Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 42 (2007) 386–393
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Original article

α-Amino acid derivatives as proton pump inhibitors and potent anti-ulcer agents☆

Preeti Sharma [a], Sangeeta Singh [a], Tanveer I. Siddiqui [a], Vishnu S. Singh [a], Bijoy Kundu [a], Philip Prathipati [a], Anil K. Saxena [a], Dinesh K. Dikshit [a,*], Leena Rastogi [b], Chetna Dixit [b], M.B. Gupta [b,✠], G.K. Patnaik [b,✠], Madhu Dikshit [b,*]

[a] *Medicinal Chemistry Division, Central Drug Research Institute, Chattar Manzil Palace, Lucknow 226 001, India*
[b] *Pharmacology Division, Central Drug Research Institute, Chattar Manzil Palace, Lucknow 226 001, India*

Received 4 August 2006; received in revised form 30 August 2006; accepted 31 August 2006
Available online 11 October 2006

Abstract

In a program to identify new anti-ulcer compounds, a series of *N*-acyl derivatives of α-amino acids were screened for their in vitro H^+/K^+ ATPase inhibitory activity, and in vivo efficacy in Pylorus ligation model. 3D-QSAR studies were carried out and a representative compound **13** was studied for the nature of its proton pump inhibition.

Keywords: Amino acids; Pylorus ligation; H^+/K^+ ATPase; Anti-ulcer

1. Introduction

The peptic ulcer and related diseases encompass a broad spectrum of clinical disorders ranging from intense burning, pain to severe complications such as deep ulcers, strictures etc. The mammalian stomach is a specialized organ of the digestive tract that serves to store and process the food for absorption by intestine. The physiological studies [1] regarding acid secretory pathway have proved that proton pump being the ultimate mediator of acid secretion, is localized in specialized acid secreting tubulovesicular system of the parietal cells in the gastric mucosa. Upon stimulation, however, this system undergoes various morphological changes accompanying oxygen consumption, which elicit acid secretory response. Besides histamine, which acts through H_2-receptors as the major stimulant, gastrin and acetylcholine also have receptors on the parietal cells [2]. The presence of proteins and their breakdown products in the food is reported to be the major stimulatory factor for gastric acid secretion. In a quite old report, anti-ulcer effects of various amino acids have been described where glutamine, tyrosine and some other amino acids have been shown to be protective [3,4]. In another study, intravenous infusion of mixture of amino acids led to the potentiation of acid secretion [5] but contrary to this, intraduodenal administration of amino acids resulted in the inhibition of peptone stimulated acid release. Studies conducted with individual amino acids have also yielded varying results. Amino acids such as leucine, isoleucine, valine and proline augment the acid release [6,7]. On the contrary, L-glutamic acid and tyrosine inhibited amino acid-induced and leucine-induced acid secretion, respectively [8]. The observation that omeprazole but not H_2-blockers can control amino acid-induced acid secretion, pointed to the gastric proton pump as the site of their action [9]. However, using ^{14}C aminopyrine accumulation, Schmidtler et al. were unable to observe direct effect of amino acids namely Arg,

☆ Communication No. 6326 from Central Drug Research Institute, Lucknow.
* Corresponding authors. Tel.: +91 522 2612411/18; fax: +91 522 2623405.
E-mail addresses: dk_dikshit@yahoo.com, dk_dikshit@cdri.res.in (D.K. Dikshit), madhudikshit@yahoo.com (M. Dikshit).
✠ Now deceased.

0223-5234/$ - see front matter © 2006 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2006.08.016

Trp, and Phe on the parietal cells [10]. L-Serine has been reported to show a dose dependent inhibition of acid release and also protected against stress, indomethacin and ethanol-induced mucosal lesions [11]. Similarly, L-tryptophan, L-arginine and dimethylglycine have been shown to provide protection against gastric ulcers. L-Tryptophan has been reported to protect against stress-induced ulcers as well as accelerate ulcer healing through increased gastric blood flow and prostaglandin synthesis [12,13]. L-Arginine has also been reported to protect against stress-induced ulcers via preservation of gastric mucin levels and NO secretion while it provides protection against ethanol-induced ulcers through increased mucosal blood flow [14,15]. Dimethylglycine is known to protect against pyloric-ligation, ibuprofen and stress-induced ulcers by free radical scavenging activity along with cytoprotection of gastric mucosa [16]. Studies on the mechanism of amino acids dependent acid release modulation have indicated that amino acids act via both gastrin dependent and independent pathways [17], while others have shown the involvement of histamine dependent pathway [18].

With this background, studies were therefore, undertaken to elucidate the SAR and QSAR of *N*-acyl derivatives of amino acids (Fig. 1) for inhibition of gastric proton pump using gastric microsomal vesicles, and their effect on pylorus ligation-induced ulcers in rats. Though *N*-acylated amino acid derivatives, mostly analogs of proglumide [19], benzotript [20] and rebamipide [21], have been shown to have potent anti-ulcer properties, there is no report of their direct effect on gastric proton pump. We have found that a significant number of *N*-acylated amino acids show good degree of inhibition of gastric proton pump. We have also tested all of these compounds for their ability to control acid secretions in pylorus-ligated rats. The most potent compound from this study, *cis*-5-(2-phenylethenyl)-2-oxo-oxazolidine-4-carboxylic acid (**13**) was subjected to detailed investigation for other pharmacological activities.

