9. CONCLUSION, FUTURE PERSPECTIVES AND LIMITATIONS

Chapter IX

9. Conclusions, Future Perspectives and Limitations

Conclusions

Due to the sedentary lifestyle associated with the industrialized world, a rapid gap in the energy imbalance from the diet is common across the globe. The condition puts a hazardous effect on human health *via* imposing various lifestyle associated diseases such as obesity, diabetes, cardiovascular diseases etc. These conditions are not only affecting the health but are also leading to a negative impact on socio-economic status of the individuals. Obesity and its associated co-morbid conditions are one of the important lifestyle diseases, that is emerging in a pandemic proportion. Hence, scientific communities have introduced a new term "globesity". Modification of diet and physical activity are the key management process for the prevention of obesity. Although obesity can be prevented by these key approaches, over 4 million people die due to obesity and its associated conditions. Hence, along with the key management approaches, pharmacotherapy has also been considered for the enhancement of wellness of the obese patients. Currently, six therapeutic drugs that focus on numerous targets are used for the treatment of obesity. Amongst these targets, inhibition of PL is considered as one of the most promising strategy. Orlistat is a PL inhibitor approved for the long-term treatment of obesity. However, current reports indicate that orlistat possess various adverse effects such as hepatotoxicity, nephrotoxicity etc. This highlights the urge to develop safer and effective anti-obesity therapeutics for the treatment of obesity and its associated conditions.

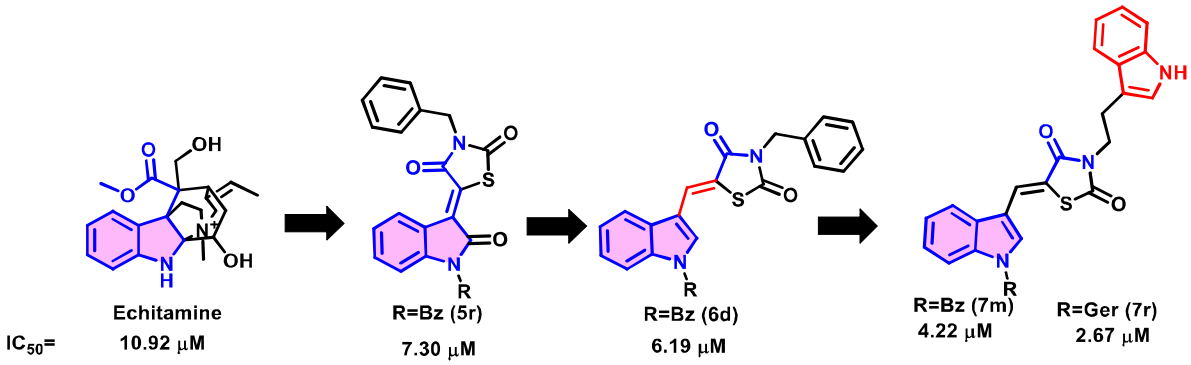
Natural products are always the center of attraction in the scientific community due to the large structural diversity, lesser side effects and availability to the common communities. Hence, the thesis work was initiated with the literature survey of various natural sources used for the management of obesity. Among the numerous sources explored, we selected a pool of 15 plants, that were screened for PL inhibition. The study highlighted the methanol extract of *Alstonia scholaris* stem bark as a promising source for potent PL inhibition (IC_{50} = 12.85 µg/mL). Bioassay guided fractionation of this extract resulted in the identification of the echitamine as a potential PL inhibitory lead (IC_{50} =10.92 µM). Further, an attempt was made to deduce a correlation with the echitamine content and PL inhibition *via* a new validated HPTLC-HRMS method. The study revealed a positive correlation between PL inhibition and echitamine content.

