ANTI-ULCER ACTIVITY OF 1-SUBSTITUTED IMIDAZOLES AGAINST GASTRIC ULCERS IN RATS

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Summary

A variety of 1-substituted imidazoles (1a-1d, 2a-2d) were synthesized and characterised by FTIR, ^{1}HNMR, DART-MS and Elemental spectral data. Synthesized compounds were screened for their antiulcer activity by Indomethacin with pylorus ligation induced ulcer and absolute alcohol induced ulcer model. Parameters like gastric volume, pH, free acidity, total acidity, ulcer index and % inhibition were measured for antiulcer activity at 20 and 40 mg/kg and compared with the standard drug Ranitidine at 20mg/kg. Histopathological studies of stomachs were performed for all groups and interpreted. The ulcer index in the test group was found to be significantly less ($P<0.01$) when compared to control group and comparable with that of standard. At higher dose (40 mg/kg) of both the model, compounds 1b and 2b reduced these ulcer lesions as evidenced by a significant ($P < 0.01$) reduction in the ulcer index when compared with the control group.

Key words: Synthesis; 1-Substituted Imidazoles; Indomethacin with Pylorus Ligation; Absolute Alcohol; Gastric Acidity.

Introduction

The stomach and the surrounding portions of the gastro intestinal tract provide a precisely balanced environment to process food. Strong acids (HCl) and other chemicals (Acetylcholine and Histamine) in the stomach break down food into more basic components that can move through the digestive system. These chemicals, or gastric juices, are also strong enough to damage the lining that protects the stomach and other gastro intestinal organs. A complex, multilayered coating forms a barrier to protect the lining. This barrier is composed of many elements, including mucus, bicarbonate and chemicals called prostaglandins. Any change among the balance of these elements can weaken the barrier and allowing gastric juices to damage the underlying tissue. At first this damage may only irritate or inflame the lining, a condition called gastritis. Eventually enough corrosion forms a sore called a peptic ulcer.

Imidazole nucleus has proved to be an abundant source for a number of medicinal agents and associated with many activities viz, antiprotozoal, mutagenic properties, anticancer, antiviral, enzyme inhibition, H_2-antagonism, α-adrenergic agonism and β-blocking, anticonvulsant, broad spectrum antibacterial and antifungal activities [1-11].

Cimetidine is a H_2-receptor antagonist containing imidazole moiety and renowned to be the most active compound for the treatment of gastric ulcer. It is well known that imidazoles are very much effective on Histamine receptors which are found principally in the parietal cells. In view of this an attempt has been made to study the antiulcer activity of some newly synthesized 1-substituted imidazoles (1a-1d, 2a-2d). Therefore in the present revision a search of these 1-substituted imidazoles possibly led to the development of compounds with probable Histaminic (H_2) antagonistic activity, especially to relieve pain in smooth muscles in conditions like ulcer.

Materials and Methods

Animals:

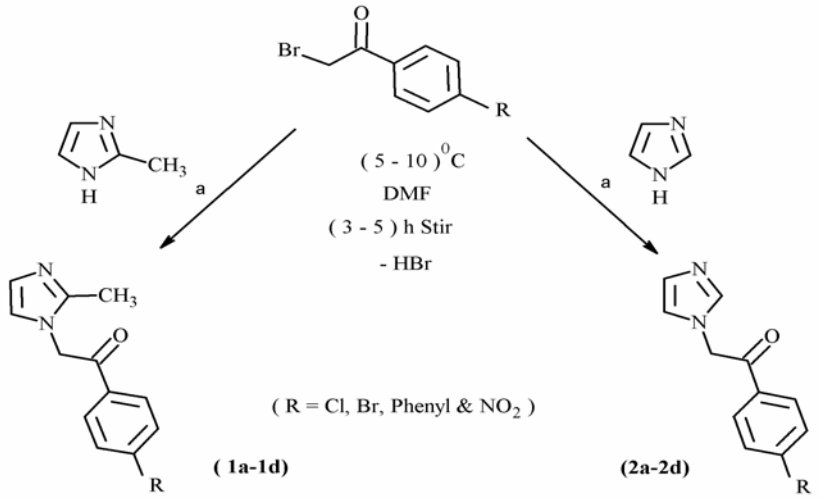
Inbred albino rats of either sex weighing between 200±20 g were used. The animals were housed in standard cages in a controlled temperature and humidity (22±3°C and 60±5%, respectively) on a 12 h light/dark cycle and with standard lab chow and tap water *ad libitum*. The animals were deprived of food for 24 h before experimentation, but had free access to drink water.

