H_2-RECEPTOR ANTAGONISTIC ACTIVITY OF SOME N-SUBSTITUTED 2-METHYL IMIDAZOLES

*L. Baskar, Papiya Mitra Mazumder, D. Sasmal and Swastika Ganguly

Department of Pharmaceutical Sciences,
Birla Institute of Technology, Mesra, Ranchi, Jharkhand-835215, India
lbaskii@gmail.com

Summary

Cimetidine is the prototype antiulcer drug having the imidazole nucleus and acts by blocking histamine H_2 receptors. Keeping this context in mind, an attempt has been made to study the antihistaminic activity of some novel N-substituted 2-methyl imidazole derivatives on isolated guinea pig ileum to reveal their desired pharmacological effects. In the present revision, some N-substituted 2-Methyl Imidazoles 1(a-d) were synthesized and confirmed by their FTIR, ^{1}HNMR, MASS and Elemental spectral data. Antagonistic activity of all prototypes were tested in this bioassay at various concentrations (10, 50 and 100 µg/ml), and Concentration-response curves were plotted to check their ability to reverse the activity of histamine on prior contact with the ileum. All the compounds 1(a-d) were producing a competitive antagonistic action at (10 µg/ml), and at higher concentrations (50 and 100 µg/ml) the curves shifted to the right showing maximum inverse agonistic activity which is probably mediated through H_2-receptors.

Key words: N-substituted 2-methyl imidazole, Antihistaminic activity, Guinea pig ileum, H_2-receptor, Concentration-response curve and Histamine.

Introduction

Imidazole nucleus (1) has proved to be a prolific source for a number of medicinal agents. The various activities associated with the imidazole nucleus are antiprotozoal, mutagenic properties, anticancer, antiviral, enzyme inhibitory activities, H_2-Antagonist, α- Adrenergic agonist and β -blocking, anticonvulsant, broad spectrum antibacterial and antifungal activities (2-12). Cimetidine (13) is the prototype antiulcer drug containing imidazole nucleus that acts by blocking histamine H_2-receptors. It is well known that Imidazoles are very much effective on H_2 histamine receptors (14) which are found principally in the parietal cells of the gastric mucosa. Keeping this context in mind, an attempt has been made to investigate the antihistaminic activity of some novel N-substituted 2-methyl imidazoles on isolated guinea pig ileum. Therefore in the present revision, a search of these novel N-substituted 2-methyl imidazole derivatives possibly led to the development of compounds with probable H_2-receptor antagonistic activity

Materials and Methods

Chemicals

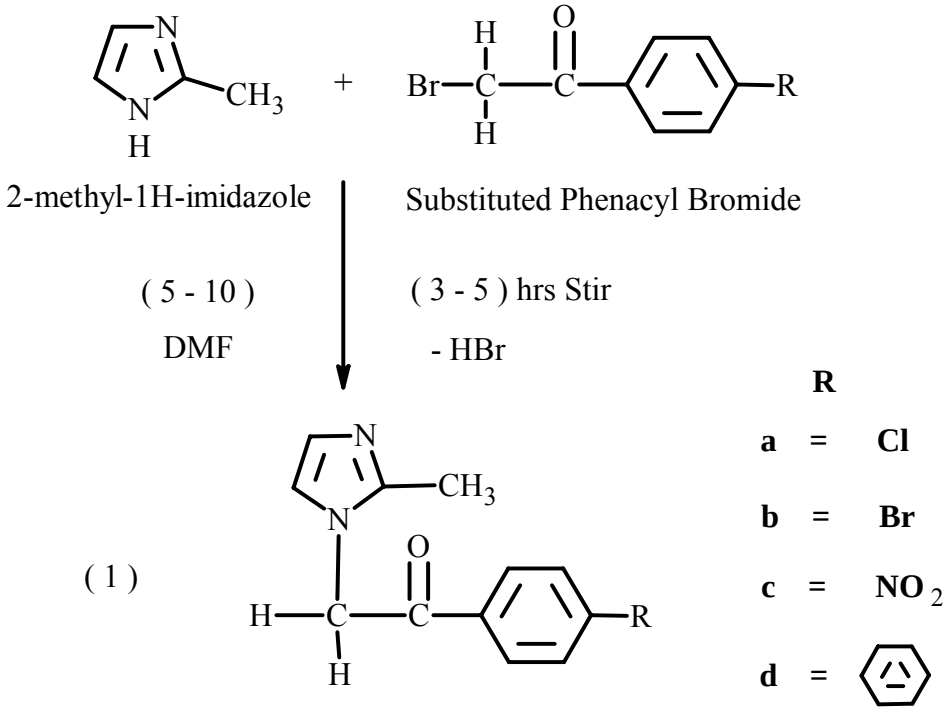
The following drugs and chemicals were used. Drugs: 2-methyl imidazole (Aldrich), phenacyl halides (Aldrich), dimethyl formamide (Sigma), sodium chloride (CDH).

