1.2. Review of Literature

1.2.1. Pathophysiology of ulceration

Secretion of acid in stomach is an indispensable physiological process. This is because it induces activation of pepsinogen leading to digestion. This also kills ingested microbes and ensures a stable intragastric environment (Herling, 1996).

![Fig-1.8. Mechanism involved in gastric ulcer formation](image)

Gastric HCl secretion (Fig- 1.8) is neurally stimulated and vagal excitation increases enhanced release of gastrin, histamine as well as gastric HCl (Uvnas B., 1942; Code CF et al., 1956; Sandvik AK et al., 1991; Welsh NJ et al., 1994). Histamine, a non-nervous factor, produces the most powerful gastric acid stimulation and contributes to the development of gastric physiology. Calcium also plays a major role in the regulation of gastric acid secretion in the parietal cells leading to activation of H^+K^+-ATPase and secretion of HCl. Although secretion of acid is essential, its secretion in excess destroys the gastro duodenal mucosal barrier leading to ulcerations (Nelson et al., 2005).
1.2.2. Therapeutic Strategies of antiulcer drugs

Therapeutic strategy for treating ulcer generally involves reducing acid secretion by inhibiting receptors/ mediators at the initial level, intermediate level and final level of acid secretion. In the initial level the strategy mainly aims to reduce secretion by preventing stimulation to transmitters including histamine, acetylcholine and gastrin. The intermediate level mainly involves the role of carbonic anhydrase in promoting acid secretion. In the final stage it is the gastric ATPase which has been the target for inhibition to reduce acid secretion.

1.2.2.1. Developments in strategy reducing gastric parietal cell stimulation in the primary level of acid secretion process

1.2.2.1.1. Acetyl choline inhibitors

As per the reports of Pfeiffer et al (1995), secretion of acid in stomach is mediated by stimulation of muscarinic receptors and in patients suffering from duodenal ulcer M₃ receptors are over expressed. Accordingly, muscarinic receptor blockers can have potential for application in ulceration. Based on this approach pirenzepine and telenzepine were demonstrated to be effective antiulcer agents (Dammann et al., 1989). However incomplete inhibition of acid secretion and related parasympathetic side effects including dry mouth, blurred vision and constipation limited their application (Lazzaroni et al., 1986).

1.2.2.1.2. Histamine inhibitors

Histamine stimulates H₂ receptor in gastric parietal cells leading to acid secretion. Thus, H₂ receptor blockers including burimamide and metiamide demonstrated more potent antiulcer effect than muscarinic receptor blockers (Black et al., 1972; Wyllie et al, 1980). However owing to improper pharmacokinetics and toxicity they were withdrawn from trial and then in 1978, Price reported cimetidine as a suitable alternative in this category of drugs. This propelled development of several other drugs with improved pharmacological profile including ranitidine, famotidine, nizatidine and roxatidine(Hirata et al., 1981; Berardi et al., 1998; Pioch et al., 1985). Although these drugs are generally very safe, in chronic as well as acute cases of ulceration, their effectiveness is far from satisfactory. Because of this they are increasingly replaced by proton pump inhibitors.
1.2.2.2. Developments in strategy reducing carbonic anhydrase activity in the intermediate level of acid secretion

Carbonic anhydrase (CA II and CA IV) plays a crucial role in gastric acid secretion (Fig-1.9). This was evident in a work by Puscas et al. (1971), where gastric acid secretion was inhibited in humans after oral administration of carbonic anhydrase inhibitor, acetazolamide in therapeutic doses of 25 mg/kg of body weight. Acetazolamide exhibited antiulcer action in acute experiments because of inhibition of CA-II, but its effect on Gastric ATPase was not clear. Since then Puscas et al., have carried extensive work on these enzymes and inhibitors of CA has been widely reported to reduce HCl secretion and heal gastric and duodenal ulcers (Puscas et al., 1984, 1987).

![Fig-1.3. Gastric hydrogen secretion](image)

Omeprazole was demonstrated to have dual mode of action to reduce acid secretion (I Puscas et al., 1999). It irreversibly binds to H+/K+ ATPase and reduces acid secretion. It also inhibited CA II and CA IV, isozymes present in large quantities in the cytosol, in the walls of the secretory canaliculi, and in the parietal cell membrane contributing to inhibition of acid secretion. This makes CA an important target in developing antiulcer agents.

1.2.2.3. Developments in strategy inhibiting gastric ATPase in the final level of acid secretion

Acid secretion in parietal cells is finally mediated by proton pump (Okamoto et al., 2001). Thus H⁺/K⁺-ATPase is the ultimate target to prevent acid secretion. Accordingly, inhibitors of this proton pump (PPIs) have received wide attention in development of effective antiulcer agents. The PPIs bind irreversibly to the gastric proton pump on the
parietal cell membrane and prevent release of hydrogen ions from the parietal cells into stomach.