2. Chemistry

The amino acids' derivatives **1**–**10** were synthesized according to the standard methodologies or procured from Nova Biochem. Syntheses of substituted serine derivatives **11**, **12**, and pyroglutamate derivatives **36**, and **37**, **38**, have been previously reported [22,23]. *N*-Substituted pyroglutamate derivatives were synthesized using reported procedures [24]. Compound **13** was synthesized by the hydrolysis of the erythro (2*R*, 3*R*) β-styryl serine ester (**11**) [25]. *N*-Acylated proline derivatives **15**–**20** were prepared using standard methodologies [26]. Compound **14** was synthesized according to the reported procedure [27] (Fig. 1).

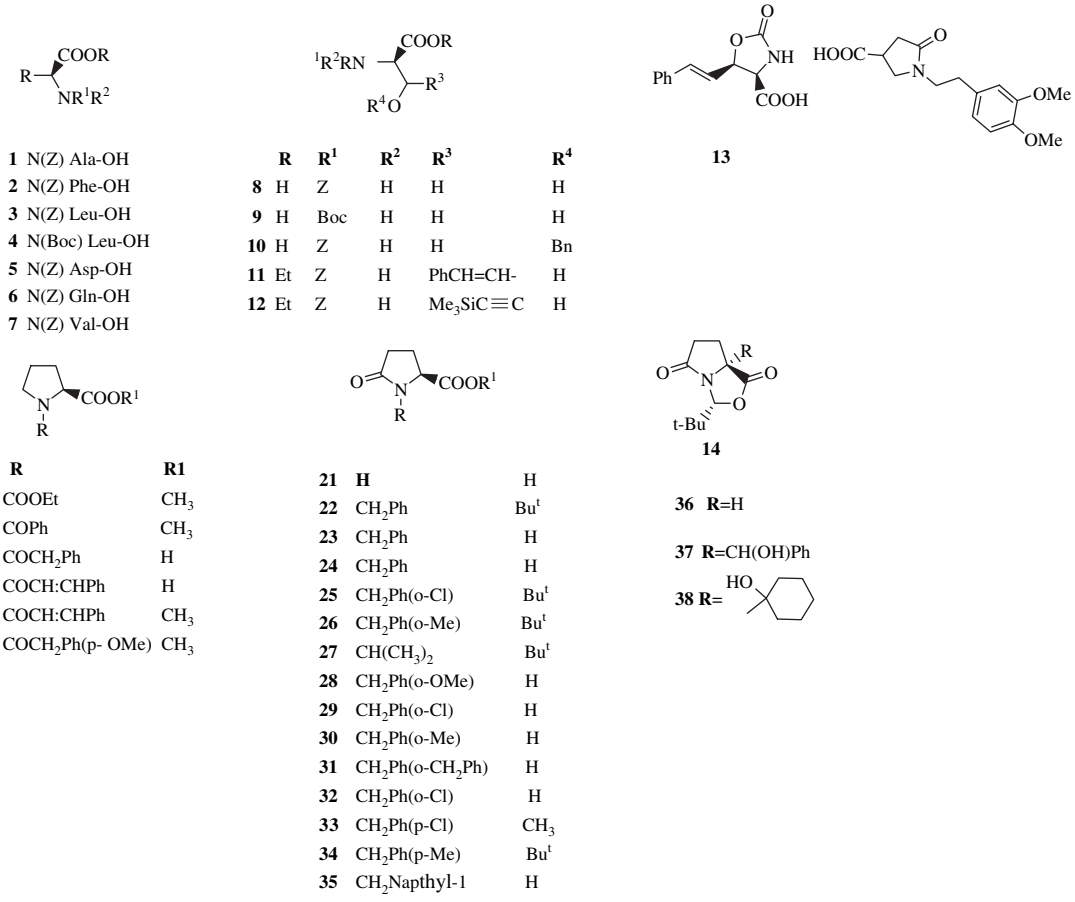

Fig. 1. Compounds synthesized.

3. Results and discussion

The test compounds were initially evaluated in vitro for their proton pump inhibitory activity in gastric microsomal vesicles. These compounds were also evaluated for their anti-secretory activity in pylorus ligation-induced ulcers in rats. Results obtained are summarized in Table 1. Even though some of the amino acid derivatives were highly effective in inhibiting the enzyme activity in an in vitro assay, their in vivo potencies were mostly modest. Though various natural amino acids and their *N*-acyl derivatives with increased hydrophobicity exhibited improved inhibition profile, effect of chirality was not evidenced. Interestingly substituted serine derivative, notably *cis*-5-(2-phenylethenyl)-2-oxo-oxazolidine-4-carboxylic acid showed better order activity in vivo. This corroborates with the recent report that serine per se has a protective effect [11]. Moreover, the amide group with an aromatic moiety like benzoyl or carbobenzyloxy had improved profile. For example, *cis*-5-(2-phenylethenyl)-2-oxo-oxazolidine-4-carboxylic acid (**13**) at 20 mg/kg dose exhibited inhibition of FA and TA similar to that of omeprazole at 10 mg/kg. Since compound **13** exhibited inhibition profile similar to omeprazole in vitro and was also effective in vivo, anti-secretory activity of this compound was evaluated in other

Table 1
Experimental, calculated ($-\log IC_{50}$) for gastric proton pump inhibitors in the model along with the secondary site parameters

