Synthesis and Properties of [*R*-(*Z*)]-(+)-α-(1-Azabicyclo[2.2.2]oct-3-yl)-α-(methoxyimino)acetonitrile, a Novel Functionally Selective Muscarinic Partial Agonist

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The (*Z*)-*N*-methoxy imidoyl nitrile functionality is a novel methyl ester bioisostere, which, when substituted onto the quinuclidine ring system gives the title compound **1**, a stable, brain penetrant and functionally selective muscarinic partial agonist; X-ray studies confirm the configurational assignment and reveal that the imino and cyano bond lengths are consistent with those expected for formal double and triple bonds, respectively.

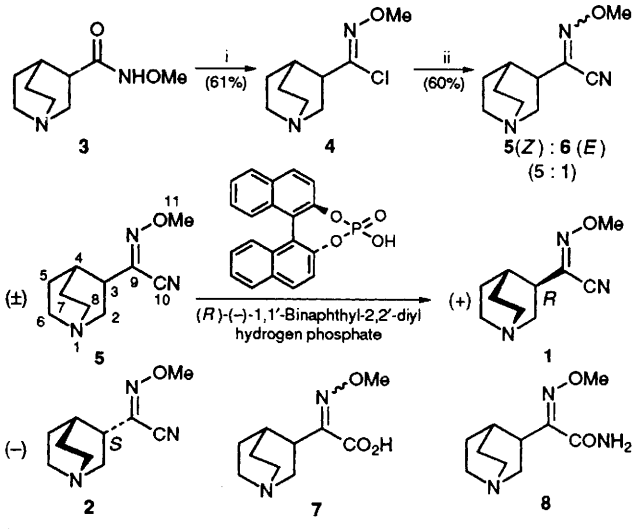
The hypothesis that the enhancement of muscarinic cholinergic transmission at cerebral cortical sties would be of benefit in the treatment of senile dementia of the Alzheimer type has led to an upsurge of interest in the design, synthesis and pharmacological evaluation of muscarinic agonists.[1] Unfortunately, owing to the widespread distribution and multiplicity of muscarinic receptor subtypes,[2] the potential utility of many of these compounds is compromised by undesirable side effects.

In previous reports we described the design and synthesis of compounds with high affinity for muscarinic receptors by the bioisosteric replacement of the ester group of methyl quinuclidine-3-carboxylate and related azabicyclic esters with metabolically more stable groups such as heteroaromatic rings, for example oxadiazoles,[3] and non-heteroaromatic groups such as oxime ethers.[4] These compounds had a range of efficacies that spanned antagonist to full agonist character.† We found that, whereas full agonists showed a propensity to induce deleterious peripheral effects, certain partial agonists did not produce many of these side effects but were capable of eliciting central effects predictive of cognitive enhancement.[4] Here, we report the use of the (*Z*)-*N*-methoxy imidoyl nitrile functionality as a methyl ester bioisostere in [*R*-(*Z*)]-(+)-α-(1-azabicyclo[2.2.2]oct-3-yl)-α-(methoxyimino)acetonitrile **1** resulting in a novel muscarinic partial agonist that shows favourable central selectivity.

Reaction of the *N*-methoxycarboxamide **3**, itself available from the corresponding ethyl ester,[3] with triphenyl phosphine and carbon tetrachloride in refluxing MeCN afforded (*Z*)-1-azabicyclo[2.2.2]oct-3-yl-*N*-methoxycarboximidoyl chloride **4** as a single geometric isomer in 61% yield[5] (Scheme 1). Treatment of **4** with NaCN in Me_2SO at 95 °C gave the imidoyl nitrile as a 5 : 1 mixture of (*Z*) and (*E*) isomers in 60% yield. The geometric isomers **5** and **6** were separated‡ by column chromatography and were found to be chemically stable in 1 mol dm^{-3} DCl in D_2O with no sign of isomerization over several days at room temperature. However, in 5.5 mol dm^{-3} DCl in D_2O both **5** and **6** slowly isomerized to a 5 : 1 equilibrium mixture of (*Z*) and (*E*) isomers (halflife ~15 h by NMR experiments). This isomerization was accompanied by slow hydrolysis of the cyano group to give the carboxylic acid **7** which was present at a level of 25% after 650 h. The relative stability of **5** and **6** in aqueous acid, compared to simple aldoxime and ketoxime ethers,[6] is presumably due to the effect of the electron-withdrawing cyano group on the basicity of the imino nitrogen. Treatment of **5** with 3 equiv. of KOH in refluxing methanol–water for 2 h hydrolysed the cyano group to give the carboxamide **8** in quantitative yield. The absence of products resulting from nucleophilic attack at the imino bond is presumably due to the interaction of the oxygen lone pair with the adjacent π system. The (*Z*) isomer **5** was resolved into the corresponding *R*-(+) **1** and *S*-(−) **2** enantiomers by the sequential use of (*R*)-(−)- and (*S*)-(+)-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate.[7] The hydrochloride of **1**, mp 218–219 °C, exhibited $[\alpha]_D^{20}$ +25.3 (*c* 1.00, EtOH) and ^1H NMR (400 MHz, D_2O)§ δ 1.96 (2H, m, 8-CH_2), 2.13 (2H, m, 5-CH_2), 2.55 (1H, m, 4-CH), 3.25–3.45 (5H, m, 3-CH, 6-CH_2 and 7-CH_2), 3.56 (1H, m, 2-CH_{ax}), 3.80 (1H, m, 2-CH_{eq}), 4.13 (3H, s, OMe). Optical purity was determined by HPLC on a Chiracel OB column (>98% e.e.) and chemical purity by HPLC on a Rainin Microsorb column (99.2%).

