Synthesis, Physicochemical and Conformational Properties of (3*R*,4*R*)-3-(3-Cyclopropyl-1,2,4-oxadiazol-5-yl)-1-azabicyclo[2.2.1]heptane, a Novel M_1 Selective Muscarinic Partial Agonist

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The cyclopropyloxadiazole derivative described in the title has been shown to be a functionally selective M_1 partial agonist with antagonist properties in M_2 and M_3 muscarinic receptor assays; conformational studies indicate free rotation around the oxadiazole–azanorbornane bond, whilst X-ray studies reveal that the cyclopropyl group is in conjugation with the oxadiazole C=N bond.

The 'cholinergic hypothesis' of senile dementia of the Alzheimer's type is based on both clinical and neurochemical evidence,[1–3] which indicates that the marked deficits in cognitive function that accompany the disease are accompanied by degeneration of cholinergic neurones from the nucleus basalis of Meynert into cortical and hippocampal regions. Two approaches have been followed to accentuate cholinergic transmission[4,5] and one of these, the inhibition of acetylcholinesterase, is currently under extensive evaluation in the clinic. The second approach involves the design of agonists to act directly at postsynaptic muscarinic receptors in the cortex. The major difficulty with this approach is the wide distribution of muscarinic receptors throughout the body and the multiplication of receptor sub-types. Three muscarinic receptors (M_1, M_2 and M_3) have been reliably defined by binding and in functional assays.[6,7]

In previous studies we have shown that the two enantiomers *exo*-3-(3-methyl-1,2,4-oxadiazol-5-yl)-1-azabicyclo[2.2.1]heptane[8,9] **1a** are both full agonists at M_1, M_2 and M_3 muscaranic receptors. We have also proposed a binding model for interaction of ligands to the muscarinic receptor.[10] In a series of substituted oxadiazole derivatives we proposed that affinity to the receptor and 'efficacy'† were affected by the size and nature of the substituent R **1**. Optimal binding at the agonist site was found with substituents with a blend of hydrophilic and electon donating properties but the size of the substituent should be small (*e.g.*, Me, NH_2). In contrast, large hydrophobic substituents were required for an antagonist profile (*e.g.*, **1c**). In this communication we report that (3*R*,4*R*)-3-(3-cyclopropyl-1,2,4-oxadiazol-5-yl)-1-azabicyclo[2.2.1]heptane **2** is a novel muscarinic agent that is a functionally selective, partial agonist at M_1 receptors in rat superior cervical ganglion (SCG), but a competitive antagonist at M_2 and M_3 receptors‡.

† The 'efficacy' of a ligand determines the maximum response achievable by that ligand on occupation of a specific receptor in a given tissue.

‡ Agonists are agents that can elicit a maximum response, partial agonists are agents that elicit a response that at its maximum is less than the maximal response to a full agonist, antagonists are agents that can occupy a receptor but do not elicit a response.

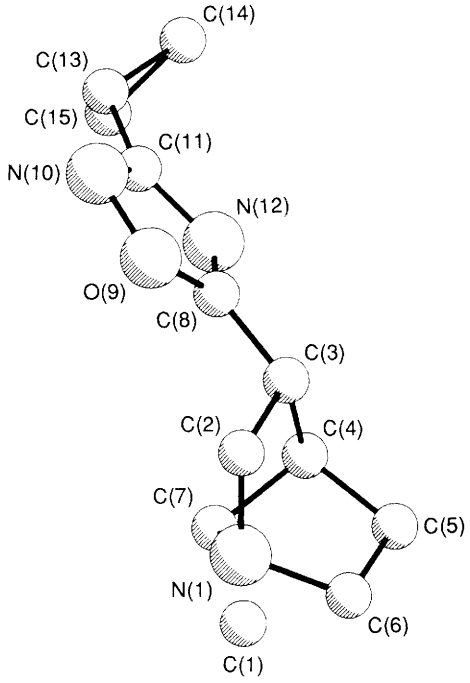
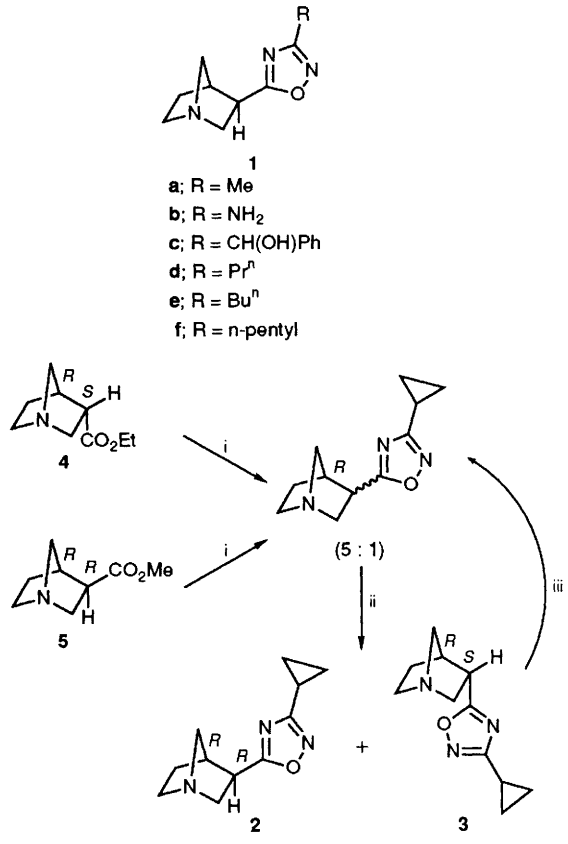