Drugs:

Histamine dihydrochloride (Hi-media) was dissolved in distilled water and desired concentrations were prepared. All the prototypes were dissolved in minimum quantity of 2% v/vTween80 and then the volume was adjusted to 10 ml with normal saline for making the concentration of (10, 50 and 100µg/ml).

Chemical Synthesis

In the present scheme, N-substituted 2-Methyl Imidazole derivatives of the type 1 (Scheme:1) have been synthesized by treating 2-methyl imidazole and various para substituted phenacyl bromides (chloro, bromo, phenyl and nitro) in presence of dry DMF(dimethylformamide) with the cold stirring for about (3-6) hrs. This yielded a solid mass which was recovered from benzene extraction and finally recrystallised and purified and confirmed on the basis of their FTIR, ^{1}HNMR, MASS spectral data. The data were found to be comparable with the earlier report (15).



Scheme:1: Syntheisis of N-Phenacyl- 2-methyl imidazoles

Pharmacological Evaluation

Male albino guinea pig weighing 175–225 g was kept in fasting condition 18 hours prior to commencement of experiment and given water ad libitum. It was then sacrificed by a blow to the head and exsanguinated as per CPCSEA recommended guidelines (Animal house Reg no: - 621/02/ac/CPCSEA). The caecum was lifted and the ileocaecal junction was identified (16). The ileum was cut at this point and transferred to a dish containing tyrode solution (17). A terminal segment of ileum about 1-1.5 cm was cut, and intestinal contents were removed and freed from mesenteric attachments. A thread was tied at each end of the tissue taking care that ileum is left open and the thread does not close the lumen (18). The tissue was mounted in 30 ml organ baths filled with tyrode solution. The temperature was maintained at 37°C and oxygenated continuously. Initial tension was 1 g and stabilization time was 45–60 min. Load was adjusted to 0.5g; the magnification of 5-7 folds and bath volume of about 15ml was maintained. The preparation was washed every 10 min with tyrode solution.

After an initial equilibration period of about 30–45 min, Increasing concentrations of histamine (0.1, 0.2, 0.4, 0.8, 1.6, 3.2µg/ml) were added to the bath and the concentration–response curve was recorded with a contact time of 90 seconds.

In addition, the antihistaminic effect of prototype 1(a-d) were tested in this bioassay at various concentrations (10, 50 and 100 µg/ml), in term of their ability to prevent the histamine contractions when they were added to the bath 5 min before histamine. Responses to histamine were recorded as changes in height from baseline and expressed as percent of maximum response of the histamine (19). The CRC was constructed till ceiling effect to histamine was obtained.

Six graded–response curves were obtained for each preparation, with a 20 min-rest between each (20). The mean maximal response obtained from the first concentration–response curve (in the absence of lead compounds) was taken as the 100% response value (21). After completing the CRC of histamine, contractions were recorded using frontal writing lever on kymograph. The kymogram was fixed with fixing solution containing shellac and colophony in alcohol.

Analysis of Results

Contractions were expressed as a percentage of the maximal contraction obtained from the corresponding control curve; each point represents the mean ± S.E.M. of four experiments. The histamine concentration–response curves with and without the antagonists were plotted and compared. The statistical analyses were obtained by the ANOVA test, followed by the Dunnett's test where necessary (21). $P<0.05$ or $P<0.01$ were considered significant.