The X-ray structure¶ of the hydrochloride of **1** revealed two independent molecules (A and B) in the crystallographic asymmetric unit, which are illustrated in Fig. 1. The two molecules differ conformationally, the principal difference being in the rotational orientation of the imidoyl nitrile substituent. The imino nitrogen nearly eclipses the adjacent methylene group in Molecule B (C2′–C3′–C7′–N2′ torsion angle = −6°) but is in a more *gauche*-like orientation in Molecule A (C2–C3–C7–N2 torsion angle = 36°). Analysis of the C3–C7 bond rotation by the semi-empirical AM1 method[8] within the MOPAC program[9] predicted several low-energy conformations and a barrier to rotation of only 2 kcal mol^{-1} (1 cal = 4.184 J), suggesting free rotation about this bond. As a consequence of the observed and predicted conformational flexibility it is difficult to draw conclusions about conformations involved in binding. The C7–N2 (C7′–N2′) bond distances of 1.266(5), 1.281(4) Å are consistent with the imino bond length observed in simple oximes. Similarly, the C8–N3 (C8′–N3′) and C7–C8 (C7′–C8′) distances of 1.133(5), 1.130(5) Å and 1.445(6), 1.451(5) Å are consistent with formal triple and single bonds, respectively. These observations suggest that the introduction of a cyano group into the oxime functionality does not lead to extensive additional resonance stabilisation as has been proposed by others.[10]

The *R*-(+) enantiomer **1** is a potent partial agonist with an affinity for muscarinic receptors comparable to that of the natural neurotransmitter acetylcholine and 16-fold greater than methyl quinuclidine-3-carboxylate thus demonstrating the value of the (*Z*)-*N*-methoxy imidoyl nitrile group as an



Scheme 1 *Reagents and conditions*: i, PPh_3–CCl_4, MeCN, reflux 5 min; ii, NaCN, Me_2SO, 95 °C, 5 h

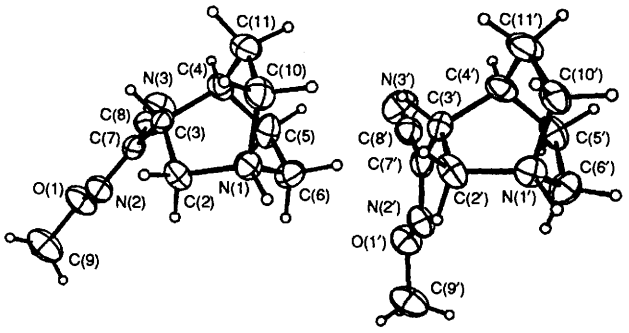


Fig. 1 ORTEP II drawing of **1** hydrochloride (molecule A on the left and B on the right) derived from the crystal structure. Non-hydrogen atoms are drawn as principal ellipses at the 50% probability level; hydrogen atoms as spheres of arbitrary radius.

ester bioisostere in this context. In addition to chemical stability **1** also showed improved metabolic stability *in vivo* relative to the corresponding ester. The *S*-(−) enantiomer **2** and the racemic (*E*)-isomer **6** have 10- and 18-fold lower affinities, respectively. The ready penetration of **1** into the CNS[11] was confirmed by its ability at low doses to induce rhythymical slow wave activity in the EEG measured in the CA1 region of the hippocampus in urethane anaesthetised rats.[4] This is a well characterised central muscarinic effect which we believe to be predictive of cognitive enhancement, and indeed **1** proved to be highly effective in animal models of learning and memory at similar doses.[12] Compared with full muscarinic agonists, such as arecoline, **1** produced significantly reduced effects on peripherally mediated cardiovascular effects in the dose ranges required to induce RSA, indicating that it is remarkably centrally selective. This functional selectivity may provide a unique opportunity to test the cholinergic hypothesis, without causing the limiting side-effects associated with full agonists.

