Chapter II:

Designing Strategy Of DPP-IV inhibitors

"An investment in knowledge pays the best interest."

-Benjamin Franklin

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 **[125,134]**. 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) and SAR study of DPP-IV inhibitors, which are in clinic or in clinical development **[221-224]**.

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 **[32,36,42,46,49,50]**.

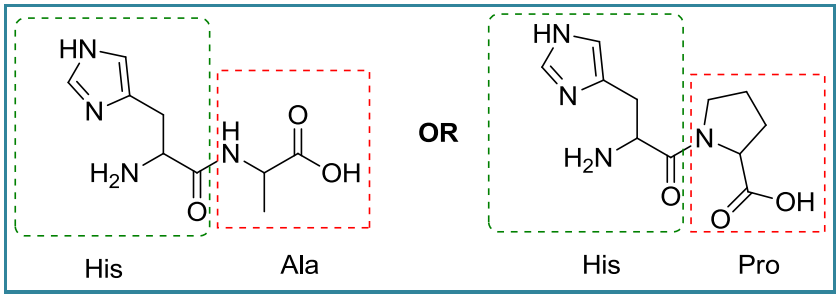


Figure 16: Structure of dipeptide substrate recognized by DPP IV enzyme

Earlier several attempts have been made to develop dipeptide based DPP-IV inhibitors **[203,225-227]**. 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 17**). To overcome ring cyclisation problem under basic condition, sterically hindered bulky substitution were introduced in new class of DPP-IV inhibitors.

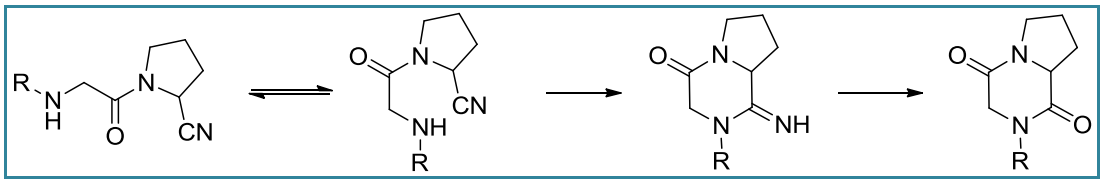


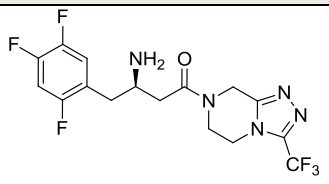
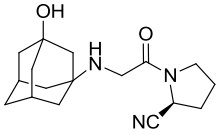
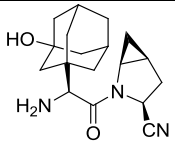
Figure 17: Cyclic amide or diketopiperazine formation

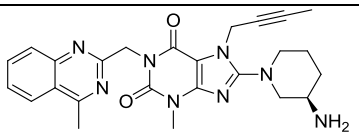
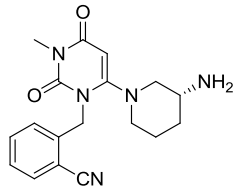
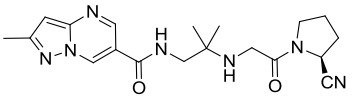
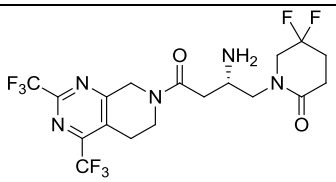
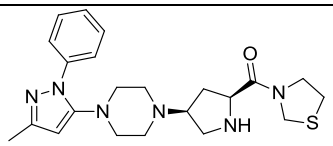
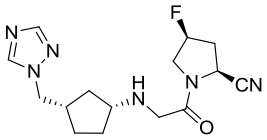
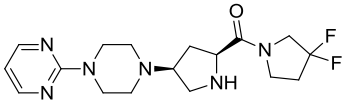
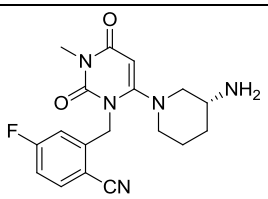
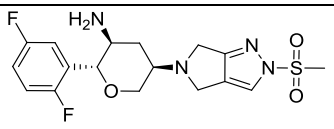
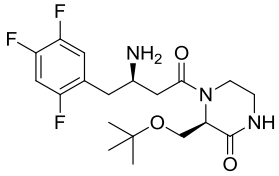
Among the DPP-IV inhibitors launched or in active development (**Table 6**), vildagliptin, saxagliptin, anagliptin, teneligliptin, melogliptin and gosogliptin are peptide mimetic compounds, which have been discovered by replacing segments of peptide-based substrates **[228]**. All these compounds contains pyrrolidine ring system bearing either –CN and/or –F substituent at specific positions on the basis of SAR **[229-230]**. 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 **[228]**. Therefore, their chemical structures are diverse, suggesting that each of their binding modes in DPP-IV would be unique **[231]**. 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 **[232]**.