Nevertheless, echitamine exerted lower PL inhibitory potential in comparison to orlistat (IC_{50} = 0.86 µM). Molecular modelling studies of echitamine revealed the lack of some essential structural features required for potential PL inhibition. Hence, based on the lead optimisation approach, an attempt was made to develop potent PL inhibitors inspired from echitamine. A hybrid drug design approach was utilised wherein indole functionality (inspired from echitamine) and TZD functionality (from various drugs/ drug candidates used for metabolic diseases) was conceptualised. Initially analogues containing the indole scaffold directly linked to TZD were designed and synthesized (**5a** to **5ag**). Amongst the Series I, analogue **5r** exerted a potential PL inhibitory activity with IC_{50} of 7.30 µM. The analogue **5r** exhibited a potential activity than echitamine but a lesser activity than that of orlistat. Molecular modelling studies revealed that the incorporation of additional carbon linker between indole-TZD scaffolds might be helpful for the enhancement of PL inhibitory activity. The study resulted in the design and synthesis of new series of analogues (Series II), wherein analogue **6d** revealed a higher PL inhibitory potential (IC_{50} = 6.19 µM) than the previous series. Although the PL inhibitory potential of the hybrid analogues was enhanced, there was further scope to enhance the potential *via* various structural modifications. Further structural modifications were done by mainly focussing to reduce the interaction distance of reactive functionalities with the Ser 152, improving the interaction ability with Arg 256 and increasing the overall hydrophobicity of the indole-TZD scaffold. These structural modifications resulted in analogue **7r**, with a comparable (IC_{50} = 2.67 µM) PL inhibitory potential to that of orlistat (IC_{50} = 0.86 µM).

The synthesized analogue(s) that exhibited potential *in-vitro* PL inhibitory activity were further planned for evaluation in the *in-vivo* conditions. Prior to that, ADMET properties of the topmost active analogues were evaluated by using numerous *in-silico* tools (SwissADME, OSIRIS property explorer and GUSAR). Analogues with IC_{50} less than 10 µM (Series I & II) and 6 µM (Series III) were selected for *in-silico* ADMET predictions. All the evaluated analogues were found to devoid of the toxicities. Further, these analogues exerted comparable LD_{50} values to that of orlistat. Nevertheless, the incorporation of isoprene functionality caused decrement in GI absorption properties of the analogues. Based on the ADMET predictions as well as the *in-vitro* PL inhibitory profile, **7r** was selected for the *in-vivo* pharmacological conditions. Three different sets of *in-vivo* evaluations used for the anti-obesity activity were: oral triglyceride tolerance test (OTTT), anti-obesity activity (four-week treatment) and faecal triglyceride quantification. Overall,

7r (20 mg/Kg) exerted a comparable anti-obesity activity to that of orlistat with respect to bodyweight, triglycerides, total cholesterol and high-density lipids. Faecal triglyceride quantifications further indicated that the anti-obesity mechanism of the synthesized analogue (**7r**) was mainly due to the inhibition of PL.

In conclusion, the present work resulted in the identification of potent Indole-TZD hybrid analogue **7r**, that was the result of collaborative efforts of various techniques namely bioassay guided fractionation of natural products, molecular modelling followed by structural modifications using synthetic methodologies.



Future Perspectives

The present work mainly focused on the identification and generation of a structurally diverse library of new/novel chemical entities using an integrated approach comprising of exploration of natural products, *in-silico* drug design, synthetic chemistry, *in-vitro* and *in-vivo* biological studies. Following are the future perspectives of the work

- A further exploration of un-investigated plants in terms of a larger pool, detailed structural understanding of the natural products etc. might result in the more potent PL inhibitory leads
- The most potent synthetic analogue **7r** exhibited an IC_{50} of 2.67μM comparable to orlistat. The potency of **7r** and related hybrid analogues can further be enhanced by various structural modifications such as substitution on the indole functionality attached to TZD and addition of various electron donating substituents on the benzyl ring substituent.
- Substitution on the indole scaffold with benzyloxy functionality resulted in the decrements of PL inhibitory potential which can be further investigated

- The mechanism of interaction amongst the synthesized analogues with PL can be evaluated by using various other techniques such as X ray crystallography, Cryo-electron microscopy, Isothermal Titration Calorimetry etc
- Furthermore, evaluation against various lipases (such as lingual, gastric, hepatic etc) might be helpful in understanding the effect of the hybrid analogues on the overall digestion process of the triglycerides
- *In-vivo* studies in other animal models of obesity can validate the antiobesity potential of the PL inhibitor "**7r**".
- Numerous other scaffolds/pharmacophores can be hybridised with indole or TZD for the discovery of novel potent PL inhibitors

Limitations

Few limitations were identified in the present thesis, that are as follows.

- The present experiments were limited to the *in-vitro* and *in vivo* (preclinical) evaluation of the synthesized analogues and no clinical study was performed.
- The study did not evaluate the metabolic stability of hybrid analogues under stimulated and physiological conditions
- *In-silico* analysis were used to study the interaction of the potent analogue with PL. Advanced techniques such as, Cryo-electron microscopy, X-ray crystallography etc were not performed
- The present work involved synthesis of a small library of indole-TZD hybrid analogues. A larger set of indole-TZD hybrid analogues can be synthesized for detailed understanding of the effect of various substituents on the PL inhibition