Chapter IV:

Overall summary

and

Future prospects

"The scientist is not a person who gives the right answers, he's one who asks the right questions." — Claude Lévi-Strauss

4. Overall Summary and Future Plan

4.1 Overall summary of current investigation

T2DM is a complex multifactorial disease affecting the length and quality of life of diabetic patients. T2DM is characterized by hyperglycemia, insulin resistance and/or impaired insulin secretion. T2DM remains a leading cause of cardiovascular disorders, blindness, end-stage renal failure, amputations and hospitalizations. The economic burden for healthcare systems is rising sharply, owing to the costs associated with the treatment of diabetes and its complications. Treatment of patients with T2DM strives for normalization of hyperglycemia by a combination of medical nutrition therapy, physical activity and pharmacological agents. Patient education and empowerment are essential components of therapy **[281]**. Most patients require continuous treatment in order to maintain normal or near-normal glycemia. T2DM is a progressive disorder accompanied by deterioration in β cell function and insulin resistance. Despite this fact, there is now clear evidence that tight control of blood glucose significantly reduces the risk of complications of diabetes.

The DPP-IV inhibitors are well known oral hypoglycemic drugs which have been in clinical use for the past 8 years. The DPP-IV inhibitors are safe, weight neutral and widely prescribed. There are currently eight gliptins registered worldwide with several more in advanced stages of development. However no gliptin is there in market for long term treatment of T2DM. Further, In vivo evidence in experimental animal models and in initial clinical trials has demonstrated that DPP IV inhibitors have therapeutic potential in the long term chronic treatment of T2DM, delaying disease progression and decreasing the circulating levels of glycated hemoglobin (HbA1c), There are two gliptins in advanced stages of clinical development which can be registered for once-weekly dosing regimen.

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 in 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. 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, by introducing suitable spacers (substituted α-amino acid) of reduced chain length to link cyanopyrrolidine (ring A) with *para*-cyanobenzoic acid (ring B) of the lead molecule (**17c** of the first series) **[235]**. 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 potent

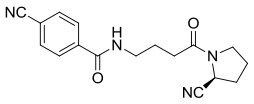
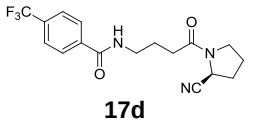
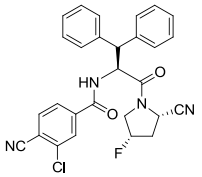
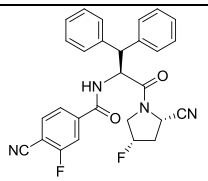
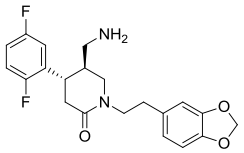
compounds **34d** and **34i**. The high potency of compounds **34d** and **34i** could be because, introduction of sterically less bulky halo atoms (i.e. –F/-Cl) specifically at meta position of the benzamide ring (might be best fit in S3 site and imparts tight binding of the molecule with DPP-IV enzyme in all the three binding sites). Again based upon the literature presidencies **[229-230]**, 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 from this 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 100µM 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, we identified progressive lead compound **68v** in this series.

Compound **68v** showed *in vitro* DPP-IV inhibitory activity 2-fold more than Sitagliptin and >5000 fold selectivity over DPP2, DPP8 and DPP9 enzyme. Further PD study of compound **68v** revels prolonged suppression of pre-and post-prandial blood glucose levels (*in vivo*), which correlates with its extended PK profile.

Shortlisted lead compounds from all the three series are listed in **Table 14**. 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.

Table. 14 Short listed lead compounds from all the three series.

Series	Lead molecule	*In vitro* DPP-IV (IC_{50} nM)	*In vitro* fold selectivity			*In vivo* IPGTT (OGTT) %Glucose reduction	PK $T_{1/2}$ (h) (%F)
			DPP2	DPP8	DPP9		
1	 **17c**	2.3±0.9	>25,000	>15,000	>15,000	54.9±3.86 (33.5±7.4) at 20mpk	7.99±0.3 (72.5%)
	 **17d**	3.8±0.5	>25,000	>15,000	>15,000	17.4±5.35 at 20 mpk	0.99±0.1 (63.1%)
2	 **34l**	4.2±0.7	>25,000	>15,000	>15,000	NA	NA
	 **34m**	2.7±0.3	>25,000	>15,000	>15,000	NA	NA
3	 **68v**	8.5±0.4	>5000	>10,000	>10,000	(38.9±5.2) at 3 mpk	8.99±0.3 (79.5%)