Inhibition of $\text{H}^+\text{K}^+$-ATPase has received wide attention with discovery of benzimidazole sulfoxide class of antisecretory agents. Timoprazole was the first well-defined PPI of this class (Ruwart et al., 1973), following which several others have been developed leading to clinically successful drugs including Omeprazole, Lansoprazole, Rabeprazole, Pantoprazole and Esomeprazole. Irreversible inhibition of $\text{H}^+\text{K}^+$-ATPase occurs following acid activation of these compounds within the acidic compartments in the parietal cells and covalent binding (Fig-1.10), with C813 residue of gastric H+/K+-ATPase (Ife et al., 1989).

![Fig-1.4.Binding of omeprazole to ATPase](image-url)

Irreversible inhibition as depicted in Fig-1.4 leads to extreme suppression on long term use leading to achlorohydria. This condition may disturb the intragastric stability and lead to enteric infections like typhoid, cholera, and dysentery (Jain et al., 2007). This destabilization of gastric environment may result in drug interactions and improper absorption of some drugs like griseofulvin, ketoconazole, vit.B$_{12}$, iron salts. Besides long term use of PPIs are associated with side effects including abdominal pain, diarrhea, nausea, and headache.

Limitations of PPIs because of irreversible proton pump inhibition have fuelled research for development of reversible inhibitors of proton pump. This has produced imidazopyridine derivative SCH28080 (Chiu et al., 1983; Keeling et al., 1988; Wallmark et al., 1987). It binds non-covalently with gastric H+/K+-ATPase and prevents acid secretion. However serious hepatotoxicity of this molecule prevented its further use.
1.2.2.4. Alternative therapeutic strategy against ulceration

1.2.2.4.1. Anti *H. pylori* drugs

*H. pylori* are gram-negative microbes. Following infection they colonize in the mucus on the luminal surface of gastric epithelium and damage the mucosal barrier leading to inflammation, ulceration and sometimes gastric lymphoma and adenocarcinoma. However infection with *H. pylori* does not always lead to ulceration. Besides the stimulating factor leading to its damaging action on stomach is not yet clear. Accordingly, antimicrobials against *H. pylori* including metronidazole, bismuth tetracycline or amoxicillin (triple therapy) may not always address ulceration. Besides effectiveness of these agents is limited by their side effects (Labenz et al., 2000).

1.2.2.4.2. Antacids

Antacids are used to neutralize the acid already secreted in the gastric cells, so as to minimize the damage by acid. Substances used as antacids include carbonates, potash, bismuth, aluminum, magnesium (Kromer et al., 2000). Surfactants including simethicone are used along with these antacids to decrease foaming and bloating. Being mostly alkali compounds they suffer from side effects including alkalosis, belching, nausea, abdominal distension, flatulence, diarrhea, and constipation. Besides use of antacid is limited to acid neutralization only and do not affect hyper acid secretion.

1.2.2.4.3. Stress modulators as antiulcer agent

Stress causes both sympathetic and parasympathetic stimulation of stomach leading to local hypoxia (near or actual “ischemia”). The ischemic condition caused an increase in the levels $\text{H}_2\text{O}_2$ which in conjunction with $\text{O}_2$ generates $\text{OH}^-$ ions which oxidized various cellular constituents such as proteins, membrane lipids and depletes glutathione. Lipid peroxidation causes loss of membrane fluidity and loss of cellular function (Tandon R et al., 2004). Present studies have implicated the role of free radicals on lipid peroxidation in the development of ulcer (Gutteridge JM, 1995).

Wide research has been undertaken in order to establish natural product as stress modulators against ulceration. Treatment with ethanolic extract of seeds of *E. jambolana* significantly decreased the LPO levels of gastric mucosa against CRS-induced changes in LPO in rats (Chaturvedi A et al., 2007). The phenolic compound rosmarinic acid from
Rosmarinus officinalis Linn has shown therapeutic potential in treatment of peptic ulcer disease (Al-Sereiti et al., 1999). The protective effect of liquorice and its derivatives including deglycyrrhized form was reported by Dehpour et al., (1994). The preventive effect of natural xanthanolides on ulcer formation in rats was confirmed by Favier et al., (2005). Solon, a synthetic isoprenyl flavonoid derived from sophoradin from the root of an ancient Chinese plant Sophora tonkinensis, administered orally to rats prevented dose-dependently the formation of acute gastric lesions (Konturek et al., 1987). Aparisthman from Aparisthmium cordatum reduced significantly the formation of gastric lesions as well as volume of gastric juice as compared with control in the pylorus-ligature model (Hiruma et al., 2001).

