ABSTRACT

Obesity is defined as a chronic relapsing disease that results in accumulation of fat in the body further leading to severe health impairments. The prevalence of obesity across the globe is rising at an alarming rate, hence it is recognised as one of the most important public health concerns. More than 200 co-morbidities are associated with obesity. The demography of obese and overweight population is found to be higher than that of underweight and malnutrition population. These all events clearly highlight that obesity is growing in a pandemic dimension, which not only is affecting the health status of individuals but also the socio-economic status of the current and future world. Hence, a proper strategy for fighting against obesity is essential for the current scenario.

Amongst the numerous interventions explored for the treatment of obesity, inhibition of Pancreatic Lipase (PL) is considered as a viable strategy. PL is the major metabolic enzyme responsible for the breakdown of dietary triglycerides into monoglycerides. Since the energy imbalance between the intake and expenditure plays a major role in the obesity condition, inhibition of energy intake by inhibiting PL is considered as a promising target for obesity. Orlistat is one of the drugs of choice used for the long-term management of obesity, that acts *via* PL inhibition. However, recently it was reported to possess severe adverse events such as hepatotoxicity and acute pancreatitis on long-term treatment. By considering the advantages of PL inhibition, (*i.e*., a peripheral target that does not require any kind of systemic absorption) and adverse events of Orlistat, there is an urge to develop safer and effective PL inhibitory anti-obesity drugs.

Traditionally natural products have been considered as one of the reliable sources for the management of various human ailments. Vast structural diversity and information regarding the various pharmacological activities obtained from the traditional knowledge highlights the importance of natural products. Interestingly, anti-obesity effects of numerous plants have been recorded in ancient literature. Inspired from these promising facts, a pool of Indian medicinal plants was selected, extracted and evaluated for their PL inhibitory activity. Amongst the various screened extracts, methanol extract of *Alstonia scholaris* (stem bark) prepared *via* continuous hot percolation exhibited comparatively greater PL inhibitory potential (IC_{50} = 12.85 µg/mL). Since crude extract consists of a mixture of natural products, a bioassay guided fractionation approach was used for the identification of bioactive constituent(s) responsible for PL inhibition. These studies resulted in the identification of echitamine as a potential PL inhibitory lead (IC_{50} = 10.92

μM). Further, a new HPTLC-HRMS method was developed and validated for the identification and quantification of echitamine in extracts prepared from various extraction techniques. A direct correlation between the echitamine content and PL inhibition was demonstrated.

Though echitamine exhibited a potential PL inhibition, its activity was lower than the orlistat (IC_{50} = 0.86 μM). An *in-silico* analysis of echitamine in the active site of Human PL (PDB ID: 1LPB) highlighted a high degree of unfavourable steric interactions. Moreover, echitamine possessed less reactive ester functionality (methyl ester). These might be the putative reasons attributed to the comparatively lower PL inhibition of echitamine.

Thiazolidinedione (TZD) are the privileged fragments in the drug discovery program due to their diverse pharmacological activities as well as the possibility of incorporation of numerous active pharmacophores (on imidic nitrogen). Apart from this, TZD scaffold has been explored for its potential role in various metabolic disorders. Previously, our group explored the PL inhibitory activity of diaryl substituted pyrazolyl substituted TZD, wherein the titled analogues demonstrated good PL inhibitory activity. These results suggested the utility of TZD based analogues as potential PL inhibitors.

Thus, motivated by the above results, molecular modelling-based lead optimization approach was used to design hybrid analogues of indole and TZD, that were then synthesized and evaluated for their PL inhibitory potential. In the hybrid analogues the indole scaffold was inspired from echitamine, while TZD was conceived from the commercially available drugs used for the metabolic conditions. These analogues also contained the various essential structural features required for PL inhibition based on our previous studies.