Results and Discussion

Antagonistic activity of all prototypes were tested in this bioassay at various concentrations (10, 50 and 100 µg/ml), and Concentration-response curves were plotted to check their ability to reverse the activity of histamine on prior (5 min) contact with the ileum (20).

When evaluated against histamine (0.1, 0.2, 0.4, 0.8, 1.6, 3.2µg/ml) all the compounds 1(a-d) at 100µg/ml) significantly antagonized the contraction of guinea pig ileum, in a competitive and concentration-dependent manner. Fig.1 represents the contractile response elicited by histamine on guinea pig ileum in presence and in absence of the experimental compounds 1(a-d). This is manifest on plotting the –log M values (6.2676, 6.9665, 6.6655, 6.3645, 6.0634, 6.7624) against % maximal response (21).

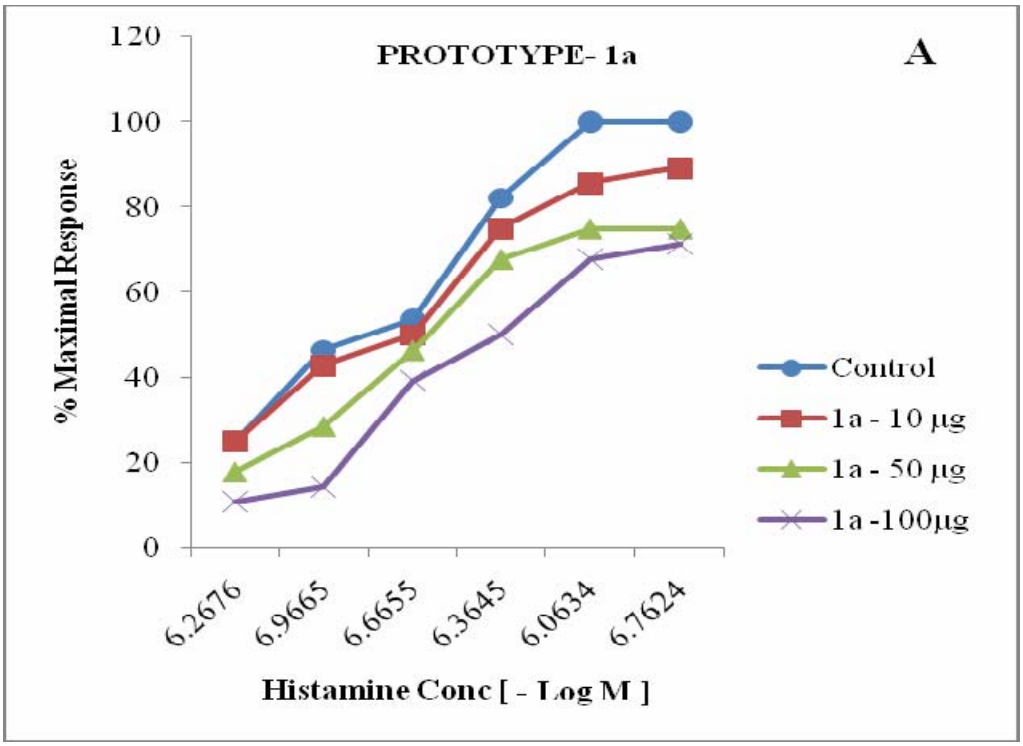
In conclusion, the exposure of guinea pig isolated ileum to prototypes (10, 50 and 100 µg/ml) for a period of 5 min produced a parallel, rightward shift of the histamine concentration-response curve as is evident from the Fig. 1.

All the compounds 1(a-d) were producing a competitive antagonistic action at (10 µg/ml), and at higher concentrations (50 and 100 µg/ml) the curves shifted to the right showing maximum inverse agonistic activity which is probably mediated through H_2-receptors.

The chloro and bromo substituted phenacyl imidazoles showed significant antagonistic action against histamine only at (100µg/ml). The nitro and phenyl substituted phenacyl imidazoles were found to be more effective in their antagonism against histamine at (50µg/ml) as compared to the chloro and bromo substituted compounds. It is probably because of possessing an electron with-drawl groups at their para position (nitro and phenyl).