#	*	Biological activity			log ($P/100 - P$)		Secondary site parameters				
		P[a]	FA[b]	TA[c]	Observed	Calculated	ss1[d]	ss2[e]	ss3[f]	ss4[g]	ss5[h]
1	*S*	30	0.57	1.13	−0.37	−0.24	0.00	−0.31	–	–	–
2	*S*	30	0.53	0.52	−0.07	−0.08	0.00	0.22	–	–	–
3	*S*	41	0.75	0.69	0.10	0.36	0.00	−0.16	–	–	–
4	*S*	44	0.21	0.43	0.16	−0.05	0.34	−0.16	0.44	0.48	
5	*S*	33	0.49	0.38	−0.18	−0.24	0.31	−0.16	–	0.48	–
6	*S*	40	0.79	0.83	−0.75	−0.61	0.00	0.22	–	–	0.74
7	*S*	15	0.45	0.57	0.12	−0.08	0.42	0.22	–	–	–
8	*R*	46	0.36	0.49	−0.29	−0.17	0.00	−0.16	–	–	–
9	*S*	34	0.71	0.72	−0.16	−0.09	0.00	−0.31	0.44	–	–
10	*S*	59	0.48	0.50	−0.31	−0.17	0.00	−0.16	–	0.48	–
11	*S*	65	0.93	0.98	0.27	−0.46	0.00	0.22	–	–	0.74
12	*S*	10	1.01	1.0	−0.95	−0.46	0.00	0.22	–	–	0.74
13	*S*	59	0.96	1.07	0.16	−0.17	0.00	0.22	–	–	–
14	*S*	50	0.94	0.96	0.00	−0.17	0.00	0.22	0.44	–	–
15	*S*	68	0.59	0.64	0.21	0.30	0.00	0.22	0.44	–	–
16	*S*	91	0.90	1.14	1.00	0.73	0.19	−0.31	–	–	0.37
17	*S*	92	0.41	0.38	1.06	0.73	0.00	0.22	0.44	–	–
18	*S*	58	0.65	0.76	0.14	0.20	0.00	0.22	–	–	–
19	*S*	62	0.47	0.59	0.21	0.36	0.00	−0.31	0.44	–	–
20	*S*	52	0.81	0.86	0.03	0.36	0.00	−0.31	–	–	0.37
21	*S*	67	0.69	0.75	0.31	0.34	0.00	0.22	–	–	0.74
22	*S*	92	0.34	0.52	0.87	0.66	0.00	−0.31	0.44	–	–
23	*S*	44	0.86	0.93	0.33	0.66	0.00	−0.31	0.44	–	–
24	*R*	55	0.46	0.51	0.03	−0.08	0.15	−0.31	–	–	0.37
25	*S*	88	0.17	0.27	0.33	0.66	0.00	−0.31	0.44	–	–
26	*S*	68	0.63	0.62	1.06	0.66	0.00	0.22	–	0.82	–
27	*S*	52	0.72	0.69	−0.12	−0.08	0.00	0.22	0.44	–	–
28	*S*	49	0.61	0.64	0.00	−0.17	0.00	0.22	0.44	–	–
29	*S*	32	0.66	0.51	−0.33	−0.17	0.00	0.22	–	0.82	–
30	*S*	46	0.92	0.89	−0.07	0.17	0.00	−0.31	–	0.82	–
31	*S*	71	0.48	0.33	0.39	0.36	0.00	−0.16	–	0.82	–
32	*S*	52	0.54	0.59	0.03	0.36	0.00	−0.31	0.44	–	–
33	*S*	46	0.67	1.01	−0.07	−0.08	0.00	0.22	–	–	–
34	*S*	79	0.48	0.52	0.58	0.36	0.00	−0.16	–	0.82	–
35	*S*	53	0.35	0.91	0.05	0.36	0.00	−0.31	–	–	0.37
36	*S*	92	61	49	1.08	0.81	0.00	0.22	–	–	–
37	*S*	44	0.17	0.27	−0.10	0.02	0.38	0.22	–	–	–
38	*S*	55	0.20	0.22	0.09	0.33	0.00	−0.31	0.44	–	–

[a] P – percentage inhibition data of the Gastric H^+/K^+ at 10 μmol concentration.
[b] FA – free acidity.
[c] TA – total acidity.
[d] ss1 – HOMO in vicinity of nitrogen of pyrrolidin-2-one.
[e] ss2 – hydrophobicity in the vicinity of carbonyl oxygen of 5-oxo-pyrorolidine-2-carboxylic acid derivatives or ether oxygen of dihydro-pyrrolo[1,2-*c*]oxazole-1,5-dione.
[f] ss3 – presence of H-acceptor in the vicinity of carbonyl at the 5th position of dihydro-pyrrolo[1,2-*c*]oxazole-1,5-dione.
[g] ss4 – hydrophobicity in the vicinity of 3rd carbon of pyrrolidin-2-one in some of the compounds.
[h] ss5 – steric effects in terms of refractivity in the vicinity of 3rd carbon of pyrrolidin-2-one in some of the compounds.

experimental models, and further experiments to define the nature of inhibition were also conducted (Table 2).