Although the mechanism underlying this anti ulcerogenic effect of antioxidants remains unknown, it seems to be related to an increase of the defensive mechanisms of the stomach and acting as scavengers against free radicals and prevention of lipid peroxidation.

1.2.3. Rationale for development of antiulcer agents.

Wide literature search in section 1.2.2 shows that in spite of the progress in understanding the etiology of ulcer, the attempts are far from successful. There are many issues that need to be addressed based on the basic pathophysiology of ulceration. Although the agents discussed have shown potential, none of them has till date matched the effectiveness of omeprazole and its analogues. However omeprazole and its analogues bind covalently and irreversibly inhibit gastric ATPase up to 16-18 hrs following a single dose. Normalization usually takes about 96 hours. This makes them unsuitable for long term use. Attempts at finding suitable alternative are far from over. A good antiulcer agent should be able to reduce acid secretion effectively and safely for a prolonged period. Unfortunately none of the agents match these expectations. Thus considering, ever increasing incidences of ulcer and ulcer leading to cancer, there is an urgent need to attempt to develop alternative agents to them.

1.2.3.1. Amino acids as antiulcer agent

Amino acids make up 75% of the human body and are essential for normal function of cell. Biochemical pathways are mediated either by amino acids or proteins made up of these amino acids. Their role as neurotransmitter or their precursor helps transmission of
signals for normal bodily function. They enable other nutrients, vitamins and minerals to perform their jobs properly.

L-serine inhibits gastric secretion and protects the gastric mucosa against stress and chemically induced ulcers (Tariq M et al., 1997). Pretreatment with serine attenuated the formation of stress, indomethacin and necrotizing agents-induced gastric lesions. Glycine, a neutral amino acid has been reported to inhibit gastric secretion and to protect the gastric mucosa against chemically and stress-induced ulcers in rats (Tariq M et al., 1997). L-glutamic acid (0.1 M) administered to the blood also produces marked inhibition of gastric secretion. Pretreatment with L-arginine markedly reduces the degree of gastric injury. It elevates NO bioavailability in the gastric mucosa, which is a gastric mucosal protective factor that contributes significantly to maintaining normal gastric mucosal integrity (Zhang et al., 2011). The effectiveness of simple and mixed copper (II) complexes of L-tryptophan and L-phenylalanine on the gastric acid secretion was reported in Shay rats by Alberghina (1982). The gastric inhibitory effect of melatonin and L-tryptophan on ulcer healing was accompanied by a significant rise in the gastric blood flow at ulcer margin and an increase of plasma melatonin, luminal NO$\textsubscript{2}^{-}$/NO$\textsubscript{3}^{-}$ and plasma gastrin levels (Brzozowska et al., 2002). Gastric acid and pepsin outputs were significantly inhibited during the ulcer healing in melatonin-treated gastric mucosa.

Intraduodenal administration of mixed AA solution resulted in significant inhibition of gastric acid secretion and gastrin release stimulated by intragastric perfusion of peptone (M. Ikeda et al., 1997). L-tryptophan on account of its ability to inhibit formation of free oxygen radicals showed protective effect against gastric mucosal damage in ischemia-reperfusion injury (N Bulbuller et al., 2003).

1.2.3.2. Calcium channel blockers as antiulcer agent

Hypercalcaemia is associated with increased gastric acid secretion and calcium appears to be critical for stimulus secretion coupling in gut (Glavin, 1989). The release of histamine, acetyl choline, gastrin and hydrochloric acid are all calcium dependent (Mccoll et al., 1987). Histamine release dependent upon both extracellular and intracellular calcium indicates the fact that development of gastric ulcer is a calcium dependent process.

Gastric stimulation results in increased calcium influx which can be blocked by calcium antagonist not by Histamine (H$\textsubscript{2}$) blockers (Szenyi et al., 1980). Accordingly,
calcium channel blockers verapamil and its analogues devapamil (desmethoxyverapamil) and gallopamil (methoxyverapamil) prevented ulcerogenesis induced by cold-restraint stress and promoted spontaneous ulcer healing (Alica et al., 1994). Nitrendipine, diltiazem and verapamil, three chemically-distinct calcium channel blockers, all reduced cold-restraint stress gastric lesions (Glavin, 1989). Nitrendipine significantly decreased stress-induced gastric lesions, but was far less efficacious against 100% ethanol-induced lesions (Vaughy et al., 1987). It also significantly reduced basal gastric acid output. Verapamil inhibited acid secretion from isolated parietal cells under basal, histamine and dibutyryl cyclic AMP stimulated condition (Sewing and Hennemann, 1983). Calcium channel antagonists also block the acid secretion by inhibiting parietal cell H+-K+-ATPase. The influence of verapamil on stress and bethanechol induced gastric effects in rats, showed a dose dependent decrease in gastric glandular secretion and also increased gastric wall contraction (Wo Clive et al., 1985). Stress ulcer antagonism by verapamil is associated with inhibition of gastric glandular mucosal mast cell degranulation and reduced stomach contractions. The Combined Cholinergic, gastrinergic and histaminergic influence results on M1 (neuronal) and M2 (Parietal), gastrin and histaminic H2 receptors respectively leading to reduced stimulation of H+K+ ATPase exchange pump which involved in the terminal step of hydrochloric acid secretion (Wai Shiu et al., 1990).