Table 6. DPP-IV inhibitors in clinic and in active development.

Sr. No.	Name	Structure	Company	Clinical status	Ref.
1	Sitagliptin (MK-0431)		Merck	Launched	**[31]**
2	Vidagliptin (LAF-237)		Novartis	Launched	**[32]**
3	Saxagliptin (BMS-477118)		Astra-zeneca / BMS	Launched	**[36]**

4	Linagliptin (BI-1356)		Boehringer Ingelheim / Eli Lilly	Launched	**[39]**
5	Alogliptin (SYR-322)		Takeda	Launched	**[41]**
6	Anagliptin (SK-0403)		Kowa JW Pharma	Launched	**[42]**
7	Gemigliptin (LC15-0444)		LG Life Sciences	Launched	**[44]**
8	Teneligliptin (MP-513)		Mitsubishi Tanabe	Launched	**[46]**
9	Melogliptin (GRC-8200)		Glenmark	Phase II	**[49]**
10	Gosogliptin (PF-734200)		SatRx	Phase II	**[50]**
11	Trelagliptin (SYR-472)		Takeda / Furiex	Phase III	**[53]**
12	Omarigliptin (MK-3102)		Merck	Phase III	**[56]**
13	Evogliptin (DA-1229)		Dong-A Pharma	Phase II	**[57]**

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 **[204]**.

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) **[233]**. 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 **[234]**.

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 18**).

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.

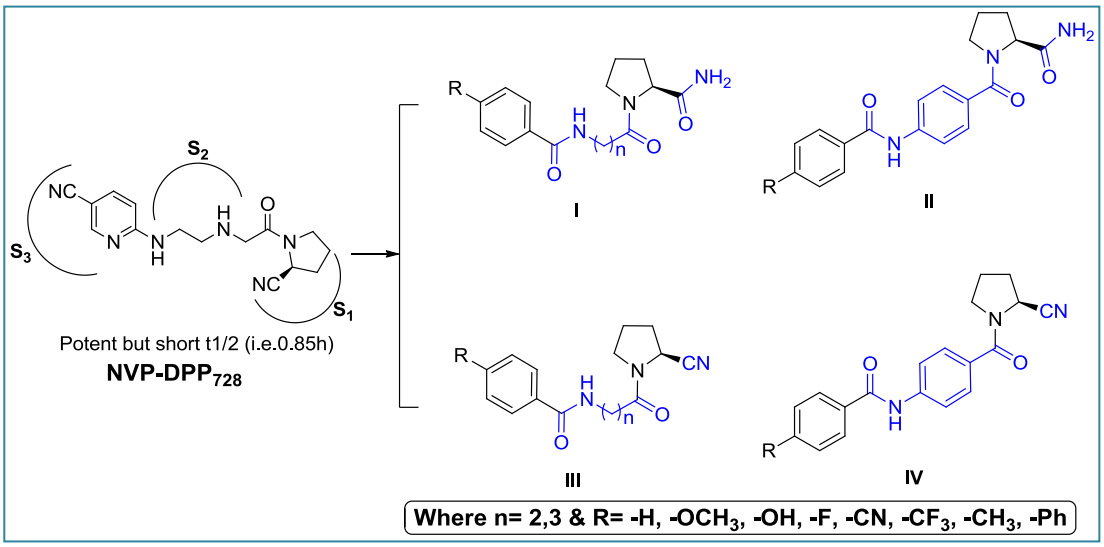


Figure 18: Design strategy of cyanopyrrolidine containing peptidomimetic based DPP-IV inhibitors.

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 **[235]**. 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 19**). 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 **[229-230]**.

