Synthesis and Muscarinic Activities of Quinuclidin-3-yltriazole and -tetrazole Derivatives

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The synthesis of 15 methyl or unsubstituted 1,2,3-triazoles, 1,2,4-triazoles, and tetrazoles additionally substituted with a 1-azabicyclo[2.2.2]octan-3-yl group is described. The potency and efficacy of these compounds as muscarinic ligands were determined in radioligand binding assays using [^{3}H]oxotremorine and [^{3}H]quinuclidinyl benzilate. Potency and efficacy were found in compounds in which the azole moiety was attached to the azabicyclic ring either through a carbon atom or a nitrogen atom. Electrostatic potential maps of both the C-linked and the novel N-linked series of compounds were calculated. A relationship between position and depth of the electrostatic minima relative to the azabicyclic ring and the potency and efficacy of the compounds was determined.

Introduction

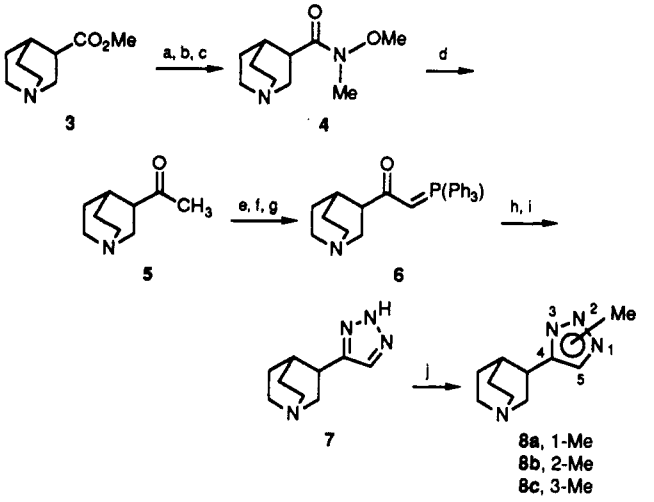
Memory loss and impaired cognition are characteristic symptoms of Alzheimer's disease (AD). It has been postulated that the appearance of these symptoms is associated with deficits in the central cholinergic pathways seen in postmortem studies of affected individuals. As a result there has been much attention given to efforts to design cholinomimetics which could reverse the effects of the neurochemical deficit with a consequent reversal of some symptoms of the disease.[1-4]

We have previously described a series of muscarinic agonists based on 1-azabicyclic oxadiazoles as a first step in the search for selective muscarinic agonists.[5] In these compounds the azabicycle is reversibly protonated at physiological pH and so is able to mimic the quaternary amine of acetylcholine (1) but, unlike acetylcholine, the unprotonated form is able to cross the blood–brain barrier. In particular the 3-methyl-1,2,4-oxadiazol-5-yl moiety was found to be a potent ester bioisostere.[5] We now propose that the electrostatic minima of the oxadiazole ring correspond with the electrostatic minima adjacent to the two oxygen atoms of the acetate unit of acetylcholine (Figure 1). Finally, the oxadiazole ring is more resistant to biological and chemical hydrolysis than the readily catabolized acetylcholine.

Muscarinic agonists mediate a variety of functions in animals, and patients treated with such compounds experience a number of side effects.[6] Consequently, selectivity of compounds for particular subtypes[7] of muscarinic receptors could offer potential advantages in therapy. We have therefore sought to develop an alternative series of muscarinic agonists in order to define the structural characteristics required of muscarinic agonists in general and in particular of compounds selective for the M_1 subtype of receptor which is abundant in the cortex.

Starting with the 3-methyl-1,2,4-oxadiazol-5-yl compounds previously described[5] as leads, we considered alternative heterocycles as potential ester bioisosteres. The use of 2-methyl-1,2,3-triazol-4-yl and 3-methyltetrazol-5-yl moieties as ester mimics in an arecoline-based series of muscarinic agonists has been described.[8] A comparison of some of the electrostatic potential maps of the oxadiazole, triazoles, and tetrazoles suggested a degree of similarity. Also within the triazole and tetrazole series, any

Scheme I[a]



[a] Reagents: (a) H^+; (b) $SOCl_2$; (c) NH(Me)OMe; (d) MeLi; (e) HCl, Cl_2; (f) $(Ph_3)P$; (g) K_2CO_3; (h) 3-nitrobenzoyl azide, CH_3CN; (i) Al_2O_3; (j) CH_2N_2.

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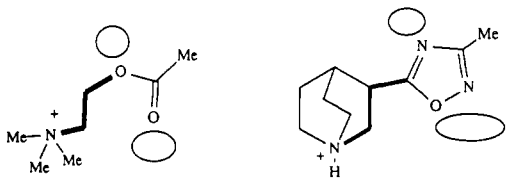


1 2

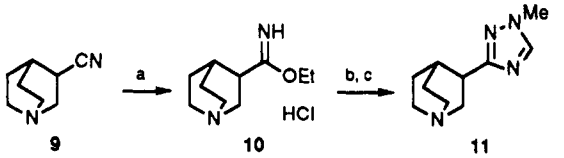
Figure 1. The circles diagrammatically represent the areas of electrostatic minima taken from ref 5.

combination of the position of attachment of the 1-azabicyclo[2.2.2]oct-3-yl (quinuclidin-3-yl) substituent and the methyl substituent was chemically possible. This offered wide scope for variety in both structure and biological activity. Azabicyclic triazoles and tetrazoles, and in particular those linked through nitrogen, are novel. Study of the electrostatic potential maps (see Results and Discussion) of 1,2,4-triazoles and tetrazoles suggested that the substitution of the azabicyclic moiety at the 2 position of both azoles most closely resembled the electrostatic potential map derived from the 3-methyl-1,2,4-oxadiazol-5-yl ring. We have devised synthetic routes to a range of structures in which the 1,2,3-triazoles, 1,2,4-triazoles, and the tetrazoles, linked through carbon or nitrogen, replace the oxadiazole in structure **2** in order to define further the factors controlling potency and efficacy of muscarinic agonists.

Synthetic Chemistry

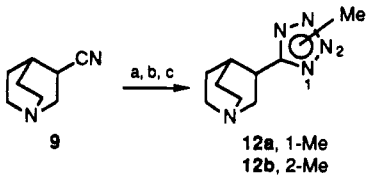
A commonly used literature method for the synthesis of 1,2,3-triazoles is the cycloaddition of an acetylene to an azide.[9] This requires forcing conditions and proceeds in poor yield unless the acetylene is activated with electron withdrawing groups such as alkoxycarbonyl. An alternative synthesis (Scheme I) previously used to prepare 4-methyl-1,2,3-triazole[10] is based on the cycloaddition of an electron-rich acetylene equivalent, in this case a α-keto phosphorus ylide, and an electron-deficient azide.[11] Applying this concept to the synthesis of **7** (Scheme I) would require a viable route to the α-keto phosphorus ylide **6**. The methyl ketone **5** was most conveniently prepared from methyl quinuclidine-3-carboxylate **3**[5] via the methoxyamide **4** by the method of Weinreb.[12] The methyl ketone thus derived was chlorinated in methanol under acid conditions, directing halogenation to the methyl group. Treatment of the crude reaction mixture with triphenylphosphine in acetonitrile yielded the required α-keto phosphorus ylide **6** which was contaminated with some methyl ketone from attack of the phosphine at the halogen rather than the carbon atom.[13] Use of the bromomethyl ketone gave exclusively halogen attack. The α-keto phosphorus ylide **6** was readily purified by column chromatography, and treatment with 3-nitrobenzoyl azide[14] in acetonitrile at reflux gave a (3-nitrobenzoyl)triazole. During purification on an alumina column, deprotection occurred and the free triazole **7** was isolated. Treatment with diazomethane afforded all three possible isomers,[15] which were separated by a combination of column chromatography and crystallization. The position of methylation in **8a**, **8b**, and **8c** was assigned using NOE difference experiments. Irradiation of the *N*-methyl resonance in **8a** at δ 4.10 gave a large NOE to H-5 of the triazole at δ 7.94. Irradiation of the *N*-methyl resonance at δ 3.92 in **8c** gave a large NOE to the azabicyclic ring protons at δ 1.83 and δ 2.88 but no NOE to H-5 of the triazole. Irradiation of the *N*-methyl resonance at δ 4.14 in **8b** gave only a very small NOE to H-5 of the triazole at δ 7.61. The 2-methyl isomer **8b** was, as expected, the major product.[15] The very low yields of **8a**, **8b**, and **8c** reflect two factors. The crude yield of the three isomers on alkylation with diazomethane was reduced by significant alkylation at the azabicyclic nitrogen atom. The resulting quaternary salt was lost on column chromatography. Yields were further reduced by the difficulty of separation of the isomers. Crystallization of the mixtures gave only a small portion of the total as crystallized pure isomers.

Scheme II[a]



[a] Reagents: (a) HCl; (b) $MeNHNH_2$; (c) HCO_2H.

Scheme III[a]



[a] Reagents: (a) TMS-N_3; (b) MeOH; (c) CH_2N_2.