4.2 Future Plan

From the third series, compound **68v** showed excellent DPP-IV inhibitory activity (*in vitro*) & antidiabetic activity (*in vivo*). The PK profile of **68v** was found to be satisfactory to represent it as a promising long acting DPP-IV inhibitor to work on. Future work includes some additional safety study and pre-clinical studies before it has been subjected for clinical development. Compound **68v** should be subjected for chronic efficacy studies and for long term toxicological evaluation, along with its PK profile in higher animals such as dog or monkey.

Furthermore, during the development of compound **68v**, it has been observed that compounds of this series accommodate more flexibility with the halo aromatics which

binds in a far region of DPP-IV enzyme S3 site. Further modification can be accomplished by incorporating alicyclic ring systems that can mimic the size of the acyclic part in halo-aromatic of **68v**. This new structural aspect may lead to change in molecular conformation analogues to **68v** but with more effective binding interaction with the enzyme. So by incorporating heterocycles which have been used widely for the development of DPP-IV inhibitors, in the form fused with alicyclic ring systems expands further scope for new series development. Main focus of developing new series will be towards improving the PK profile to get batter DPP-IV inhibitor that can turn as a once a week regimen (**Figure 37**).

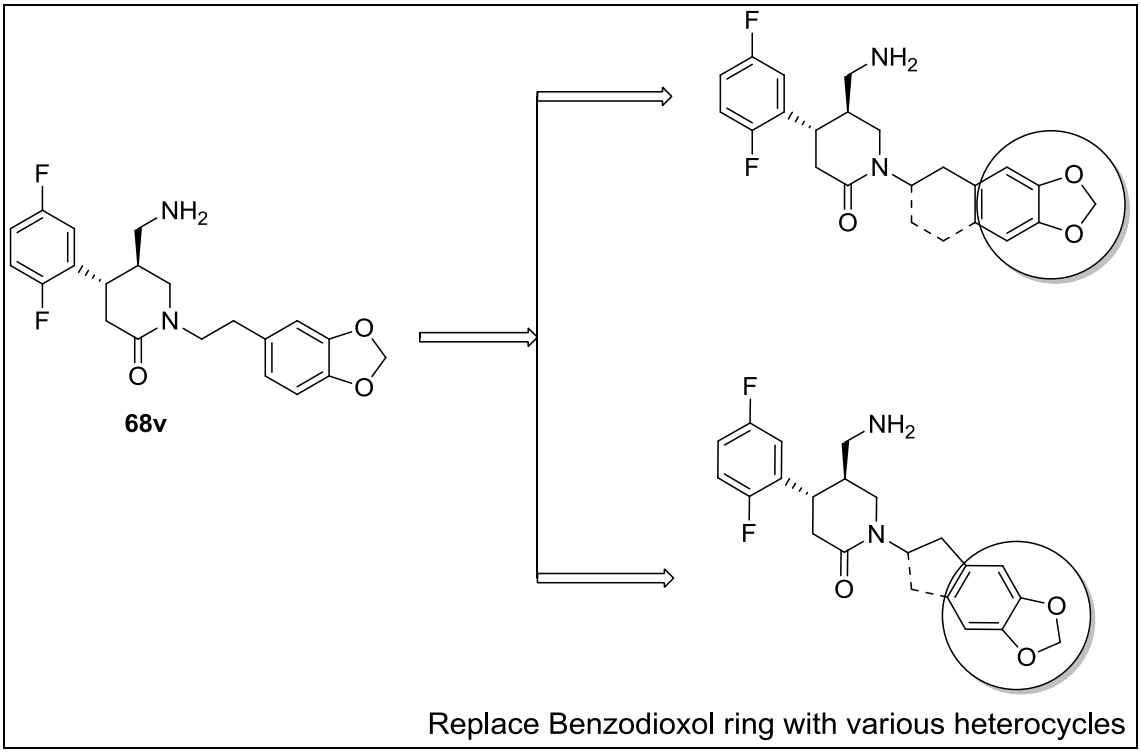


Figure 37: New series development based on lead molecule **68v**