Various amino acids have been found to be protective against gastric ulcers [28–30]. Proton pump is associated with three activities (e.g. vesicular H^+ transport, H^+/K^+-ATPase activity and *p*-NPPase activity) depending on potassium and substrate concentration. In vitro experiments were thus designed to evaluate its mechanism of proton pump inhibition (vesicular H^+ transport, H^+/K^+-ATPase activity and *p*-NPPase activity) by compound **13**. Different optimum concentrations and substrates were used during mechanistic evaluation for vesicular H^+ transport, H^+/K^+-ATPase and *p*-NPPase activity. Substrate concentrations were varied (3–1000 μM) to evaluate the mechanism of action of compound **13**. This compound, which had significant inhibitory effect on rat vesicular H^+ transport, was tested for its *p*-NPPase and H^+/K^+-ATPase activity in rat gastric microsomes (Tables 4 and 5). It was observed that **13** is a potent inhibitor and its IC_{50} for vesicular H^+ transport, *p*-NPPase and H^+/K^+-ATPase were 10 μM, 300 μM and 300 μM, respectively, whereas IC_{50} of standard drug omeprazole is 10 μM for all the three activities. In addition **13** was tested for its nature of proton pump inhibition by evaluating vesicular H^+ transport before and after washing the microsomal vesicles with or without compound treatment (Table 3). It was observed that upon incubation with compound **13**, the proton pump activity was inhibited by 60% but was restored to 90% after several washings of the microsomes pretreated with **13**, suggesting that binding of **13** was reversible in nature. On the other hand omeprazole, a standard anti-ulcer drug, exhibited 71% inhibition of vesicular H^+ transport before as well as after washing the microsomes. This suggests that in contrast to omeprazole, an irreversible inhibitor, **13** is a reversible inhibitor. In order to find out the binding sites for the test compound, dose dependent effects of compound **13** and omeprazole were studied on ATPase and *p*-NPPase activities of gastric proton pump at different substrate concentrations (Tables 4 and 5). Compound **13** showed dose dependent inhibition of ATPase and *p*-NPPase activities at 1 mM ATP and 0.3 mM *p*-NPP, respectively. It was observed that inhibition was more at lesser substrate concentration and vice versa thereby indicating that compound was competing with substrate for the binding while inhibitory effect of omeprazole was not influenced by change in substrate concentration. This also reaffirms the reversible nature of inhibition of proton pump by **13** which seems to compete for the substrate-binding site different to that for omeprazole.

In order to better elucidate the structure and gastric proton pump inhibitory activity (*P*) of the 38 compounds, molecular modeling and 3D-QSAR studies were undertaken. All the compounds were considered for the development of 3D-QSAR models, which relate the spatial and physicochemical properties as independent variables and log ($P/100 - P$) values as dependent variables (Table 1). Among several 3D biophoric models for all the molecules of training set, one model was selected based on the following criterion: correlation coefficient $r^2 > 0.65$, the difference between RMSE and RMSP < 0.04, chance ≤ 0.01, number of variables < 6, match > 0.30 and compounds > 36. This model has three biophoric (pharmacophoric) sites: one being nitrogen at position **A** capable of donating electrons and the second and third being the oxygen of the carbonyl group at positions **B** and **C**, respectively, (Figs. 2 and 3). The spatial dispositions of these biophoric sites (pharmacophores) which are present in each compound of the training set important for proton pump inhibitory activity, depends not only on the physicochemical properties of the biophoric centers corresponding to sites **A** (charge_heteroatom: -0.4112 ± 0.0174), **B** (charge_heteroatom: -0.3556 ± 0.0226, Don_01: 8.304 ± 0.0814) and **C** (charge_heteroatom: -0.3012 ± 0.0301, Don_01: 8.4016 ± 0.1434) but also on their spatial arrangements; the mean distances between the biophoric descriptor centers being **A**, **B** and **C** are **A**–**B** (2.2678 ± 0.032), **B**–**C** (3.0738 ± 0.303), and **A**–**C** (4.6567 ± 0.383).

In addition to the identification of the three key structural features described above as biophoric (pharmacophoric) sites for all the molecules, 3D multiparameter equations were

Table 2
Statistical details of the best selected model

Model no.	RMSA[a]	RMSP[b]	r^2	Chance	Size	Match	Variable	No. of compounds
1.	0.27	0.30	0.69	0.0	3	0.35	5	38

[a] Root mean square error of approximation.
[b] Root mean square error of prediction "leave one out".

Table 3
Proton pump (vesicular transport) inhibitory activity of compound **13** and omeprazole treated microsomes before and after washing the microsomes

Treatment	Enzyme activity/ 10 min/μg protein (before washing)	Enzyme activity/ 10 min/μg protein (after washing)
Control enzyme activity	29.60 ± 1.02	28.75 ± 0.52
Enzyme + omeprazole (10 μM)	8.66 ± 2.63*	10.88 ± 1.2*
Enzyme + **13** (10 μM)	14.68 ± 1.41*	25.33 ± 1.23

*$p < 0.05$ in comparison to control.

Table 4
Effect of omeprazole and compound **13** on H^+/K^+-ATPase activity at various concentrations of ATP

Compound	Concentration (μM)	% Inhibition from control at varying concentrations of ATP (mM)		
		0.3	1.0	3.0
Omeprazole	3	38 ± 2	38 ± 5	34 ± 5
	10	56 ± 2	51 ± 5	53 ± 5
	30	75 ± 2	71 ± 0.6	76 ± 7
13	3	20 ± 4	12 ± 2	25 ± 8
	10	23 ± 1	28 ± 7	30 ± 7
	30	27 ± 2	12 ± 2	34 ± 5
	100	32 ± 0.3	16 ± 0.5	28 ± 6
	300	51 ± 3*	51 ± 3	46 ± 6
	1000	67 ± 3*	55 ± 3	50 ± 8

*$p < 0.05$ as compared to inhibition at 3 mM ATP concentration.

