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In Vitro Antihistaminic Activity of Some 1-Substituted Imidazoles: H_2-Receptor Antagonism

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Abstract

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 1-substituted imidazole derivatives on isolated guinea pig atria to reveal their desired pharmacological effects. In the present revision, some 1-substituted Imidazoles (1a-1d, 2a-2d) 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 atria the results have been compared with standard Ranitidine. All the compounds were producing a competitive antagonistic action at 10µg/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: 1-substituted imidazoles, Antihistaminic activity, Guinea pig right atria, 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-Antagonism, 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 receptorswhich are found principally in the parietal cells of the gastric mucosa [14] and in many tissues, including vascular and bronchial smooth muscle and the right atrium. Keeping this context in mind, an attempt has been made to investigate the antihistaminic activity of some novel 1-substituted imidazoles (1a-1d, 2a-2d) on isolated guinea pig right atrium. Therefore in the present revision, a search of these novel 1-substituted imidazole derivatives possibly led to the development of compounds with probable H_2-receptor antagonistic activity.

MATERIALS AND METHODS

Chemicals

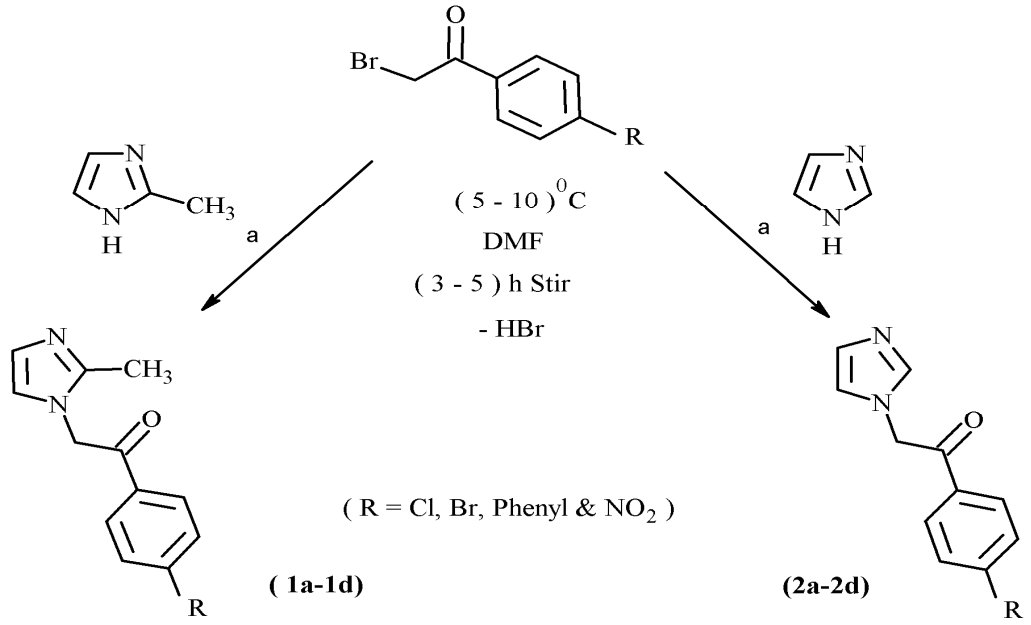
The following drugs and chemicals were used. 2-methyl imidazole, imidazole phenacyl bromides, dimethyl formamide all were procured from Sigma-Aldrich, India. All other chemicals used were analytical grade and obtained from Merck, India.

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/v Tween80 and then the volume was adjusted to 10 ml with normal saline for making the concentration of 10, 50 and 100 µg/ml.

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

To a solution of Imidazole/2-methyl imidazole (0.03mol, 2.46 g) in dry DMF (10 ml) was added dropwise to a solution



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

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 [15].

Pharmacological Evaluation

Male albino guinea pig weighing 350–400g 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 right atria are dissected and suspendedin a 25 ml organ bath with Krebs-Henseleit solution [16], containing [mM] NaCl 118, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgSO_4$ 1.6, glucose 6.2, bubbled with carbogen (5% CO2/95% O2). The temperature was maintained at 32.5˚ C and oxygenated continuously. Initial tension was 0.7 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 Krebs-Henseleit 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, 3.2 ml of 1 µg/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 individual prototypes (1a-1d, 2a-2d) were tested in this bioassay at various concentrations (10, 50 and 100 µg/ml), in terms of their ability to prevent the histamine contractions when they were added to the bath 5 min before histamine and compared with the standard drug Ranitidine (10µg/ml). Responses to

histamine were recorded as changes in height from baseline and expressed as percent of maximum response of the histamine [17]. 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 [18]. The mean maximal response obtained from the first concentration–response curve (in the absence of lead compounds) was taken as the 100% response value.

