6.1 Summary

Treatment for T2DM is very complex. Almost all patients need multiple medications at a time to meet minimum required glycaemic control. So, newer and better effective therapies are in demand than currently available conventional drugs. DPP-4 has been proved as well established and promising target for T2DM since last decade. Many diverse molecules with various P1 and P2 heterocycles under both peptidomimetic and non-peptidomimetic series have been reported for DPP-4 inhibition and still research is going on for further better and novel molecules. Their common SAR suggests that binding into the proline mimic S1 pocket is not restricted only with substrate like pyrrolidine fragment. Phenyl/Substituted phenyl or similar P1 fragment can also occupy the proline pocket of the enzyme to give novel and potent non-peptidomimetic inhibitors. The amino group in P2 fragment is essential for activity. The S2 pocket is bigger in size compared to S1 pocket, so extended P2 fragments increases the overall affinity. These structurally diverse DPP-4 inhibitors act through common pathway. They increase half-life of incretin hormone, GLP-1 and prolong its actions. They efficiently lower the elevated blood sugar level through increased insulin release and reduce HbA1c level up to 1%. Also, they are safer drugs with minor side effects due to the residual inhibition of DASH proteins, CYP enzymes and hERG channel. On the basis of these notions, research has to be focused on the discovery of potent, selective, safe and long acting DPP-4 inhibitors. To develop such more novel longer acting molecules, new hits or leads with novel heterocyclic scaffold are required which can be further modified/optimized to desired candidate. Although many fused heterocycles with multiples nitrogens in the ring have been reported so far but triazolotriazine scaffold has not been explored for DPP-4 inhibition till date so we considered as novel heterocycle for designing a new hit. With this rationale, a new series of triazines derivatives have been designed using CADD tools, pharmacophore modelling and 3D-QSAR. Based on the docking scores, in-silico ADMET predictions and synthetic feasibilities, total 17 molecules were actually synthesized in good yield and characterized using spectroscopic techniques like FT-IR, Mass (ESI-MS) and NMR. Single crystal X-ray crystallography analysis of one compound was also carried out to support the structure elucidation. Purity of all the compounds were determined using HPLC and found >95%. Results of In-vitro screening of all the compounds in DPP-4 enzyme inhibition assay indicated that all molecules are having very weak inhibitory action against DPP-4 enzyme compared to standard drug, sitagliptin. Compound 5q and 5c gave acceptable 53.3% and 48.3% DPP-4 inhibition, respectively. The other compounds like 5p and 5l gave 30% inhibition. The SAR of synthesized compounds suggested that electron
releasing groups (i.e. –OH, CH$_3$ and OCH$_3$) either at 4$^{th}$ (para), 3$^{rd}$ (meta) or 2$^{nd}$ (ortho) positions of phenyl ring decreases the activity as found in compounds 5a, 5b, 5g, 5i, 5o etc. while electron withdrawing groups (i.e. –Cl, -CN, -NO$_2$) at the same positions increases the activity as found in compounds 5d, 5h, 5j, 5l and with 5c. Also, it has been concluded that compared to substituted phenyl ring as P1 fragment, the fused heterocyclic ring, compound 5q as P1 fragment produced more inhibition of enzyme due to the better fit of ring with more surface area into the S1 pocket compared to phenyl ring. Also its lower logP value (2.04) indicates its improved aqueous solubility compared to other compounds. Compound 5c was also found good and might give interaction with Ser630 of catalytic triad in the S1 pocket and thereby giving better affinity. This cyano substituted phenyl ring represents the same P1 fragment as found in marketed drug, alogliptin. The IC$_{50}$ of compound 5q and 5c was determined in-vitro against DPP-4, DPP-8 and DPP-9. For compound 5q, they were found as 28.05 µM, 230 µM, and 275 µM respectively while for compound 5c, they were found as 166.4 µM, 1176.5 µM and 1923.4 µM respectively which suggested that both compounds are more selective towards DPP-4 compared to DPP-8 and DPP-9. According to the results of in-silico study also, compound 5q was found most active in pharmacophore mapping as well as second highest in docking score. Thus, in-silico designing was proved in line with the actual activity data.

In further study, both compounds were checked for cytotoxicity in normal Vero cell line by in-vitro MTT assay and found safe and non-cytotoxic. During in-vivo OGTT in C57BL/6 mice, compound 5q produced significant reduction in blood glucose levels and glucose-induced blood glucose excursion was significantly inhibited by 19.63%, 33.59% and 47.14%, respectively, in a dose-dependent manner from 5mg/kg, 10 mg/kg and 20 mg/kg dose. Compound 5c was found to produce moderate reduction in blood glucose by 12.01%, 19.21%, and 23.72% at a dose of 5mg/kg, 20 mg/kg and 50 mg/kg respectively. The dose dependent effect was significant and potent in compound 5q than in compound 5c. These results suggested that compound 5q effectively improves the blood glucose tolerance in vivo and the proposed dose of compound 5q can be 20 mg/kg in mice.
6.2 Future Prospects

- Looking into the results of the present research done so far, many further ideas can be explored in future.
- The compound 5q can be further screened *in-vivo* into type II diabetes animal model to check the effect in T2DM.
- Also, before structural optimization, its pharmacokinetic properties can also be explored *in-vivo* to check mainly its %bioavailability, clearance and $T_{1/2}$. These all data will collectively give an idea about the overall efficacy, safety and duration of action of the molecule.
- After careful study of SAR, the preliminary novel hit molecule 5q can be structurally optimized further to occupy the extended S2’ pocket of DPP-4 which increases selectivity against DPP-8/9 as well as DPP-4 binding affinity. Many synthetic modifications can be tried out to increase this occupancy of S2 and S2’ sub sites of DPP-4 binding pocket which can end up into potent, safe, selective and longer acting inhibitor.
- The other alternative synthetic routes can be worked out to synthesize scaffolds 6, 5r and 7.