The C-linked 1,2,4-triazole **11** may be prepared through a standard procedure[16] from the nitrile **9** (Scheme II). Treatment with HCl in EtOH gave the ethyl imidate **10** under standard Pinner conditions.[17] Reaction of **10** with $MeNHNH_2$ gave the amidrazone, which is unstable above 30 °C. Treatment with formic acid, first at room temperature and then at reflux resulted in ring closure to give the 1-methyl isomer **11**. In an NOE experiment, irradiation of the methyl resonance at δ 3.95 produced a strong positive NOE to the triazole proton H-3 at δ 8.40, but none to quinuclidine protons, confirming this assignment of regiochemistry. Thus, nucleophilic attack on the Pinner intermediate by the hydrazine had occurred primarily at the unsubstituted nitrogen atom.[18]

Tetrazoles are commonly synthesized by the cycloaddition of hydrazoic acid with nitriles under forcing conditions.[19] A convenient alternative (Scheme III) in-

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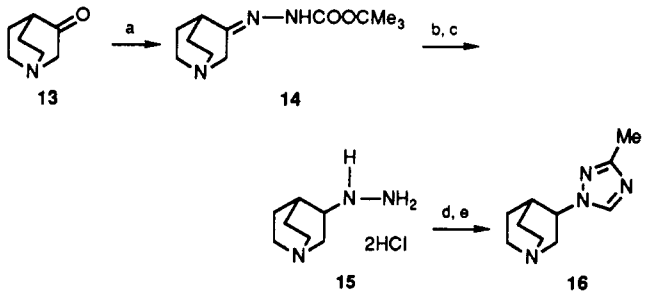
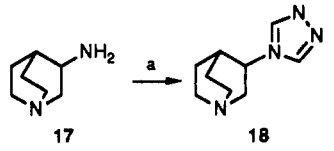
Scheme IV[a]



[a] Reagents: (a) $Me_3COCONHNH_2$; (b) H_2, Pd/C; (c) HCl/MeOH; (d) methyl acetimidate hydrochloride, Et_3N; (e) $(EtO)_3CH$, pyridine.

Scheme V[a]



[a] Reagents: (a) $(-N{=}CHNHMe)_2$.

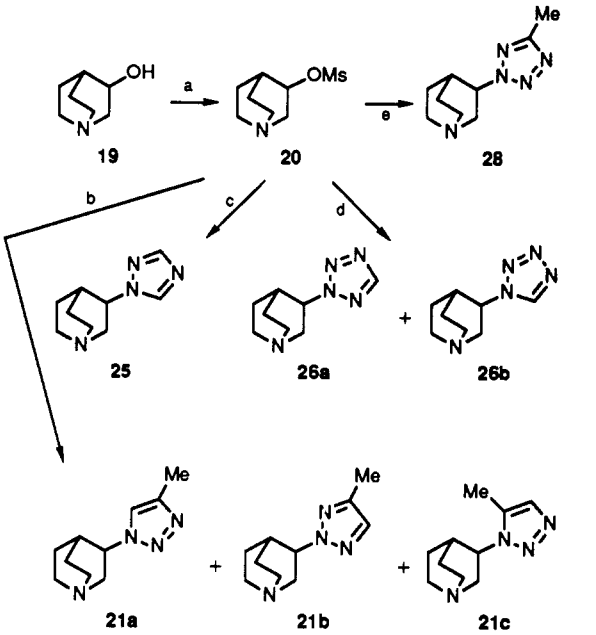
volves reaction of trimethylsilyl azide[19] with **9** followed by deprotection with MeOH. Methylation with ethereal diazomethane gave a 1:2 mixture of the 1-methyl- and 2-methyltetrazoles (**12a,b**). These isomers were readily separated by column chromatography and could be distinguished by ^1H NMR in which the 1-methyl group at δ 4.14 is more shielded than the 2-methyl group at δ 4.36.[20] This assignment was confirmed by NOE experiments, in which irradiation of 1-methyl protons gave a positive NOE to protons on the azabicyclic ring, an effect which was absent on irradiation of 2-methyl protons in a similar experiment.

The synthesis of the novel series of triazoles and tetrazoles linked through nitrogen was then approached. Two routes to 1,2,4-triazol-1-yl moieties were investigated with initially a stepwise assembly of the triazole ring being used (Scheme IV). Reaction of the ketone **13** with ^{t}butylcarbazate gave the protected hydrazone **14**. Subsequent hydrogenation and deprotection with HCl/MeOH gave a good yield of the hydrazine **15**. Regioselective synthesis of the 3-methyl-1,2,4-triazole **16** (rather than the 5-methyl isomer) was achieved by reaction of **15** with methyl acetimidate hydrochloride which afforded the amidrazone,[21] stable below 30 °C. Subsequent addition of triethyl orthoformate as a formaldehyde equivalent[22] completed the synthesis. A direct displacement route was also developed for the synthesis of N-linked azoles and this will be discussed later.

The isomeric 1,2,4-triazol-4-yl **18** was readily prepared from commercially available 3-aminoquinuclidine (**17**) using a standard literature method[23] by treatment with *N,N'*-dimethylformamide azine (Scheme V).

Stepwise construction of 2-substituted 1,2,3-triazoles and

Scheme VI[a]



[a] Reagents: (a) MsCl, pyridine; (b) sodium 4-methyl-1,2,3-triazolate (**22**), DMF, Δ; (c) sodium 1,2,4-triazolate (**23**), DMF, Δ; (d) sodium tetrazolate (**24**), DMF, Δ; (e) sodium 5-methyltetrazolate (**27**), DMF, Δ.

tetrazoles was not possible. Direct displacement of a leaving group at the 3-position of quinuclidine with an azole anion seemed a feasible alternative.[19] The 3-position of quinuclidine is sterically hindered with respect to nucleophilic displacement, but displacement of the 3-(methylsulfonyl)oxy group from **20** with azide anion is possible.[24]

Commercially available **19** can be readily converted to the methanesulfonate (mesylate) **20** (Scheme VI). The mesylate, which is surprisingly stable at room temperature, when treated with the sodium salt of 4-methyl-1,2,3-triazole[10] (**22**) in DMF at reflux afforded all three possible isomers **21a–c**, with the isomer **21b** predominating. Separation of this isomer was achieved by column chromatography and **21a** and **21c** (which eluted together) were separated by crystallization from petrol/ether. The regiochemistry of all three isomers was assigned by NOE experiments. Irradiation of H-3 of the quinuclidine moiety in **21a** at δ 5.20 gave a large positive NOE to H-5 of the triazole at δ 8.18. No NOE was observed to the methyl proton. In a similar experiment, irradiation of H-3 of the quinuclidine moiety in **21c** at δ 5.05 gave a large positive NOE to the methyl at δ 2.41. No NOE to the triazole proton was observed. Irradiation of H-3 of the quinuclidine moiety in **21b** at δ 5.09 resulted in no NOE to the methyl resonance and only a small NOE to the triazole proton H-5 at δ 7.68, while NOEs to quinuclidine protons between δ 1.00 and 4.00 were observed.

Likewise 1-substituted 1,2,4-triazole (**25**) was prepared by treatment of the mesylate **20** with the sodium salt of 1,2,4-triazole (**23**) in DMF at reflux (Scheme VI). The two distinct triazole proton resonances distinguished this product from the isomeric 4-substituted-1,2,4-triazole **18**.

Likewise N-1 and N-2-substituted tetrazoles were also prepared by this direct displacement method. Treatment of **20** with the sodium salt of tetrazole (**24**) gave a 4:1 mixture of N-2 and N-1 isomers **26a** and **26b**, respectively, which could be separated by column chromatography.

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Structural assignment was made using ^{1}H NMR. The H-3 proton of quinuclidine in **26a** at δ 5.66 is deshielded with respect to the equivalent proton in **26b** which is at δ 5.35 and is consistent with literature assignments.[20]

Treatment of **20** with the sodium salt of 5-methyltetrazole (**27**) gave exclusively **28**, rather than the 1-substituted isomer, presumably due to the steric influence of the 5-methyl group. The assignment was confirmed using ^{1}H NMR by the absence of an NOE between H-3 of the quinuclidine moiety and the methyl substituent. Although yields are not high in these displacement reactions, the directness makes them attractive routes.

Results and Discussion

The IC_{50} values for inhibition of tritiated muscarinic ligand binding by the triazoles and tetrazoles prepared in this study at rat cerebral cortex muscarinic receptor were determined as described previously.[25] We have shown that [^{3}H]oxotremorine-M ([^{3}H]OXO-M) binding is inhibited by both muscarinic agonists and antagonists with high potency. Conversely, [^{3}H]quinuclidinyl benzilate ([^{3}H]-QNB) binding is inhibited by muscarinic antagonists with high potency, whereas agonists are always much less potent. In consequence, the ratio of the (IC_{50} [^{3}H]QNB)/(IC_{50} [^{3}H]OXO-M) values is characteristic of the potential efficacy of muscarinic compounds. A ratio of greater than 100 is usually associated with a full agonist, and antagonists have ratios close to unity. Intermediate ratios are suggestive of partial agonism. Partial and full agonists which we required would therefore potently displace OXO-M binding while being less active against QNB binding. The results are shown in Table I.

The IC_{50} QNB/IC_{50} OXO-M ratios of compounds in Table I have values which suggest either partial or full agonism. Some of the compounds have potencies for the displacement of [^{3}H]OXO-M greater than that for the standards arecoline and pirenzepine, but all are less potent than the natural ligand acetylcholine and the standards oxotremorine and oxotremorine-M. All the compounds have IC_{50} QNB/IC_{50} OXO-M ratios less than the standards acetylcholine, arecoline, oxotremorine, and oxotremorine-M, implying that they are all less agonistic. Some of the compounds are however significantly more agonistic than the putative M_1 antagonist pirenzepine. As with the related oxadiazoles, the more potent compounds against OXO-M binding (**2**, **8b**, **12a**, **16**, and **28**) have a 1,3 relationship between the azabicyclic ring and a methyl substituent. An estimate of the lipophilic interaction between the methyl substituent and the receptor can be made by comparing **26a** to **28**, and **25** to **16**. The approximately 5-fold increase in affinity on introduction of methyl substitution is presumably associated with the binding pocket for the methyl of the acetyl group of acetylcholine. This comparison was not possible for ref 2 where the instability of the 3-unsubstituted 1,2,4-oxadiazole precluded its observation. No lipophilic contribution to binding was therefore noted. A subsequent publication,[3] in which the unsubstituted thiadiazole was stable, noted the contribution of the methyl substituent. A second even more important factor for determining affinity of agonists for the OXO-M labeled muscarinic receptor site is the position of the heteroatoms in the azole. The most potent compounds (**2**, **8b**, **12a**, **16**, and **28**) have nitrogen or oxygen atoms at positions 2 and 4 using the numbering system of Table I, where the methyl substituent is put in position 3. This has been rationalized[2] in terms of hydrogen bond acceptor capabilities of positions 2 and 4 contributing to the binding at the site labeled by OXO-M.