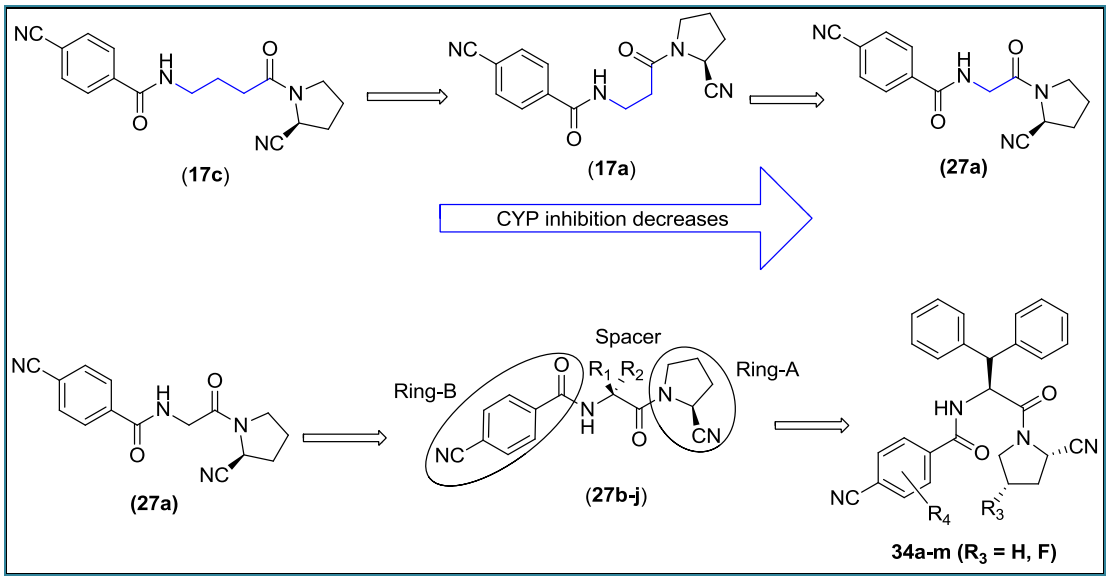


Figure 19: Design strategy of peptidomimetic based DPP-IV inhibitors, devoid of CYP liabilities.

R_1 and R_2 together represent substituted α-amino acids with absolute (S) stereo configuration (**Figure 20**). Substituent R_4 in ring-B together represent substituted 4-Cyano benzoic acids selected from the group given in **Figure 21**.

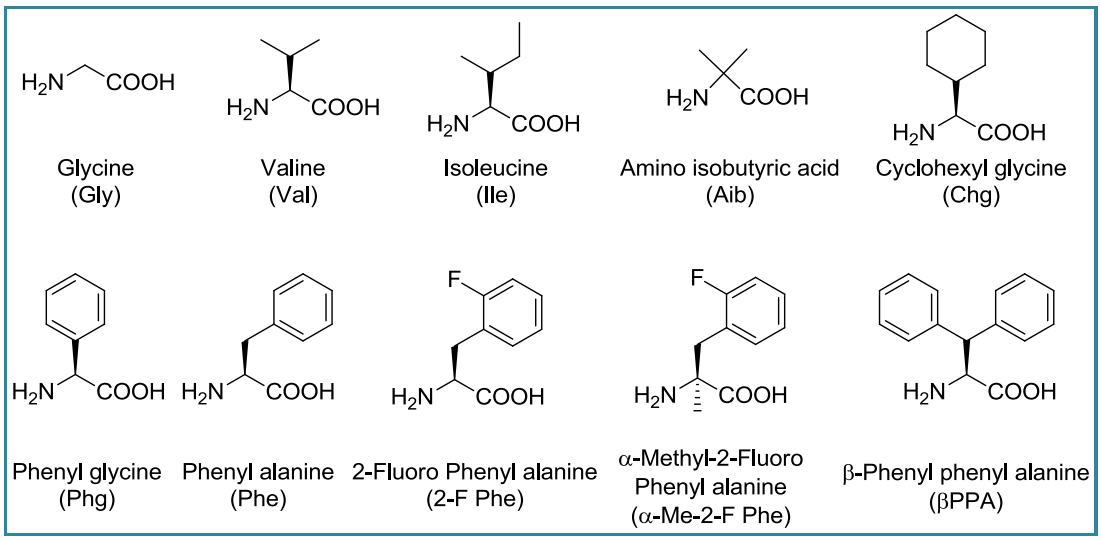


Figure 20: Structures of α-amino acids used as a linker.

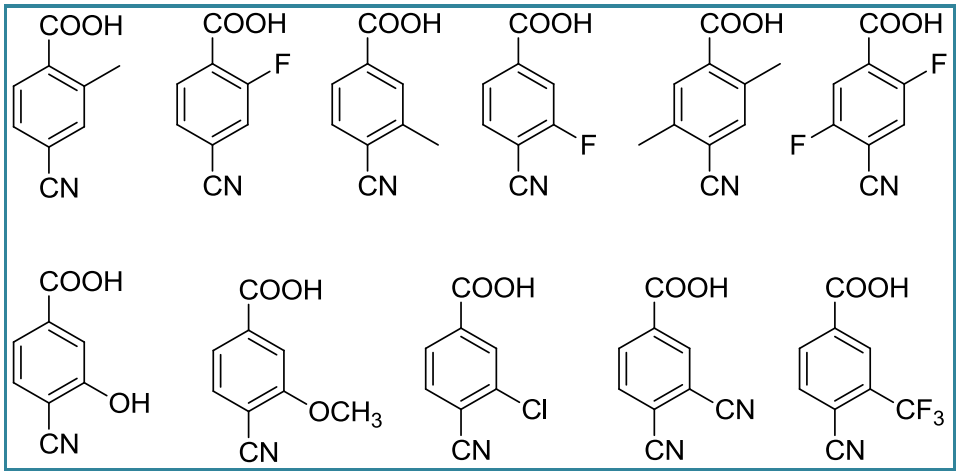


Figure 21: Structures of substituted 4-Cyano benzoic acids

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 22**).