Drugs and chemicals used:

Tween 80 was purchased from Merck. Indomethacin was obtained from Lupin Pharmaceuticals, Mumbai, India and Ranitidine hydrochloride from Glaxosmithkline Pharmaceuticals ltd, India. All other standard drugs and chemicals used in this study were obtained commercially from Sigma-Aldrich and were of analytical grade. All the prototypes were dissolved in a minimum quantity of 2 %w/v Tween80 and then the volume was adjusted to 10 ml with normal saline for making the desired concentrations of 20 and 40 mg/kg.

General procedure for Synthesis of 1-substituted imidazoles (1a-1d & 2a-2d) (12)

To a solution of Imidazole/2-methyl imidazole (0.03mol, 2.46 g) in dry DMF (10 ml) was added drop wise to a solution of appropriate para substituted phenacyl bromides (0.002 mol, 0.46 g) in DMF (10 ml) at a temperature of 5-10 ˚C with stirring. The stirring was continued for another 3-6 h at the same temperature. Then the mixture was poured into cold water (20ml) and stirred for further 1 h. The precipitate obtained was removed by filteration and the filterate was extracted with benzene. Upon evaporation of organic layer compounds 1a-1d & 2a-2d were obtained as crystalline mass and are recrystallised from benzene-ethanol. The purity of all compounds was established by single spot on the TLC plates.



Scheme 1. Reagents: a) 2-methyl imidazole, Imidazole, P-substituted phenazyl bromides.

Pharmacological Evaluation

Treatment protocol for Antiulcer activity

The animals were divided into their respective groups each containing six animals. In which one group of normal non ulcer induced (Indomethacin not treated) animals receiving the vehicle 2 %w/v of Tween 80 (10 ml/kg) denoted as normal control and another group of ulcer induced (Indomethacin treated) animals receiving the vehicle 2 %w/v of Tween 80 (10 ml/kg) named as Indomethacin control. All the protoypes [1(a-d) and 2(a-d)] were given at the doses of 20 mg/kg, and 40mg/kg p.o in 2 %w/v of Tween 80 (10 ml/kg) for a period of 7 days to different groups of animals. Indomethacin an ulcer induced drug was given at the doses of 20 mg/kg p.o in 2 %w/v of Tween 80 (10 ml/kg). Standard drug Ranitidine hydrochloride was given at the dose of 20 mg/kg p.o dissolved in distilled water.

Treatment protocol

Group I	:	Normal animals received 2 %w/v Tween 80 (10 ml/kg p.o.) in distilled water (Normal control).
Group II	:	Indomethacin challenged animals received 2 %w/v Tween 80 (10 ml/kg p.o.) in distilled water (Indomethacin control).
Group III	:	Indomethacin challenged animals received Ranitidine at a dose of 20 mg/kg p.o.
Groups (IV-VII)	:	Indomethacin challenged animals received prototypes [1a-1d] at a dose of 20 mg/kg p.o.
Groups VIII-XI	:	Indomethacin challenged animals received prototypes [2a-2d] at a dose of 20 mg/kg p.o.
Groups XII-XV	:	Indomethacin challenged animals received prototypes [1a-1d] at a dose of 40 mg/kg p.o.
Groups XVI-XXI	:	Indomethacin challenged animals received prototypes [2a-2d] at a dose of 40 mg/kg p.o.