Conclusion

From the present findings, it is evident that the synthesized N-substituted imidazole derivatives 1(a-d) are showing marked H_2 blocking activity in isolated tissue (20). Thus this may facilitate to design further in vivo studies to check their effect in protecting against ulcer.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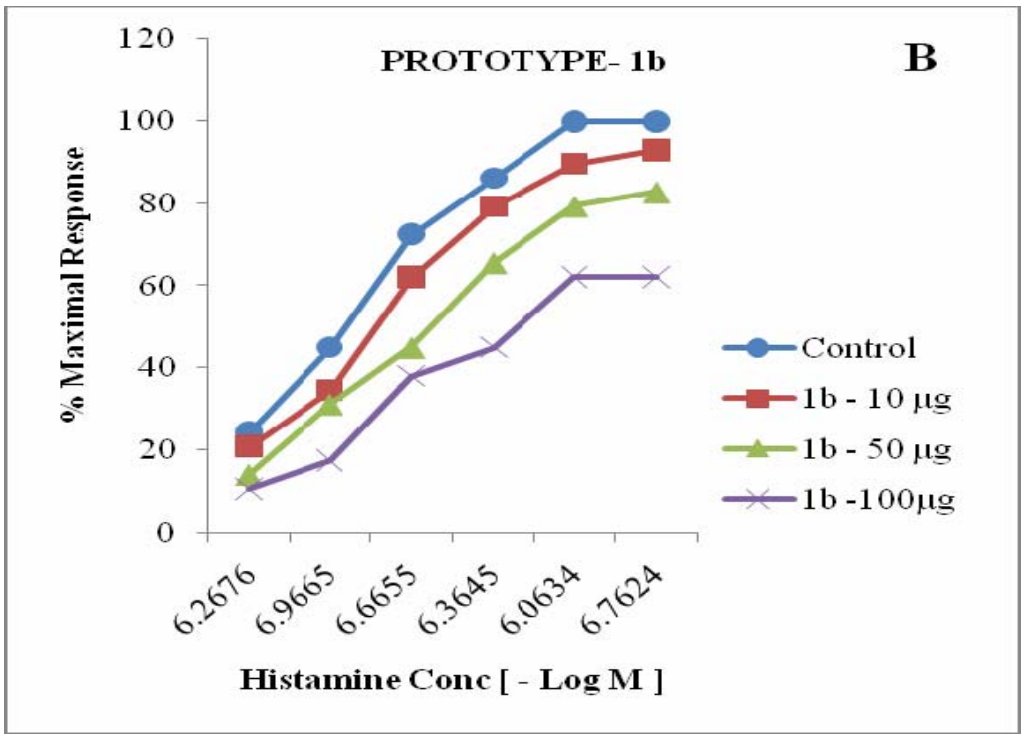
A
% Maximal Response
120
100
80
60
40
20
0
6.2676
6.9665
6.6655
6.3645
6.0634
6.7624
Histamine Conc [- Log M]
Control
1a - 10 µg
1a - 50 µg
1a -100µg