Initially, indole scaffold was directly linked to the TZD scaffold. Since PL inhibitors require hydrophobic interactions with the lid domain amino acids, various substituted aromatic functionalities were attached to the imidic nitrogen of TZD and indole nitrogen. This resulted in the formation of 33 analogues (**5a** to **5ag**), wherein **5r** was found to be the most active inhibitor of PL with an IC_{50} of 7.30 μM, followed by **5t** (IC_{50} = 9.51 μM). A competitive mode of enzyme inhibition was revealed in the enzyme kinetics analysis, while fluorescence spectroscopy further supported this fact.

Molecular modelling studies of **5a** to **5ag** revealed that the increment in the carbon linker between the indole and TZD scaffold can exert a possibility for the enhancement of PL inhibitory property. Thus, a new series of indole-TZD analogues was designed by incorporating one additional carbon linker, and the study resulted in the synthesis of 28 analogues (**6a** to **6ab**). Among the synthesized analogues, **6d** was found to be the most active inhibitor of PL with an IC_{50} of 6.19 μM, followed by **6e** (IC_{50} = 8.96 μM). A competitive mode of enzyme inhibition similar to the previous analogues was demonstrated along with the fluorescence quenching studies that supported this fact.

Further structural expansion of the hybrid analogues was performed based on various literature reports and molecular modelling studies resulting in synthesis of 21 analogues (**7a** to **7u**). The ability for the formation of π-cation interaction by analogues with Arg 256 was reported as an essential structural feature for potent PL inhibition. To achieve this, initially, an additional carbon linker between the imidic nitrogen of TZD and aromatic functionality was incorporated (in series II analogues), which exerted a potential PL inhibition (**7d**; IC_{50} = 5.01 μM). Moreover, considering the potential role of indole scaffold in the formation of higher π-cation interactions, the aromatic functionality was further replaced with a denser indole scaffold. This resulted in a potential analogue **7m** (IC_{50} = 4.22 μM). Additionally, numerous unsaturated long-chain alkyl chains (prenyl/geranyl) were also incorporated in the hybrid analogues for enhancing the overall hydrophobic interactions. Amongst the synthesized analogues, **7r** was found to be the most active PL inhibitor with an IC_{50} of 2.67 μM, Furthermore, enzyme inhibition kinetics study revealed a competitive mode of inhibition by these analogues.

A total of 14 candidates, comprising of analogues with IC_{50} less than 10 μM (from Series I & II) and 6 μM (from Series III) were selected and subjected to the *in-silico* ADME predictions by using various tools such as SwissADME, OSIRIS Property explorer, GUSAR. The studies highlighted that the synthesized analogues were devoid of toxic effects and a higher LD_{50} revealed a safe profile of these screened candidates. Based on the ADMET profiles and *in-vitro* PL inhibitory profiles, **7r** was finally selected for the *in-vivo* pharmacological evaluation. Three different approaches were utilised in the *in-vivo* experiments, that resulted in the identification of various aspects of anti-obesity effects of the **7r** analogue. Oral triglyceride tolerance test (OTTT) of **7r** was performed at three doses (5, 10 and 20 mg/Kg) and the study revealed that the 10 and 20 mg/Kg dose of **7r** inhibited the absorption of triglyceride content. Thus, these doses (10 and 20 mg/Kg) were

selected for further *in-vivo* experiments. A four-week treatment of **7r** in the High Fat Diet (HFD) feed mice provided information regarding its anti-obesity effect with respect to parameters such as bodyweight, triglycerides, total cholesterol and high-density lipids. Quantification of the faecal triglyceride contents highlighted the potential role of **7r** in the PL inhibition. Overall, the *in-vivo* pharmacological evaluation in mice suggested that the synthesized analogue **7r** exerted an anti-obesity effect comparable to orlistat.

In conclusion, the work presented in the thesis opened a new avenue for the exploration of potent PL inhibitors inspired from echitamine using structural modification and molecular modelling approaches. Overall, all these results might inspire for the exploration of other bioactive indole based natural products and TZD like privileged scaffolds in the search for newer antiobesity drugs/drug candidates.