Experimental procedures

Indomethacin + pylorus ligation induced ulcer model

Each group received the respective treatment for 4 days without giving the challenge (Indomethacin). From 5th day to 7th day the animals in each group were administrated Indomethacin orally as an aqueous suspension at a dose of 20 mg/kg, 2 hrs later the administration of respective drug treatment. After the last treatment, animals in each group were fasted for 18 h and were anaesthetized with anesthetic ether. The abdomen was opened by a small midline incision below the xiphoid process and pylorus portion of stomach was lifted out and ligated (13) Precaution was taken to avoid traction to the blood supply. The stomach was sutured with interrupted sutures. Six hours after pylorus ligation the rats were sacrificed and the stomach was removed (14). The gastric contents were collected, centrifuged and the volume of the supernatant was expressed as ml/6 h.

Free and total acidity were determined by titrating with 0.01 N NaOH using Topfer's reagent and phenolphthalein as indicator (15). The free and total acidity were expressed as μequiv/6 h. The stomach was then incised along the greater curvature and observed for ulcers. The number of ulcers was counted using a magnifying glass and the diameter of the ulcers were measured using vernier calipers. The following arbitrary scoring system (16) was used to grade the incidence and severity of lesions: (i) score 0 = no ulcer, (ii) score 1 = denuded epithelium; (iii) score 2 = petechial and flank haemorrhages; (iv) score 3 = one or two ulcers; (v) score 4 = multiple ulcers; (vi) score 5 = perforated ulcer. Ulcer index (UI) was then calculated from the above scorings as follows:

$$\mathbf{UI} = \boldsymbol{U}_{\mathbf{N}} + \boldsymbol{U}_{\mathbf{s}} + \boldsymbol{U}_{\mathbf{p}} \times \mathbf{10}^{-\mathbf{1}}$$

where U_N is the average of number of ulcers per animal, U_s is the mean severity of ulcer score and U_p is the percentage of animals with ulcer incidence. Stomachs were again immersed in 10 % formalin for 24 h and histopathological examinations were carried out subsequently. The results have been presented in Table 1, 2 and Fig 1.

The percentage change with respect to normal control group (% Inhibition) has been calculated using the following formula,

% Inhibition = (UI control group − UI treated) / UI control group × 100

Absolute alcohol induced ulcer model

A similar protocol were been followed for the absolute alcohol induced ulcer model. Animals in all the groups were fasted for 18 h after the respective assigned treatment and were anaesthetized with anaesthetic ether. Prototypes (1a-1d, 2a-2d) and Ranitidine hydrochloride were administered orally 30 min before the oral administration of 1ml/200g of absolute alcohol (17). After an hour the animals were sacrificed and their stomachs excised and gastric contents were aspirated.

Stomachs were removed and immersed in 10 % formalin for 5 min. Each stomach was incised along the greater curvature and examined for linear haemorrhagic lesions in the glandular region. The length (mm) of each lesion was determined at 10X magnification with pair of dividers and each length was summed per stomach. The sum of length (mm) of all lesions for each stomach was used as the ulcer index (UI). Stomachs were again immersed in 10 % formalin for 24 h and histopathological examinations. The results have been presented in Table 3 and Figure 2.

The percentage change from control group (percentage inhibition) has been calculated using the same formula as discussed in Indomethacin + pylorus ligation induced ulcer model.

Statistical analysis

Results were expressed as mean ± S.E.M; n = 6 in each group Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Dunnett's test, with the level of significance at $P<0.001$, $P<0.01$ and $P<0.05$. The percent inhibition of was calculated by (mean control – mean treated) / mean control × 100.

Results and Discussion

In both the models administration of prototypes produced significant decrease in ulcer index in a dose dependent manner (20 mg/kg and 40 mg/kg). The prototypes also significantly reduced the gastric volume, total and free acidity, and increased the pH of the gastric fluid, proving its antisecretory activity

Indomethacin + pylorus ligation induced ulcer model

In Indomethacin + pylorus ligation induced ulcer model compounds 1b, 2b, 2c and 2d significantly reduced the ulcer index at 20mg/kg concentration against control group. In which compounds 1b, 2c and 2d significantly reduced the ulcer index against control group. Compound 2b shows highly significant activity (**$P<0.01$, Table 1) against gastric volume, pH elevation, ulcer index, free acidity and total acidity when compared to control group.