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 six 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 [19]. $P<0.05$ or $P<0.01$, $P<0.001$ were considered significant.

RESULTS AND DISCUSSION

Antihistaminic activity of all prototypes (1a-1d, 2a-2d) were tested in this bioassay at various concentrations of 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 atria. When evaluated against Histamine (0.1, 0.2, 0.4, 0.8, 1.6, 3.2 ml of 1 µg/ml) all the compounds (1a-1d, 2a-2d) at 100µg/ml significantly ($P<0.05$) antagonized the contraction of guinea pig atria, in a competitive and concentration dependent manner. Fig.1 & 2 represents the contractile response elicited by Histamine on guinea pig atria in presence and in absence of the experimental compounds (1a-1d, 2a-2d) with the comparison of Ranitidine (10µg/ml). This is evident on plotting the –log M values (6.2676, 5.9665, 5.6655, 5.3645, 5.0634, 4.7624) against% maximal response [20].

Compound 1b showed its moderate significant antagonism ($P<0.05$) only at 100 µg/ml concentration when compared to control and the % maximal response wasn't decrease at lower concentrations of 10 and 50 µg/ml. Rather while increase the volume of same concentration significantly reduce the % maxima of the contractile response. Compound 1c showed its mild significant antagonism ($P<0.05$) at both 100 and 50 µg/ml concentrations respectively. Compound 1d showed its antagonism ($P<0.05$and $P<0.01$) at 100 µg/ml by concentration dependent manner when compared to control.

Rather in second series (2a-2d), compound 2a showed its mild antagonism ($P<0.05$, $P<0.01$) at both 50 and 100 µg/ml concentration. Compound 2b, 2c and 2d showed utmost significant antagonism ($P<0.01$, $P<0.001$) with respective concentrations of 50 and 100 µg/ml. Moreover these compounds (2b, 2c and 2d) had shown their mild antagonism ($P<0.05$) against the contractile response elicited by guinea pig atria even at the lower concentration of 10 µg/ml when compared to control. In addition they (2b, 2c and 2d) were quiet comparable with that of standard ranitidine by concentration dependent manner.

Thus the exposure of guinea pig isolated atria 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 & 2.

All the compounds (1a-1d, 2a-2d) were producing a competitive antagonistic action 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-[21].

Figure: 1 Concentration-response curves of Histamine in the absence and presence of compounds (1a-1d) and Ranitidine following 5minutes pre incubation time. Each point represents the Mean± S.E.M of four experiments.