PROTOTYPE- 1b
B
% Maximal Response
120
100
80
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6.2676
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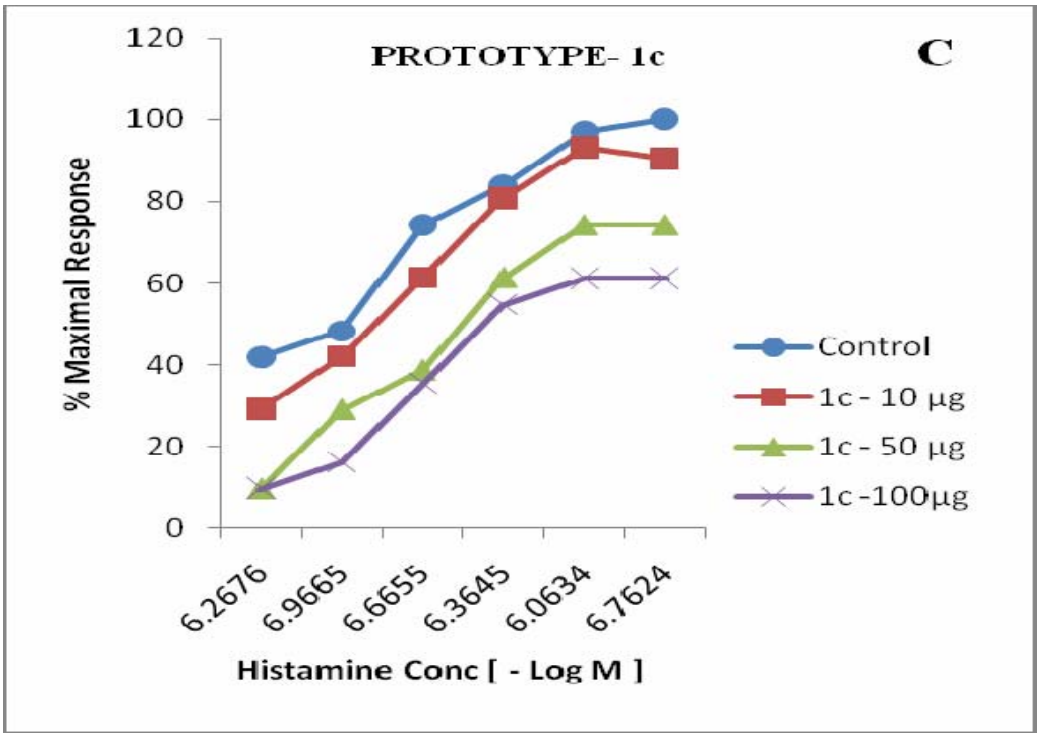
Histamine Conc [- Log M]
Control
1b - 10 µg
1b - 50 µg
1b -100µg



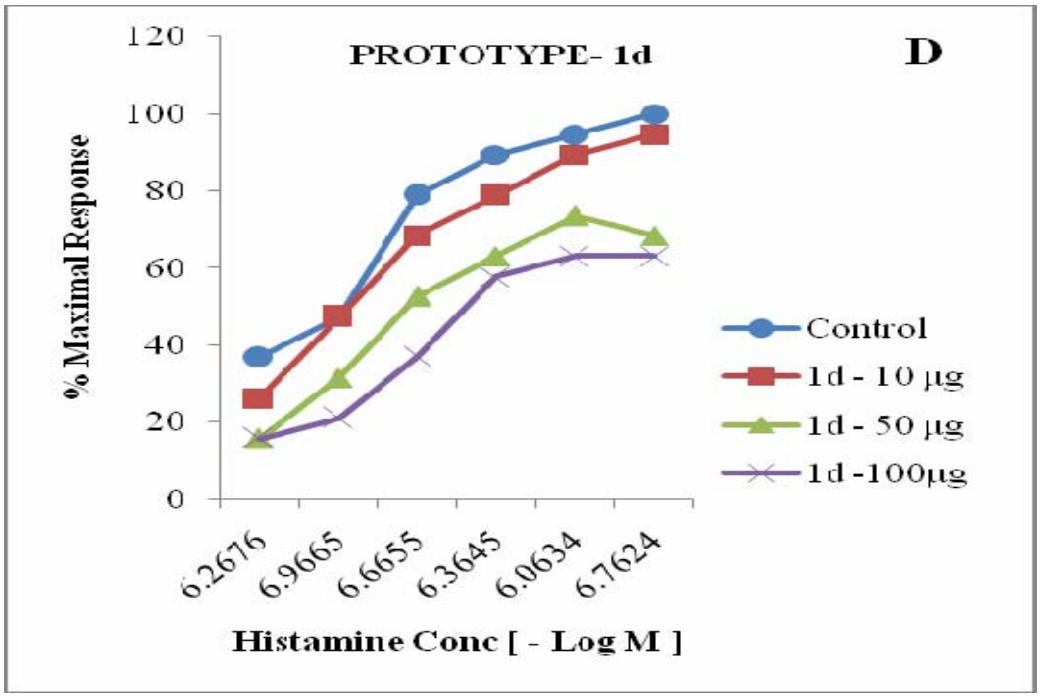


Figure: 1(A-D)
Concentration-response curves of histamine in the absence and presence of compounds (1a-d), following 5-min pre incubation time. Each point represents the mean± S.E.M of four experiments. ($P < 0.05$)

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