Summary of Thesis

SUMMARY OF THE THESIS

To be submitted for the degree of

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To

The Maharaja Sayajirao University of Baroda.

Name of the Student : **Pradipsinh Arjunsinh Jadav**

Title of the Thesis : **"Design, Synthesis and Biological Evaluation of Novel and Selective DPP-IV Inhibitors for the Treatment of Type-2 Diabetes''**

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Date of Registration : **5th June 2009**

The thesis entitled **"Design, Synthesis and Biological Evaluation of Novel and Selective DPP-IV Inhibitors for the Treatment of Type-2 Diabetes''** will be presented consisting of the following sections.

CHAPTER I (Introduction)

1.1. Diabetes

The term "Metabolic syndrome" (MS) refers to combination of medical disorders such as, hyperinsulinemia, dyslipidemia, high blood pressure, insulin resistance and obesity, when occurring together, increase the risk of developing cardiovascular diseases and diabetes.

Obesity, is a medical condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health and is a major risk factor for developing the cluster of these metabolic diseases while diabetes and cardiovascular disease are the major manifestations of metabolic syndrome. The incidence of metabolic syndrome has reached global epidemic proportions **[1]**.

Diabetes mellitus is a group of metabolic diseases in which hyperglycemia arising as a result of a relative or absolute deficiency of insulin secretion, resistance to insulin action, or both **[2]**. The cause of diabetes continues to be anonymity, although both genetics and environmental factors such as obesity and lack of exercise appear to play roles. Diabetes mellitus is a major and growing public health problem throughout the world, with an estimated worldwide prevalence of 220 million people in 2010 and it is expected to increase to 366 million people by 2030 **[3]**. 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 condition are summarized in **Table 1**.

Majority of diabetic patients can be treated with the agents that reduces hepatic glucose production (gluagon antagonist), reduce glucose absorption from gastrointestinal track (GIT), stimulate β-cell function (insulin secretagogues) or with agents that enhance the tissue sensitivity of the patients towards insulin (insulin sensitizers). The drugs presently used to treat diabetes include α-glucosidase inhibitors, insulin sensitizers, insulin secretagogues and K_{ATP} channel blocker **[4]**. However, almost one-half of diabetic subjects loss their response to these agents, over a period of time and thereby require insulin therapy. Insulin treatments has several drawbacks, it is injectable, causes hypoglycemia and weight gain **[5]**.

Table 1. Diagnostic Criteria for Diabetes Mellitus and Prediabetes conditions

Types of Diabetes	Pre prandial fasting plasma glucose mg/dL (mmol/L)	Post-prendial plasma glucose mg/dL (mmol/L)
Normal	**< 110 (< 6.1)**	**< 140 (< 7.8)**
Impaired fasting glucose (IFG)	**≥ 100 (≥ 6.1) & < 120 (< 7.0)**	**< 140 (< 7.8)**
Impaired glucose tolerance (IGT)	**< 126 (< 7.0)**	**≥ 140 (≥ 7.8)**
Diabetes mellitus	**≥ 126 (≥ 7.0)**	**≥ 200 (≥ 11.1)**

1.1.1 Types of diabetes

Although several pathogenic processes may be involved in the development of diabetes, the vast majority of cases fall into two main categories: Type I diabetes and Type II diabetes. Recently gestational diabetes, a new type of diabetes is also diagnosed in pregnant women.

1.1.1.1. Type 1 diabetes mellitus (T1DM)

Type I diabetes is occurs usually due to an immune-mediated destruction of pancreatic islet β-cells with consequent insulin deficiency, and hence T1DM patient rely on insulin injections for survival. T1DM is usually diagnosed in children and young adults, it is also called as juvenile diabetes or insulin-dependent diabetes mellitus (IDDM). Condition associated with T1DM include hyperglycemia and ketoacidosis (**Figure 1**). T1DM increases risk for many serious complication, such as heart disease (cardiovascular disease), blindness (retinopathy), nerve damage (neuropathy), kidney damage (nephropathy), foot and skin complications and depression.

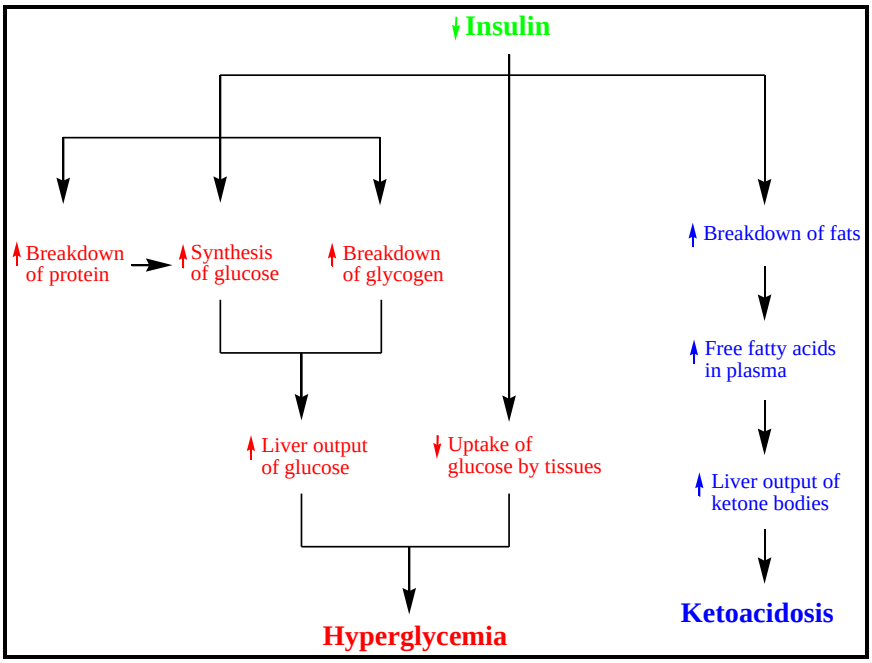


Figure 1. Metabolic Changes in T1DM

1.1.1.2. Type 2 diabetes mellitus (T2DM)

Type II diabetes mellitus (T2DM), the most common type of diabetes usually occurs due to insulin resistance, defect in the insulin production or increase in the hepatic glucose production and is usually associated with dyslipidemia, hypertension and obesity **[6]**. In T2DM, >50% of β-cells are already lost at the time of diagnosis and continues to decline throughout the course of T2DM. As depicted in **Figure 2**, insulin resistance arises as a consequence of multiple factors such as sedentary lifestyle, aging and obesity which results in hyperglycemia, blood pressure elevation, and dyslipidemia.

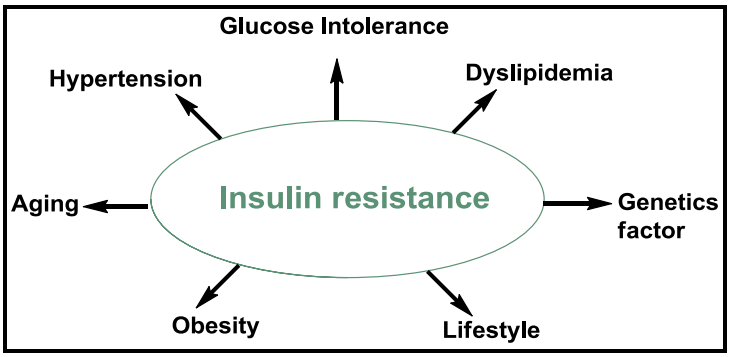


Figure 2. Causes and consequences of Insulin resistance

In T2DM, patients begin with insulin resistance and often treated with various oral antihyperglycemic agents; however, over a period of time, almost one-half of T2DM

subjects lose their response to these agents and thereby require insulin therapy **[7]**.

1.1.1.3. Gestational diabetes mellitus (GDM)

Gestational diabetes mellitus (GDM) is a condition in which women without previously diagnosed diabetes exhibit high blood glucose levels during pregnancy (especially during third trimester of pregnancy).

1.2. Pathogenesis of T2DM

The pathological sequence of T2DM is complex and involves many different elements that act together and makes T2DM condition more complex (**Figure 3**).

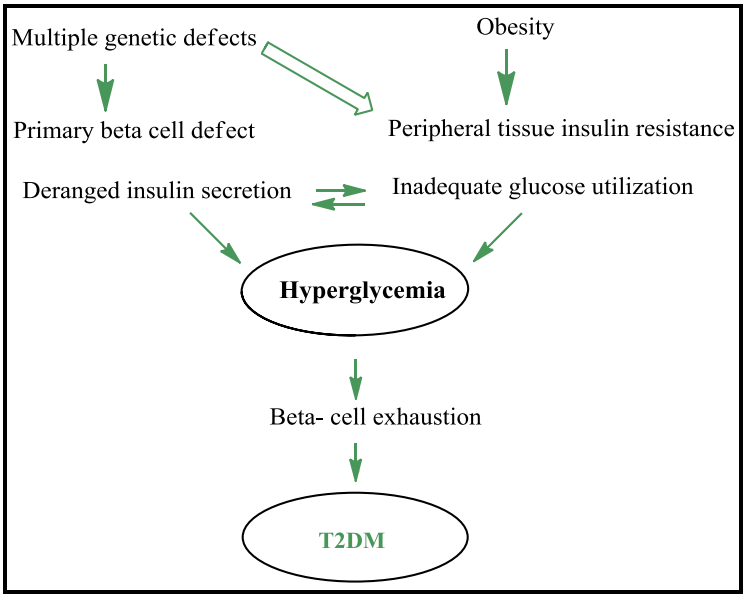


Figure 3. Proposed Pathogensis of Type 2 Diabetes

Along with different factors, both endogenous hormone Glucagon Like Peptide-1 (GLP-1) and glucagon play an important role in pathogenesis of T2DM **[8]**. GLP-1 (7-36) amide is a product of the preproglucagon gene, which is secreted from intestinal L-calls, in response to the ingestion of food. Endogenous GLP-1 binds to a membrane GLP-1 receptor. As a consequence of this, insulin release from the pancreatic β-cell is increased. The major problem of GLP-1 is its shorter half life. Glucagon (29 amino acid peptide) hormone processed from proglucagon in pancreatic α-cell by PC2. The main physiological role of glucagons is to stimulate hepatic glucose output, thereby leading to hypoglycemia. Therefore two defects, insulin resistance and insulin deficiency are responsible factors for the development of T2DM.

1.3. Current & Newer therapies for the treatment of T2DM

1.3.1. Current therapies for the treatment of T2DM

The cornerstone of treatment and prevention of T2DM is lifestyle modification through increased physical activity and attention to food intake, particularly among the subjects, where in weight loss is the principal goal. When lifestyle modifications do not result in normalization or near normalization of metabolic abnormalities, pharmacological therapy is required. Based on route of administration, current therapy are divided in two groups, (1) Injectable and (2) Oral therapies.

1.3.1.1. Injectable therapies for the treatment of T2DM

Insulin facilitates glucose entry into adipose tissues, muscles, and liver by stimulating several enzymatic reactions that start at the insulin receptors. Currently available injectable analogues are divided into two groups, (1) insulin analogues and (2) incretin mimetics.

