MS/MS were a breakthrough and most widely used for quantification of multiple compounds simultaneously and showed high sensitivity [91]. In this study we have developed and validated simple, selective and sensitive LC-MS/MS method for quantitative determination of S002-853 S002-857 and S001-469 in rat biological matrix.

Considering the promising results of S002-853, S002-857 and S001-469 at the pharmacological level, its preclinical pharmacokinetic studies (in-vitro and in-vivo) were planned in rats, so as to determine its absorption, distribution, metabolism and excretion (ADME) profile. ADME profile of the compounds will helpful for further development. As a requisite of drug development process, the studies on systemic bioavailability, protein binding, and in-vitro stability studies were undertaken in rats. Urinary and fecal excretion studies were planned in rats to establish the amount of unchanged S002-853, S002-857 and S001-469 excreted in urine and feces.
References


47. Singh R P, Sabarinath S, Singh SK, Gupta RC. A sensitive and selective liquid chromatographic tandem mass spectrometric assay for simultaneous quantification of novel trioxane antimalarials in different biomatrices using sample-pooling