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Footnotes

† The affinity and efficacy of the compounds were assessed by a two-stage binding assay designed to measure both parameters. The ability of the compounds to inhibit the binding of the muscarinic agonist [^{3}H]oxotremorine-M (OXO-M) provided a measure of affinity for the high affinity agonist state of the receptor. The ratio of the IC_{50} values for inhibition of binding of [^{3}H]quinuclidinyl benzilate (QNB), a muscarinic antagonist, and OXO-M was used to predict efficacy.[3] Ratios greater than 100 are associated with full agonists; antagonists give ratios close to unity and intermediate values indicate partial agonists.[3] The 'efficacy' of a ligand determines the maximum response achievable by that ligand on occupation of a specific receptor in a given tissue. Full 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 response of the full agonist, antagonists are agents that occupy receptors but do not cause a response. Partial agonists can produce selective effects by exploiting regional differences in receptor reserve. The 'receptor reserve' reflects the receptor density and the efficiency of coupling in various tissues. In tissues where receptor reserve is high low efficacy agonists can elicit a response. However, where the reserve is low the level of occupancy achievable by a low efficacy agonist may not be great enough to produce a response.

‡ The isomers were separated on silica gel using 15% methanol in diethyl ether as eluant. The ^{1}H and ^{13}C NMR spectra of the isomers were similar, the most notable differences being in certain signals in the ^{13}C NMR: the less polar (*E*)-isomer showed signals at δ 22.0 (CH_2), 24.4 (4-C), 27.2 (CH_2), 35.0 (3-C), 46.9 (CH_2), 47.2 (CH_2), 51.2 (CH_2), 64.1 (9-C), 114.3 (8-C), 142.4 (7-C) compared to δ 21.2, 25.2, 27.1, 29.7, 39.6, 47.0, 47.2, 49.3, 63.7, 110.4, 134.3 in the case of the (*Z*)-isomer. Irradiation of the (OMe) protons at δ 4.13 in the ^{1}H NMR of the (*Z*)-isomer showed no NOE effects to any other protons in the molecule, whereas irradiation of the corresponding signal at δ 4.05 in the case of the (*E*)-isomer gave a small positive NOE to signal at δ 2.04 corresponding to (4-CH).

§ The numbering system used for NMR assignments is as shown in Scheme 1 and differs from that used in the ORTEP II drawing and the X-ray supplementary data.

¶ *Crystal data* for **1** hydrochloride: $C_{10}H_{15}N_3O.HCl$, M = 229.71, monoclinic, a = 13.546(2), b = 6.742(2), c = 14.490(3) Å, β = 115.57(2)°, V = 1193.7(7) Å^3 [determined from the diffractometer angles of 25 reflections well distributed in reciprocal space, λ(Mo-Kα) = 0.71073 Å], T = −50 °C, space group $P2_1/No.$ 4, Z = 4, D_c = 1.278 g cm^{-3}. Colourless plate. Crystal dimensions: 0.48 × 0.18 × 0.05 mm, μ = 2.974 cm^{-1}. An expanded quadrant of intensity data were collected on an Enraf-Nonius CAD-4 diffractometer using a variable speed ω scan technique, graphite monochromated molybdenum radiation; 4442 reflections measured ($2 \leqslant 2\theta \leqslant 54°$, h, ± k, ± l), 3573 unique [merging R = 0.036], giving 2397 with $I \geq 3\sigma(I)$; linear and approx. isotropic crystal decay, *ca.* 6.7% corrected during processing. The structure was solved by direct methods using the SHELXS program series and refined by full-matrix least squares analysis. Final crystallographic residuals (on F) were R = 0.0367, wR = 0.0405, GOF = 1.120; final difference peaks between ±0.274 e Å^{-3}. The absolute configuration was assinged by the method of Rogers[13a] (η parameter refined to +1.0(1)) and cooberated with a statistical test based on R-factors[13b] which indicated significance at better than the 99.95% confidence level (wR for the enantiomer was 0.0419). Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre, see Information for Authors, Issue No. 1.

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5 Stereochemistry was assigned on the basis of ^{1}H and ^{13}C NMR and in the absence of X-ray data remains tentative.
6 In related studies we found that 1-azabicyclo[2.2.2]oct-3-ylcarboxaldehyde *O*-methyloxime reference 4(*a*) underwent rapid isomerization accompanied by hydrolysis in 1 mol dm^{-3} aqueous HCl. Similarly, 3-acetyl-1-azabicyclo[2.2.2]octane *O*-methyloxime was readily hydrolysed under these conditions, S. M. Bromidge: unpublished results. For more general references see C. G. McCarty, in *The Chemistry of the Carbon–Nitrogen Double Bond*, ed. Patai, Wiley, 1970, pp. 383–392 and references cited therein.
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11 The Log P (at pH 11.9) of **1** was determined as +1.51 and the apparent pKa values as 8.96. The calculated log D value (for pH 7.3) of −0.11 suggests that the compound will readily penetrate the CNS.
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