Table 5
Effect of omeprazole and compound **13** on *p*-NPPase activity at various concentrations of *p*-NPP (mM)

Compound	Concentration (μM)	% Inhibition from control at different concentrations of *p*-NPP (mM)		
		1.0	3.0	5.0
Omeprazole	3	28 ± 2	28 ± 3	ND
	10	54 ± 3	52 ± 9	48 ± 5
	30	77 ± 11	78 ± 7	79 ± 9
	100	ND	ND	96 ± 4
13	3	13 ± 5	26 ± 11	ND
	10	16 ± 3	16 ± 7	5 ± 2
	30	33 ± 5*	8 ± 2	13 ± 3
	100	42 ± 7*	18 ± 4	16 ± 7
	300	56 ± 6*	35 ± 1	ND
	1000	85 ± 0.7*	38 ± 8	ND

ND = not done; * $p < 0.05$ as compared to % inhibition at 3 mM/5 mM *p*-NPP concentration.

derived using these pharmacophores as a template for superimposition. The percentage inhibition activity data log $(P/100 - P)$ was related to five secondary site parameters (variables) (Figs. 2 and 3) (Tables 1 and 2): HOMO (**ss1**) [(2.293 ± 0.147), (0 ± 0), (3.074 ± 0.302) Å from the biophoric sites **A**, **B**, **C**, respectively], hydrophobicity (**ss2**) [(4.678 ± 0.423), (3.074 ± 0.302), (0 ± 0) Å from the biophoric sites **A**, **B**, **C**, respectively], presence of H-acceptor (**ss3**) [(5.694 ± 0.084), (3.507 ± 0.135), (2.398 ± 0.396) Å from the biophoric sites **A**, **B**, **C**, respectively], hydrophobicity (**ss4**) [(2.629 ± 0.179), (1.789 ± 0.426), (4.09 ± 0.189) Å from the biophoric sites **A**, **B**, **C**, respectively] and steric effect in terms of refractivity (**ss5**) [(2.376 ± 0.047), (2.292 ± 0.029), (3.156 ± 0.477) Å from the biophoric sites **A**, **B**, **C**, respectively] (Eq. (1) and Table 2). The positively contributing parameters include presence of H-acceptor in the vicinity of carbonyl at the 5th position of dihydro-pyrrolo[1,2-*c*]oxazole-1,5-dione (**ss3**) and steric effect in terms of refractivity in the vicinity of 4th carbon of 5-oxo-pyrrolidin-2-carboxylates in some of the compounds (**ss5**), while the negatively contributing parameters were HOMO in the vicinity of nitrogen of pyrrolidin-2-one (**ss1**), hydrophobicity in the vicinity

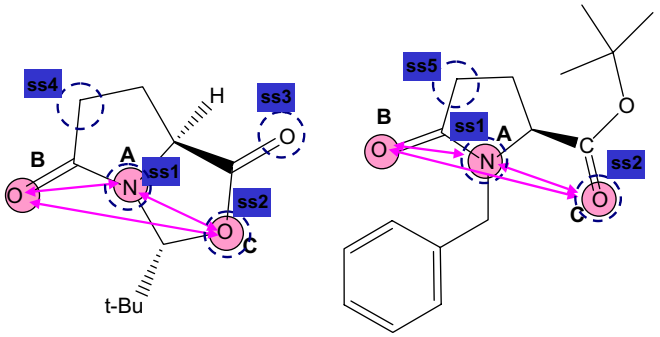

Fig. 2. Schematic representation of the best APEX-3D model, the filled spheres represent biophoric sites and the broken lined circles represent the secondary sites (ss).

of carbonyl oxygen of 5-oxo-pyrorolidine-2-carboxylic acid derivatives or ether oxygen of dihydro-pyrrolo[1,2-*c*]oxazole-1,5-dione (**ss2**) and hydrophobicity in the vicinity of the carbon adjacent to amide function in other classes of compounds (**ss4**).

$$\log(P/100-P) = 0.767[\text{HOMO}](\pm 0.296) - 0.624 \times(\text{Hydrophobicity})(\pm 0.116)+0.440[\text{H-Acceptor}] \times(\pm 0.111)-0.960[\text{Hydrophobicity}](\pm 0.163)+0.254 \times[\text{Refractivity}](\pm 0.054) - 0.3 \quad (1)$$

4. Conclusion

We have demonstrated that *N*-acyl derivatives of α-amino acids are effective inhibitors of gastric proton pump, and have identified a series of amino acid derivatives with improved biological activity using both in vitro and in vivo assays. The hydrophobicity of compounds seems to positively correlate with the in vitro proton pump inhibition. However, the high order of in vitro inhibition by simple natural amino acid derivatives, and potent in vivo activity of more complex molecules are quite baffling and seem to suggest that the latter compounds could be binding promiscuously to other targets and role of other parameters such as bioavailability could not be ruled out. The study on the mode of inhibition of **13** suggests it to be a competitive inhibitor. However, in view of its low order of inhibition in *p*-NPPase assay which is known to reflect more truly the therapeutic efficacy, its in vivo efficacy appears not to be entirely due to proton pump inhibition. Finally, the 3D-QSAR studies have led to the identification of essential structural features for gastric proton pump inhibitory activity in terms of the physicochemical properties (charge_heteroatom, and Don_01) and their spatial dispositions. It is revealed that the carbonyl oxygen atoms of either carbonyl oxygen of 5-oxo-pyrorolidine-2-carboxylic acid derivatives or ether oxygen of dihydro-pyrrolo[1,2-*c*]oxazole-1,5-dione and the carbonyl oxygen atom of pyrrolidin-2-one and the nitrogen atom of these compounds in proper spatial disposition are essential and crucial for the activity in the present set (training set) of thirty-eight (38) compounds. The equations obtained for the model also suggest that the inhibition of gastric proton pump activity is influenced by HOMO, hydrophobicity, presence of H-acceptor groups and steric refractivity at the various secondary sites. The hydrophobicity (in the vicinity of **ss2** and **ss4**) contributes negatively while HOMO (in the vicinity of **ss1**), presence of H-acceptor (**ss3**) and steric effect in terms of refractivity (**ss5**) contribute positively to gastric proton pump inhibitory activity.