Secreted insulin binds to its receptor, which in turn starts many protein activation cascades, such as translocation of Glut-4 transporter to the plasma membrane and influx of glucose, glycogen synthesis, glycolysis and fatty acid synthesis.

Exenatide and liraglutide belong to group of incretin mimetics. Exenatide is a 39-amino-acid peptide, an insulin secretagogue, with glucoregulatory effects. It bears a 50% amino acid homology to GLP-1 and it has a longer half-life. Liraglutide is an acylated human GLP-1 receptor agonist, with a 97% amino acid sequence identity to endogenous human GLP-1(7-37). Liraglutide is stable against metabolic degradation by both peptidases, dipeptidyl peptidase IV (DPP-IV) and neutral endopeptidases (NEP).

These injectable therapies & incretin mimetics have several drawbacks, it is injectable, produces hyperglycemia and causes weight gain, which is believed to be a potential cause for the development of diabetes complications **[9]**. Thus, there is an urgent need to develop some oral antihyperglycemic agents that can complement with the existing injectable therapies.

1.3.1.2. Oral antidiabetic agents for the treatment of T2DM

Before 1995, sulfonylureas were the only oral antidiabetic agents available for the treatment of T2DM. Currently available oral antidiabetic therapies include agents which causes insulin production (sulfonylureas, secretagogues), agents which

decreases hepatic glucose production (biguanides), agents which acts as insulin sensitizers (glitazones), and R-glucosidase inhibitors.

However, most of the oral antihyperglycemic agents are also associated with side effects and adverse events, such as digestive manifestation (nausea, epigastic pain, liver pain) and of hematological manifestations (pancytopenia, autoimmune hemolytic anemia, thrombocytopenia).

1.3.2. Newer therapies for the treatment of T2DM

Knowing the side effects of the injectable and oral therapy, such as hypoglycemia, GI side effect, lactate production, fluid retention, hepatoxicity, allergic reaction and cardiovascular effects. Currently several new therapies are in various stages of clinical development for the treatment of T2DM.

- GLP-1 agonist
- DPP-IV inhibitors
- FBPase inhibitors
- GSK-3 inhibitors
- 11β-HSD-1 inhibitors
- SGLT2 inhibitors
- PPARγ dual agonist
- PTP-1B inhibitors

1.4. Introduction to Dipeptidyl Peptidase IV (DPP-IV) inhibitors

1.4.1. DPP-IV and their importance

The incretin mimetics glucagon-like peptide 1 (GLP-1) and glucose-dependent gastric inhibitory polypeptide (GIP) are released from the L-cell of intestine upon injestion of food **[10-11]**. These 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, get inactivated by DPP-IV enzyme **[12-13]**.

In vivo, DPP-IV enzyme inactivates both the incretin hormones (GLP-1 & GIP), which in-turn stimulates glucose dependent insulin secretion. DPP-IV enzyme selectively cleaves first two amino acids (His-Ala) of 29 amino acid GLP-1 peptide and thereby

makes it inactive which are eliminated via kidney **[14]**.

1.4.2. Dipeptidyl peptidase family

The DPP family (family S9), a subfamily of the prolyl oligopeptidase superfamily, includes four enzymes, DPP-IV, FAP, DPP-8 and DPP-9, and two non-enzymes, DPP-IV-like protein-6 (DPP-6, DPL-1 or DPP-X) and DPP-10 (DPL-2) **[15]**.

Members of the DPP-IV family preferentially cleave Xaa-Pro- and Xaa- Ala-dipeptides (where Xaa is any amino acid except proline) from the N-terminus of proteins **[16]**. The DPP-IV family differentiates itself from the prolyl oligopeptidase superfamily by the presence of two glutamate residues located within the catalytic pocket, which are essential for enzymatic activity **[17]**.

1.4.3. Role of DPP-IV in metabolic diseases

As discussed earlier DPP-IV enzyme rapidly degrades bioactive incretin hormones GLP-1, and GIP to their inactive metabolites. Both these incretins are important regulator of glucose metabolism. Competitive inhibition of DPP-IV increases the half-life and bioavailability of active incretin hormones, enhancing their biological effect **[18]**.

Other than regulating the levels of endogenous incretin hormones such as GLP-1 and GIP, DPP-IV enzyme play crucial role in controlling the lymphocyte and cell growth, T-cell activation, metastasis, inflammation and immune function of body**.**

1.5. Crystal structure of DPP-IV

The seven DPP-IV crystal structures reported till date reflect tremendous global interest in the pharmaceutical design of DPP-IV inhibitors **[19-20]**. Human DPP-IV has a short cytoplasmatic tail of 6 amino acids, a 22-amino acid hydrophobic transmembrane region, and a 738-amino acid extracellular domain with ten potential glycosylation sites **[21]**. The DPP-IV glycoprotein is a homodimer. The main DPP-IV structural features include (A) catalytic or *α*/*β*-hydrolase domain (B) *β*-propeller domain (C) active site and (D) substrate binding site.

1.6 Mechanism of action of DPP-IV inhibitors

As described earlier DPP-IV enzyme selectively cleaves the N-terminal dipeptide from the penultimate position of GLP-1 and GIP thus makes them inactive

[22]. Competitive inhibition of the DPP-IV enzyme blocks the degradation of these incretin hormones and extend the duration of action of endogenous GLP-1, thereby stimulating insulin secretion, inhibiting glucagon release and slowing gastric emptying..
Inhibition of DPP-IV enzyme activity, using suitable DPP-IV enzyme inhibitor likely to increase the levels (prolong half-life) of endogenous intact and bioactive GIP and GLP-1 peptides, which in-turn increase the insulin secretion and decrease the blood glucose, thereby, it acts as antidiabetic agents **[23-24].**

1.7. Challenges in developing potent and selective DPP-IV inhibitors

DPP-IV enzyme resembles with several other closely related serine proteases so development of small molecules as selective inhibitors of DPP-IV become a major challenge. Although the in vivo function of other members of DPP family, that is, DPP-2, DPP-8, DPP-9 etc. are largely unknown, the physiological effects of their inhibition has been documented in the literature **[25]**. However, extensive SAR work has proved that desired selectivity for DPP-IV inhibition can be achieved via introducing appropriate substituents or groups that can attribute all favorable interactions with DPP-IV enzyme.

1.8. Overview on DPP-IV inhibitors under recent development

Several excellent reviews have been published covering the development of potential new therapeutic DPP IV inhibitors **[26-29]**. Eight gliptins are in the market. Although the bibliography on DPP-IV inhibitors is rich, active research continues on this subject. Currently 35 compounds are in preclinical development having IC_{50}≤6nM. Major players include AstraZeneca Plc., Boehringer Ingelheim GmbH, Bristol-Myers Squibb, Eli Lilly and Company, Merck & Co Inc., Mitsubishi Tanabe Pharma Corp., Novartis AG, Takeda Pharmaceutical Company Limited, Cadila Healthcare Ltd., Phenomix, Lupin limited, L G Life Sciences etc.

1.9. Introduction to Cytochrome P450 (CYPs) and its importance

The cytochrome P450 system is a group of enzymes, found mainly in the liver and gut mucosa that plays a crucial role in controlling the concentrations of many endogenous substances and drugs. CYPs enzymes are mainly essential for the detoxification and the metabolism of drugs. CYP subfamilies involved in drug metabolism includes CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4. However CYP2D6 and CYP3A4 are the major drug-metabolizing enzymes in humans.

CYP inhibition/ induction can have significant consequences on other antidiabetic drugs that are metabolized by these enzymes, which may result in drug-drug interactions (DDI) and idiosyncratic drug toxicity (IDT) **[30-31]**.

1.9. Conclusion

Diabetes mellitus is the most prevalent and serious metabolic disorder. Among the T1DM & T2DM, T2DM is one of the major public health challenges of 21st century. Currently available therapy have several drawback. Therefore various new therapies are being developed, among which DPP-IV inhibitors are the most promising approach for the safe and effective treatment of Type 2 diabetes. However achieving selectivity and oral bioavailibity with longer duration of action are major challenges with the design & development of DPP-IV inhibitors. To address this concern, in the next section, we have designed novel series of DPP-IV inhibitors to develop second generation therapies for the treatment of T2DM.

CHAPTER II (Designing Strategy)

2. Design strategy of DPP-IV inhibitors

2.1. Orally active, potent and selective DPP-IV inhibitors

As mention earlier, DPP IV enzyme selectively cleaves the N-terminal dipeptides (X-Ala or X-Pro) from target polypeptides, such as GLP-1 and GIP **[16]**. Also, structure of DPP-IV enzyme resembles with several other protease enzymes and it exhibit broad substrate specificity. Thus, in order to develop selective DPP-IV inhibitor, we decided to design dipeptide based DPP-IV inhibitors, based upon the sequence homology of first two amino acids of GLP-1 peptide (His-Ala/His-Pro) and SAR study of DPP-IV inhibitors, which are in clinic or in clinical development **[32-33]**.

Most of the DPP-IV inhibitors, which are in clinical development were designed based upon the SAR study of dipeptide substrate recognized by DPP-IV enzyme (**Figure 16**). A key feature in most of the DPP-IV inhibitors, which are under development include incorporation of cyanopyrrolidine ring system, attached to sterically hindered group such as adamantyl, with -CH_2- spacer/linker.

Earlier several attempts have been made to develop dipeptide based DPP-IV inhibitors **[26,34-36]**. In general, pyrrolidine or thiazolidine based DPP-IV inhibitors were found to be very potent and selective but under in vivo condition, they were found to be

metabolically unstable, mainly due to cyclisation (**Figure 4**). To overcome ring cyclisation problem under basic condition, sterically hindered bulky substitution were introduced in new class of DPP-IV inhibitors.

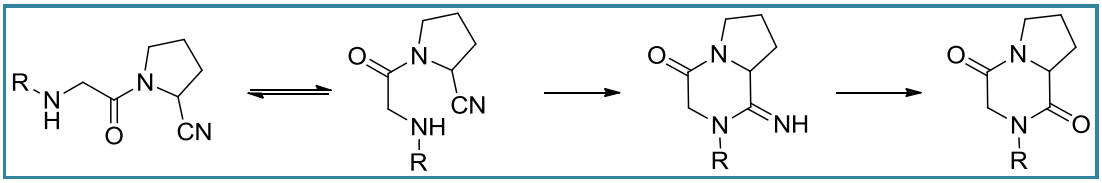


Figure 4: Cyclic amide or diketopiperazine formation

Among the DPP-IV inhibitors launched or in active development, vildagliptin, saxagliptin, anagliptin, teneligliptin, melogliptin and gosogliptin are peptide mimetic compounds, which have been discovered by replacing segments of peptide-based substrates **[37]**. All these compounds contains pyrrolidine ring system bearing either –CN and/or –F substituent at specific positions on the basis of SAR **[38-39]**. As nitrile and fluoro groups are important for high potency; analogs with similar structures, but without a nitrile/fluoro in this position are 2-3 orders of magnitudes less active. Since pyrrolidine best fits in S1 pocket and nitrile group covalently interact with the catalytically active serine hydroxyl (Ser630), imparts high potency.

