05. SUMMARY AND CONCLUSION

Diabetes mellitus is one of the most common endocrine disorders affecting almost 6% of the world’s population. The number of diabetic patients will reach 300 million in 2025. More than 97% of these patients will have type II diabetes. The projected increase in the number of diabetic patients will strain the capabilities of healthcare providers the world over. Thus it is of paramount importance to revisit the causes and epidemiology of diabetes mellitus.

Diabetes is always associated with degenerative long-term complications (retinopathies, nephropathies, neuropathies, angiopathies, atherosclerosis and cataracts) that make it one of the leading causes of blindness, renal failure and neuronal pathologies. The increased flux of glucose through the polyol pathway that occurs in hyperglycaemic conditions in tissues possessing insulin-independent glucose transport (nerve, retina, lenses and kidney) is a well-examined factor involved in the onset and progression of such chronic complications. Aldose reductase (EC 1.1.1.21, ALR2) is the first enzyme of the polyol pathway and catalyses the NADPH-dependent reduction of glucose to sorbitol. Dual agonist of Peroxisomal proliferated activated receptor \( \alpha/\gamma \) represents the novel class of agents having a therapeutic application for the treatment of Type 2 diabetes. PPAR\( \gamma \) agonist were reported to exhibit an anti-diabetic effect which enhance the catabolism of glucose while PPAR\( \alpha \) exhibit an anti-hyperlipidemic effect their by control the increase in body weight by increasing the catabolism of lipid.

Many structurally different compounds have been shown to inhibit Aldose Reductase enzyme with various degrees of efficacy and specificity. However, many appear to be promising during \textit{in vitro} studies or in trials with animal models. But often fail to proceed for clinical trial either because of undesirable side effects or as a result of poor efficacy. Only epalrestat is currently marketed one. Literature revealed that a large number of thiazolidinedione derivatives have been reported as potent inhibitors of Aldose Reductase and PPAR \( \alpha/\gamma \) dual agonist without any side effect. But yet, little work has been carried
out on these analogs. Therefore, it was thought worthwhile to rationally design some thiazolidinedione derivatives.

The structural characteristic common to all TZDs is a thiazolidinedione ring, to which divergent molecular moieties are attached. The thiazolidinedione ring is assumed to relate to antihyperglycaemic TZD action. Besides, there is a great interest in 2,4 Thiazolidinediones derivatives as aldose reductase inhibitors. TZDs free from hypersensitivity reactions, which are linked to hydantoin system. On the other hand, it was reported that some 2,4-TZDs have been patented as antihyperglycemic and AR inhibitors. Therefore it is worthwhile to design and develop new TZDs analogs as potent aldose reductase inhibitors with devoid of toxicity and adverse action.

Therefore, it was thought worthwhile to rationally design some selective Aldose reductase inhibitors which could enhance the potency of TZD ring containing dual agonist of PPAR-α and PPAR-γ. The thesis work was planned on the following heads:

1. Literature review of various anti-diabetic molecules.
2. Rational designing of new chemical entities as selective Aldose Reductase inhibitors and dual PPAR α and γ agonist.
3. Selectivity and activity of the designed compound were studied using docking.
4. Synthesis of designed compounds
   - Development of synthetic pathway for the compounds.
   - Synthesis of designed compounds.
   - Purification of synthesized compounds.
5. Characterization of the synthesized compounds.
   - Determination of melting point.
   - Determination of $R_f$ value.
   - Determination of $\lambda_{\text{max}}$.
   - Infra Red spectroscopic Analysis.
   - $^1$H NMR spectrum Analysis.
   - Mass spectroscopy.
6. Biological evaluation of synthesized compounds.
   - In vitro analysis
     i. Evaluation for Aldose Reductase inhibitory activity
     ii. Evaluation for dual PPAR α and γ agonist activity
   - In vivo analysis
     i. Evaluation for anti-hyperglycemic activity
     ii. Evaluation of body weight index

Docking Study of Designed compound

Docking study of proposed compounds revealed key interaction of hydrogen bonds with the amino acids. Docking interaction studies for all the proposed 72 compounds summarized as follows.

- Thiazolodine or rhodanine nucleolus as an pharmacophore for selectivity
- N-acetic acid derivatives shows high selectivity and potency for all the proteins while N-acetate derivative are less active due steric hindrance
- G-score of all the compounds reveals that 5-Arylidene moiety fix in an aromatic center between the carbonyl linker gr. and the TZD/Rhodanine moiety.
- Substituted phenyl as an tail is important for potency and the specificity of the entity. Electron releasing gr. Improve the Inhibitory effect against AR and agonist effect against PPAR receptors.
- Compounds containing 2-Cl, 2,4-CH₃ and para –OCH₃ groups are to be more potent.
- G-score for synthesized compounds were found within significant range of -19 TO -1.9 while N-acetate derivatives were rejected due to less interaction.
General Synthetic Method

Selected substituted 5-Arylidene derivatives having Thiazolidinedione and Rhodanine groups showing significant G-scores were synthesized by conventional method. The synthesis of designed compounds was carried out by chlorination of derivative of benzoic acid followed esterification with 4-hydroxyl benzaldehyde. The substituted 4-fluoro-methylphenylsulfones further undergo for condensation with thiazolidinedione in presence of sodium acetate to get end product.

