Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Stereoselective synthesis of oxazolidinonyl-fused piperidines of interest as selective muscarinic (M_1) receptor agonists: a novel M_1 allosteric modulator

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Supplementary Data

Studies of the allosteric effect of piperidine **64**.

Report on M_1 receptor activity of piperidine 64 on rat duodenum

Aims

1. To evaluate piperidine **64** as an allosteric agonist at M_1 muscarinic receptors of rat duodenum.
2. To evaluate piperidine **64** as an antagonist of muscarinic M_3 receptors in guinea-pig ileum

Methods

M_1 receptor-mediated functional responses were measured as the relaxation responses of rat duodenum as characterized in this laboratory previously (Hamrouni, Gudka and Broadley, 2006). Isolated segments (1-2cm) of rat duodenum or guinea-pig ileum were set up in tissue baths containing Tyrodes solution (mM): NaCl, 137; KCl, 2.68; $CaCl_2$, 1.82; $NaHCO_3$, 5.9; $MgCl_2$, 1.0; NaH_2PO_4, 0.42; glucose, 5.6 gassed with O_2 95% and CO_2 5% and maintained at 37^{o}C. Isometric tension was recorded by connecting one end of the tissue to a tissue holder and the other to a transducer, by means of a cotton thread. Duodenum was progressively stretched to a resting tension of 1.5g while ileum had a resting tension of 0.5g applied. Isometric tension was measured by force transducers (Ormed, Welwyn Garden City, Hertfordshire, UK) coupled to a PowerLab/4SP computer system (AD Instruments, Charlgrove, Oxfordshire, UK) for data collection. Data was analysed using Chart v.4.1.1 software (AD Instruments, Charlgrove, Oxfordshire, UK).

Concentration-response curves were constructed in the duodenum by adding either McN-A-343 or piperidine **64** to the bath non-cumulatively in increasing half logarithmic concentrations. Each dose was left in the bath for 1 min or until a maximum effect was produced. It was then washed from the bath and a 10 min interval allowed before the next dose was introduced. To examine the effect of piperidine **64** on responses to McN-A-343, a concentration-response curve for McN-A-343 was obtained first and in the same tissue repeated in the presence of piperidine **64** (10^{-7} M). Piperidine **64** was added to the bath 15 min before each dose of McN-A-343.

Responses of the duodenum were measured as the maximum fall in tension (g) from the maximum baseline tension observed prior to addition of McN-A-343.

Concentration-response curves in the ileum to methacholine, a muscarinic agonist, were constructed by cumulative addition of increasing doses until the maximum contraction was achieved. Piperidine **64** (10^{-7}M) or its vehicle (DMSO) were added to the tissue bath and allowed to equilibrate for 15 min before a second curve was constructed in their presence. Responses of the ileum were measured as the increase in contraction above the pre-concentration-response curve base line.

Results

Piperidine **64** (10^{-7}M) did not affect the resting rhythmic activity of the rat duodenum, indicating that there was no direct agonist (orthosteric) acivity at M_1 receptors. However, in its presence there was a shift of the dose-response curve for the relaxation by McN-A-343 to the left (Fig 1). This indicates POTENTIATION of the responses. By contrast, the vehicle for piperidine **64**, DMSO, had no effect on the dose-response curves for the M_1 receptor agonist (Fig 2).

The same concentration of piperidine **64** (10^{-7}M) had a small inhibitory effect on the concentration-response curve for methacholine contractions on the guinea-pig ileum (Fig 3). However, this shift to the right was not significant.

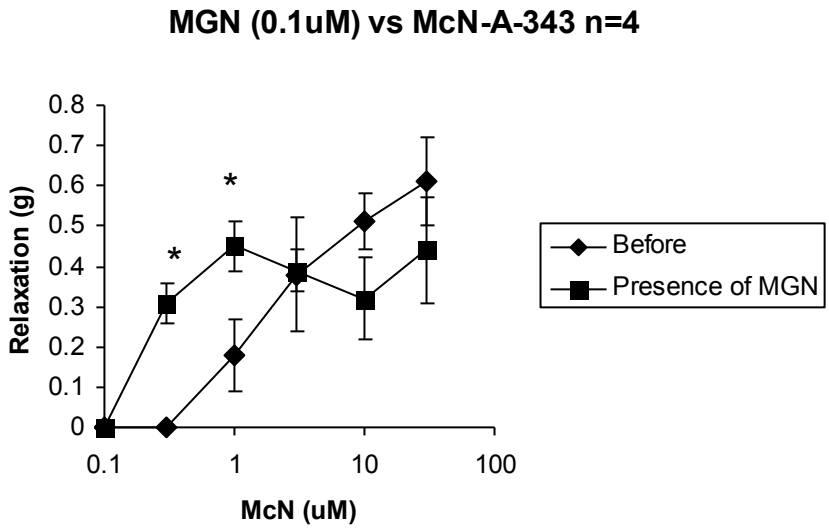

Figure 1 Effect of piperidine **64** (MGN, 10^{-7}M) on relaxation responses of rat duodenum to the selective M_1 receptor agonist McN-A-343. * Significantly different from values in the absence of piperidine **64** $p<0.05$ Student's paired t-test.