However, sitagliptin, alogliptin and linagliptin are non-peptide mimetic compounds, which have been discovered by optimization of the initial lead compounds identified by random screening **[37]**. Therefore, their chemical structures are diverse, suggesting that each of their binding modes in DPP-IV would be unique. Fluoro or nitrile substituted phenyl ring in sitagliptin, alogliptin, trelagliptin, omarigliptin and evogliptin occupies the S1 pocket, where it provide tight binding by additional interaction with Tyr666.

Binding of DPP-IV inhibitors to S3 site play important role in increasing the selectivity of the inhibitor over other related enzymes. Compounds which are recently launched and in active development, contains various hetero aromatic substituents, which fit in to S3 site and give binding interaction with Phe357 and Arg358.

Hence, keeping in mind the SAR study of DPP-IV inhibitors developed and in active development, novel DPP-IV inhibitors are designed as illustrated in sections **2.1.1. -2.1.3.**

2.1.1. Rational for designing cyanopyrrolidine containing peptidomimetic based DPP- IV inhibitors. (First series)

As discussed above to develop dipeptide based DPP-IV inhibitors 2-cyano-pyrrolidine based DPP-IV inhibitors have been studied most extensively because cyanopyrrolidine ring system not only mimic the proline ring system, but also the presence of nitrile on the five membered ring provides nanomolar inhibition of DPP-IV and the metabolic stability favors oral administration of cyanopyrrolidine based DPP-IV inhibitors. Hence to overcome ring cyclisation problem under basic condition and chemical instability, sterically hindered bulky substitution and pyrrolidine ring with various substituents were introduced into the new class of dipeptide based DPP-IV inhibitors **[27]**.

Among various DPP-IV inhibitors reported in the literature, NVP-DPP728, closely resembles with dipeptide substrate and therefore it was found to be very potent and selective (reported EC_{50} value 7 nM and > 15000 fold selective) **[40]**. Furthermore, crystal structure of DPP-IV enzyme interaction with derivative of NVP-DPP728 at catalytic site, is reported in the literature indicated that that there are two hydrophobic binding pockets located at catalytic site and electrophilic groups are essential for hydrogen binding **[41]**.

Thus based upon SAR study of NVP-DPP728 and past developmental scenario, we have designed new series of dipeptide based DPP-IV inhibitors, which mainly consist of five member proline ring system, attached to sterically hindered aromatic acid, with suitable linker. These Novel dipeptide based DPP-IV inhibitors were prepared, either by varying the length of linker with carbon chain or aromatic ring as a spacer and electrophilic functionality on proline ring (amide/nitrile) or electron withdrawing / donating groups on sterically hindered aromatic ring system (**Figure 5**).

Compounds represented by general structure **I** and **II** were designed in close analogy to NVP-DPP728 for the reason that it attenuate all the favorable enzyme interactions for binding and activation to achieve nanomolar potency. Pyrrolidinecarboxamides **I** and **II** were specifically designed to minimize the intra-molecular cyclisation (i.e cyclic amide or diketopiperazine formation). However it is known that phenomenon is more prone in dipeptidic smaller compounds so knowing the fact that the presence of nitrile on the five membered proline ring provides nanomolar inhibition of DPP-IV we designed pyrrolidinecarbonitriles **III** and **IV** tripeptide based peptidomimetics.

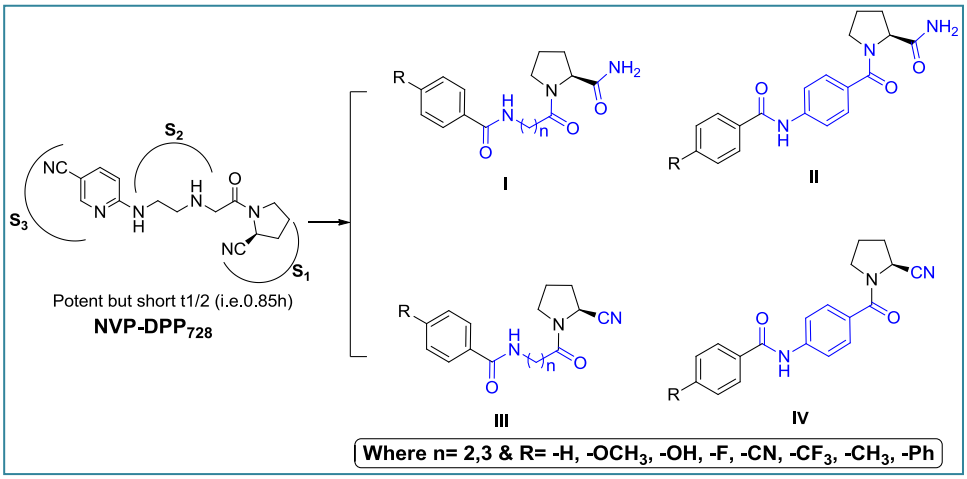


Figure 5: Design strategy of cyanopyrrolidine containing peptidomimetic based DPP-IV inhibitors.

Thus, in this series, all favorable structure components of compound NVP-DPP728 essential for the key interaction with DPP-IV were retained, except suitable changes were made to improve potency, selectivity and pharmacokinetic profile. In this, series, we planned to prepare total thirty compounds **11a-h**, **12a-h, 16a-h, 17a-d** and **18a-b**, their synthetic schemes are explained in **Chemistry section 3.1.1**.

2.1.2. Rational for designing peptidomimetic based DPP-IV inhibitors, devoid of CYP liabilities. (Second series)

However, upon secondary profiling of lead compound of the first series **17c**, CYP3A4 and CYP2D6 inhibitions (IC_{50}: 1.1 and 1.9 mM respectively) were observed, which halted its further preclinical development because CYP inhibition/ induction can have significant consequences on antidiabetic drugs that are metabolized by these enzymes, which may result in drug-drug interaction (DDI) and idiosyncratic drug toxicity (IDT). Hence new series have been designed and synthesized based upon the following rationale.

Affinity of a molecule for CYP can be attenuated by increasing / decreasing the carbon chain length **[42]**. So to overcome CYP liabilities, amino-alkyl spacer (-$(CH_2)_3$-; 3C) of compound **17c** was specifically reduced from 3C to 2C (-$(CH_2)_2$-) (i.e. **17a**) and 1C (-CH_2-) (i.e. **27a**) and the resulting molecules were examined for CYP inhibitions. Here the chain length has been reduced for the reason that all DPP-IV inhibitors reported in literature are very small molecules in bulk with shorter length to preserve the potency such as Vildagliptin, Saxagliptine, Alogliptin etc. The reduction of amino-alkyl

spacer attenuates CYP inhibitions but led to a significant drop in DPP-IV inhibitory activity.

Further to improve DPP-IV inhibitory activity of **27a**, two series (**27b-j** and **34a-m**) of structurally constrained cyanopyrrolidine containing peptidomimetic based DPP-IV inhibitors were designed (**Figure 6**). In the first series suitable modifications were carried out on 1C amino-alkyl spacer of **27a** and altogether nine compounds (**27b-j**) were prepared by linking ring A with ring B, using various α-substituted amino acids as spacers. In the second series, thirteen compounds (**34a-m**) were prepared by modifying the best compound obtained from first series (i.e. **27j**), specifically by carrying out suitable changes over ring -A and –B, taking in to consideration the effect of substituents extensively being used in the DPP-IV drug discovery research.

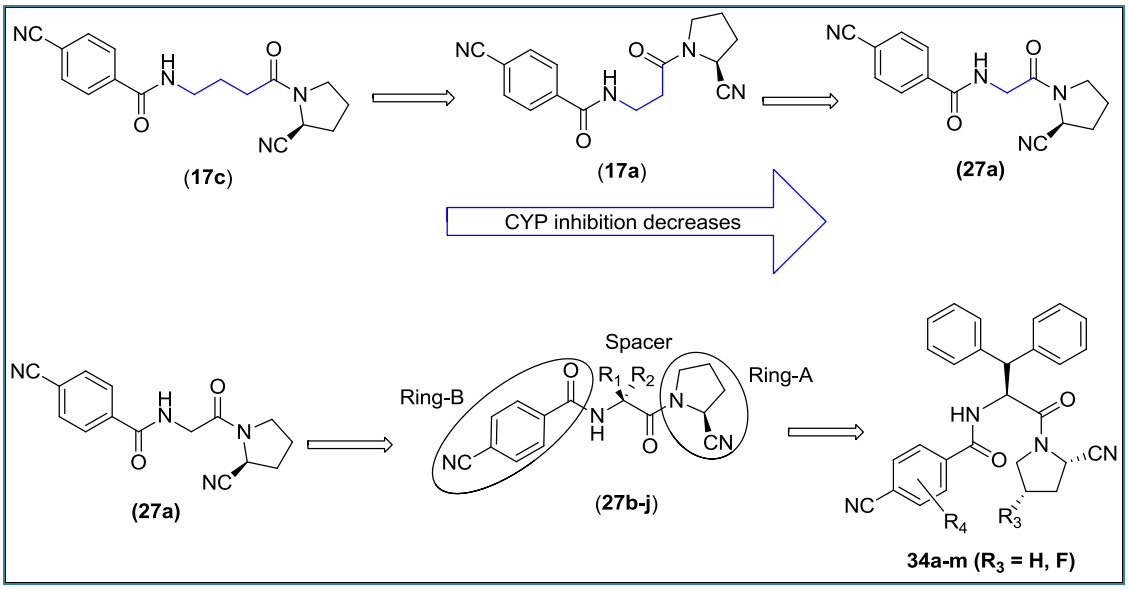


Figure 6: Design strategy of peptidomimetic based DPP-IV inhibitors, devoid of CYP liabilities.

R_1 and R_2 together represent substituted α-amino acids with absolute (S) stereo configuration (**Figure 7**). Substituent R_4 in ring-B together represent substituted 4-Cyano benzoic acids selected from the group given in **Figure 8**.

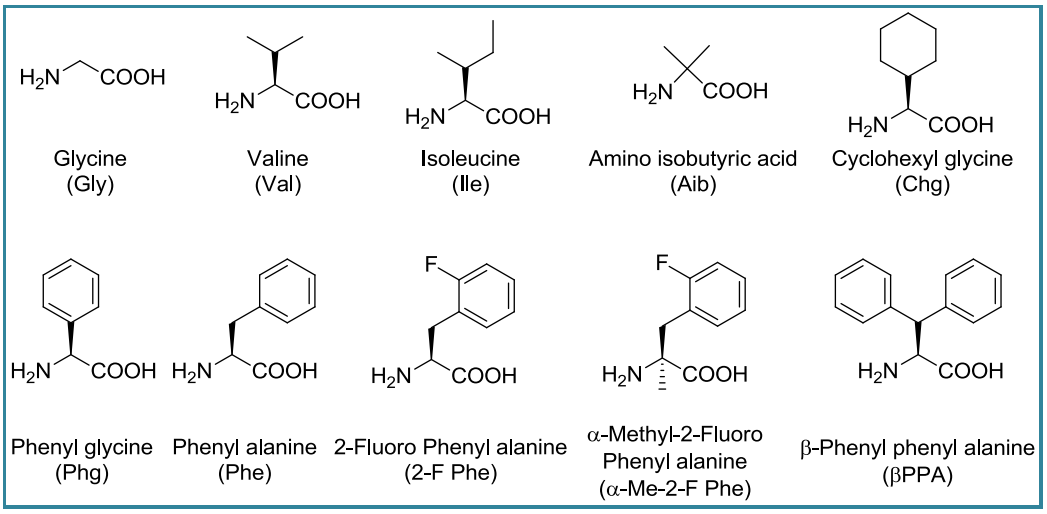


Figure 7: Structures of α-amino acids used as a linker.

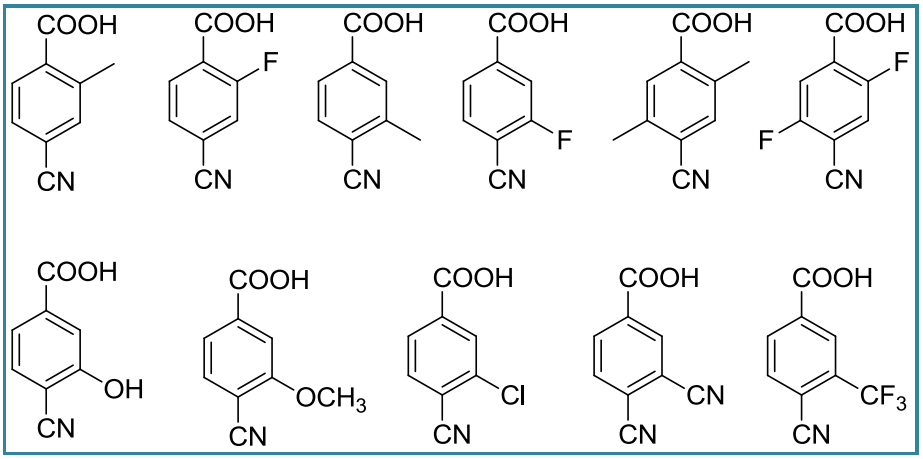


Figure 8: Structures of substituted 4-Cyano benzoic acids

In this series, we planned total twenty-three compounds as two different series **27a-j** and **34a-m**, their synthetic methodology and chemical characterization are explained in details in **Chemistry section 3.2.1**.

2.1.3. Rational for designing of aminomethylpiperidone based DPP-IV inhibitors. (Third series)

Taking into consideration journey of Merck Sharp & Dohme Corp. to develop potent and selective DPP-IV inhibitors with improved pharmacokinetic profile, we have designed aminomethylpiperidone based DPP-IV inhibitors (**Figure 9**).

During their journey to improve PK profile Merck has come up with compound **VII** having extended $t_{1/2}$ and almost double bioavailability as compared to Sitagliptin **[43-46]**. So considering all structural modification done by Merck and extending the scope of novelty with rationale, compounds (**68a-v**, **69a-e** and **70a-e)** were designed based on the piperidone skeleton and anticipated that the aminomethyl and the amide groups of the piperidone ring may contribute improved pharmacokinetic and pharmacodynamic effects, along with the potent and selective DPP-IV inhibitory activity.

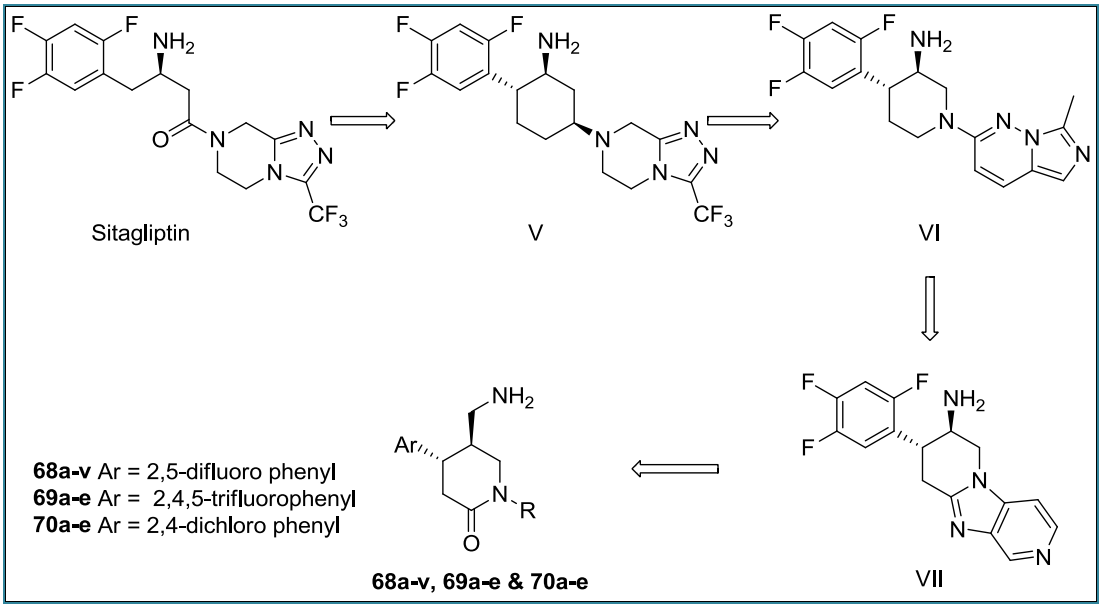


Figure 9: Design strategy of aminomethylpiperidone based DPP-IV inhibitors.

Substutuent R in novel aminomethylpiperidones **68a-v**, **69a-e** and **70a-e** was selected from the following group (**Figure 10**).

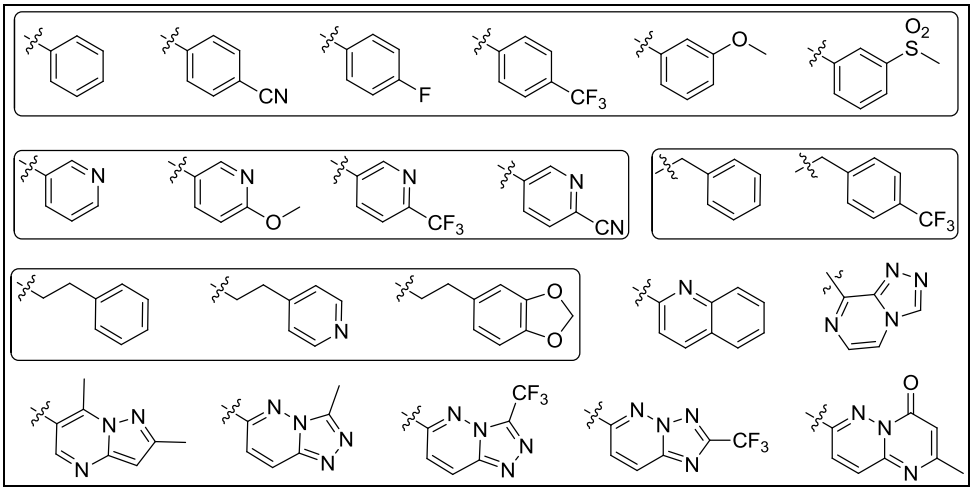


Figure 10: Substituents used for synthesis of aminomethylpiperidone based DPP-IV inhibitors.

In this series we planned to prepare total thirty two compounds as three different series viz **68a-v**, **69a-e** and **70a-e**, their synthetic methodology and chemical characterization are explained in details in **Chemistry section 3.3.1**.

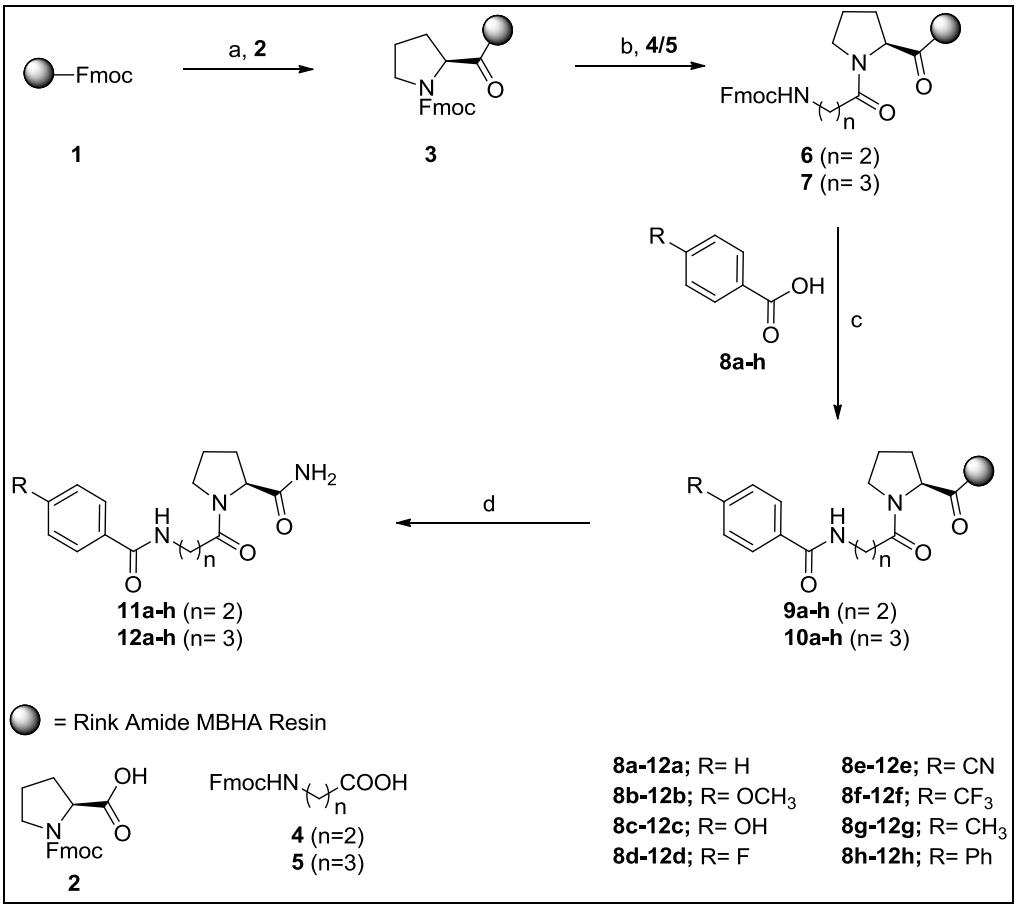
2.1.4. Conclusion

In the present investigation, total three series (First series: cyanopyrrolidine containing peptidomimetic based DPP-IV inhibitors, second series: peptidomimetic based DPP-IV inhibitors, devoid of CYP liabilities and Third series: aminomethylpiperidone based DPP-IV inhibitors) were designed as potent and selective DPP-IV inhibitors for the safe and effective treatment of metabolic diseases such as T2DM. Altogether eighty five compounds were planned, mainly as DPP-IV inhibitors. All the test compounds were synthesized and well characterized, subjected for *in vitro*, *in vivo* and pharmacokinetic (PK) studies and results are summarized in the results and discussion section.

CHAPTER III (Results and discussion)

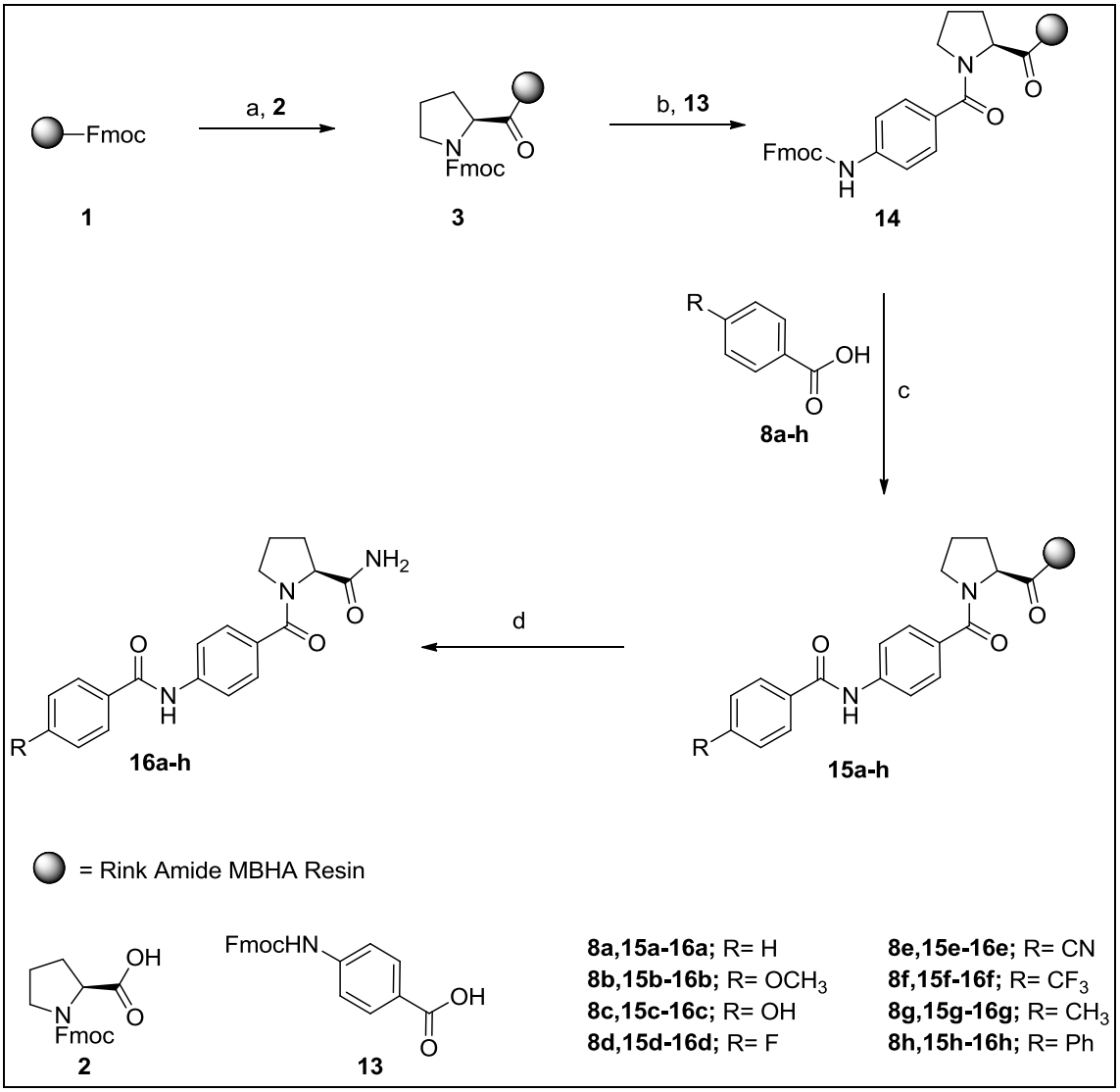
3.1.1. Cyanopyrrolidine containing peptidomimetic based DPP-IV inhibitors (First series)

Synthesis of designed compounds **11a-h**, **12a-h, 16a-h, 17a-d** and **18a-b** is illustrated in **Schemes 1-3.** Novel peptidomimetics were synthesized using Fmoc-based Solid Phase Peptide Synthesis (SPPS) approach **[47]**



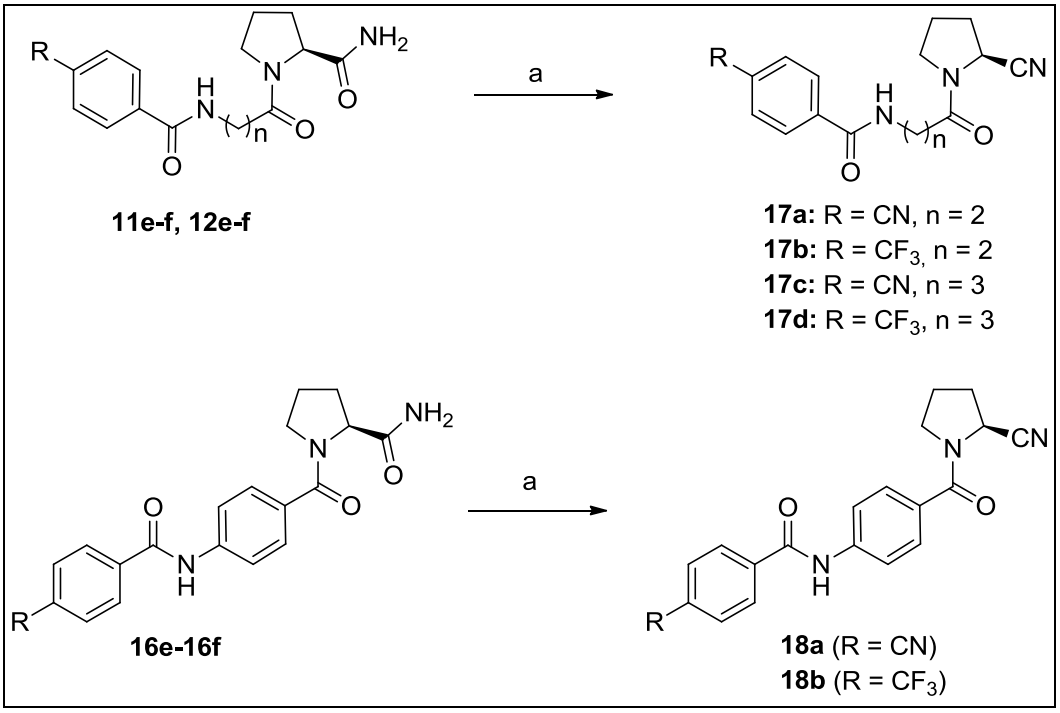
Reagents and conditions: (a) i. 20% Piperidine in DMF ii. Fmoc-Pro-OH (**2**), HOBt, DIC, DMF, N_2 (b) i. 20% Piperidine in DMF ii. Fmoc-NH-$(CH_2)_n$-COOH (**4**/**5**), HOBt, DIC, DMF, N_2 (c) i. 20% Piperidine in DMF ii. Substituted benzoic acids (**8a-h**), HOBt, DIC, DMF, N_2 (d) TFA: H_2O: Triisopropylsilane (95:2.5:2.5), 25°C, 3h.

Scheme 1. Synthetic methods for the preparation of title compounds **11a-h** and **12a-h**



Reagents and conditions: (a) i. 20% Piperidine in DMF ii. Fmoc-Pro-OH (**2**), HOBt, DIC, DMF, N_2 (b) i. 20% Piperidine in DMF ii. Fmoc-PABA-COOH (**13**), HOBt, DIC, DMF, N_2 (c) i. 20% Piperidine in DMF ii. Substituted benzoic acids (**8a-h**), HOBt, DIC, DMF, N_2 (d) TFA: H_2O: Triisopropylsilane (95:2.5:2.5), 25°C, 3h.

Scheme 2. Synthetic methods for the preparation of title compounds **16a-h**.



Reagents and conditions: (a) TFAA, CH_2Cl_2, 25°C, 6h.

Scheme 3. Synthetic methods for the preparation of title compounds **17a-d** and **18a**

Using above synthetic routes (**Scheme 1**, **2** and **3**), thirty compounds (**11a-h**, **12a-h, 16a-h, 17a-d** and **18a-b**) were prepared. Compounds were purified by preparative HPLC method and characterized using spectroscopic analysis. Compounds **11a-h**, **12a-h, 16a-h, 17a-d** and **18a-b** were evaluated for their *in vitro* DPP-IV inhibition activity using enzymatic assay (details experimental protocol is given in **experimental Section 5.2.1.**). Based on these results few compounds were selected and evaluated for their selectivity, *in vivo* antidiabetic activity and pharmacokinetic parameters.

3.1.2. Conclusion

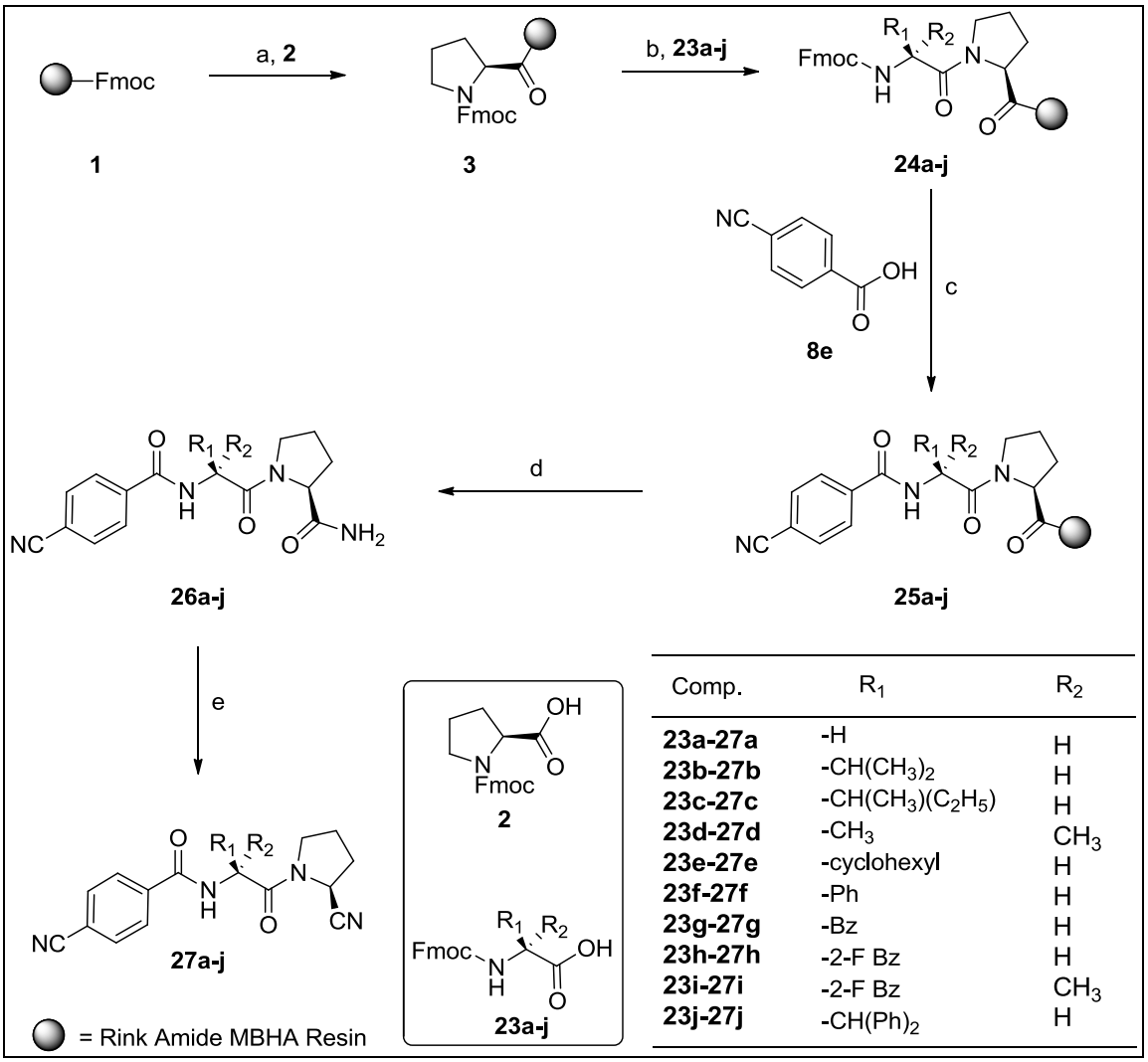
To overcome the ring cyclization problem (piperazine ring formation) associated with the existing dipeptide based DPP-IV inhibitors (to improve stability), carboxamide based peptidomimetics were prepared (**11a-h**, **12a-h** and **16a-h**). Here new compounds are consist of tripeptide bearing substituted benzamide rings which can fit in to S3 binding site of DPP-IV enzyme, responsible for improving selectivity. However new compounds contain peptidic amide bonds and no free amine group so there is no

chances of cyclization even if carboxamide is replaced with the nitrile group. Knowing this fact novel cyanopyrrolidine based peptidomimetics **17a-d** and **18a-b** were synthesize by doing dehydration of most potent carboxamide based peptidomimetics. These compounds were tested for their *in vitro* DPP-IV inhibitory activity, selectivity over other serine proteases and *in vivo* antidiabetic activity in animal models. Establishing the evidence for our hypothesis of incorporating the peptidic amide bond to improve stability, nitrile functionality in pyrrolidine ring to improve potency and benzamide ring as P3 site of the molecules in order to develop novel DPP-IV inhibitors with good selectivity, we identified the lead compound **17c** consisting of *para*-nitrile benzamide attached to cyanopyrrolidine ring with GABA spacer, which showed excellent *in vitro* potency and selectivity over other serine protease, due to its favorable orientation across all the three binding sites. Compound **17c** exhibited excellent anti-hyperglycemic effects in animal models, along with improved oral bioavailability. Thus discovery of cyanopyrrolidine based peptidomimetics series suggests that this class of compounds could be useful approach towards the safe and effective prevention of T2DM and need to subject for further pre-clinical evaluation.

3.2.1. Peptidomimetic based DPP-IV inhibitors, devoid of CYP liabilities (Second series)

Synthesis of designed peptidomimetics **27a-j** and **34a-m** was carried out using Fmoc-based Solid Phase Peptide Synthesis (SPPS) approach illustrated in **Schemes 4-5.**

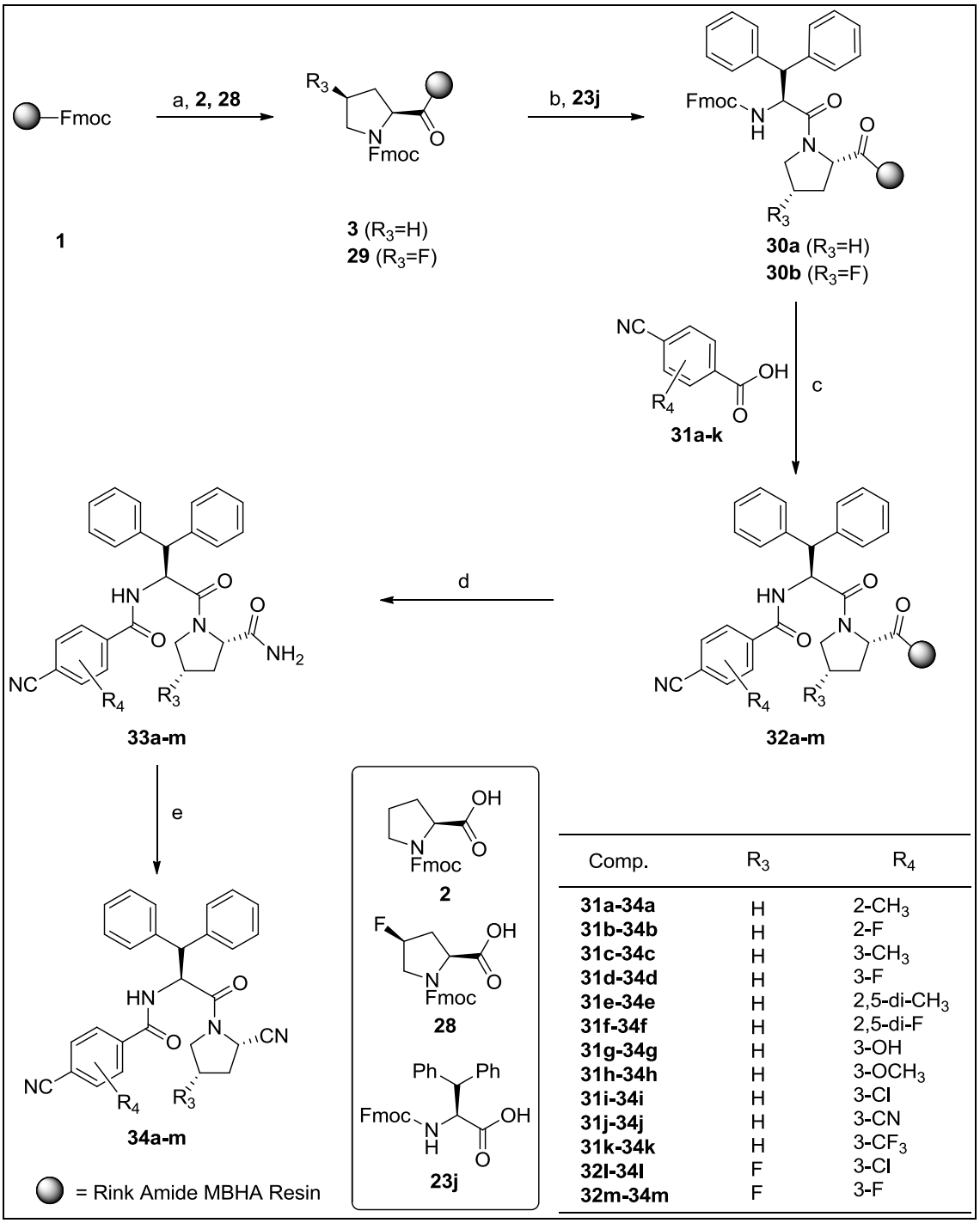
Using described synthetic routes (**Scheme 4-5**), twenty three compounds **27a-j** and **34a-m** were prepared. Compounds were purified by preparative HPLC method and characterized using spectroscopic analysis. All the compounds were evaluated for their *in vitro* DPP-IV inhibitory activity using enzymatic assay (details experimental protocol is given in **experimental Section 5.2.1.**). Based on these results few compounds were selected and evaluated for their selectivity over other serine protease and CYP inhibition study (*in vitro*). *in vivo* antidiabetic activity and pharmacokinetic parameters.



Comp.	R_1	R_2
23a-27a	-H	H
23b-27b	-$CH(CH_3)_2$	H
23c-27c	-$CH(CH_3)(C_2H_5)$	H
23d-27d	-CH_3	CH_3
23e-27e	-cyclohexyl	H
23f-27f	-Ph	H
23g-27g	-Bz	H
23h-27h	-2-F Bz	H
23i-27i	-2-F Bz	CH_3
23j-27j	-$CH(Ph)_2$	H

Reagents and conditions: (a) i. 20% Piperidine in DMF ii. Fmoc-Pro-OH (**2**), HOBt, DIC, DMF, N_2 (b) i. 20% Piperidine in DMF ii. Fmoc-NH-(CHR_1R_2)-COOH (**23a-j**), HOBt, DIC, DMF, N_2 (c) i. 20% Piperidine in DMF ii. *p*-cyano benzoic acid (**8e**), HOBt, DIC, DMF, N_2 (d) TFA: H_2O: Triisopropylsilane (95:2.5:2.5), 3h. (e) TFAA, CH_2Cl_2, 25°C, 6h.

Scheme 4. Synthetic methods for the preparation of peptidomimetics **27a-j**



Comp.	R_3	R_4
31a-34a	H	2-CH_3
31b-34b	H	2-F
31c-34c	H	3-CH_3
31d-34d	H	3-F
31e-34e	H	2,5-di-CH_3
31f-34f	H	2,5-di-F
31g-34g	H	3-OH
31h-34h	H	3-OCH_3
31i-34i	H	3-Cl
31j-34j	H	3-CN
31k-34k	H	3-CF_3
32l-34l	F	3-Cl
32m-34m	F	3-F

Reagents and conditions: (a) i. 20% Piperidine in DMF ii. Fmoc-Pro-OH (**2**)/Fmoc-4-F-Pro-OH (**28**), HOBt, DIC, DMF, N_2 (b) i. 20% Piperidine in DMF ii. Fmoc-NH-($CHPh_2$)-COOH (**23j**), HOBt, DIC, DMF, N_2 (c) i. 20% Piperidine in DMF ii. Substituted benzoic acids (**30a-k**), HOBt, DIC, DMF, N_2 (d) TFA: H_2O: Triisopropylsilane (95:2.5:2.5), 3h. (e) TFAA, CH_2Cl_2, 25°C, 6h.