The effect of nifedipine and cimetidine on cold or stress-induced gastric ulcers and glandular wall mast cell count were studied in rats (Waleed, 1991). Nifedipine in three doses (1, 5 and 10 mg/kg) administered intra peritonealy prevented gastric ulceration and mast cell degranulation. This was attributed to inhibition of histamine stimulated acid secretion and ability to reduce acetyl choline induced gastric secretion in which calcium is the most likely final common denominator.

1.2.3.3. Free radical scavengers as antiulcer agent

Free radicals, especially reactive oxygen species in living cells leads to pathogenicity of various diseases (Scott G, 1995; Foye WO et al., 1995) including hepatic diseases, cardiovascular diseases, atherosclerosis, diabetes, cancer and ulcers in gastrointestinal tract. Fig-1.11 explains about the pathogenesis of ulcer stimulation due to activation of free radicals which causes mucosal ischemia. Due to increased lipid peroxidation mucosal cell integrity decreases leading to ulcerogenesis in GI tract.
In review of literature section 1.2.2.4.3. Several works have been reported where natural products with good antioxidant action have shown strong antiulcer activity. Although these reports do not elucidate the mechanism, a holistic approach is believed to explain those findings.

In a recent work N-aroyl urea developed by Amornraksa et al., (2009) was reported with good antioxidant properties. It was developed as a derivative from N,N'-dicyclohexylcarbodiimide (DCC). This molecule is used as oxidizing agents and coupling agents for amides. Besides it is known to inhibit bacterial F_0F_1-ATPase reacting with the small F_0 subunit (proteolipid) in the V alginolyticus membrane vesicles (Krasnoselskaya et al., 1991). Considering the resemblance of this ATPase to gastric ATPase, N,N'-dicyclohexylcarbodiimide analogues may have potential to reduce gastric ATPase. Hence, it needs to be considered for further evaluation.

1.2.3.4. Sulfonamides as antiulcer Agent (as carbonic anhydrase inhibitors)

Sulfonamides with the general formula RSO_2NH_2 constitute a wide class of inhibitors of the zinc enzyme carbonic anhydrase (CA). Acetazolamide a classic, sulfonamide drug has also been reported to reduce gastric acid secretion commensurate with gastric carbonic anhydrase inhibition (Puscas I et al., 1971). In the mechanism of antiulcer action of omeprazole it has been found that, CA inhibitory action against gastric mucosa
CA I, II, and IV is induced by its active sulfenamide form (Liljas et al., 1994). Thus this class of compound can be interesting leads for further exploration of antiulcer action.

1.2.4. Rationale for development of amino acid conjugates

In our approach to find suitable alternatives to existing antiulcer agent we found that amino acids provide some interesting clues. They are innocuous and desirable for normal physiological process. So, development of active agents will have less probability of rejection. However chemically they possess reactive features and if not used judiciously, may lead to undesirable results.

Keeping these insights and challenges in view, we selected amino acids which either have a previous history of antiulcer action or other effect that is conducive to antiulcer action (Section 1.2.3.1). For optimization of their antiulcer action we selected leads which have previously been reported to act at different pathophysiological process of gastric acid secretion and ulceration.

Nifedipine was selected because it is the prototype of calcium channel blockers which earlier has been reported with significant antiulcer action because of prevention of stimulation of gastric cells (Section. 1.2.3.2). N-aroyl urea as a derivative of N, N’-dicyclohexylcarbodiimide has been a good antioxidant. Besides N, N’-dicyclohexylcarbodiimide was found to inhibit an ATPase that resembles gastric ATPase (Section. 1.2.3.3). This makes it a promising moiety to be further evaluated on conjugation with amino acids. Gastric carbonic anhydrase have been proposed to be functionally coupled to gastric ATPase for acid secretion (Section. 1.2.3.4). Accordingly, sulfonamide, a prototype of carbonic anhydrase will be a useful entity to be conjugated to amino acid to enhance their antiulcer action.

Keeping the above in view, amino acid conjugates are to be designed and synthesized following adaptation of established method. Since multiple leads are involved in the design, the property of conjugate may undergo some change with respect to their original property. Accordingly it will be useful to use similar models for screening irrespective of the individual property of the lead. Methods for preliminary screening followed by screening in final level acid secretion (gastric ATPase) will establish the potential of these conjugates to be potential candidates for future agents against ulceration.