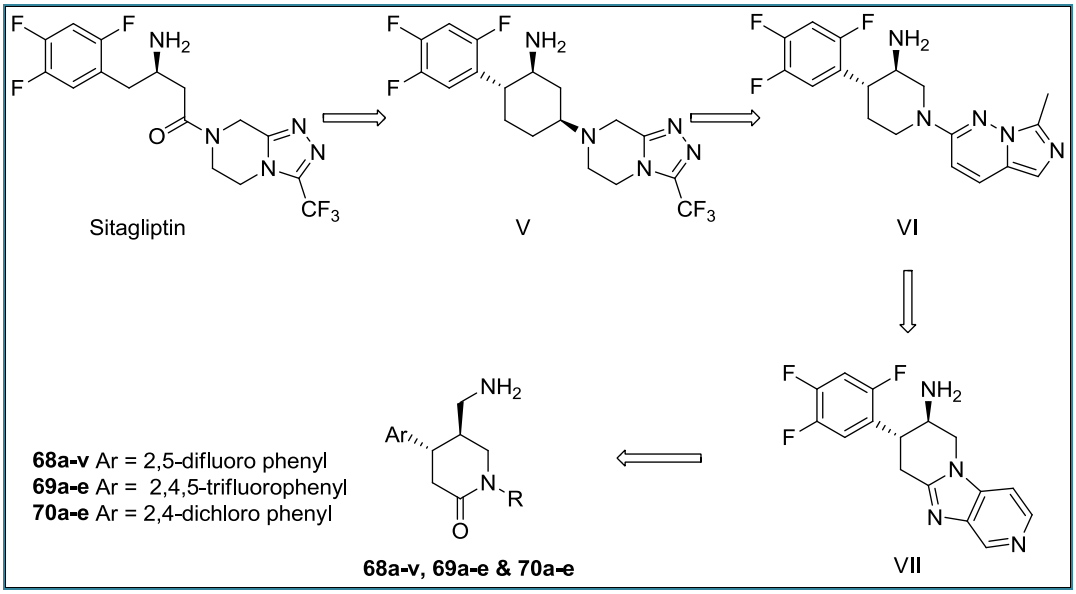


Figure 22: Design strategy of aminomethylpiperidone based DPP-IV inhibitors.

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 **[195,209-210,236]**. 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.

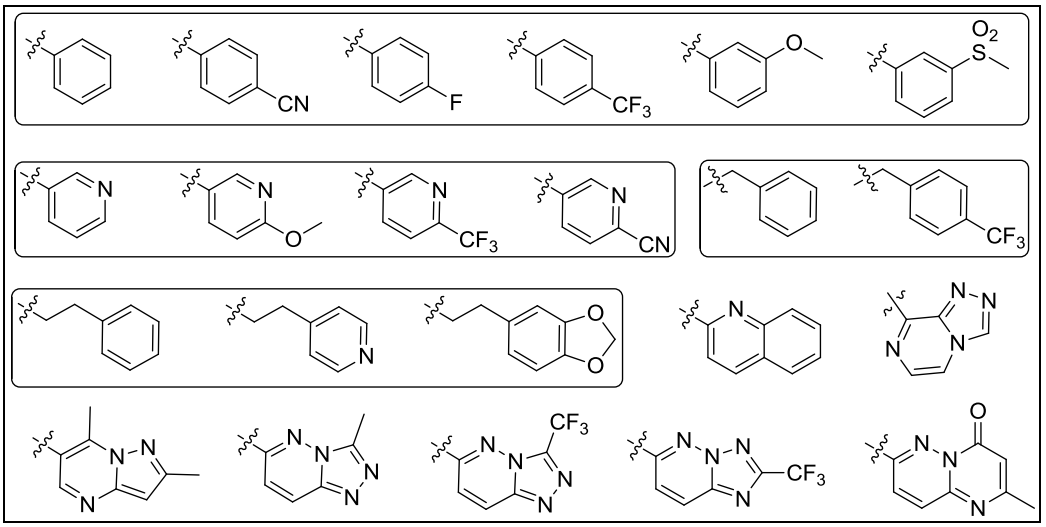


Figure 23: Substituents used for synthesis of aminomethylpiperidone based DPP-IV inhibitors.

Substituent R in novel aminomethylpiperidones **68a-v**, **69a-e** and **70a-e** was selected from the given group (**Figure 23**).

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.

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