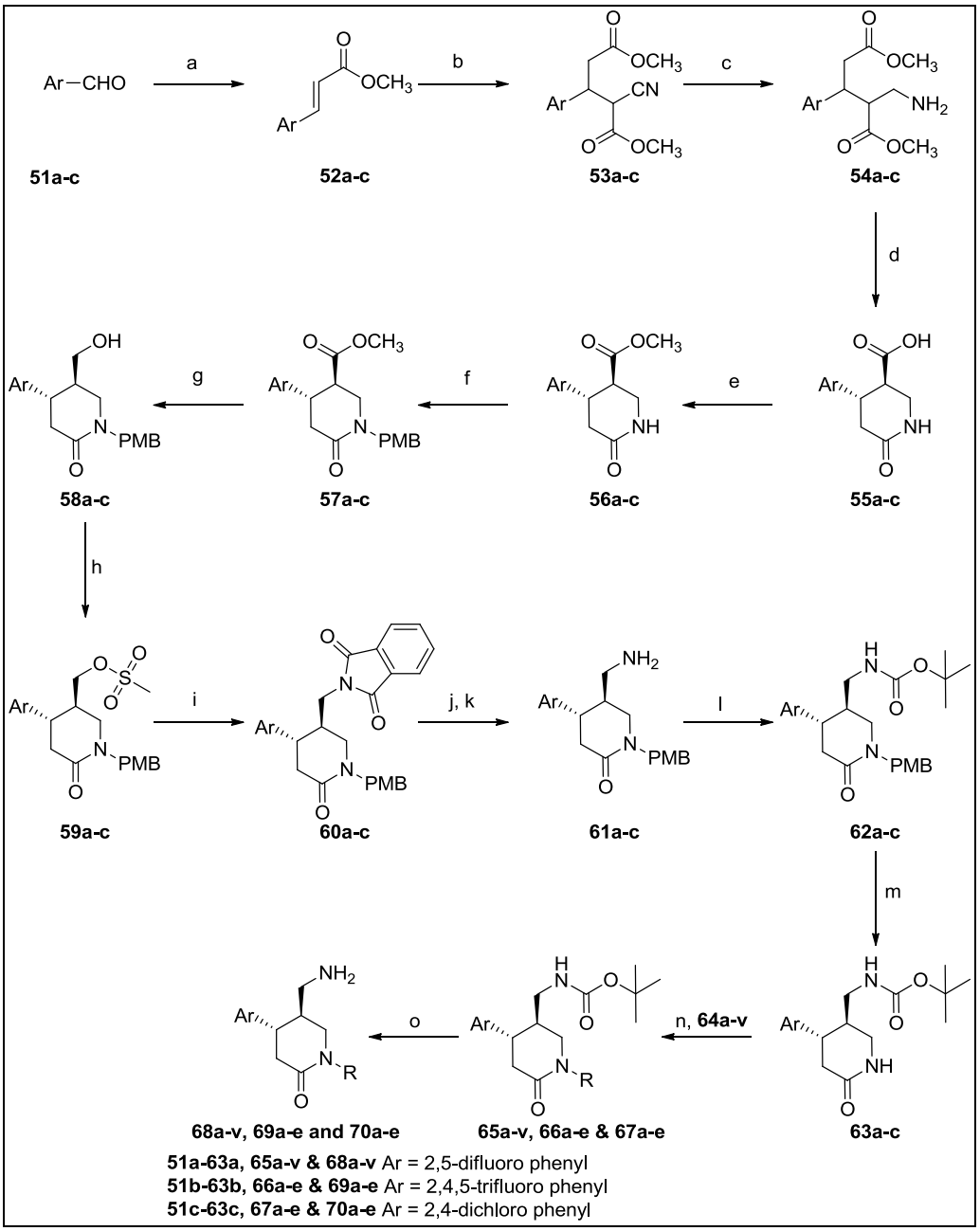
Scheme 5. Synthetic methods for the preparation of peptidomimetics **34a-m**

3.2.2. Conclusion

As discussed in designing section, this series was specially designed to overcome CYP activity problem associated with the lead compound **17c** of the first series. Based upon the literature evidence that by reducing chain length CYP activity can be modulated, we synthesized compounds **27a-j** by doing suitable changes in lead molecule **17c**. Here we identified compound **27j** as the most potent compound. Further to improve DPP-IV inhibitory activity, suitable changes were carried out in benzamide ring as well as in cyano pyrrolidine ring and total thirteen compounds **34a-m** were prepared. Here we identified peptidomimetic based cyanopyrrolidines derivatives **34l** and **34m** as potent and selective inhibitors of DPP-IV and devoid of CYP liabilities. Novel peptidomimetics **34l** and **34m** showed excellent *in vitro* potency and selectivity over other serine proteases, due to their favorable orientations across all the three binding sites. Thus we successfully overcome the CYP inhibition problem arise with the lead compound **17c** of the first series by modifying it to novel peptidomimetic **34m** with no CYP inhibition up to 100 mM concentrations.

3.3.1. Aminomethylpiperidone based DPP-IV inhibitors (Third series)

Using above synthetic route (**Scheme 6**), thirty two compounds **68a-v**, **69a-e** and **70a-e** were prepared. Compounds were purified whenever required and characterized using spectroscopic analysis. Compounds **68a-v**, **69a-e** and **70a-e** were evaluated for their *in vitro* DPP-IV inhibitory activity and selectivity using enzymatic assay (details experimental protocol is given in **experimental Section 5.2.1.**). Based on these results few compounds were selected and evaluated for their *in vivo* antidiabetic activity and pharmacokinetic parameters.



Reagents and conditions: (a) $(Et_2O)_2POCH_2COOMe$, Na_2CO_3, EtOH (b) $NCCH_2COOMe$, NaOMe, MeOH (c) H_2, PtO_2, HCl, MeOH (d) K_2CO_3, Toluene/MeOH (e) Me_3SiCHN_2, Et_2O/MeOH (f) PMB-Br, NaHMDS, THF/DMF(4:1), -78°C (g) $LiAlH_4$, THF, 0°C (h) CH_3SO_2Cl, NEt_3, DCM, 0°C (i) Pottassium phthalimide, DMF, 90°C (j) NH_2-NH_2, EtOH, 25°C (k) Chiral resolution: D-tartaric acid, MeOH. (l) Boc_2O, NEt_3, THF/H_2O(3:2), 25°C (m) CAN, CH_3CN/H_2O(3:1), 25°C (n) R-X (**64a-v**), CuI, K_2CO_3/K_3PO_4, N,N'-dimethylethylenediamine, Toluene, Reflux *or* R-X (**64a-v**), NaH, DMF, 0°C-25°C (o) Conc. HCl/EtOAc(1:3), -50°C, 2h, 0°C, 1h.

Scheme 6. Synthetic methods for the preparation of aminomethylpiperidones **68a-v**, **69a-e** and **70a-e**

3.3.2. Conclusion

Considering all structural modification done by Merck and extending the scope of novelty with rationale, compounds (**68a-v**, **69a-e** and **70a-e)** were designed based on the piperidone skeleton and anticipated that the aminomethyl and the amide groups of the piperidone ring may contribute improved pharmacokinetic and pharmacodynamic effects, along with the potent and selective DPP-IV inhibitory activity. Upon exploration of our hypothesis we identified lead compound **68v**, which demonstrated added advantages over currently practiced gliptins and appears to serve as long-acting DPP-IV inhibitors. Thus we discover novel aminomethyl-piperidone derivative **68v** as potent, selective and long acting DPP-IV inhibitors for the treatment of T2DM. The lead compound **68v** showed prolonged suppression of pre-and post-prandial blood glucose levels (*in vivo*), which correlates with its extended PK profile.

CHAPTER IV (Overall summary)

4.1 Overall summary of current investigation

In the present investigation altogether three series of DPP-IV inhibitors were designed. In the first series, cyanopyrrolidine containing peptidomimetic based DPP-IV inhibitors, total thirty compounds were prepared. In the second series, peptidomimetic based DPP-IV inhibitors, devoid of CYP liabilities, total twenty three compounds were prepared. In the third series, aminomethylpiperidone based DPP-IV inhibitors, total thirty two compounds were prepared. Altogether eighty five compounds were synthesized, purified, characterized and subjected for *in vitro* DPP-IV inhibitory activity. The most potent selected DPP-IV inhibitors from each series were further subjected for the *in vitro* selectivity over other serine proteases (especially over DPP-2, DPP-8 and DPP-9). From each series, the most potent and selective compounds were subjected for the *in vivo* antidiabetic activity followed by PK studies. Compounds of all the three series were found to be potent and selective DPP-IV inhibitors.

In the first series, pyrrolidine carboxamide compounds **11e**, **11f**, **12e**, **12f**, **16e** and **16f** (*para*-nitrile/ trifluoromethyl benzamide) were identified as primary lead compounds. These lead compounds were transformed to their respective nitrile derivatives to give potent DPP-IV inhibitors **17a**-**d** and **18a**-**b**. Among these cyanopyrrolidene based peptidomimetics, compounds **17c** and **17d** showed excellent

DPP-IV inhibition (*in vitro*) along with selectivity over other related serine proteases. Therefore **17c** and **17d** was considered as optimize lead for this series.

Results of *in vitro* DPP-IV inhibitory activity, *in vivo* pharmacodynamic study and molecular docking studies of **17c** and **17d** clearly demonstrated that the potency of cyanopyrrolidine containing peptidomimetic based DPP-IV inhibitors can be modulated by introducing nitrile group (-CN) at the C2 carbon of the pyrrolidine ring, which binds to the S1 pocket of the DPP-IV enzyme. Further selectivity can be modulated by introducing -CN or -CF_3 group at *para* position of the benzamide ring which binds to S3 site of the DPP-IV enzyme. Furthermore, it was observed that introduction of suitable spacer (i.e. GABA: γ-amino butyric acid), which links the S1 and S3 pocket binding component of the ligand, contributed significantly towards improvement in the *in vivo* DPP-IV inhibitory activity, which could be correlated with its improved oral bioavailability. The pharmacodynamic study of **17c** demonstrated excellent *in vitro* DPP-IV inhibitory activity and >15,000 fold selectivity against related enzymes with sustained suppression of pre- and post-prandial blood glucose levels (*in vivo*). In PK studies, compound **17c** showed higher oral bioavailability with extended $T_{1/2}$, indicating that compound **17c** can be considered as the promising candidate for effective treatment of T2DM and need to subject for further pre-clinical evaluation.

As discussed earlier in designing section second series was planned to overcome CYP activity associated with the lead compound **17c** of the first series. In this regard initial attempts were made to reduce CYP activity, for which based upon the literature evidence we introduced suitable spacers (substituted α-amino acid) of reduced chain length for linking cyanopyrrolidine (ring A) with *para*-cyanobenzoic acid (ring B) of the lead molecule **17c** of the first series. Compound **27j** was identified as primary hit from this series. Further to improve DPP-IV potency of **27j** changes were done in *para*-cyanobenzamide ring, which lead to give potent compounds **34d** and **34i**. The high potency of compounds **34d** and **34i** could be because, introduction of sterically less bulky halo atoms (i.e. –F/-Cl) specifically at meta position of the benzamide ring might be best fitting in S3 site and imparts tight binding of the molecule with DPP-IV enzyme in all the three binding sites. Again based upon the literature presidencies **[38-39]**, we made change in pyrrolidine ring system by introducing *cis*-4 fluoro substituent and thereby we identified compounds **34l** and **34m** as the most potent compounds of the series. Lead compound **34m** showed in vitro DPP-IV inhibition equivalent to lead compound **17c** of

the first series with >15,000 fold selectivity over other related serine protease (DPP2, DPP8 and DPP9) and showed no CYP inhibition up to 100mM concentration.

In the third series, modification of the lead compound **VII** developed by Merck Sharp & Dohme Corp. was carried out by enhancing the spatial position of $–NH_2$ group by introducing methylene group (i.e. amino methyl group) and rupturing tricyclic ring from the 5-membered imidazole ring to get flexible structure keeping other component intact. We anticipated that the presence and the position of the primary amine might be critical for the inhibitory activity. By making this suitable scaffold changes and incorporating widely used halo-aromatics and halo-heterocycle for the development of DPP-IV inhibitor, we identified progressive lead compound **68v**.

Among all the series, compound **68v** from the third series turn out as the best compound in terms of preclinical profiling. Compound **68v** showed extended $T_{1/2}$ of ~9 h and bioavailability of ~80% with prolong suppression of serum glucose levels ~20% up to 24h, which reports discovery of compound **68v**, a novel aminomethyl-piperidone derivative as potent, selective and long acting DPP-IV inhibitor for the treatment of T2DM.

CHAPTER V (Experimental)

5.1 Chemistry

5.1.1. Materials and Methods

All the reagents used for the synthesis were purchased from Sigma Aldrich Company Limited, Dorset and were used without further purification. Solvents were procured from commercial source and used after distilling or drying according to the known methods. All the air and/or moisture sensitive reactions were carried out in dry solvents under nitrogen atmosphere. Melting points were recorded on open glass capillaries, using scientific melting point apparatus and are uncorrected.

The ^1H NMR spectra were recorded on a Bruker Avance-400 (400 MHz) spectrometer. The chemical shifts (δ) are reported in parts per million (ppm) relative to TMS either in CD_3OD, DMSO-d_6 or $CDCl_3$. Signal multiplicities are represented by s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), bs (broad singlet), and m (multiplet). ^{13}C NMR spectra were recorded on Bruker Avance-400 at 100 MHz either in $CDCl_3$ or Acetone-d_6. Mass spectra (ESI-MS) were obtained on Shimadzu

LCMS 2010-A spectrometer. Elemental analyses were carried out using a Perkin-Elmer 2400 CHN analyzer.

HPLC analyses were carried out at λ_{max} 220 nm using column. Progress of the reactions was monitored by TLC using precoated TLC plates (E. Merck Kieselgel 60 F254) and the spots were visualized by UV and/or iodine vapors. The chromatographic purification was performed on silica gel (200-400 mesh). Few compounds directly used for next step without purification and analysis. Detailed synthetic procedures and characterization data of all the final compounds and intermediates are described in next section.

5.2. Biology

5.2.1. DPP-IV inhibitory activity and selectivity over other serine protease (*in vitro*)

Enzyme activity was determined by a fluorescence-based assay, adapted from the work of Blackmon et al **[48]**. H-Glycine-Proline-7-amino-4-methyl coumarin (Gly-Pro-AMC, 200 µM) (Bachem, PA) was used as substrate (which is cleaved by the enzyme to release the fluorescent aminomethylcoumarin (AMC)), and soluble human protein (DPP IV enzyme) produced in a baculovirus expression system (Bac-To-Bac; Life Technologies), was used as the enzyme source. H-Gly-Pro-AMC (200 µM) was incubated with DPP-IV enzyme in the presence of various concentrations of test compounds. Reaction was carried out at pH 7.8 (HEPES buffer 25 mM containing 1.0% BSA, 140 mM NaCl, 16 mM $MgCl_2$, 2.8% DMSO) in a total volume of 100 µl at 25°C for 30 min., in the dark. Reaction was terminated with acetic acid (25 µl of 25% solution). Activity (fluorescence) was measured (expressed as fluorescent units (FU)) in a Spectra Max fluorometer (Molecular Devices, Sunnyvale CA) by exciting at 380 nm, and measuring emission at 460 nm. IC_{50} values were determined for test compounds using Graph Pad prism software.

DPP8.

Compounds were tested against human DPP8 (baculovirus) in a continuous fluorescent assay in 50 mmol/l sodium phosphate buffer, pH 8.0, and 0.1 mg/ml BSA, using Ala-Pro-7-amino-4-trifluormethylcoumarin as substrate at 100 µmol/l at 37°C for 15 min (excitation/emission: 400/505 nm).

DPP9.

Compounds were tested against human DPP9 (baculovirus) in a continuous fluorescent

assay in 100 mmol/l Tris/HCl buffer, pH 7.4, and 0.1 mg/ml BSA, using Gly-Pro-AMC as substrate at 100 µmol/l at 37°C for 30 min (excitation/emission: 360/460 nm).

QPP/DPP2.

Compounds were tested against human QPP (baculovirus) in a continuous fluorescent assay in 100 mmol/l cacodylate buffer, pH 5.5, and 0.1 mg/ml BSA, using Nle-Pro-AMC as substrate at 5 µmol/l at 37°C for 15 min (excitation/emission: 360/460 nm).

Data analysis:

To measure the inhibition constants, serial dilutions of inhibitor were added to reactions containing enzyme and substrate. IC_{50} values were determined by a fit of the reaction rates to a three-parameter Hill equation by nonlinear regression. The data are reported as percentage inhibition calculated as follows: %Inhibition = 100 (1 – (V_t/V_c)), where V_t is the rate of reaction of treated sample and V_c is the rate of reaction of control sample.

5.2.2. CYP inhibition study (*in vitro*)

For CYP1A2, CYP2C8, CYP2C9, CYP2D6, CYP2C19 and CYP3A4 inhibition studies, Human liver microsomes (0.2 mg/ml), Testosterone (50 µM) / Dextromethorphan (5 µM) respectively, as probe substrates, potassium phosphate buffer (0.1 M; pH 7.4) and NADPH (1mM) were incubated with different concentrations of test compounds (@1, 10 and 100 µM concentrations) at 37^{o}C for 10 min., enzyme activity (% of control) was determined and IC_{50} values were calculated **[49]**.

5.2.3. Pharmacodinemic study (Antidiabetic activity) (*in vivo*)

The *in- vivo* glucose lowering properties of some of the test compounds and standards were evaluated in db/db animal models as described below. Study was conducted in male C57BL/6J (using IPGTT protocol) or without glucose load, in db/db mice (age 8-12weeks). All animal experiments were conducted according to the internationally valid guidelines following approval by the 'Zydus Research Center Animal Ethical Committee'. Two days prior to the study, the animals were randomized and divided into 2 groups (n = 6), based upon their fed glucose levels. Animals were left for 2 days under acclimatization and maintained on a standard diet. On the day of experiment, food was withdrawn from all the cages, water was given *ad-libitum* and were kept for overnight fasting. Briefly, in IPGTT protocol (C57 mice) overnight fasted mice were dosed orally (p.o.) with the test compounds (x mg/kg), 0.5 h prior to the intraperitoneal (i.p.) glucose load (1.5 g/kg), while in db/db mice, fed mice were dosed orally (p.o.) with the test compounds (x mg/kg) and the blood samples were collected at various time

points. Blood samples were centrifuged and the separated serum was immediately subjected for the glucose estimation. The glucose estimation was carried out with DPEC-GOD/POD method (Ranbaxy Fine Chemicals Limited, Diagnostic division, India), using Spectramax-190, in 96-microwell plate reader (Molecular devices Corporation, Sunnyvale, California). Mean values of duplicate samples were calculated using Microsoft excel and the Graph Pad Prism software (Ver 4.0) was used to plot an area under the curve (0-240 min AUC). The AUC obtained from graphs were analyzed for two-way ANOVA, followed by Bonferroni post test, using Graph Pad prism software **[50-51]**.

5.3. Pharmacokinetic study (PK study)

Briefly, for single dose PK study, test compounds were administered orally / iv on a body weight basis (x mg/kg) to overnight fasted male C57BL/6J mice. Serial blood samples were collected in micro centrifuge tubes containing EDTA at pre-dose, 0.15, 0.3, 0.5, 0.75, 1, 2, 4, 6, 8, 24, 36 and 48h post-dose after compounds administration. Approximately 0.2 ml of blood was collected at each time point and centrifuged at 4 °C. The obtained plasma was frozen, stored at -70 °C and the concentrations of compounds in plasma were determined by the LC-MS/MS (Shimadzu LC10AD, USA), using YMC hydrosphere C18 (2.0 x 50 mm, 3 μm) column (YMC Inc., USA). The pharmacokinetic parameters, such as Tmax, $t_{1/2}$, Cmax, AUC and %F were calculated using a non-compartmental model of WinNonlin software version 5.2.1.

5.4. Docking study

The molecular docking analysis of potent compounds from all the three series and standard compounds was carried out using extra precision (XP) Glide docking method, to understand its critical interactions with all the three binding sites (S1, S2 and S3) of DPP-IV enzyme. The crystal structure of the DPP-IV enzyme (PDB ID: 2I03/ 2AJL/2OQI) was obtained from the protein data bank and the protein structure was prepared using protein preparation wizard module of Schrödinger. After protein structure was prepared, the bound ligand of receptor was defined as grid binding box.

For docking experiments all the compounds were geometry optimized using the Optimized Potentials for Liquid Simulations-all atom (OPLS-AA) force field with the steepest descent followed by a truncated Newton conjugate gradient protocol as implemented in Macro model. DPP-IV was optimized for docking using the protein

preparation wizard provided by Schrodinger LLC **[52]**. Partial atomic charges for compounds as well as protein were assigned according to the OPLS-AA force field. The extra precision (XP) Glide docking method was then used to dock all compounds into the catalytic site of DPP-IV **[53]**. Grids for Glide docking were calculated using the bound inhibitor as the reference of catalytic site in the DPP-IV. Upon completion of each docking calculation, 50 poses per ligand were allowed to generate. The top-scored pose was chosen using a Glidescore (Gscore) function. Another scoring function used by Glide is Emodel, which itself is derived from a combination of the G-score, Coulombic, van der Waals, and the strain energy of the ligand. The docking method was further validated by docking NVP-DPP728 and the binding mode was found similar as reported earlier.

6. References

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7. Spectral data

Scanned copies of Spectra of selected compounds are included in this section.

8. Publication

List of Publications from the PhD work

1. **Pradip Jadav**, Rajesh Bahekar, Shailesh R. Shah, Dipam Patel, Amit Joharapurkar, Samadhan Kshirsagar, Mukul Jain, Mubeen Shaikh, Kalapatapu V. V. M. Sairam. "Long-acting peptidomimetics based DPP-IV inhibitors" *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3516-3521.

2. **Pradip Jadav**, Rajesh Bahekar, Shailesh R. Shah, Dipam Patel, Amit Joharapurkar, Kiran Shah, Shruti Bhardwaj, Kishan Patel, Kaushil Patel, Rajendra Chopade, Mubeen Shaikh, Kalapatapu V. V. M. Sairam and Mukul Jain. "Design of Peptidomimetics Based DPP-IV Inhibitors, Devoid of CYP liabilities" *Letters in Drug Design & Discovery* **2012**, *9*, 867-873.

3. **Pradip Jadav**, Rajesh Bahekar, Shailesh R. Shah, Dipam Patel, Amit Joharapurkar, Mukul Jain, Kalapatapu V. V. M. Sairam and Praveen Kumar Singh. "Design, Synthesis and Biological Evaluation of Novel Aminomethyl-piperidones based DPP-IV Inhibitors" *Bioorg. Med. Chem. Lett.* **2014**, *24*, 1918-1922.