ANTISPASMODIC EFFECTS OF SOME N-SUBSTITUTED 2-METHYL IMIDAZOLE DERIVATIVES ON GUINEA PIG ILEUM

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Abstract: A series of novel N-substituted 2-methyl imidazole derivatives have been synthesized, and their in vitro antispasmodic activity was assessed on contractions of isolated guinea pig ileum, induced by acetylcholine (5×10^{-6} M to 1×10^{-8} M), and compared with the effect of atropine. All the prototypes were synthesized and confirmed by their FTIR, ¹H NMR, MASS and elemental spectral data. Antispasmodic activity of all prototypes were tested by 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 acetylcholine on prior contact with the ileum. All the compounds (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 significant antagonism (P<0.05, P<0.01 and P<0.001) which is probably mediated through muscarinic receptors.

Key words: Antispasmodic activity, Acetylcholine, Concentration-Response Curve, Guinea Pig Ileum, N-Substituted 2-Methyl Imidazoles.

Introduction
Irritable bowel syndrome is a disorder associated with muscarinic acetylcholine receptors (M₃) and characterized by cramping, abdominal pain, bloating, constipation, and diarrhea. M₃-selective antimuscarinic agents should have therapeutic potential for the treatment of altered smooth muscle contractility and tone, for example, as seen in intestinal spasm associated with smooth muscle in stability. Improper functioning of the intestinal muscles results in painful intestinal spasms. It is an especially common condition for individuals with chronic colon conditions such as irritable bowel syndrome, diverticulitis, and colitis. 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, H₂-Antagonism, α-Adrenergic agonist and β-blocking, anticonvulsant, broad spectrum antibacterial and antifungal activities. It is well known that imidazoles are effective on muscarinic receptors which are found principally in the peripheral tissues (e.g., glands and smooth muscle). Here a study of the synthesis and antispasmodic activity of some novel N-substituted 2-methyl imidazole derivatives on isolated guinea pig ileum has been performed which possibly led to the development of compounds with probable muscarinic antagonistic activity especially those relieving pain in smooth muscles in conditions like diarrhea.

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
Acetylcholine hydrochloride (Hi-media) and Atropine sulphate (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 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 cold stirring for about (3-6) hrs. This yielded a solid mass which was recovered from benzene extraction and finally purified by recrystallization and confirmed on the basis of their FTIR, $^1$H NMR, MASS spectral data. The data were found to be comparable with the earlier report$^{16}$.

Pharmacological Evaluation
Male albino guinea pig weighing (370-450) 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$^{17}$. The ileum was cut at this point and transferred to a dish containing tyrode solution$^{18}$. 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$^{19}$. 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 acetylcholine (1, 2, 4, 8, 16, 32µg/ml) were added to the bath and the concentration–response curve was recorded with a contact time of 90 seconds.

In addition, the antispasmodic (anti cholinergetic) effect of synthesized compounds 1(a-d) were tested in this bioassay at various concentrations (10, 50 and 100 µg/ml) and atropine (10µg/ml), in term of their ability to prevent the acetylcholine induced contractions when they were added to the bath 5 min before administration of standard drug acetylcholine. Responses to acetylcholine were recorded as changes in height from baseline and expressed as percent of maximum response of the acetylcholine$^{20}$. The CRC was constructed till ceiling effect to acetylcholine was obtained.
Six graded–response curves were obtained for each preparation, with a 20 min-rest between each. The mean maximal response obtained from the first concentration–response curve (in the absence of lead compounds) was taken as the 100% response value. After completing the CRC of acetylcholine, 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 acetylcholine concentration–response curves with and without the antagonists were plotted and compared. The statistical analyses were obtained by the ANOVA test, followed by the Dennett’s test where necessary. P<0.05, P<0.01 and P<0.001 were considered significant.

Results and Discussion

Antispasmodic 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 acetylcholine on prior (5 min) contact with the ileum.

When evaluated against acetylcholine (1, 2, 4, 8, 16, 32µg/ml) all the compounds 1(a-d) at 100 µg/ml significantly antagonized the contraction of guinea pig ileum, in a competitive and concentration-dependent manner. Fig.1 represents the contractile response elicited by acetylcholine on guinea pig ileum in presence and in absence of the experimental compounds 1(a-d) with the comparison of atropine (10µg/ml). This is evident on plotting the –log M values (5.2577, 4.9567, 4.6555, 4.3556, 4.0545, 3.7544) against % maximal response.

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 acetylcholine 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 M₁-receptors.

The chloro and bromo substituted phenacyl imidazoles showed significant antagonistic action (P<0.05 and P<0.01) against acetylcholine only at (100µg/ml) and less potent when compared with standard drug atropine (10µg/ml) The nitro and phenyl substituted phenacyl imidazoles (P<0.001) were found to be more effective in their antagonism against acetylcholine at (50µg/ml) as compared to the chloro and bromo substituted compounds. Also the nitro and phenyl substituted phenacyl imidazoles produced almost equipotent responses only at 100µg/ml when compared with that of the standard antagonistic drug atropine sulphate. It is probably because these compounds of possess an electron with drawing groups at their para position (nitro and phenyl).
Figure: I: Concentration-response curves of acetylcholine in the absence and presence of compounds (1a-d), following 5-min pre incubation time. Each point represents the mean ± S.E.M of six experiments ($P < 0.05, P < 0.01$ and $P < 0.001$).

**Conclusion**

From the present findings, it is evident that the synthesized N-substituted imidazoles 1(a-d) are showing marked muscarinic anticholinergic activity in isolated guinea pig ileum. Thus this may help to design further in vivo studies to check their antispasmodic effect in abdominal disorders like spasm, bloating, constipation, and diarrhea with probable muscarinic antagonistic activity particularly with the subtype of M$_3$-receptors which is responsible for smooth muscle spasm.

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**References**

8. Vitali T, Impicciatore M, Ferrari C, Morini G., Imidazole H2-antagonists and H2- angorists:


