Synthesis of Azabicyclic Pyrazine Derivatives as Muscarinic Agonists and the Preparation of a Chloropyrazine Analogue with Functional Selectivity at Sub-types of the Muscarinic Receptor

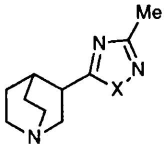
Raymond Baker,* Leslie J. Street, Austin J. Reeve and John Saunders

Merck Sharp and Dohme Research Laboratories, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR, UK

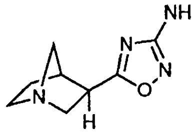
The synthesis of quinuclidine and azanorbornyl pyrazine derivatives has yielded highly potent and efficacious muscarinic agonists; chloro-substitution in the pyrazine ring of the quinuclidine analogue resulted in the formation of a derivative with both enantiomers displaying partial agonist character but, more importantly, functional selectivity at the M_1, M_2 and M_3 sub-types of the muscarinic receptor.

Numerous studies have indicated that the loss of cognitive and memory faculties in patients with senile dementia is linked with deficits in cerebral cholinergic transmission.[1,2] Several approaches to improving cholinergic transmission have been followed but the main effort has focused on the study of acetylcholinesterase inhibitors such as 9-amino-1,2,3,4-tetrahydroaminoacridine[3] and physostigmine[4] and directly acting agonists for post-synaptic muscarinic receptors in the cortex.[2,5] The critical issue surrounding the second of these approaches is targeting specific muscarinic receptor sub-types in the brain that are associated with cognition whilst minimising activation of other muscarinic receptors that are widespread in tissues outside the central nervous system. At least five different muscarinic proteins (termed m1 to m5) have been identified.[6] Only three of them have been discriminated to date in binding or pharmacological studies. In general, however, the M_1 sub-type is found in neuronal tissue, whilst additionally M_2 is associated with cardiovascular function and M_3 receptors are found in glandular and smooth muscle tissue.[7] Selective muscarinic antagonists for individual sub-types have been well defined, however, programmes to discover selective agonists have made extremely limited progress.

In previous reports we described the synthesis and biochemical evaluation of a series of azabicyclic-based ligands for the muscarinic receptor. The 1,2,4-oxadiazole[8–10] and thiadiazole[11] rings were found to be excellent bio-isosteres for an ester and compounds of high affinity and efficacy at the muscarinic receptor were discovered **1**–**3**. The remarkable potency of these derivatives was interpreted as resulting from the hydrogen-bond capability of the pharmacophore and the conclusion that high efficacy requires two H-bond acceptor sites in a precise location on the heterocycle. In the current work we demonstrate that muscarinic agonists of high affinity and efficacy can be obtained by replacing the oxadiazole group by a pyrazine moiety. In addition the resolution of a chloro-substituted analogue has yielded two enantiomers with the remarkable property of displaying functional selectivity at the M_1, M_2 and M_3 receptors.



1 X = O
2 X = S

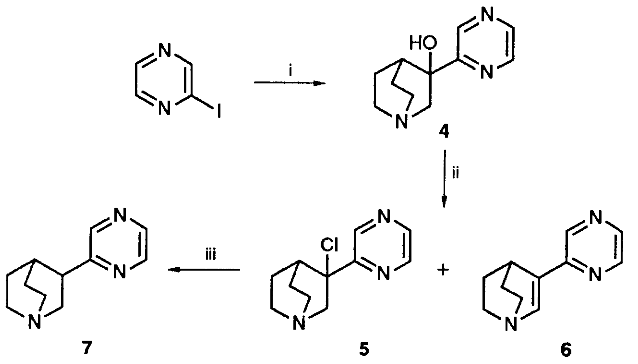


3

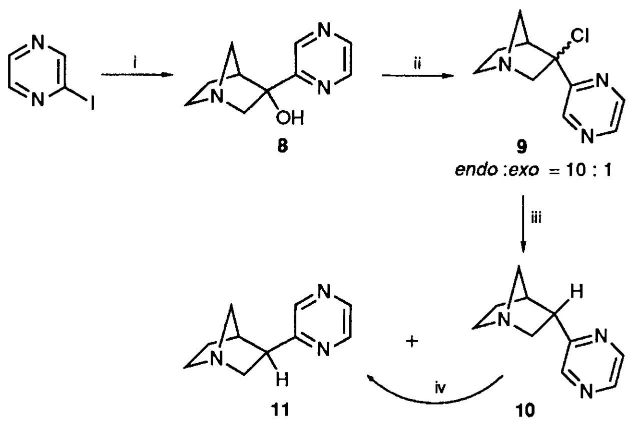
Molecular modelling had indicated that areas of significant electrostatic potential are associated with the 1,4-pyrazinyl moiety, these areas being in the same location as in the 1,2,4-oxadiazole, and it was of interest to compare compounds in this series with the oxadiazole and thiadiazole derivatives previously prepared. Reaction of quinuclidin-3-one with 2-lithiopyrazine, prepared by addition of Bu^tLi to 2-iodopyrazine in diethyl ether, gave the crystalline alcohol **4** in 27% yield (Scheme 1). Treatment of **4** with thionyl chloride gave an equal mixture of chloride **5** and elimination product **6**, which could either be separated or hydrogenated as a mixture to give **7**, in 59% overall yield.[†] The analogous 1-azabicyclo-

---

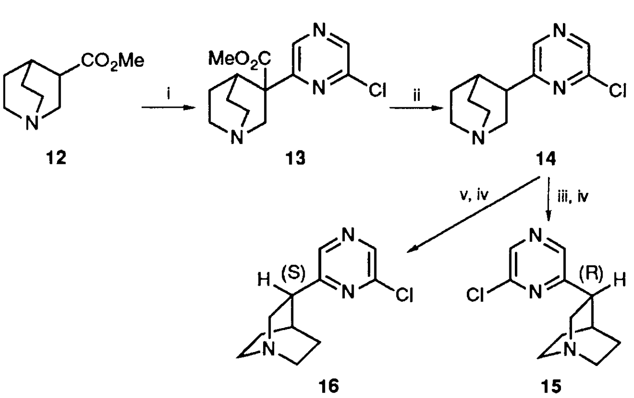
[†] All new compounds have been fully characterised and assessed by ^1H NMR spectroscopy at 360 MHz, mass spectrometry, elemental analysis (C,H,N) and chromatography (TLC, HPLC and GC where possible).



Scheme 1 *Reagents and conditions*: i, Bu^{t}Li(2 equiv.), diethyl ether, −45 °C, 15 min, then quinuclidin-3-one, diethyl ether, −65 °C → + 25 °C; ii, $SOCl_2$, CH_2Cl_2, 0 °C; iii, 10% Pd/C, H_2, MeOH



Scheme 2 *Reagents and conditions*: i, Bu^t Li(2 equiv.), diethyl ether, −45 °C, 15 min., then 1-azabicyclo[2.2.1]heptan-3-one, diethyl ether, −65 °C → + 25 °C; ii, $SOCl_2$, CH_2Cl_2, 0 °C; iii, 10% Pd/C, H_2, MeOH; iv, NaOMe, MeOH, reflux 2 h



Scheme 3 *Reagents and conditions*: i, Lithium diisopropylamide, THF, −50 °C, 1.5 h, then 2,6-dichoropyrazine, THF, −50 °C → 0 °C, 16 h; ii, concentrated HCl, 130 °C, 3 h; iii, (+)-di-*O*,*O*′-*p*-toluoyl-D-tartaric acid (0.25 equiv.), EtOH; iv, EtOH, reflux (× 2); v, (−)-di-*O*,*O*′-*p*-toluoyl-L-tartaric acid (0.25 equiv.), EtOH; (THF = tetrahydrofuran)

[2.2.1]heptane pyrazine derivatives were also prepared (Scheme 2). Thus, treatment of 1-azabicyclo[2.2.1]heptan-3-one[10] with 2-lithiopyrazine at −50 °C gave alcohol **8**‡ in 30% yield and as a single diastereoisomer. Reaction with thionyl chloride at room temperature gave chlorides **9** as a 10 : 1 *endo* : *exo* mixture with only trace quantities of the elimination product being isolated. Hydrogenation of **9** over 10% Pd/C gave a 10 : 1 mixture of **10** and **11**, respectively, which were readily separated by alumina chromatography. The thermodynamically more stable *exo*-isomer **11** could be obtained as the major product by reaction of **10** with NaOMe in refluxing methanol.