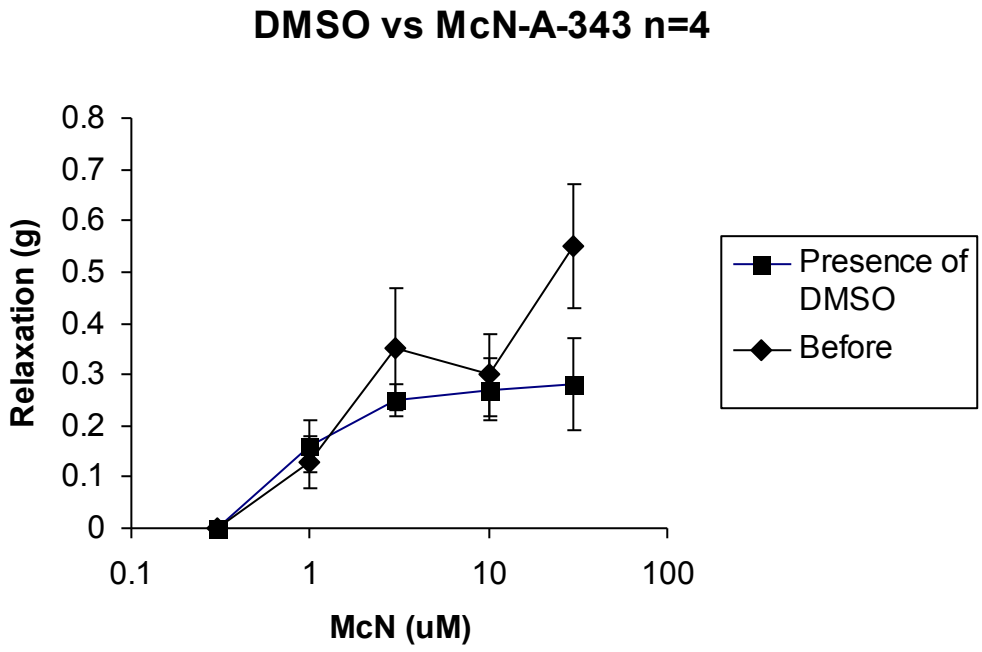

Figure 2 Effect of DMSO on relaxation responses of rat duodenum to the selective M_1 receptor agonist McN-A-343.

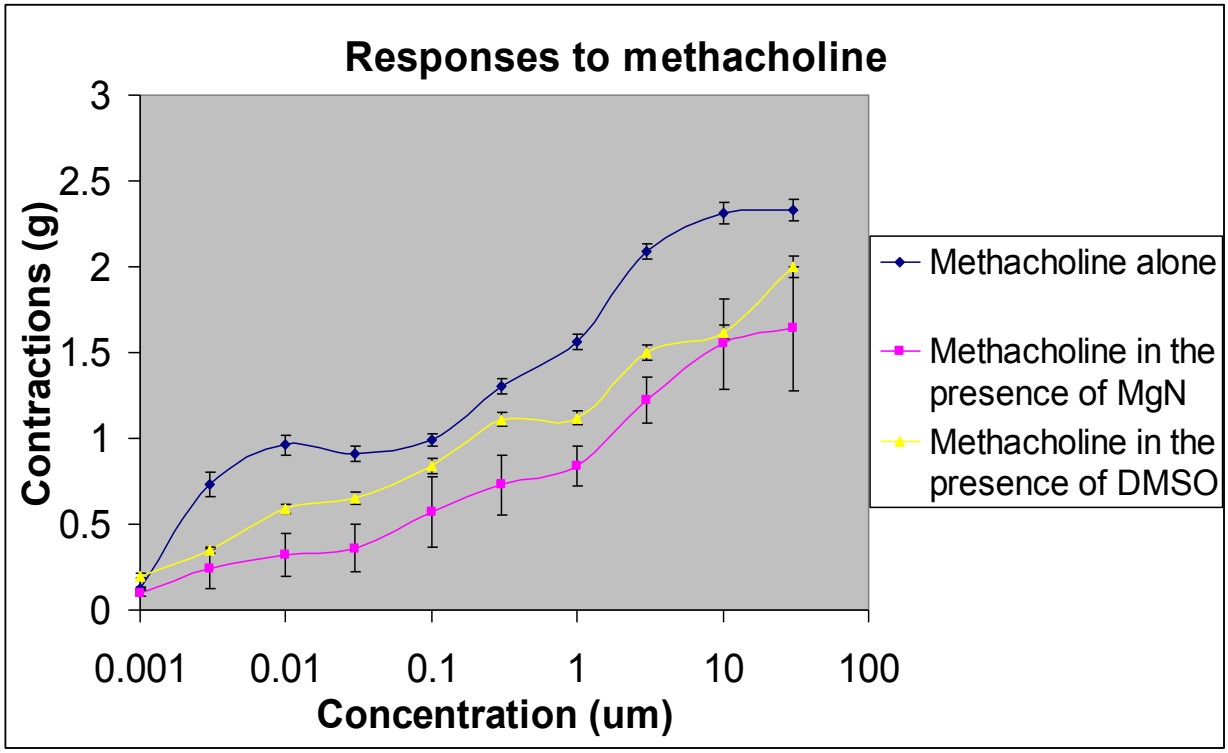

Figure 3 Contractions of isolated guinea-pig ileum to methacholine, added cumulatively. Effects of piperidine **64** (MGN,10 $^{-7}$M) and DMSO on the concentration-response curve for methacholine. After addition of piperidine **64** or DMSO, methacholine was added to the bath cumulatively.

Conclusion

Piperidine **64** (MGN) appears to be a positive allosteric modulator of the muscarinic M_1 receptor as it potentiates the effects of an M_1 receptor agonist without causing agonist activity on its own or antagonistic activity. There was minimal activity at M_3 receptors of the guinea-pig ileum as the contractions to methacholine were only slightly shifted to the right by piperidine **64**.

Reference

Hamrouni, AM, Gudka N & Broadley KJ (2006) Investigation of the mechanism for the relaxation of rat duodenum mediated via M_1 muscarinic receptors, *Auton. Atacoid. Pharmacol*., **26**, 275-284.