5. Experimental protocols

*5.1. Synthesis of cis-5-(2-phenylethenyl)-2-oxo-oxazolidine-4-carboxylic acid (**13**)*

To a cooled (0 °C) solution of ethyl *E*-2,3-*anti*-2-(benzyloxycarbonylamino)-3-hydroxy-5-phenylpent-4-enoate [22] (3 g,

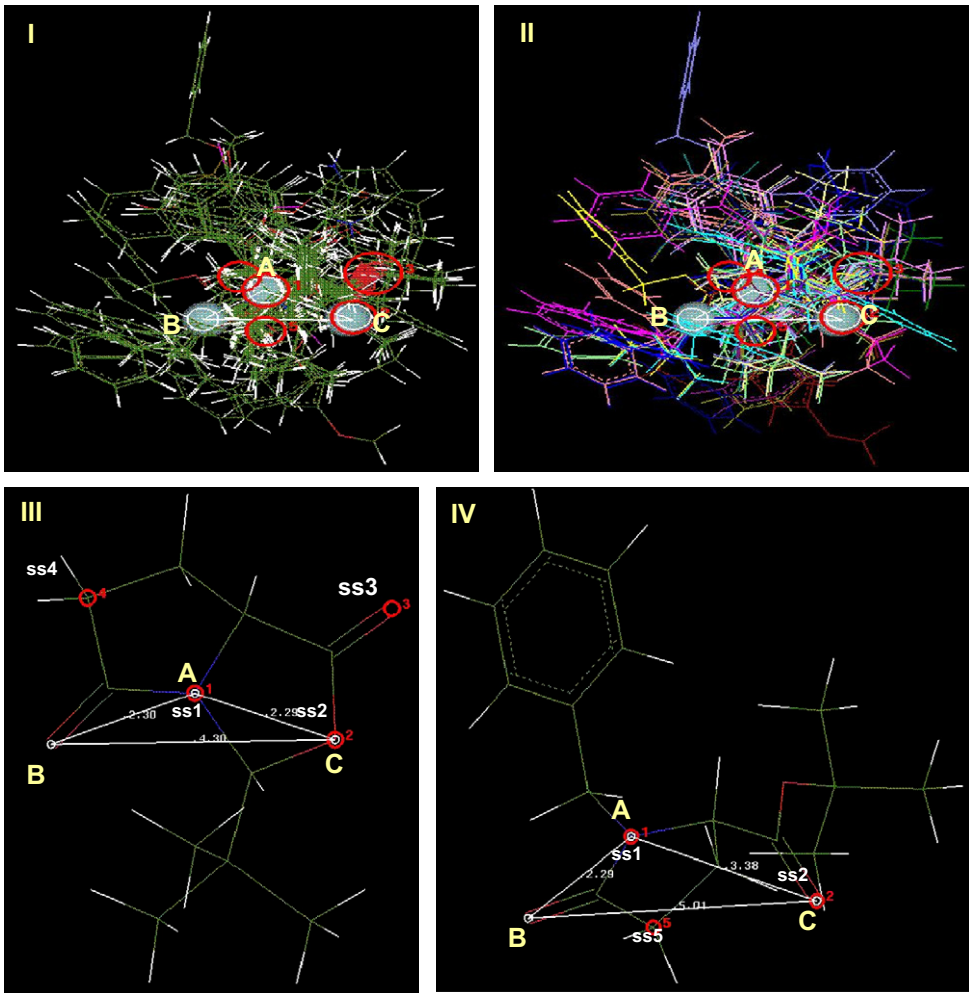

Fig. 3. Models of all 38 molecules superimposed according to a common pharmacophore (solid spheres): (**I**) the molecules are color coded by atom types (left) and (**II**) different molecules are indicated in different colors (right). Red circles represent the secondary sites. (**III**) Molecule **36** (left) and (**IV**) molecule **22** (right) mapping to the pharmacophore (white circles) and depicting the positions of the secondary sites (red circles).

0.0081 mol) in methanol–water (100 ml, 2:1), NaOH (1.8 g dissolved in 5 ml of water) was added slowly. The reaction mixture was stirred at 0–5 °C for 15 min and allowed to warm up to room temperature and stirred for additional 1 h. It was then concentrated under aspirator vacuum, the residue made acidic by slow addition of conc. HCl under ice cooling and extracted with ethyl acetate (3 × 100 ml). The organic layer was washed with chilled saturated brine, dried over anhyd. Na_2SO_4 and concentrated to about 20 ml. Addition of hexane (40 ml) to the concentrate and cooling gave **13** (1.35 g, 71%) as white amorphous solid. m.p. 141–143 °C; IR (KBr) 1700–1740, 3000, 3400 cm^{-1}, *m/z* 233 (M^+), PMR (DMSO-d_6) 90 MHz: δ 4.40 (1, d, $J = 9$ Hz, 4-*H*), 5.35 (1, t, $J = 7$ Hz, 5-H), 6.10 (1, d, $J = 6$ and 17 Hz, PhCH=CH), 6.72 (1, d, $J = 17$ Hz, PhCH=CH) 7.31(5, bs, Ar-*H*); analyzed satisfactorily for $C_{12}H_{11}NO_4$.

5.2. Molecular modeling

All molecular modeling and 3D-QSAR studies were performed on Silicon graphics *INDY* R-4000 workstation employing Insight II (Builder, Discover, MOPAC, APEX-3D) software of Molecular Simulation Incorporation (MSI) [31]. The molecular structures of all thirty-eight (38) compounds were constructed using the sketch menu in the builder module and were optimized for their geometry (net charge 0.0) by keeping the force field potential action, partial charge action and formal charge action fixed. The energy minimization method for each compound employed the steepest descent, conjugate gradients and Newton–Raphson's algorithms in sequence followed by Quasi-Newton optimized procedure with a maximum number of iterations set at 1000 and using energy tolerance value of 0.001 kcal/mol. The molecular structures were stored in MDL format for computation of MOPAC version 6.0 (MNDO Hamiltonian) [32] based indexes, such as atomic charge, π-population, electron donor and acceptor index, HOMO and LUMO coefficients and hydrophobicity and molar refractivity based on atomic contributions.

The percentage inhibition data of the Gastric H^+/K^+ at 10 μmol concentration (*P*) was used by APEX-3D program for automated biophore identification and 3D-QSAR model

building. The 3D-QSAR equations were derived with the site radius set at 0.80, occupancy at 8, sensitivity at 1.0 and randomisations at 100. The biophoric sites and secondary sites combined to global properties (total hydrophobicity, total refractivity, and indicator) were used to obtain an equation to predict the pharmacological activity. The biophoric sites were set to charges, π-population, HOMO, LUMO, hydrogen acceptor, hydrogen donor, refractivity and hydrophobicity. The secondary sites were set to hydrogen acceptor, presence; hydrogen donor, presence; heteroatom, presence; hydrophobic, hydrophobicity; steric, refractivity; ring, presence.

Quality of each model was estimated from the observed r^2 (correlation coefficient), RMSA (root mean square error of approximation), RMSP (root mean square error of predicted on 'leave one out' basis), chance (ratio of the equivalent regression equations to the total number of randomized set; 0.00 being the best corresponding to 0% chance correlation) and match parameter.

5.3. Pharmacological assays

All the tests/evaluations viz. H^+-vesicular transport, H^+/K^+-ATPase activity, *p*-nitrophenyl phosphatase (*p*-NPPase) activity, induction of ulcers (pylorus ligation), and free and total acidity, were done according to the ethical guidelines of the institute.

5.3.1. Preparation of gastric microsomes [33]

Gastric microsomes were prepared by standard density gradient method. Sprague Dawley rats (130–150 g) were fasted 24 h prior to the pyloric ligation in mesh bottom cages and water was provided ad libitum. Stomach was collected under ether anesthesia. Gastric lining was scrapped with the help of a scalpel blade in Harm's buffer (10 mM triethanolamine, 10 mM acetic acid, 150 mM KCl and 5 mM $MgSO_4$; pH 7.4) containing 0.25 M sucrose, 5 μg/ml PMSF and 2 μg/ml leupeptin. The scrapings were homogenized using Ultra Turrax-T25 and homogenate was used to isolate microsomal vesicles containing H^+/K^+-ATPase using differential centrifugation. Microsomal pellet was washed and used for measuring H^+ vesicular transport.

5.3.2. H^+-vesicular transport [34]

The microsomal pellet was homogenized in acidification buffer (20 mM HEPES, 150 mM KCl and 5 mM $MgSO_4$; pH 7.4) using glass homogenizer. Assay system contained 5–10 μg enzyme, 10 μM valinomycin and 2.5 μM acridine orange (AO). Alterations in AO fluorescence at excitation wavelength 493 nm and emission wavelength 530 nm were monitored continuously for 20 min after the addition of ATP (5 mM). Vesicles were preincubated for 30 min at 37 °C in the presence of omeprazole or test compounds. Results have been expressed as percent inhibition in comparison to the control activity.

5.3.3. H^+/K^+-ATPase activity [34]

H^+/K^+-ATPase activity in gastric microsomes was measured by the method of Lee and Forte [34]. The assay medium for H^+/K^+-ATPase activity contained 40 mM Tris–HCl buffer pH 7.4, 2 mM $MgCl_2$, 10 mM KCl or H_2O (blank), 50 μg vesicular protein and drugs/test compound in a total volume of 1 ml. The assay mixture was incubated at 37 °C for 30 min followed by the addition of Tris–ATP (3 mM) and reaction was terminated after 20 min by adding 1 ml chilled 10% trichloroacetic acid. Liberation of inorganic phosphate (Pi) from ATP was measured at 530 nm. Percent inhibition of enzyme activity in the presence of compounds was calculated as compared to control activity. In order to assess the nature of inhibition of the test compound, the proton pump activity was measured in compound or omeprazole treated microsomes before and after washing the microsomal vesicles. Besides, proton pump activity was also measured at variable concentrations of ATP (0.3, 1.0 or 3 mM).

5.3.4. *p*-Nitrophenyl phosphatase (*p*-NPPase) activity [35]

p-NPPase activity in gastric microsomes was measured as described by Keeling et al. [35]. The assay medium contained 10 mM PIPES/Tris buffer (pH 7.0), 5 mM KCl and microsomal suspension or H_2O (blank) in total volume of 1 ml. The reaction was started by addition of *p*-nitrophenyl phosphate (*p*-NPP, 5 mM) to the assay mixture, after 20 min of incubation at 37 °C the reaction was stopped by adding 0.5 ml NaOH (0.5 N) and hydrolysis of *p*-nitrophenyl phosphatase was measured spectrophotometrically at 410 nm. Drugs/test compounds were preincubated at different concentrations with assay mixture for 30 min at 37 °C prior to addition of *p*-NPP. Results have been reported as percent inhibition of enzyme activity in the presence of compounds in comparison to control. Experiments were also conducted at different concentrations of *p*-NPP (1.0, 3.0 or 5.0 mM) to evaluate the nature of inhibition of the test compound.

5.3.5. Induction of ulcers (pylorus ligation) [36]

Rats were fasted for 24 h before pylorus ligation and provided free access to water. Abdomen was opened by a small midline incision below xiphoid process under ether anesthesia. Pyloric end of the stomach was lifted slightly and ligated. Standard drug or test compounds were administered orally 1 h prior to ligation. After 4 h of ligation, animals were sacrificed, gastric contents were collected and stomach was washed with chilled saline. The stomach was opened along the greater curvature and used for assessment of gastric ulcers.