While increasing the concentration (40mg/kg, Table 2), compounds 1b, 1c, 2b, 2c and 2d significantly reduced the ulcer index against control group. In which compounds 1b, 1c and 2d shows their mild inhibition (**$P<0.05$) on ulcer index when compared to control group. Compounds 2b and 2c shows their significant inhibition (**$P<0.01$, Fig 1) on gastric volume, pH elevation, ulcer index, free acidity and total acidity against control group. Whereas the standard drug, ranitidine hydrochloride showed its ulcer protection (***$P<0.001$) against control group.

Table 1. Effect of Prototypes (20mg/kg) and Ranitidine Hydrochloride (20mg/kg) on Gastric Secretion Using Indomethacin + Pylorus Ligation Rat Model

Treatment (p.o)	Dose (mg/kg) p.o	Gastric volume/ml	pH	Free acidity (µ equiv/6h)	Total acidity (µ equiv/6h)	Ulcer index	% Inhibition
Normal control	1ml/kg	01.33±0.02	04.33±0.18	09.83±0.03	09.83±0.03	00.83 ± 0.21	-
Indomethacin Control	1ml/kg	10.50±0.12	1.68±0.13	32.86±0.03	34.14±0.03	29.41 ± 0.93	-
1a	20	7.66±0.32	2.20±0.05	25.58±0.09	27.41±0.07	25.66 ± 0.60	12.74
1b	20	5.4±0.27*	3.75±0.25*	18.93±0.07*	20.26±0.07*	16.50 ± 0.56*	43.9
1c	20	5.56±0.31	3.04±0.35	21.05±0.04	22.38±0.14	20.16 ± 0.77*	31.44
1d	20	5.73±0.25	2.75±0.17	23.06±0.09	24.07±0.10	22.00 ± 0.79	25.21
2a	20	5.58±0.13	2.87±0.12	23.07±0.04	15.30±0.12	21.58 ± 0.80	26.62
2b	20	5.3±0.17**	3.95±0.16**	18.27±0.04**	19.59±0.04**	14.33 ± 0.60**	51.27
2c	20	5.43±0.20*	3.21±0.25*	19.13±0.01*	21.53±0.08*	14.91 ± 0.87*	49.29
2d	20	5.41±0.44*	3.05±0.23*	20.48±0.01*	24.00±0.05*	17.16 ± 1.10*	41.64
Ranitidine	20	1.76±0.033***	4.02±0.09***	10.66±0.033***	11.98±0.023***	02.50 ± 0.25***	91.5

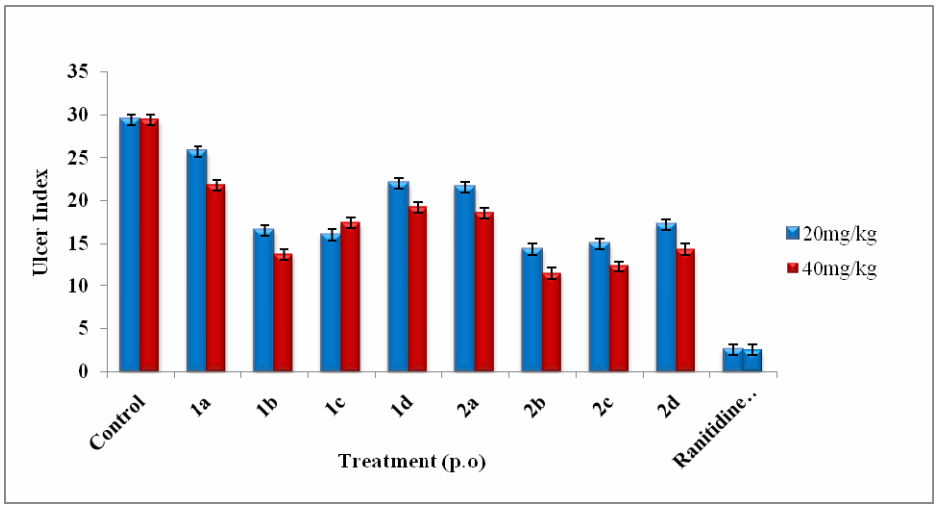
Data are expressed as the mean ± S.E.M; $n = 6$ in each group. *P<0.05, *P<0.01, *** P<0.001 when compared to control group (one-way ANOVA followed by Dunnett's test). Normal control = 2 %w/v Tween 80; Indomethacin Control = Indomethacin + 2 %w/v Tween 80.