Results from binding assays indicated that **7**, **10** and **11** all had high affinity[12]§ and displayed high efficacy¶ at cortical muscarinic receptors approaching that of acetylcholine itself. Furthermore, the results indicated that **11** had affinity and predicted efficacy comparable to the aminooxadiazole **3**[10] and thus is one of the most potent and efficacious nonquaternary muscarinic agonists ever discovered. The full agonist profile of these compounds is consistent with maximum interactions with the receptor. In previous work with the oxadiazole derivatives we had concluded that the correct alignment and strength of two hydrogen-bond interactions (normally associated with the ester oxygens of acetylcholine) between receptor and ligand are essential for the full agonist properties of these derivatives. Indeed, it was shown that affinity for the agonist binding site correlated directly with the magnitude of the negative potential in the vicinity of the oxadiazole ring nitrogens. It is now also clear that a pyrazine group is a suitable bio-isostere for the ester group of the endogenous ligand, acetylcholine.

The nature of the pyrazine ring also provide an opportunity to examine the effect of substitution on the efficacy and selectivity of these derivatives against the M_1, M_2 and M_3 sub-types of the muscarinic receptor. To this end the 6-chloropyrazinyl quinuclidine **14** was prepared and resolved into its enantiomers **15** and **16** (Scheme 3). Treatment of 3-carbomethoxyquinuclidine **12**[13] with lithium diisopropylamide at −78 °C followed by 2,6-dichloropyrazine gave quinuclidinyl pyrazine **13** in 60% yield. Refluxing **13** in concentrated HCl resulted in hydrolysis and decarboxylation to give **14** in 63% yield. Resolution of **14** was achieved using both enantiomers of di-*O*,*O*′-*p*-toluoyltartaric acid. The (*R*)-enantiomer **15**[14] was obtained in 99.2% e.e. (99.6% optically pure) by treatment of **14** with 0.25 equiv. of (+)-di-*O*,*O*′-*p*-toluoyl-D-tartaric acid followed by trituration of the resulting tartrate salt with refluxing ethanol. In the same way the (*S*)-enantiomer **16** was obtained using (−)-di-*O*,*O*′-*p*-toluoyl-L-tartaric acid.

To our surprise and delight the biological assay of **14** and **15** showed that these two compounds had a unique pharmacological profile. Thus, at M_1 and M_3 receptors, the compounds were shown to be agonists whereas at M_2, **14** and **15** were antagonists.[15] In contrast, the (*S*)-enantiomer **16** was significantly less potent and displayed little efficacy at the M_3 receptor. The profile of **14** and **15** would, therefore, allow the compounds to be used *in vivo* animal studies resulting in activation of the M_1, M_3 receptors whilst minimising effects on the cardiovascular system. The basis of this functional sub-type selectivity is associated with the partial agonist nature of the two compounds, which may be related to changes in the electronic distribution consequent to substitution in the pyrazine group.

---

‡ The relative stereochemistry of these molecules was assigned by ^1H NMR COSY and NOE experiments by Dr R. Herbert.

§ Binding assays involved displacement by the test ligand of [^3H]-oxotremorine-M from the high affinity state (the 'agonist binding site') of the receptor and [^3H]-*N*-methylscopolamine from predominantly the low-affinity state (the 'antagonist binding site'). The ratio of these dissociation constants gives a prediction of the cortical efficacy of the ligand since this ratio has been shown to correlate directly with the ability of the given ligand to stimulate PI hydrolysis.

¶ Used in this context, the 'efficacy' of a ligand determines the magnitude of the maximum response achievable by that ligand on occupation of a specific receptor in a given tissue; low efficacy compounds are unable to induce a full pharmacological response irrespective of their affinity for the receptor.

We thank Drs S. B. Freedman, R. Hargreaves, N. Newberry and A. T. McKnight of the Biochemistry and Pharmacology Departments for permission to disclose the biological results, full details of which will be reported elsewhere.

Received, 25th March 1991; Com. 1/01410B

References

1 S. Iversen, *ChemB.*, 1988, 338; J. H. Growdon, *Med. Rev. Res.*, 1983, **3**, 237; E. K. Perry, *Br. Med. Bull.*, 1986, **42**, 63; B. S. Greenwald, R. C. Mohs and K. L. Davis, *J. Am. Geriatr. Soc.*, 1983, **31**, 310; *Trends Pharmacol. Soc.*, eds. B. I. Hirschowitz, R. Hammer, A. Giachetti, J. J. Keirns and R. R. Levine, Supplement 1983; T. Archer and C. J. Fowler, *Trends Pharmacol. Soc.*, 1985, **6**, 61.

2 W. H. Moos, R. E. Davis, R. D. Schwarz and E. R. Gamzu, *Med. Res. Rev.*, 1988, **8**, 353; R. Baker and J. Saunders, *An. Rep. Med. Chem.*, 1989, **24**, 31; E. Hollander, R. C. Mohs and K. L. Davis, *Br. Med. Bull.*, 1986, **42**, 97; F. M. Hershenson and W. H. Moos, *J. Med. Chem.*, 1986, **29**, 1125.

3 W. K. Summers, L. V. Majovski, G. M. Marsh, K. Tachiki and A. Kling, *New Eng. J. Med.*, 1986, **315**, 1241.

4 J. E. Christie, A. Shering, J. Ferguson and A. I. M. Glen, *Br. J. Psychiat.*, 1981, **138**, 46.

5 M. M. Mouradian, E. Mohr, J. A. Williams and T. N. Chase, *Neurology*, 1988, **38**, 606; R. D. Caine, *New Engl. J. Med.*, 1980, **303**, 585.

6 T. L. Bonner, N. J. Buckley, A. C. Young and M. R. Brann, *Science*, 1987, **237**, 527; E. G. Peralta, A. Ashkenazi, J. W. Winslow, D. H. Smith, J. Ramachandran and D. Capon, EMBO. *J.*, 1987, **6**, 3923.

7 Editorial comment, Subtypes of Muscarinic Receptors in *Trends in Pharmacol.*, 'Science Supplement,' Dec. 1989, p. VII; M. Waelbroeck, M. Tastenoy, J. Camus, R. Feifel, E. Mutschler, C. Strohmann, R. Tacke, G. Lambrecht and J. Christophe, *Trends in Pharmacol.*, 'Science Supplement,' Dec. 1989, p. 65.

8 J. Saunders, A. M. MacLeod, K. Merchant, G. A. Showell, R. J. Snow, L. J. Street and R. Baker, *J. Chem. Soc., Chem. Commun.*, 1988, 1618.

9 J. Saunders, M. Cassidy, S. B. Freedman, E. A. Harley, L. L. Iversen, C. Kneen, A. M. MacLeod, K. J. Merchant, R. J. Snow and R. Baker, *J. Med. Chem.*, 1990, **33**, 1128.

10 L. J. Street, R. Baker, T. Book, C. O. Kneen, A. M. MacLeod, K. J. Merchant, G. A. Showell, J. Saunders, R. H. Herbert, S. B. Freedman and E. A. Harley, *J. Med. Chem.*, 1990, **33**, 2690.

11 A. M. MacLeod, R. Baker, S. B. Freedman, S. Patel, K. J. Merchant, M. Roe and J. Saunders, *J. Med. Chem.*, 1990, **33**, 2052.

12 S. B. Freedman, E. A. Harley and L. Iversen, *Brit. J. Pharmacol.*, 1988, **93**, 437.

13 C. A. Grob and E. Renk, *Helv. Chim. Acta.*, 1954, **37**, 1689.

14 The absolute stereochemistry of **15** and **16** was obtained from the X-ray study of the bromo analogue of **16**, T. Willson, unpublished results.

15 N. Newberry, A. T. McKnight and M. J. Gilbert, unpublished results.