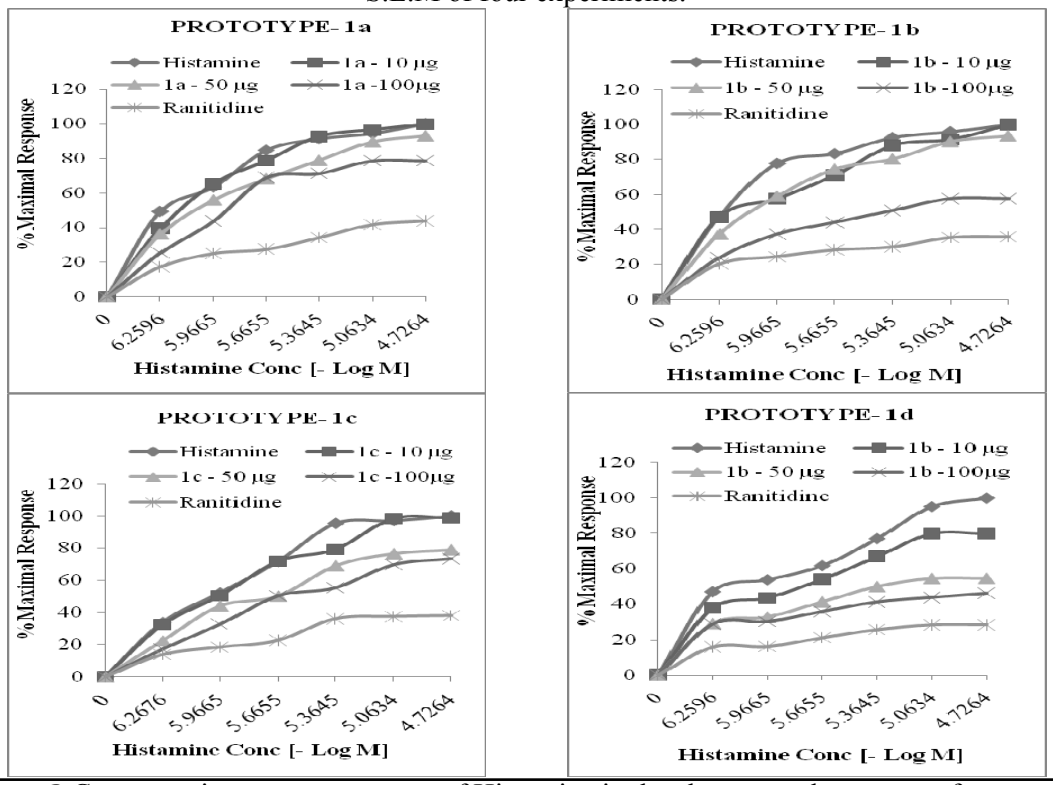
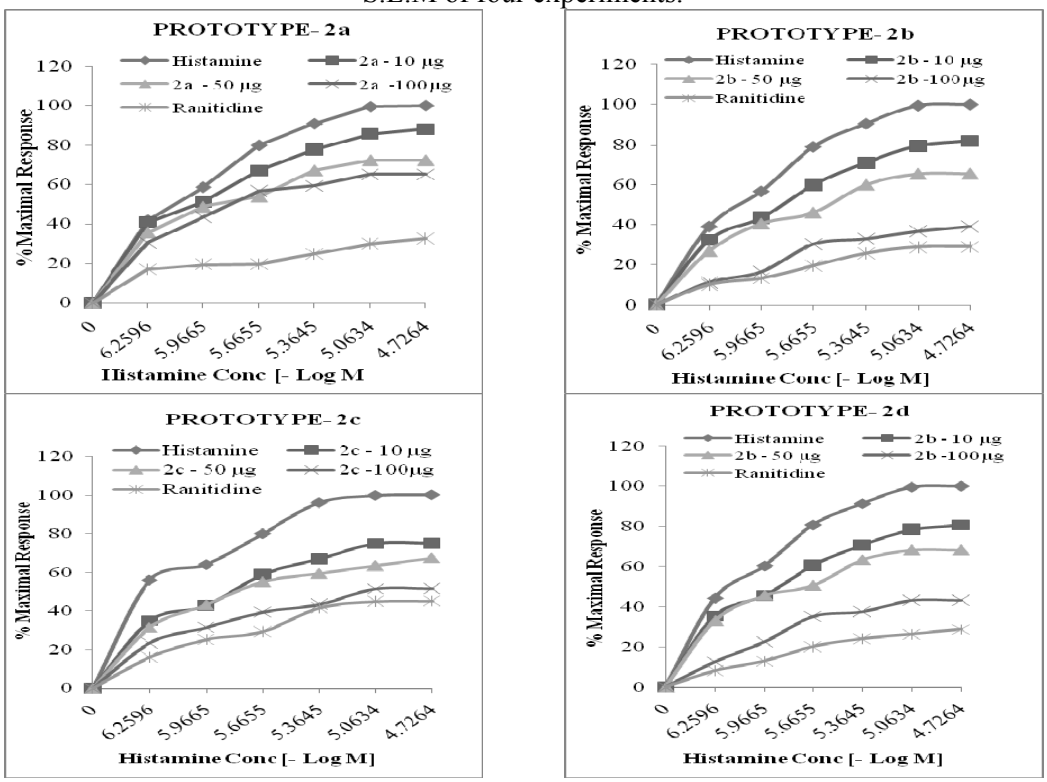


Figure: 2 Concentration-response curves of Histamine in the absence and presence of compounds (2a-2d) and Ranitidine following 5minutes pre incubation time. Each point represents the Mean± S.E.M of four experiments.



Conclusion

In conclusion, the bromo, phenyl and nitro substituted phenacylimidazoles (1b, 1c, 1d, 2b, 2c and 2d; $P<0.05$, $P<0.01$, $P<0.001$) were found to be more effective in their antagonism against histamine at 50 and 100 µg/ml when compared with that of the standard antagonistic drug ranitidine. It is probably because these compounds possess strong electron with drawing groups at their para position. Rather the chloro substituted phenacylimidazoles (1a and 2a) showed low ($P<0.05$) or no antagonistic action against histamine even at 100µg/ml and less potent when compared with other derivatives as well as standard drug ranitidine.

From the present findings, it is manifest that the synthesized 1-substituted imidazoles (1a-1d, 2a-2d) had shown marked antihistaminic activity or H_2 blocking activity in isolated tissue of guinea pig right atria. Hence this may help to design further in vivo studies to check their antihistaminic effect against ulcer [22] like an imidazole derivative cimetidine.

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