The progress of reactions was monitored with the help of thin layer chromatography using pre-coated silica gel-G on TLC slides. Iodine vapor was used as detecting agent. Purification of synthesized compounds was carried out by column chromatography technique. The melting point of synthesized compounds and intermediates was determined by open capillary method and which is uncorrected. The absorption maxima of the synthesized compounds were determined using Shimadzu UV-1700 spectrophotometer. The Infrared spectra of the synthesized compounds showed absorption bands which is characteristic for their structure. All compounds were in conformity with the structure envisaged. The structures were proved on the basis of UV, IR, H1NMR and Mass spectroscopy. All compounds gave the satisfactory results.

Biological Evaluation

*In-vitro* evaluation as AR Inhibitor

All the synthesized compounds were evaluated for their ability to inhibit the In-vitro reduction of D,L-glyceraldehydes by partially purified ALR2 from bovine lenses; Sorbinil was used as a reference drug. The inhibitory activity data of the compounds are shown in figure 5.1-5.4.
Chapter No. 05   Summary and Conclusion

Figure 5.1: Graphical Representation of AR Inhibitory Activity (YPA-01-YPA12)

Figure 5.2: Graphical Representation of AR Inhibitory Activity (YPA-13-YPA24)
In-vitro enzymatic assay as AR Inhibitors for synthesized compounds were performed merely by using a 10 μM concentration of each drug, IC$_{50}$ values of
compounds were studied and found between 1.82 to 22.75. The compounds (YPA-02, YPA-06, YPA-08, YPA-14, YPA-20, YPA-26, and YPA-38) exhibited significant AR inhibitory activity.

**In-vitro evaluation as PPAR γ and PPAR α agonist**

22 compounds amongst the 48 having significant selectivity and potency for Aldose Reductase enzyme was further screened against PPAR α and γ using Rosiglitazone and WY14643 as reference drug. The values were calculated as percent maximal activity (Emax) of PPAR α and γ.

![Graphical Representation of PPAR γ and PPAR α agonist Activity](image)

**Figure 5.5:** Graphical Representation of PPAR γ and PPAR α agonist Activity (S-01-S-10)
Figure 5.6: Graphical Representation of PPAR $\gamma$ and PPAR $\alpha$ agonist Activity (S-11-S-22)

Percent of maximal efficacy ($E_{\text{max}}$) of all compounds was calculated comparing to reference compounds (WY-14643, Rosiglitazone) normalized to 100%. The agonist study was performed by using 10 $\mu$M concentration of each drug, $E_{\text{max}}$ values of compounds were studied and found between 18% to 89%. The compounds (YPA-02, YPA-06, YPA-08, YPA-16, YPA-20, YPA-26, and YPA-32) exhibited significant PPAR $\alpha$ and PPAR $\gamma$ agonist activity.

*In-vivo* anti-diabetic activity: Evaluation for anti-hyperglycemic activity

Antidiabetic activity of the synthesized compounds were tested using alloxan induce diabetic model *In-albino* rat. The dose of the synthesized compounds (15 mg/kg body weight) and Rosiglitazone (4 mg/kg body weight) were administered orally in 2% acacia. The blood glucose level was monitored at different times 0, 1, 3, and 6h respectively.
To establish safety profile of synthesized compounds, the body weight index of the compounds was assessed and compared with standard WY14643 (4mg/kg body weight) dose. It was observed that there was no significant change in body weight of standard and test compounds with respect to control. The compounds showed low value of body weight index as compared to standard.

*Figure 5.7: Graphical Representation of Antidiabetic Activity*

*In-vivo anti-diabetic activity: Evaluation of body weight index*

To establish safety profile of synthesized compounds, the body weight index of the compounds was assessed and compared with standard WY14643 (4mg/kg body weight) dose. It was observed that there was no significant change in body weight of standard and test compounds with respect to control. The compounds showed low value of body weight index as compared to standard.
Figure 5.8: Graphical Representation of body weight index
Conclusion

We conclude from the observations, that ….

- The compounds containing a Thiazolidinedione nucleus showed significantly high \textit{In-vivo} antidiabetic activity over Rhodanine analogs.
- The electron withdrawing group ($o$ & $p$) shows less potency as compare to electron releasing group.
- Substitution on 2\textsuperscript{nd} and 4\textsuperscript{th} position with electron releasing group is more favourable.
- Bi-substitution on aromatic ring reduces the activity as compare to mono-substituted analogs.
- Bulkiness favour at 4\textsuperscript{th} position while polarizability non-favorable for the activity.
- The \textit{In-vivo} activity data of all compounds have P value less than 0.001 after 6h.
- The results of Aldose Reductase inhibitory, agonist of PPAR $\gamma$ and PPAR $\alpha$ as anti-diabetic activity of compounds were found to be statistically significant.

Future Scope

The field is further open for study of (YPA-02, YPA-06, YPA-08, YPA-16, YPA-20, YPA-26, and YPA-32) compounds with respect to toxicity studies, pharmacokinetic studies and clinical studies so as to establish these analogs as better and safer drug molecules.

The other 3D QSAR techniques like CoMFA, CoMSIA, Pharmacophoric Mapping, Receptor Surface Model Generation, Molecular Shape Analysis could be utilized to further explore the physicochemical properties required to design more potent drug molecules devoid of side effects shown by conventional antidiabetic agents for the treatment and management of Diabetes Mellitus.