Fig. 1 Computer generated drawing of **2** hydrochloride derived from X-ray coordinates

Reaction of either ethyl (3*S*,4*R*)-1-azabicyclo[2.2.1]heptane-3-carboxylate[11] **4** or methyl (3*R*,4*R*)-1-azabicyclo[2.2.1]heptane-3-carboxylate[9] **5** with the sodium salt of cyclopropylcarboxamide oxime in anhydrous tetrahydrofuran (THF) gave a 5:1 mixture of oxadiazoles **2** and **3** in 55–60% yield. The *exo*-diastereoisomer **2** was separated by column chromatography on neutral alumina and equilibration of mixed fractions using sodium methoxide in MeOH again afforded a 5:1 mixture from which further *exo*-diastereoisomer could be isolated. The free base of **2** was an oil but both the hydrochloride and hydrogen L-tartrate salts were highly crystalline, m.p. 169–170 °C and 124–125 °C, respectively. Of the two, the latter displayed the most favourable stability profile.[12] The hydrochloride salt of **2** exhibited $[\alpha]_D^{22}$ −17.4 (*c* 1.0, CH_2Cl_2) and ^1H NMR (360 MHz, D_2O) δ 0.95–1.02 (2H, m, cyclopropyl-CH_2), 1.11–1.21 (2H, m, cyclopropyl-CH_2), 1.96–2.05 (1H, m, 5-CH), 2.08–2.16 (1H, m, cyclopropyl-CH), 2.23–2.33 (1H, m, 5-CH), 3.30–3.46 (4H, m, 4-CH, 6-CH and 7-CH_2), 3.50–3.60 (1H, m, 6-CH), 3.71–3.87 (3H, m, 2-CH_2 and 3-CH). Optical purity was determined by HPLC on an Enantiopac column (>98% e.e.) and chemical purity by HPLC on a Spherisorb ODS2 column (99.9%). Log *P* (at pH 7.4) was determined as 0.21 and **2** had a measured pK_a of 8.16.

The biological profile of **2** was both novel and intriguing. Whilst demonstrating a partial agonist character [EC_{50} 63 nmol dm^{-3}, relative maximum (RM) 0.55] in the M_1 pharmacological assay, it antagonised the effects of carbachol competitively both in M_2 (pA_2, 8.0) and M_3 (pA_2 8.15) assays.§ The interesting profile of **2** is most probably

§ The pharmacological preparations used were the depolarizing response on the rat superior cervical ganglion (mediated by M_1 receptors), the negative inotropic effect on the electrically driven guinea-pig atria (mediated by M_2 receptors) and the contraction of the guinea-pig myenteric plexus (mediated by M_3 receptors). The relative maximum quoted for M_1 is obtained by comparison of the amplitude of the test response to the maximum obtained with 1 μmol dm^{-3} (±)-muscarine. The relative maximum quoted for M_2 and M_3 is obtained by comparison of the amplitude of the test response with the maximum obtained with carbachol. Antagonist activity (pA_2) was estimated using carbachol as agonist. Full details will be published elsewhere (S. B. Freedman *et al.*, manuscript in preparation).



Scheme 1 *Reagents and conditions*: i, NaH, THF, cyclopropyl-C(=NOH)NH_2, 4A molecular sieves, reflux; ii, column chromatography on neutral alumina; iii, NaOMe, MeOH

determined, though not entirely, by the size of the cyclopropyl group. For **1a** (R = Me) the compound is a highly potent and efficacious full agonist at all the muscarinic sub-types M_1–M_3, as assessed by pharmacological experiments and the relevant tissues. As the size of the alkyl group increases through the series ethyl, n-propyl and n-pentyl, the compounds,¶ although showing no selectivity between M_1, M_2 and M_3, displayed decreasing efficacy. Thus the n-propyl derivative had agonist activity at all three sub-types, M_1 (EC_{50} 130 nmol dm^{-3}, RM 0.6), M_2 (EC_{50} 400 nmol dm^{-3}, RM 0.4) and M_3 (EC_{50} 900 nmol dm^{-3}, RM 0.9). Groups larger than these produced compounds that were muscarinic antagonists.

The interesting biological profile of **2** prompted a study of the X-ray structure of its hydrochloride salt (Fig. 1).‖ The analysis revealed that the cyclopropyl ring is eclipsed with the oxadiazole C(11)–N(12) bond and this feature appeared to be consistent in several X-ray crystal structures obtained on 3-cyclopropyl-1,2,4-oxadiazoles.[13] The orientation of the cyclopropyl ring presumably allows effective conjugation with the oxadiazole π-system. Whilst this part of the molecule

¶ Prepared in a similar manner to **2** (Scheme 1).

‖ *Crystal data* for **2** hydrochloride: $C_{11}H_{16}ClN_3O$, M = 241.72, a = 8.264(1), b = 6.788(1), c = 10.821(1) Å and β = 104.73(1)°, monoclinic, space group $P2_1$, Z = 2, D_c = 1.367 g cm^{-3}. An automatic four-circle diffractometer with Cu-Kα radiation (λ = 1.5418 Å) was used to measure 1216 reflections of which 1105 were observed [$I \geq 3\sigma(I)$]. The structure was solved by direct methods and refined with least squares and Fourier analyses.

Anisotropic temperature factors were refined for the non-hydrogen atoms while isotropic temperature factors were applied to the non-hydrogen atoms but not refined. The function $\Sigma\omega(|F_o|-|F_c|)^2$ with $\omega = 1/(\sigma F_o)^2$ was refined to an unweighted residual R = 0.060.

Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

appears to have a fixed conformation this is not the case with the oxadiazole ring in relation to the azanorbornane moiety. Although the dihedral angle C(2)–C(3)–C(8)–O(9) was measured as 29.2° in the crystal structure, analysis by the semi-empirical AM1 method[14] within the AMPAC program[15] indicated that the barrier to rotation around the C(3)–C(8) bond was 1.1 kcal mol^{-1} suggesting that free rotation would occur about this bond. The ability of the cyclopropyl group to adopt differing conformations by rotation of the oxadiazole–azanorbornane bond, whilst also allowing maximum hydrogen bond interaction of the oxadiazole ring with the receptor protein, could be an important feature in determining the biological profile of **2**.

The low efficacy of **2** confers selectivity on the basis of muscarinic receptor reserve** in the different assay systems. These characteristics provide an opportunity to probe the pharmacology of muscarinic agonists *in vitro* and *in vivo*.

** The stimulus given to a tissue by a given agonist is proportional to the intrinsic activity of the agonist, receptor density in the tissue and the efficiency of receptor coupling to stimulus response mechanisms. Assuming efficient receptor coupling, equality between fractional receptor occupancy by an agonist and the fractional tissue response would indicate no reserve. When the fractional receptor occupancy is less than the fractional tissue response the difference is termed reserve. In a given tissue, agonists with high intrinsic efficacy need to activate fewer receptors to produce a given fractional response than agonists with low intrinsic efficacy. The relative levels of a particular agonist needed in different tissues to elicit the same fractional response reflect receptor number and efficiency of coupling in the various tissues. These factors are collectively the 'effective' reserve. Low efficacy compounds, which need higher levels of occupancy to produce responses, thus appear most active in tissues with a high effective reserve. In tissues with low effective reserve the levels of occupancy achieved by a low efficacy agonist may not be great enough to produce sufficient receptor activation to elicit a response. In such tissues low efficacy compounds may, however, complete with higher efficacy agonists for the receptor sites and thus act as competitive antagonists.

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