Table 2. Effect of Prototypes (40mg/kg) and Ranitidine Hydrochloride (20mg/kg) on Gastric Secretion Using Indomethacin + Pylorus Ligation Rat Model

Treatment (p.o)	Dose (mg/kg) p.o	Gastric volume/ml	pH	Free acidity (µ equiv/6h)	Total acidity (µ equiv/6h)	Ulcer index	% Inhibition
Normal control	1ml/kg	01.33±0.02	04.33±0.18	09.83±0.03	09.83±0.03	00.83 ± 0.21	-
Indomethacin Control	1ml/kg	10.50±0.12	1.68±0.13	32.86±0.03	34.14±0.03	29.41 ± 0.93	-
1a	40	7.66±0.32	2.73±0.19	23.03±0.10	25.40±0.05	21.75 ± 0.81	26.06
1b	40	4.35±0.27*	4.98±0.32*	20.29±0.06*	21.68±0.08*	13.75 ± 0.81*	53.25
1c	40	4.98±0.31*	3.73±0.10*	21.15±0.11*	22.86±0.10*	16.00 ± 0.69*	45.6
1d	40	5.48±0.25	3.45±0.15	21.86±0.07	22.95±0.07	19.16 ± 0.51	34.84
2a	40	5.58±0.13	3.25±0.22	22.53±0.06	23.60±0.07	18.50 ± 0.89	37.11
2b	40	3.61±0.17**	5.37±0.19**	18.65±0.11**	19.96±0.11**	11.41 ± 0.80**	61.18
2c	40	4.61±0.20**	4.21±0.24**	20.24±0.08**	21.31±0.07**	12.25 ± 0.72**	58.35
2d	40	4.86±0.07*	3.59±0.07*	20.50±0.06*	22.46±0.07*	14.33 ± 0.49*	51.27
Ranitidine	20	1.76±0.033***	4.02±0.09***	10.66±0.033***	11.98±0.023***	02.50 ± 0.25***	91.5

Data are expressed as the mean ± S.E.M; $n = 6$ in each group. *P<0.05, *P<0.01, *** P<0.001 when compared to control group (one-way ANOVA followed by Dunnett's test). Normal control = 2 %w/v Tween 80; Indomethacin Control = Indomethacin + 2 %w/v Tween 80.

Fig. 1: Effect of prototypes (20mg/kg and 40 mg/kg) and Ranitidine hydrocholoride (20mg/kg) on inhibition of ulcer index in the Indomethacin + pylorus ligation rat model.



Absolute alcohol induced ulcer model

In absolute alcohol induced ulcer model, administration of ethanol produced haemorrhagic gastric lesions in the gastric mucosa of the control group. Compounds 1b and 2b reduced these lesions as evidenced by a significant ($P<0.01$, Table 3) reduction in the ulcer index when compared with the control group. Compounds 2c and 2d showed their moderate inhibition ($P<0.05$) on ulcer index when compared with the control group.

