CHAPTER 5

CONCLUSIONS
1.5. Conclusions

Growing incidences of gastric ulcer associated with lack of suitable option for effective and safe management for a prolonged period, demands search for alternative agents to the existing agents.

Amino acids are highly compatible and desirable molecules of our physiological systems. There have been lot of efforts as well as achievements in elucidating their role in different physiological process. In the process of acid secretion, there role is not clear. However many reports have been published citing their potential to block gastric acid secretion. In spite of this, the findings can be considered only preliminary and their application in management in gastric ulcer is far from practice. In this scenario we considered amino acids as leads which can be optimised for development of potent antiulcer agent.

As a process for optimisation we choose conjugation of amino acids with molecules which are known to interfere in the process of gastric acid secretion and ulceration. Calcium influx is a basic process for release of stimuli for gastric acid secretion. Accordingly nifedipine, as well known calcium ion channel blocker interferes in the primary level of stimulation for acid secretion. Conjugation of amino acids to nifedipine yielded compounds 3a-3j, which was found with good antiulcer action in the screening in Shay rat model. Two most potent compound 3b and 3f further found to inhibit gastric ATPase very strongly (79-84%), comparable to that of omeprazole (91%). Molecular docking studies showed that polar interaction residues (pockets) of compounds were different from that of omeprazole, suggesting a probable different mode of binding to gastric ATPase inhibition which needs further investigation. It suggested that binding affinity of pheyl alanine and methionine to gastric ATPase was enhanced by conjugation to nifedipine. The in silico studies predicted acceptable drug likeness for the conjugates. However, it also predicted some toxicity which was also found during its acute toxicity studies.

In continuation of the same approach, we selected N,N’-dicyclhexylcarbodiimide for conjugation to amino acids. This was based on the fact that their conjugates have good antioxidant action and it binds to another ATPase which is structurally similar to gastric ATPase. In silico studies of these amino acid conjugates 4a-4j showed good drug like
property with higher safety profile. This was validated in acute toxicity studies as the compounds were found to be very safe. Preliminary antiulcer screening suggested good antiulcer potential of the conjugates. To further explore their antiulcer action we carried out gastric ATPase inhibitory activity and found them to possess inhibition capacity (81-87%), comparable to that of omeprazole (95%). To rationalise this inhibition we carried out molecular docking studies. It showed that both compounds exhibit more polar interaction than omeprazole and they bind in the same pocket but different from that of omeprazole. Validation this study showed that conjugation with methionine and histidine has significantly enhanced their binding affinity to gastric ATPase.

Carbonic anhydrase-II plays a crucial role in HCl formation in the intermediate level of acid secretion. Sulfonamides are prototype of compounds that inhibit CA. Accordingly p-methyl benzene sulphonamide was selected for conjugation to amino acids to enhance their antiulcer properties. In silico studies of these compounds were favourable from toxicity as well as druglikeness point of view. Preliminary studies in Shay rat model showed their good antiulcer potential. Based on that 5a and 5b were screened in different ulcer model including aspirin induced, alcohol induced and cold stress induced ulcer model. As these models induce ulceration by different etiological factors, effectiveness of compounds in these models suggested their multiple mode of action. Their inhibition of gastric ATPase (72-78) was found to be less but significant in comparison to that of omeprazole (91%). Elucidation of this activity by molecular docking showed that both molecules have less binding affinity (6.5) than omeprazole (7.7). This is in accordance with the experimental finding. However the binding and gastric ATPase can be considered significant. Validation of the in silico studies suggest that binding affinity of cysteine and methionine was enhanced for gastric ATPase following conjugation to p-methyl benzene sulphonamides.

This study has been able to demonstrate that antiulcer action of amino acid has increased by conjugation to leads that have potential to interfere in the acid secretion process. The leads selected including nifedipine, N,N’-dicyclhexylcarbodiimide and p-methyl benzene sulphonamide have different properties and they are likely to influence acid secretion in different manner. So in the study preliminary antiulcer screening, followed by influence on final level of acid secretion, in terms of gastric ATPase inhibition was selected to provide a common basis of evaluation. As shown in the case of selected compounds their polar interaction residues are different and it is possible that they
possess different mode of gastric ATPase inhibition. However these need further investigation to establish the mode. In all cases it was found that conjugation of amino acids to these leads has enhanced their binding affinity which is in agreement with experimental results and justifies the rationale. As conclusion, it can be said that, this study has taken forward the research on development of amino acids as antiulcer agent.