5.3.6. Free and total acidity

Gastric contents were used for measuring free and total acidity of the gastric juice. Free and total acidity were measured by titration against 0.01 N NaOH using Topfer's reagent and phenolphthalein as indicators. The results have been expressed as the ratio of percent inhibition in experimental vs. control group.

5.3.7. Statistical analysis

The anti-ulcer ED_{50} (dose protecting 50% of animals from gastric ulceration in a group) was determined by Probit analysis. The data concerning the gastric secretion and pH were

analyzed by '*t*' test whereas; Chi-square test was used for data related to gastric ulceration.

References

[1] M. Michael Wolfe, H.S. Andrew, N. Engl. J. Med. 319 (1988) 1707.
[2] G. Sachs, Biochim. Biophys. Acta 805 (1984) 181.
[3] S. Okebe, K. Takagi, Jpn. J. Pharmacol. (1968) 18.
[4] Okebe Susumu, Koji Takeuchis, Keita Nakamura, Keijero Takagi, Jpn. J. Pharmacol. 24 (1974) 363.
[5] L.C. Baak, J.B. Jansen, C.B. Lamers, Dig. Dis. Sci. 35 (5) (1990) 596.
[6] W.B. Feig, D.L. Nahrwold, Surgery 104 (2) (1988) 412.
[7] L.G. Ignatenko, Vopr. Pitan. 3 (1984) 51.
[8] E.Y. Yang, D.L. Nahrwold, Surgery 114 (5) (1993) 915.
[9] L.C. Baak, J.B. Jansen, C.B. Lamers, Infusions therapie 17 (1990) 321.
[10] J. Schmidtler, W. Schepp, V. Schusdziarra, M. Classen, Digestion 44 (4) (1989) 211.
[11] M. Tariq, A.R. Al Mougary, Res. Commun. Mol. Pathol. Pharmacol. 97 (2) (1997) 171.
[12] N. Bulbuller, M.A. Akkus, Y.S. Ilhan, F. Baysal, I. Ozercan, E. Aygen, C. Kirkil, Ulus. Travma Derg. 9 (2) (2003) 90–95.
[13] I. Brzozowska, P.C. Konturek, T. Brzozowski, S.J. Konturek, S. Kwiecien, R. Pajdo, D. Drozdowicz, M. Pawlik, A. Ptak, E.G. Hahn, J. Pineal Res. 32 (3) (2002) 149–162.
[14] Y. Ohta, K. Nishida, Clin. Exp. Pharmacol. Physiol. 29 (2002) 32–38.
[15] N. Kalia, K.D. Bardhan, M.W. Reed, S. Jacob, N.J. Brown, J. Gastroenterol Hepatol. 15 (2000) 915–924.
[16] K. Hariganesh, J. Prathiba, J. Pharm. Pharmacol. 52 (2000) 1519–1522.
[17] J. Del Valle, T. Yamada, J. Clin. Invest. 85 (1990) 139.
[18] C.T. Richardson, M. Feldman, Regul. Pept. 10 (1995) 339.
[19] S.W. He, K.Q. Shen, B. He Yjxie, Y.M. Zhao, World J. Gastroenterol. 5 (1999) 408.
[20] R. Magnous, J.P. Bali, Regul. Pept. 7 (1983) 233.
[21] T. Kobayashi, V.S. Zinchuk, E. Garcia Del Saz, Jiang Y. Yamasaki, S. Kataoka, T. Ekeda, S. Tsunavaki, H. Seguchi, Histol. Histopathol. 15 (2000) 1067.
[22] D.K. Dikshit, Singh Sangeeta, Tetrahedron Lett. 29 (1988) 3109.
[23] D.K. Dikshit, A. Maheshweri, Tetrahedron Lett. 36 (1995) 6131.
[24] E. Campaigne, D.M. Matthews, J. Heterocycl. Chem. 12 (1975) 391.
[25] D.K. Dikshit, S. Singh, G.K. Patnaik, R.C. Srimal, B.N. Dhawan, Indian Patent No. 178841.
[26] K.S. Soai, A. Ookawa, J. Chem. Soc. Perkin Trans. 1 (1986) 759.
[27] P.L. Pavash, E. Sparrow, J.C. Gathe, J. Am. Chem. Soc. 72 (1950) 1415.
[28] R. Yan, Y. Sun, R. Sun, Zhonghua Zheng Xing Shao Shang Wai Ke Za Zhi 11 (1995) 189.
[29] A. Houdijk, P.A. Van Leeuwem, M.A. Boermeesterm, Am. J. Physiol. 267 (1994) G 1035.
[30] T. Brzozowski, S.J. Konturek, Z. Sliwowski, J. Gasterenterol. 32 (1997) 442.
[31] Insight II, Version 2.3.0, San Diego Biosym Technologies, 1993.
[32] J.J.P. Stewart, J. Comput. Aided Mol. Des. 4 (1990) 1.
[33] F.R. Zuo, S.G. Li, Z.H. Lin, Biochem. Biophys. Acta 23 (1991) 196.
[34] H.C. Lee, J.G. Forte, Biochim. Biophys. Acta 508 (1978) 339.
[35] D.J. Keeling, S.M. Laing, Senn-Bilfinger, J. Biochem. Pharmacol. 42 (1988) 123–130.
[36] M. Shay, S.A. Kamrov, D. Fels, O. Meraze, H. Gruestein, H. Siplet, Gastroenterology 5 (1945) 43.