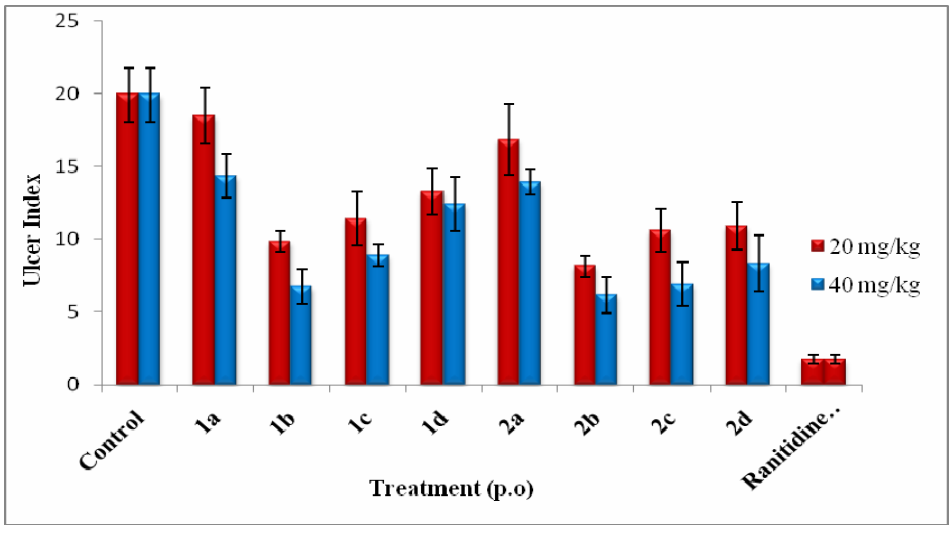
At higher dose of absolute alcohol induced ulcer model (40 mg/kg) compounds 1b and 2b reduced these lesions as evidenced by a significant ($P<0.01$) reduction in the ulcer index when compared with the control group. Compounds1c, 2c and 2d showed their moderate inhibition ($P<0.05$, Fig. 2) on ulcer index when compared with the control group.

Table 3. Effect of Prototypes (20 & 40mg/kg) and Ranitidine Hydrochloride (20mg/Kg) on Absolute Alcohol Induced Gastric Ulcer Model.

Treatment	Ulcer index		Inhibition (%)	
	20 mg/kg	40 mg/kg	20 mg/kg	40 mg/kg
Normal control	00.83 ± 0.21	00.83 ± 0.21	-	-
Absolute alcohol Control	19.91 ± 0.93	19.91 ± 0.93	-	-
1a	17.83 ± 0.91	14.33 ± 0.74	10.46	28.03
1b	09.83 ± 0.35**	06.75 ± 0.60**	50.62	66.1
1c	11.41 ± 0.93	08.91 ± 0.37*	42.67	55.23
1d	13.25 ± 0.78	12.41 ± 0.92	33.47	37.65
2a	16.83 ± 0.84	13.91 ± 0.62	15.48	30.12
2b	08.16 ± 0.35**	06.16 ± 0.62**	58.99	69.03
2c	10.58 ± 0.73*	06.91 ± 0.74*	46.86	65.27
2d	10.91 ± 0.82*	08.33 ± 0.96*	45.18	58.15
Ranitidine	01.75 ± 0.17***	-	91.21	-

Data are expressed as the mean ± S.E.M; *n* = 6 in each group. *P<0.05, *P<0.01, *** P<0.001 when compared to control group (one-way ANOVA followed by Dunnett's test). Normal control = 2 %w/v Tween 80; Absolute alcohol control = Absolute alcohol + 2 %w/v Tween 80.

Fig. 2. Effect of Prototypes (20mg/kg and 40 mg/kg) And Ranitidine Hydrocholoride (20mg/kg) on Inhibition of Ulcer Index in the Absolute Alcohol Induced Gastric Ulcer Model.



The histopathological evaluations were made under the microscope for histopathological changes such as oedema, inflammation, infiltration and erosion and photographs were taken. The rats in the control treated group showed loss of gland architecture with erosion of the epithelial layer and evident oedema and infiltration by inflammatory cell. Prototypes (20 mg/kg) treated rats showed no or less ulceration but intactness of gastric epithelium was not completely restored. Minimal oedema and infiltration was seen in the lower half of the mucosa. Prototypes (40 mg/kg) treated rats showed no ulceration in the mucosa. Glands are regular with complete restoration of gastric epithelium. Ranitidine treated groups showed no ulceration in gastric mucosa, glands were regular and no inflammation was observed.

Conclusion

From the present findings, it is evident that the synthesized 1-substituted imidazoles (1a-1d, 2a-2d) have significant antiulcer activity. These findings could be utilized further to design compounds with superior activity in biological studies to promote their antiulcer effectness which is probably due to the histaminic antagonistic activity particularly with the subtype of H_2-receptors which is one of the mediators in gastric ulcers. Therefore in the present revision a search of these 1-substituted imidazole derivatives possibly led to the development of compounds with probable histaminic (H_2) antagonistic activity, especially to relieve pain in intestinal muscles in conditions like ulcer.

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