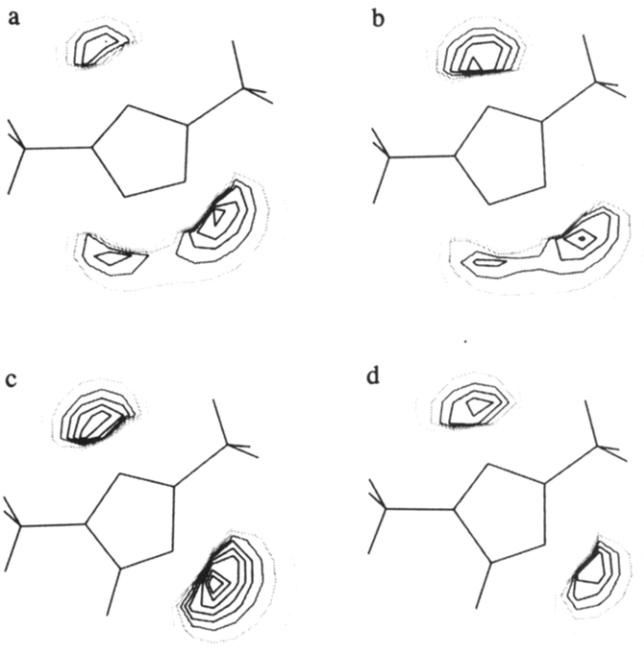

Figure 2. 2-D electrostatic potential maps for representative heterocycles. Contours at 10-kcal intervals decreasing from −10 kcal (lightest gray): (a) 3,5-dimethyl-1,2,4-oxadiazole; (b) 2,5-dimethyltetrazole; (c) 1,3-dimethyl-1,2,4-triazole; (d) 2,4-dimethyl-1,2,3-triazole.

Electrostatic Potential Maps

We have attempted to quantify the relationships between these structures and their potencies and efficacies using the electrostatic potential maps of these compounds. In order to simplify the calculations the quinuclidine substituent, which was common to all, was replaced by a methyl group. Details of this are presented in the Experimental Section. Four typical electrostatic potential maps are shown in Figure 2. The relative coordinates and depth of the electrostatic minima of compounds from Table I (excluding the hydrogen-substituted compounds for which the electrostatic minima are very similar to the methyl-substituted compounds) are presented in Table II. The coordinates of the electrostatic minima are an estimate of the optimum location a proton would adopt in forming a hydrogen bond to this ligand from the receptor. The magnitude of the potential well has been used to estimate the strength of such a hydrogen bond. The orientation of the azole was fixed in order to place the methyl substituent (not that used to mimic the azabicycle) at either position 2 or 3. The validity of this approach is seen in the subsequent analysis. A scatter diagram of the coordinates of the electrostatic minima of the compounds in Table II identified clusters of regions of electronegativity (Figure 3). Compounds with IC_{50} OXO-M < 150 nM (i.e. **2**, **8b**, **12a**, **16**, and **28**) all have electrostatic minima around x = 1.90, y = 2.24 *and* x = 4.50, y = 1.52. Three of these compounds, but not all, also have electrostatic minima close to x = 2 and y = −2.5. Although some compounds with IC_{50} OXO-M > 150 nM have electrostatic minima close to one of these two coordinates (x = 1.9, y = 2.24 and x = 4.5, y = 1.52), none have minima at both. This suggests that the optimum positions of the two electrostatic minima in structures mimicking the acetate unit of ace-

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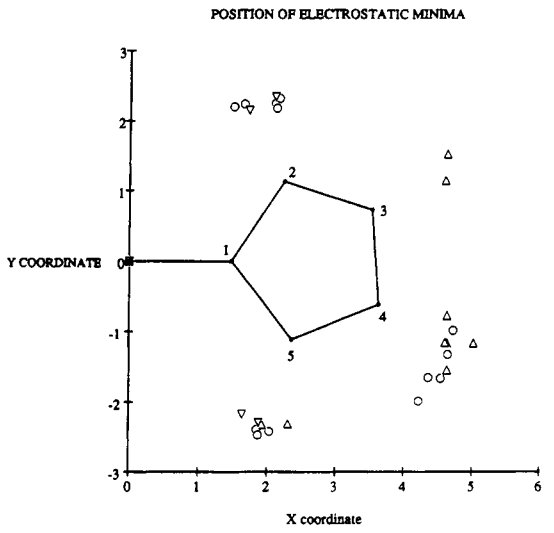


Figure 3. The position of the electrostatic minima taken from Table II. Compounds with IC_{50} OXO-M < 150 nm (**2, 18b, 12a, 16, 28**) are represented by a circle, compounds with no electrostatic minima adjacent to position 2 (**8a, 8c, 12b, 21a, 21c, 18**) are represented by a triangle, and compounds with no electrostatic minima adjacent to position 4 are represented by an inverted triangle. The units are 10^{-10} nm.

tylcholine are adjacent to positions 2 and 4 of quinuclidin-3-yl five-membered heterocycles, in agreement with previous work.[2,5]

We wished to derive a quantitative relationship between the position and depth of the electrostatic minima and the binding data. This would allow the IC_{50} OXO-M and IC_{50} QNB/IC_{50} OXO-M ratio to be calculated for all quinuclidin-3-yl substituted five-membered aromatic heterocycles. In a closely related series of muscarinic agonists based on quinuclidin-3-yl substituted five-membered heterocycles it has been reported[2] that a quantitative relationship exists between log (IC_{50} NMS)/(IC_{50} OXO-M) and the electrostatic potentials around the heterocycle [*N*-methylscopolamine (NMS) is a muscarinic ligand with properties similar to QNB]. Since a lipophilic interaction at position 3 is important in binding, and calculations based solely on electrostatic potentials could not take account of this effect, a subset of nine of our compounds was selected for a similar analysis. Compounds **2, 8a, 8b, 11, 12a, 21a, 21b, 16,** and **28**, which all have a 3-methyl substituent, were found by multiple regression analysis using RS1 to have a similar relationship (eq 1):

$$\log (IC_{50}\ QNB/IC_{50}\ OXO\text{-}M) = -0.0138\ (\pm 0.0027)(\nu_2) - 0.0128\ (\pm 0.0027)(\nu_4) - 0.062 \quad (1)$$

where ν_x = electrostatic potential adjacent to position x in Figure 3 (taken from Table II), $R = 0.925$, $f = 17.6$, SD = 0.085, and $n = 9$. No correlation was found between ν_5 and log (IC_{50} QNB/IC_{50} OXO-M). A graph of calculated log (IC_{50} QNB/IC_{50} OXO-M) vs measured log (IC_{50} QNB/IC_{50} OXO-M) is shown in Figure 4.

Equation 2 shown below derived in the previous study is of the same form, but the constants are of greater magnitude reflecting the lower affinity of NMS for the muscarinic receptor.[2]

$$\log (IC_{50}\ NMS/IC_{50}\ OXO\text{-}M) = 0.0187\ (\pm 0.0018)(\nu_2) - 0.019\ (\pm 0.0019)(\nu_4) \quad (2)$$

From these results we can therefore estimate the efficacy of this class of compounds from their electrostatic potential maps.

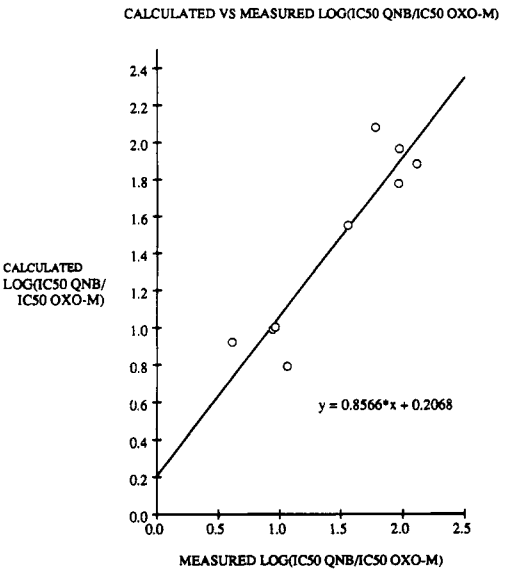


Figure 4. Plot of measured log (IC_{50} QNB/IC_{50} OXO-M) vs calculated log (IC_{50} QNB/IC_{50} OXO-M) using the equation: calculated log (IC_{50} QNB/IC_{50} OXO-M) = $-0.0138\nu_2 - 0.0128\nu_4 - 0.062$, with the line of best fit being $y = 0.857x + 0.21$.

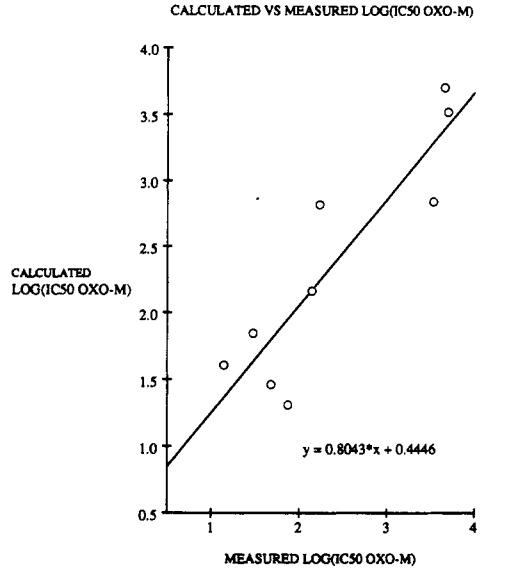


Figure 5. Plot of measured log (IC_{50} OXO-M) vs calculated log (IC_{50} OXO-M) using the equation: calculated log (IC_{50} OXO-M) = $0.027\nu_2 + 0.018\nu_4 + 4.9$ (IC_{50} OXO-M in nM) with the line of best fit being $y = 0.804x + 0.444$.

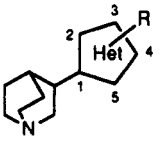
The scatter diagram shown in Figure 3 suggested a relationship might also exist between ν_2, ν_4, and the IC_{50} OXO-M binding. Regression analysis of the same series of compounds identified the following relationship (eq 3):

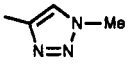
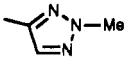
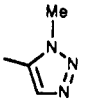
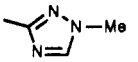
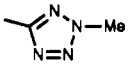
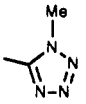
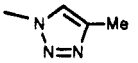
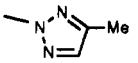
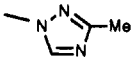
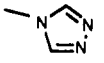
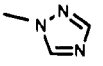
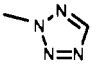
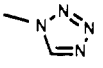
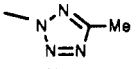
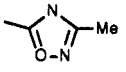
$$\log (IC_{50}\ OXO\text{-}M) = 0.027\ (\pm 0.0058)(\nu_2) + 0.018(\pm 0.0058)(\nu_4) + 4.9 \quad (3)$$

where ν_x = electrostatic potential adjacent to position x in Figure 3 (taken from Table II), $R = 0.89$, $f = 12.0$, SD = 0.18, and $n = 9$. No correlation was found between ν_5 and log (IC_{50} OXO-M). A plot of calculated log (IC_{50} OXO-M) vs measured log (IC_{50} OXO-M) binding is shown in Figure 5.

From this equation we can therefore calculate the IC_{50} OXO-M binding of this class of compounds from their electrostatic potential maps. The equation shows the greater importance of ν_2 compared with ν_4. This suggests that when this class of compounds bind to the receptor,

Table I. In Vitro Affinities for Muscarinic Receptors[a]



compd	heterocycle	[^{3}H]OXO-M IC_{50} (nM)	[^{3}H]QNB IC_{50} (nM)	IC_{50} QNB/ IC_{50} OXO-M
8a		4600 (3400–6250)	53 000 (44 000–64 000)	11.5
8b		48 (41–56)	4500 (3100–6600)	94
8c		930 (750–1150)	29 000 (22 000–38 000)	32
11		3400 (3200–3600)	30 000 (26 000–34 000)	8.8
12a		140 (95–200)	5100 (3300–6500)	36
12b		6500 (6100–7500)	60 000 (57 000–63 000)	9.2
21a		5000 (4400–5600)	20 000 (19 000–22 000)	4.1
21b		170 (115–260)	1600 (1200–2100)	9.2
21c		6700 (6500–7000)	33 000 (28 000–39 000)	4.9
16		75 (67.5–83)	4500 (3600–5600)	60
18		40 000 (35 000–46 000)	165 000 (160 000–170 000)	4.1
25		430 (350–490)	28 000 (17 500–44 000)	65
26a		240 (195–300)	16 000 (9000–28 000)	66
26b		2300 (2000–2750)	115 000 (110 000–120 000)	49
28		30 (13.5–62.5)	2800 (2600–3100)	92
2[b]		14 (6–29)	1800 (1700–2000)	130
acetylcholine[c]		12 (7–20) n = 3	24 000 (12 000–50 000) n = 3	2000
arecoline[d]		115 (75–170) n = 16	25 400 (10 000–110 000) n = 8	222
oxotremorine[d]		17 (7.5–33) n = 18	3300 (1750–4400) n = 11	190
oxotremorine-M[d]		13 (9–33) n = 10	23 000 (12 000–62 000) n = 12	1820
pirenzepine[d]		322 (80–1400) n = 30	213 (145–450) n = 31	0.7

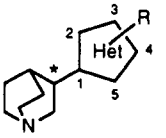
[a] Displacement of tritiated radioligand from rat corical homogenates. Each value represents the geometric mean of at least two determinations performed in separate experiments using seven concentrations. The ranges of values are given in parentheses. [b] Reference 5. [c] Assays carried out in the presence of eserine. [d] n = number of determinations.

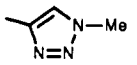
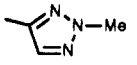
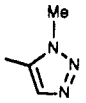
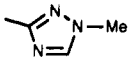
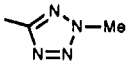
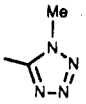
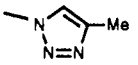
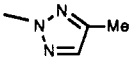
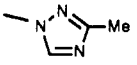
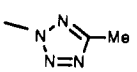
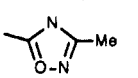
the hydrogen bond to position 2 is stronger than to position 4.

A further extention of this equation would be to allow for the contribution to binding made by the methyl substituent which has previously been noted, to be included. If the presence or absence of a methyl substituent at position 2 or 3 is denoted by a variable χ_2 or χ_3, where $\chi = 1$ when present and $\chi = 0$ when absent, a further analysis is possible. Now all compounds in Table II may be included. Multiple regression analysis identified the following relationship:

$$\log (IC_{50}\ \text{OXO-M}) = 0.031\ (\pm 0.0073)\nu_2 + 0.020\ (\pm 0.0080)\nu_4 - 1.33\ (\pm 0.57)\chi_2 - 0.95\ (\pm 0.45)\chi_3 + 6.4$$

Table II. Position and Depth of the Electrostatic Potential Minima around Azole[a]



compd	heterocycle	adjacent to 2[b]			adjacent to 4[b]			adjacent to 3 or 5[b]		
		x	*y*	V_2[c]	*x*	*y*	V_4[c]	*x*	*y*	$V_{3/5}$[c]
8a		–	–	0	4.63	−1.54	−66.7	1.93	−2.31	−81.0
8b		2.10	2.25	−77.2	4.64	−1.32	−75.2	–	–	–
8c		–	–	0	5.02	−1.16	−77.9	4.63	1.54	−71.3
11		2.10	2.34	−76.3	–	–	0	1.64	−2.17	−85.3
12a		2.16	2.32	−61.7	4.54	−1.66	−59.5	2.04	−2.42	−77.3
12b		–	–	0	4.63	−0.77	−76.5	1.93 for 5 4.63 for 3	−2.32 for 5 1.54 for 3	−76.4 for 5 −52.3 for 3
21a		–	–	0	4.63	−1.16	−76.9	2.32	−2.32	−68.3
21b		1.72	2.15	−77.2	–	–	0	1.88	−2.29	−75.2
21c		–	–	0	4.63	−1.16	−79.6	2.31	−2.31	−68.3
16		1.50	2.20	−76.3	4.72	−0.98	−85.1	–	–	0
18		–	–	0	4.60	−1.15	−95.5	4.60	1.15	−95.0
25		1.69	2.11	−84.9	4.21	−1.26	−92.0	–	–	0
26a		1.69	2.11	−54.8	4.21	−1.69	−69.1	2.11	−2.53	−48.6
26b		1.69	2.11	−56.3	4.63	−1.26	−76.1	4.63	1.26	−76.1
28		1.65	2.24	−61.7	4.36	−1.65	−77.3	1.85	−2.39	−59.5
2		2.12	2.18	−73.4	4.22	−1.98	−72.9	1.87	−2.47	−46.3

[a] The carbon atom of position * of quinuclidine was defined as $x = y = 0$. The x-axis was defined by the line joining the carbon atom at position 1 in the structure to the origin. The dimensions are 10^{-10} m. [b] The position of the electrostatic minima (calculated as described in the Experimental Section) in the plane of the azole ring adjacent to positions as defined in this table. The azole ring was oriented to place the methyl group at either position 2 or 3, whichever is possible. [c] Depth of the electrostatic minima in kcal mol^{-1}.

where v_x = electrostatic potential adjacent to position x, $n = 15$, $f = 7.4$, and $R = 0.91$. A plot of calculated versus measured log (IC_{50} OXO-M) is shown in Figure 6.

From this equation we can therefore calculate the IC_{50} OXO-M binding of all of this class of compounds with or without a methyl substituent at position 3. The coefficients of v_2 and v_4 are only marginally affected by the larger data set. A methyl substituent at position 3 is shown to increase the binding at the OXO-M receptor by a factor of (log 1), i.e. 10, close to the previous estimate of a factor of 5. Interestingly, from eq 3, a methyl substituent at position 2, of which **8c**, **12b**, and **21c** are three examples, also significantly increases binding. (The coefficient for $x_2 = 1.33$.) This result should be treated with care as rotation of the azole to put the methyl substituent at position 5 would alter the electrostatic minima. Also, the absence of an electrostatic minimum at v_2 (by definition), the most important for binding, or a methyl at position 3 may allow movement of the molecule to exploit the interaction normally found by a methyl substituent at

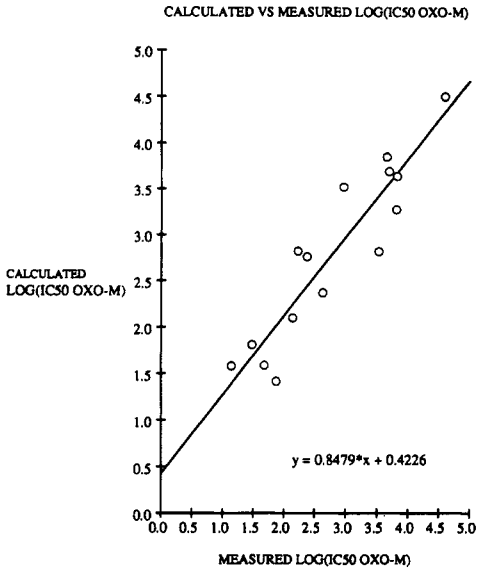


Figure 6. Plot of measured log (IC_{50} OXO-M) vs calculated log (IC_{50} OXO-M) using the equation: log (IC_{50} OXO-M) = $0.031\nu_2 + 0.020\nu_4 - 1.33\chi_2 - 0.95\chi_3 + 6.4$ (IC_{50} OXO-M in nM) with the line of best fit being $y = 0.848x + 0.423$.

position 3.

Conclusions

In a series of quinuclidin-3-yltriazole- and -tetrazole-based muscarinic ligands the positions of two electrostatic potential minima required for high affinity at the agonist binding site have been identified in agreement with previous work. A set of equations relating these minima and both log (IC_{50} QNB/IC_{50} OXO-M) and log (IC_{50} OXO-M) has been identified. From these equations it is possible to predict the potency and efficacy of a muscarinic agonist in the class of compounds described in this paper. For optimum binding to the agonist binding site, a lipophilic substituent at position 3 of the heterocycle is required. The greater importance of the electrostatic minima ν_2 compared with ν_4 in binding to the agonist binding site has been shown. We propose that the most potent muscarinic agonists in this series form two hydrogen bonds from the azole at positions 2 and 4 to the muscarinic receptor. This suggests that the acetyl group of the natural ligand also interacts through a hydrogen bond with each oxygen atom and via a lipophilic interaction with the methyl group.

Experimental Section

Melting points and boiling points are uncorrected. The elemental analyses were within 0.4% of the theoretical values. NMR spectra were recorded on a Bruker AM-400, Bruker AC-250, JEOL GX-270, or a Varian EM-360A spectrometer using Me_4Si as internal standard. All evaporations of solvents were carried out under reduced pressure, and organic solutions were dried over $NaSO_4$. For column chromatography, the silica gel used was Merck Kieselgel 60, and the alumina used was Camag Brockmann type II alkaline or BDH Brockmann type I neutral. Petrol refers to the fraction with bp 60–80 °C.

***N*-Methoxy-*N*-methyl-1-azabicyclo[2.2.2]octane-3-formamide (4).** Methyl quinuclidine-3-carboxylate3[5] (49 g, 0.268 mol) in 5 N HCl (1 L) was heated under reflux for 8 h. After evaporation of dryness, the residue was then treated with excess $SOCl_2$ (250 mL) and heated under reflux for 30 min at which time a homogeneous solution was obtained and the evolution of SO_2 ceased. The solution was then evaporated to dryness to a gum which was azeotroped repeatedly with toluene to remove the last traces of $SOCl_2$. The crystalline acid chloride hydrochloride was suspended in dry CH_3CN (250 mL) and cooled to −10 °C. To this was added dry *N*-methyl-*O*-methylhydroxylamine hydrochloride (28.5 g, 0.295 mol), and pyridine (106 g, 1.34 mol) was added dropwise at such a rate that the temperature did not exceed −5 °C. The solution was then allowed to warm to room temperature over a period of 1 h. $CHCl_3$ (500 mL) was added, and the solution was extracted with saturated aqueous K_2CO_3. The organic phase was separated, dried, and evaporated to yield 4 (17.7 g, 0.089 mol, 33%), bp 100–110 °C at 0.5 mmHg, which solidified on standing: ^1H NMR ($CDCl_3$) δ 1.3–1.45 (1 H, m), 1.5–1.7 (2 H, m), 1.75–1.9 (1 H, m), 2.0–2.1 (1 H, m), 2.62–3.08 (6 H, m), 3.2 (3 H, m), 3.23–3.33 (1 H, m), 3.7 (3 H, s). The oxalate salt crystallized from acetone as needles, mp 140–141 °C. Anal. ($C_{10}H_{18}N_2O \cdot C_2H_2O_4$) C, H, N.

3-Acetyl-1-azabicyclo[2.2.2]octane (5). A solution of 4 (16.3 g, 0.082 mol) in dry THF (250 mL) at −50 °C under N_2 was treated with methyllithium in ether (60.4 mL of a 1.5 M solution) over a period of 25 min. The reaction was allowed to warm to −20 °C, was held at this temperature for 15 min and was then recooled to −50 °C. The reaction was then quenched with glacial acetic acid (5.4 mL), poured onto saturated aqueous K_2CO_3, and extracted with $CHCl_3$ (3 × 250 mL). The combined organic extracts were dried and evaporated to dryness to yield a gum which was distilled in vacuo, bp 90–94 °C at 1 mmHg, to afford 5 (11.66 g, 0.076 mol, 92%): ^1H NMR (free base) (DMSO) δ 1.43–1.57 (1 H, m), 1.57–1.73 (1 H, m), 1.8–2.0 (2 H, m), 2.3 (3 H, s), 2.45–2.55 (1 H, m), 2.9–3.2 (6 H, m), 3.37–3.50 (1 H, m); ^{13}C NMR (free base) (DMSO) δ 20.2 (CH_2), 22.7 (CH_2), 24.5 (CH), 28.5 (CH_3), 45.8 (CH_2), 45.8 (CH_2), 46.6 (CH), 47.16 (CH_2), 208.3 (C=O). The oxalate salt crystallized from acetone, mp 130–135 °C. Anal. ($C_9H_{15}NO \cdot C_2H_2O_4$) C, H, N.

3-[[(Triphenylphosphoranylidene)methyl]carbonyl]-1-azabicyclo[2.2.2]octane (6). A solution of 3-acetyl-1-azabicyclo[2.2.2]octane (5) (5.44 g, 0.035 mol) in ether (100 mL) was treated with HCl gas until the precipitation of the HCl salt was complete. The suspension was evaporated to dryness to yield a gum which was redissolved in MeOH (50 mL). The solution was cooled to 0 °C, treated with a solution of Cl_2 (2.94 g, 0.042 mol) in MeOH (100 mL) at 0 °C, and allowed to warm to room temperature over 4 h. The solution was evaporated to dryness to yield a gum which was dissolved in dry CH_3CN (100 mL), treated with triphenylphosphine (20 g, 0.077 mol), and heated under reflux for 24 h. The reaction mixture was evaporated to yield a gum which was partitioned between $CHCl_3$ and saturated aqueous K_2CO_3. The organic phase was dried and evaporated to afford a gum. Column chromatography on basic alumina, eluting with EtOAc/MeOH (5:1), afforded 6 which crystallized from EtOAc/Et_2O (2.15 g, 0.0056 mol, 16%): mp 210–215 °C; ^1H NMR (DMSO) δ 1.15–1.30 (1 H, m), 1.43–1.60 (2 H, m), 1.60–1.75 (1 H, m), 2.04–2.13 (1 H, m), 2.42–2.75 (6 H, m), 3.14 (1 H, dd, J = 5 Hz, J = 15 Hz), 3.65 (1 H, d, J = 26 Hz), 7.48–7.74 (15 H, m). Anal. ($C_{27}H_{28}NOP \cdot 0.25H_2O$) C, H, N.

3-(2*H*-Triazol-4-yl)-1-azabicyclo[2.2.2]octane (7). A solution of 6 (8.0 g, 0.0019 mol) in CH_3CN (150 mL) under N_2 was treated with *m*-nitrobenzoyl azide[14] (7.44 g, 0.0386 mol) under reflux for 2 h. The solution was evaporated to dryness to yield a gum, which was purified by column chromatography on basic alumina, eluting with $CHCl_3$/MeOH (7:1) to afford 7 which was crystallized from EtOAc (1.95 g, 0.0109 mol, 52%) as needles: mp 172–174 °C; ^1H NMR (DMSO) δ 1.28–1.44 (1 H, m), 1.44–1.60 (1 H, m), 1.65–1.80 (2 H, m), 1.93–2.00 (1 H, m), 2.7–2.94 (4 H, m), 3.0–3.15 (2 H, m), 3.17–3.33 (1 H, m), 3.8 (1 H, br s), 7.84 (1 H, s); ^{13}C NMR (DMSO) δ 21.0 (CH_2), 26.6 (CH), 27.0 (CH_2), 32.4 (CH), 46.6 (CH_2), 47.0 (CH_2), 52.7 (CH_2), 128.6 (triazole C-5), 147.9 (triazole C-4). Anal. ($C_9H_{14}N_4$) C, H, N.

3-(1-Methyl-1,2,3-triazol-4-yl)-1-azabicyclo[2.2.2]octane (8a), 3-(2-Methyl-1,2,3-triazol-4-yl)-1-azabicyclo[2.2.2]octane (8b), and 3-(3-Methyl-1,2,3-triazol-4-yl)-1-azabicyclo[2.2.2]-octane (8c). A solution of 7 (1 g, 0.0056 mol) in MeOH (20 mL) was treated with diazomethane in ether at 0 °C until a faint yellow color persisted. The solution was allowed to warm to room temperature and then evaporated to dryness under reduced pressure to yield a gum. This was purified by column chromatography on basic alumina, eluting with EtOAc/MeOH (9:1), which afforded a fraction containing two *N*-methyl isomers (0.31 g). Crystallization from ether/petroleum ether afforded 8a as needles (25 mg, 2.3%): mp 126–129 °C; ^1H NMR ($CDCl_3$) δ 1.3–1.45 (1 H, m), 1.51–1.63 (1 H, m), 1.63–1.75 (2 H, m), 1.78–1.85 (1 H, m), 2.75–3.0 (6 H, m), 3.25–3.45 (1 H, m), 3.9 (3 H, m), 7.5 (1 H, s); ^{13}C NMR

($CDCl_3$) δ 21.0 (CH_2), 25.5 (CH), 27.3 (CH_2), 32.0 (CH), 34.5 (CH_3), 47.0 (CH_2), 47.9 (CH_2), 53.5 (CH_2), 131.3 (triazole C-5), 140.0 (triazole C-4). Anal. ($C_{10}H_{16}N_4 \cdot 2.5H_2O$) C, H, N.

The mother liquors were treated with oxalic acid in MeOH until no further precipitate was produced. Crystallization from acetone/MeOH afforded the oxalate salt of **8b** (79 mg, 5%) as needles: mp 127–132 °C; ^1H NMR (CD_3OD) 1.73–1.97 (2 H, m), 2.05–2.20 (2 H, m), 2.25–2.34 (1 H, m), 3.33–3.78 (7 H, m), 4.15 (3 H, s), 7.6 (1 H, s); ^{13}C NMR (CD_3OD) δ 19.3 (CH_2), 24.3 (CH_2), 27.2 (CH), 32.2 (CH_3), 41.9 (CH), 47.3 (CH_2), 47.8 (CH_2), 51.6 (CH_2), 133.6 (triazole C-5), 149.0 (triazole C-4), 165.4 (CO_2H)$_2$. Anal. ($C_{10}H_{16}N_4 \cdot 1.5C_2H_2O_4$) C, H, N.

Elution with EtOAc/MeOH (6:1) afforded a second fraction from column chromatography. Treatment with excess oxalic acid in MeOH afforded the oxalate salt of **8c** (30 mg, 2%) from acetone as needles: mp 97–102 °C; ^1H NMR (CD_3OD) δ 1.78–2.0 (2 H, m), 2.08–2.22 (2 H, m), 2.26–2.35 (1 H, m), 3.35–3.84 (7 H, m), 4.14 (3 H, s, CH_3), 7.95 (1 H, s); ^{13}C NMR (CD_3OD) δ 19.4 (CH_2), 24.4 (CH_2), 27.4 (CH), 32.3 (CH_3), 37.1 (CH), 47.3 (CH_2), 47.7 (CH_2), 51.6 (CH_2), 124.8 (triazole C-5), 148.5 (triazole C-4), 165.4 (CO_2H)$_2$. Anal. ($C_{10}H_{16}N_4 \cdot C_2H_2O_4 \cdot 0.1H_2O$) C, H, N.

3-(1-Methyl-1,2,4-triazol-3-yl)-1-azabicyclo[2.2.2]octane (11). 3-Cyano-1-azabicyclo[2.2.2]octane (**9**)[5] (1.0 g, 8.2 mmol) was dissolved in dry EtOH (5 mL), the solution was cooled to 0 °C, and HCl gas was bubbled through the solution to saturation. The solution was allowed to warm to room temperature under N_2, stirred for 3 h, and then evaporated to dryness to yield a crystalline solid. The solid was dissolved in dry EtOH (20 mL) under N_2, and $MeNHNH_2$ (0.6 mL, 1.3 equiv) and dry Et_3N (2.86 mL, 2.5 equiv) were added. The suspension was stirred at room temperature for 3 h and then evaporated to dryness, keeping the bath temperature below 30 °C. The resulting white solid was dissolved in formic acid (20 mL), stirred at room temperature for 30 min, and then heated under reflux for 1.5 h. The solution was cooled, poured into saturated aqueous K_2CO_3, and extracted with $CHCl_3$ (4 × 250 mL). The organic extracts were dried and evaporated to dryness. This residue was purified by column chromatography on basic alumina, eluting with EtOAc/MeOH (3%) to afford the triazole **11** (0.24 g, 15%), which was crystallized as the dihydrochloride salt: mp 220–222 °C dec (MeOH/Et_2O); MS observed 192.1377 ($C_{10}H_{16}N_4$ requires 192.1375); ^1H NMR (DMSO) δ 1.75 (2 H, m), 2.05 (2 H, m), 2.42 (1 H, m), 3.29 (4 H, m), 3.62 (3 H, m), 3.95 (3 H, s, Me), 8.84 (1 H, azole CH); ^{13}C NMR (DMSO) δ 18.3 (CH_2), 22.7 (CH_2), 24.8 (CH), 32.3 (CH), 36.2 (CH_3), 45.0 (CH_2), 45.4 (CH_2), 48.5 (CH_2), 144.9 (CH-azole), 161.7 (azole C-3). Anal. ($C_{10}H_{16}N_4 \cdot 2HCl$) C, H, N.

3-(1-Methyltetrazol-5-yl)-1-azabicyclo[2.2.2]octane (12a) and 3-(2-Methyltetrazol-5-yl)-1-azabicyclo[2.2.2]octane (12b). 3-Cyano-1-azabicyclo[2.2.2]octane (**9**)[5] (0.56 g, 4.1 mmol) was dissolved in dry THF (1 mL), and the solution was placed in a PTFE lined autoclave. Azidotrimethylsilane (1.66 mL, 6.6 mmol) was added, and the mixture was heated to 130 °C for 5 h. The resulting mixture was transferred to a flask using MeOH, excess solvent was removed under reduced pressure, and an ethereal solution of diazomethane (10 mmol) was added at 0 °C. The mixture was stirred for 2 h and then evaporated to dryness. The resulting oil was purified by column chromatography on neutral alumina, eluting with EtOAc/MeOH (3%) to give, in order of elution, the 2-methyltetrazole **12b** (120 mg, 15%) followed by the 1-methyltetrazole **12a** (63 mg, 8%). Isomer **12b** crystallized as an oxalate salt from ether/acetone: mp 164–166 °C; ^1H NMR (DMSO) δ 1.66 (2 H, m), 1.99 (2 H, bm), 2.33 (1 H, m), 3.23 (4 H, m), 3.72 (3 H, complex m), 4.36 (3 H, s, Me); ^{13}C NMR (DMSO) δ 18.4 (CH_2), 22.8 (CH_2), 24.8 (CH), 30.6 (CH), 39.5 (CH_3), 45.2 (CH_2), 45.5 (CH_2), 48.8 (CH_2), 166.1 (tetrazole C-5). Anal. ($C_9H_{15}N_4 \cdot C_2H_2O_4$) C, H, N.

Isomer **12a** crystallized as an oxalate salt from ether/methanol: mp 155–156 °C (MeOH/Et_2O); ^1H NMR (DMSO) δ 1.79 (2 H, bm), 2.03 (1 H, m), 2.18 (1 H, bm), 2.37 (1 H, m), 3.36 (4 H, m), 3.77 (3 H, complex m), 4.13 (3 H, s, Me); ^{13}C NMR (DMSO) δ 18.3 (CH_2), 22.8 (CH_2), 23.3 (CH), 28.5 (CH), 33.4 (CH_3), 45.2 (CH_2), 45.7 (CH_2), 48.9 (CH_2), 155.5 (tetrazole C-5). Anal. ($C_9H_{15}N_4 \cdot C_2H_2O_4$) C, H, N.

3-Hydrazino-1-azabicyclo[2.2.2]octane (15). Quinuclidin-3-one (**13**) (1.25 g, 10 mmol) was dissolved in petroleum ether (50 mL), and *tert*-butyl carbazate (2.0 g, 15 mmol) was added. The solution was heated under reflux for 24 h and allowed to cool overnight, and the colorless crystalline solid was isolated by filtration to yield **14** (2.30 g, 96%): mp 170–172 °C; MS observed 239.1634 ($C_{12}H_{21}N_3O_2$ requires 239.1632); ^1H NMR ($CDCl_3$) δ 1.51 (9 H, s, ^{t}Bu), 1.86 (4 H, m), 2.83 (5 H, complex m), 3.45 (0.85 H, s) with 3.55 (0.15 H, s); ^{13}C NMR ($CDCl_3$) δ 26.5 (CH_2), 28.2 (^{t}Bu), 31.0 (CH), 47.3 (CH_2), 52.7 (CH_2), 81.1 (^{t}Bu-*C*), 152.9 (C=N), 159.9 (C=O).

A solution of **14** (1.0 g, 4.18 mmol) in EtOH (100 mL) with anhydrous oxalic acid (0.37 g, 4.0 mmol) was hydrogenated at atmospheric pressure and 35 °C for 24 h using Pd/C (10%, 0.3 g) as catalyst. The suspension was filtered through Celite under N_2, and the filtrate was evaporated to dryness. The residue was dissolved in saturated aqueous K_2CO_3 and extracted with EtOAc (2 × 250 mL). The organic solution was dried, filtered, and evaporated to dryness under reduced pressure to yield the [*tert*-(butyloxy)carbonyl]hydrazine as a clear colorless oil (1.01 g, 100%) which crystallized on standing: mp 40–50 °C; ^1H NMR ($CDCl_3$) δ 1.47 (9 H, s, ^{t}Bu), 1.64 (1 H, m), 1.86 (2 H, m), 2.42 (1 H, dm), 2.78 (5 H, complex m), 3.66 (3 H, m). A portion of this (1.0 g, 4.2 mmol) was dissolved in 10% HCl/MeOH (40 mL) and heated under reflux for 30 min. The solution was evaporated to dryness and recrystallized from MeOH/Et_2O to afford **15** as a white crystalline dihydrochloride salt: mp 245–250 °C dec; MS observed 141.1265 ($C_7H_{15}N_3$ requires 141.1272); ^1H NMR (DMSO) δ 1.87 (2 H, m), 2.05 (1 H, m), 2.28 (1 H, m), 2.50 (1 H, m), 3.09 (1 H, d), 3.31 (4 H, bm), 3.62 (2 H, m); ^{13}C NMR (DMSO) δ 16.5 (CH_2), 21.0 (CH_2), 21.9 (CH), 44.7 (CH_2), 45.5 (CH_2), 49.9 (CH_2), 52.2 (CH). Anal. ($C_7H_{15}N_3 \cdot 2HCl$) C, H, N.

3-(3-Methyl-1,2,4-triazol-1-yl)-1-azabicyclo[2.2.2]octane (16). A suspension of **15** (0.78 g, 3.6 mmol) in dry MeOH (30 mL) was treated with methyl acetimidate hydrochloride (0.4 g, 3.65 mmol), followed by Et_3N (1.52 mL, 3 equiv). The solution was stirred for 3 h at room temperature under N_2 and then evaporated to dryness at a temperature not exceeding 30 °C. Anhydrous triethyl orthoformate (40 mL) and dry pyridine (4 mL) were added, and the suspension was stirred at room temperature under N_2 overnight and then heated at reflux for 1.5 h. The mixture was evaporated to dryness, and the residue was dissolved in saturated aqueous K_2CO_3 (40 mL). The product was extracted into EtOAc (2 × 300 mL), and the organic extracts were dried and evaporated to dryness. The resulting oil was purified by column chromatography on basic alumina, eluting with EtOAc/MeOH (2%). The resulting clear oil **16** was crystallized as a dihydrochloride salt from acetone/ether (110 mg, 13%); mp >250 °C (acetone/Et_2O); MS observed 192.1374 ($C_{10}H_{16}N_4$ requires 192.1374); ^1H NMR (DMSO) δ 1.85 (2 H, bm), 2.04 (2 H, bm), 2.42 (3 H, s, Me), 2.50 (1 H, m), 3.40 (4 H, m), 3.80 (2 H, bm), 5.07 (1 H, m), 9.05 (1 H, s, triazole CH); ^{13}C NMR (DMSO) δ 13.1 (CH_3), 17.2 (CH_2), 21.3 (CH_2), 26.0 (CH), 45.0 (CH_2), 45.5 (CH_2), 48.9 (CH_2), 53.4 (CH), 143.9 (triazole C-5), 157.0 (triazole C-3). Anal. ($C_{10}H_{16}N_4 \cdot 2HCl$) C, H, N.

3-(1,2,4-Triazol-4-yl)-1-azabicyclo[2.2.2]octane (18). 3-Amino-1-azabicyclo[2.2.2]octane (**17**) (2.6 g, 0.021 mol) in dry toluene (50 mL) was treated with *N,N'*-dimethylformamide azine (4.90 g, 0.042 mol) and tosic acid (100 mg) and heated under reflux for 12 h. The reaction was evaporated to dryness, and the residue was partitioned between $CHCl_3$ (200 mL) and saturated aqueous K_2CO_3. The organic phase was dried and evaporated to dryness to yield a gum. Column chromatography on basic alumina, eluting with EtOAc/MeOH (7%), afforded the 4-substituted triazole **18** (0.96 g, 26%), which was crystallized from $CHCl_3$/petroleum ether as needles: mp 151–153 °C; ^1H NMR ($CDCl_3$) δ 1.5–1.53 (2 H, m), 1.7–1.9 (2 H, m), 2.1–2.2 (1 H, m), 2.8–3.05 (4 H, m), 3.12 (1 H, dd, J = 13 Hz, J = 3 Hz), 3.57 (1 H, t, J = 11 Hz), 4.3–4.4 (1 H, m), 8.3 (2 H, m); ^{13}C NMR ($CDCl_3$) δ 20.0 (CH_2), 26.4 (CH), 28.1 (CH_2), 47.0 (CH), 47.7 (CH_2), 54.1 (CH_2), 54.4 (CH_2), 141.9 (triazole CH). Anal. ($C_9H_{14}N_4$) C, H, N.

3-(4-Methyl-1,2,3-triazol-1-yl)-1-azabicyclo[2.2.2]octane (21a), 3-(4-Methyl-1,2,3-triazol-2-yl)-1-azabicyclo[2.2.2]octane (21b), and 3-(4-Methyl-1,2,3-triazol-3-yl)-1-azabicyclo-[2.2.2]octane (21c). Quinuclidin-3-ol **19** (10.0 g, 0.079 mol) was dissolved in dry CH_2Cl_2 (200 mL) under N_2 and cooled to 0 °C. Methanesulfonyl chloride (10.8 g, 0.094 mol) and dry pyridine (7.46 g, 0.094 mol) were added, and the mixture was stirred at 0 °C for 20 min. The solution was allowed to warm to room

temperature and stirred under N_2 for 1 h. Saturated aqueous K_2CO_3 (150 mL) was added, and the product was extracted into EtOAc (3 × 200 mL). The organic solution was dried and evaporated to dryness to yield a pale yellow oil. The last traces of pyridine were removed under high vacuum to afford **20** (15.8 g, 98%): ^{1}H NMR ($CDCl_3$) δ 1.67 (4 H, m), 2.18 (1 H, pentet), 2.85 (5 H, bm), 3.03 (3 H, s, SO_2Me), 3.38 (1 H, q), 4.82 (1 H, m, *CH*OMs). Compound **20** (5.0 g, 24.4 mmol) was dissolved in dry DMF (150 mL), and **22** (5.0 g, 2 equiv) was added. The mixture was heated under reflux for 1 h, evaporated to dryness, dissolved in saturated aqueous K_2CO_3, and extracted with EtOAc (2 × 250 mL). The organic extracts were dried and evaporated to dryness, and the residue was purified by column chromatography on basic alumina, eluting with EtOAc/MeOH (36:1) to give, in order of elution, the 2-substituted triazole **21b** (1.1 g, 23.5%) which was crystallized as a hydrochloride salt: mp 181–182 °C (MeOH/Et_2O); MS observed 192.1373 ($C_{10}H_{16}N_4$ requires 192.1375); ^{1}H NMR (DMSO) δ 1.50 (1 H, m), 1.70 (1 H, m), 1.98 (2 H, m), 2.27 (3 H, s, azole Me), 3.24 (4 H, bm), 3.38 (1 H, m), 3.87 (2 H, m), 5.08 (1 H, m), 7.68 (1 H, s, triazole H); ^{13}C NMR (DMSO) δ 10.4 (CH_3), 17.3 (CH_2), 20.9 (CH_2), 26.0 (CH), 45.1 (CH_2), 45.4 (CH_2), 48.9 (CH_2), 57.5 (CH), 133.8 (triazole C-4), 143.8 (azole quaternary C). Anal. ($C_{19}H_{16}N_4$·HCl) C, H, N.

Also isolated, inseparable by chromatography, were the 1- and 3-substituted triazoles **21a** and **21c**, which were separated by repeated trituration of the residue with petroleum ether. The solid isolated was the substituted triazole **21c** (0.41 g, 9%), which was crystallized as an oxalate salt: mp 151–153 °C (MeOH/Et_2O); MS observed 192.1377 ($C_{10}H_{16}N_4$ requires 192.1375); ^{1}H NMR (DMSO) δ 1.75 (2 H, br m), 2.08 (2 H, br m), 2.36 (1 H, m), 2.41 (3 H, s, triazole Me), 3.33 (4 H, m), 3.52 (1 H, m), 3.81 (1 H, br m), 5.05 (1 H, m, quinuclidine H-3), 7.68 (1 H, s, triazole H); ^{13}C NMR (DMSO) δ 7.8 (CH_3), 17.5 (CH_2), 22.1 (CH_2), 25.7 (CH), 45.5 (CH_2), 45.8 (CH_2), 49.6 (CH_2), 52.0 (CH), 132.9 (triazole C-5), 133.8 (triazole C-4). Anal. ($C_{10}H_{16}N_4$·$C_2H_2O_4$) C, H, N.

The filtrate was evaporated to dryness, and the residue was repeatedly triturated with petroleum ether followed by decantation and evaporation to dryness to yield **21a** (0.62 g, 13%), which crystallized as an oxalate salt: mp 135–140 °C (MeOH/Et_2O); ^{1}H NMR (DMSO) δ 1.66 (1 H, m), 1.82 (1 H, m), 2.10 (2 H, m), 2.35 (3 H, s, azole Me), 2.50 (1 H, m), 3.40 (4 H, m), 3.94 (1 H, m), 4.07 (1 H, m), 5.20 (1 H, m, quinuclidine H-3), 8.18 (1 H, s, azole-CH); ^{13}C NMR (DMSO) δ 10.5 (CH_3), 17.2 (CH_2), 21.3 (CH_2), 26.5 (CH), 45.2 (CH_2), 45.4 (CH_2), 48.8 (CH_2), 54.1 (CH), 122.5 (triazole C-5), 142.5 (triazole C-4). Anal. ($C_{10}H_{16}N_4$·1.5$C_2H_2O_4$) C, H, N.

3-(1,2,4-Triazol-1-yl)-1-azabicyclo[2.2.2]octane (25). The mesylate **20** (0.6 g, 3.0 mmol) was dissolved in dry DMF (20 mL), **23** (1.0 g, 0.011 mol) was added, and the mixture was heated under reflux for 1 h. The mixture was evaporated to dryness, and the residue was partitioned between saturated aqueous K_2CO_3 and EtOAc (2 × 100 mL). The organic extracts were separated, dried, and evaporated to afford an oil, which was purified by column chromatography on basic alumina, eluting with EtOAc/MeOH (36:1) to yield a colorless oil (0.134 g, 26%). This was crystallized as a dihydrochloride salt: mp 187–190 °C; ^{1}H NMR (DMSO) δ 1.83 (2 H, m), 1.99 (2 H, m), 2.42 (1 H, m), 3.29 (4 H, m), 3.86 (2 H, bm), 5.08 (1 H, m), 8.28 (1 H, s, triazole CH), 9.02 (1 H, s, triazole CH); ^{13}C NMR (DMSO) δ 17.1 (CH_2), 21.2 (CH_2), 26.5 (CH), 44.8 (CH_2), 45.2 (CH_2), 49.0 (CH_2), 53.3 (CH), 144.0 (triazole CH), 150.2 (triazole CH). Anal. ($C_9H_{14}N_4$·2HCl) C, H, N.

3-(Tetrazol-2-yl)-1-azabicyclo[2.2.2]octane (26a) and 3-(Tetrazol-1-yl)-1-azabicyclo[2.2.2]octane (26b). Following the procedure described for **25**, the mesylate **20** (1.0 g, 4.9 mmol) in dry DMF (50 mL) was reacted with **24** (1.5 g, 3.4 equiv). The resulting oil was purified by column chromatography on basic alumina, eluting with EtOAc/MeOH (36:1) to give the 2-substituted tetrazole **26a** (80 mg, 9%), which crystallized as an oxalate salt from acetone as needles: mp 139–141 °C; MS observed 179.1173 ($C_8H_{13}N_5$ requires 179.1171); ^{1}H NMR (DMSO) δ 1.68 (1 H, m), 1.91 (1 H, m), 2.19 (2 H, m), 2.75 (1 H, m), 3.42 (4 H, m), 4.06 (2 H, m), 5.66 (1 H, m, quinuclidine H-3), 9.21 (1 H, s, tetrazole H); ^{13}C NMR (DMSO) δ 17.1 (CH_2), 20.9 (CH_2), 25.7 (CH), 45.1 (CH_2), 45.4 (CH_2), 49.0 (CH_2), 57.4 (CH), 153.4 (tetrazole CH). Anal. ($C_8H_{13}N_5$·0.8$C_2H_2O_4$) C, H, N.

Further elution gave the 1-substituted tetrazole **26b** (60 mg, 7%) which crystallized as a hydrochloride salt from acetone: mp 229–230 °C; MS observed 179.1171 ($C_8H_{13}N_5$ requires 179.1171); ^{1}H NMR (DMSO) δ 2.00 (2 H, m), 2.52 (2 H, m), 3.12 (1 H, m), 3.43 (6 H, complex m), 5.35 (1 H, m, quinuclidine H-3), 9.08 (1 H, s, tetrazole H); ^{13}C NMR (DMSO) δ 21.8 (CH_2), 22.8 (CH_2), 38.6 (CH), 49.6 (CH_2), 50.1 (CH_2), 56.2 (CH_2), 60.2 (CH), 153.1 (tetrazole C-5). Anal. ($C_8H_{13}N_5$·HCl) C, H, N.

3-(5-Methyltetrazol-2-yl)-1-azabicyclo[2.2.2]octane (28). Following the procedure described for **25**, the mesylate **20** (1.0 g, 4.9 mmol) in dry DMF (50 mL) was reacted with **27** (1.26 g, 2.5 equiv) to yield **28** (90 mg, 10%), which was crystallized as a hydrochloride salt: mp 196–198 °C; MS observed 199.1328 ($C_9H_{15}N_5$ requires 199.1328); ^{1}H NMR (DMSO) δ 1.63 (1 H, m), 1.79 (1 H, m), 2.05 (2 H, m), 2.50 (3 H, s, Me), 3.32 (5 H, m), 3.92 (2 H, m), 5.44 (1 H, m, quinuclidine H-3); ^{13}C NMR (DMSO) δ 10.6 (CH_3), 17.1 (CH_2), 20.9 (CH), 25.6 (CH), 45.1 (CH_2), 45.5 (CH_2), 49.0 (CH_2), 57.1 (CH), 162.4 (azole C-5). Anal. ($C_9H_{15}N_5$·HCl·0.5H_2O) C, H, N.

5-Methyltetrazole (29).[26,27] Acetonitrile (4.1 g, 5.22 mL, 0.10 mol), sodium azide (7.48 g, 0.115 mol), acetic acid (6.59 mL, 0.115 mol), and 2-propanol (20 mL) were mixed and heated in a PTFE-lined autoclave at 130 °C for 5 days with stirring. The reaction was allowed to cool, transferred to a round-bottomed flask with EtOH, and evaporated to dryness. The solid was extracted repeatedly with boiling *n*-butyl acetate until no further solid dissolved. The organic extracts were evaporated to a small volume, and the solid was allowed to crystallize to give the title compound **29** as a pale cream solid (3.25 g, 39%): MS observed 84.0436 ($C_2H_4N_4$ requires 84.0436); ^{1}H NMR ($CDCl_3$) δ 1.85 (3 H, s, Me), 4.12 (1 H, bs, NH).

General Synthesis of Azole Sodium Salt. Sodium metal (0.01 mol) was dissolved in EtOH (50 mL) under N_2 at 5 °C, the solution was warmed to room temperature, and the azole (0.01 mol) was added. The suspension was stirred until dissolution occurred, and the solution was evaporated to dryness to yield a white solid, which was dried in vacuo at 50 °C. The salt was then used directly. Salts made thus were sodium 4-methyl-1,2,3-triazolate (**22**),[10] sodium 1,2,4-triazolate (**23**), sodium tetrazolate (**24**), and sodium 5-methyltetrazolate (**27**) (from **29**).

Radioligand Binding. Cerebral cortex from Hooded Lister rats (Olac, U.K.) was homogenized in 2.5 volumes of ice-cold 50 mM Tris buffer pH 7.7 (at 25 °C). After centrifugation at 25000*g* at 4 °C for 15 min, the pellet was resuspended in 2.5 volumes of buffer and the wash was repeated three times more. The final resuspension was in 2.5 volumes, and the homogenates were stored in 1-mL aliquots at −20 °C. Incubations (total volume 2 mL) were prepared using the above buffer with the addition of 2 mM magnesium chloride in the [^{3}H]oxotremorine-M ([^{3}H]OXO-M) experiments. For [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB), 1 mL of stored membranes was diluted to 30 mL, and 0.1 mL was mixed with test compound and 0.27 nM (ca. 25000 cpm) [^{3}H]QNB (Amersham International). For [^{3}H]OXO-M, 1 mL of membranes was diluted to 6 mL, and 0.1 mL was mixed with test compound and 2 nM (ca. 250000 cpm) [^{3}H]OXO-M (New England Nuclear). Nonspecific binding of [^{3}H]QNB was defined using 1 μM atropine sulphate (2 μM atropine) and of [^{3}H]OXO-M using 10 μM oxotremorine. Nonspecific binding values typically were 5% and 25% of total binding, respectively. Incubations were carried out at 37 °C for 30 min, and the samples were filtered using Whatman GF/B filters (in the [^{3}H]OXO-M experiments the filters were presoaked for 30 min in 0.05% polyethylenimine in water). Filters were washed with 3 × 4 mL of ice-cold buffer. Radioactivity was assessed using a Packard BPLD scintillation counter and 3 mL of Pico-Fluor 30 (Packard) as scintillant.

Molecular Modeling. Structures were built using standard bond lengths and angles in SYBYL, developed and distributed by Tripos Associates Inc., St. Louis, MO. Geometry optimizations were carried out using the semiempirical AM1 method[28] in the

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AMPAC programm.[29] Ab initio calculations were carried out using the extended 4-31G basis set[30] in GAMESS (Generalised Atomic and Molecular Electronic Structure System, Revision A, M. F. Guest).[31] Two-dimensional potential maps were displayed on an Iris Silicon Graphics work station (Model 4D 70G) using software developed by Dr. F. E. Blaney in collaboration with Polygen Corp.

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Registry No. **3**, 38206-86-9; **4**, 139348-20-2; **5**, 93117-84-1; **6**, 133366-38-8; **7**, 133366-39-9; **8a**, 133366-07-1; **8b**, 139348-21-3; **8c**, 133366-10-6; **9**, 51627-76-0; **10**, 139348-22-4; **11**, 139348-23-5; **12a**, 133365-87-4; **12b**, 133365-85-2; **13**, 3731-38-2; **14**, 136681-83-9; **15**, 53242-77-6; **16**, 139348-24-6; **17**, 6238-14-8; **18**, 139348-25-7; **19**, 1619-34-7; **20**, 127424-06-0; **21a**, 139348-27-9; **21b**, 139348-28-0; **21c**, 139348-30-4; **22**, 139348-31-5; **23**, 41253-21-8; **24**, 13440-29-4; **25**, 139348-32-6; **26a**, 139348-34-8; **26b**, 139348-35-9; **27**, 40370-03-4; **28**, 139348-36-0; NH(Me)OMe, 1117-97-1; $MeNHNH_2$, 60-34-4; TMS-N_3, 4648-54-8; $Me_3COCONHNH_2$, 870-46-2; MeC(OMe)=NH, 14777-27-6; MeNHCH=N—N=CHNHMe, 139348-37-1; 3-nitrobenzoyl azide, 3532-31-8; 5-methyltetrazole, 4076-36-2; sodium azide, 26628-22-8; acetonitrile, 75-05-8.

Muscarinic Receptor Subtype Specificity of (*N*,*N*-Dialkylamino)alkyl 2-Cyclohexyl-2-phenylpropionates: Cylexphenes (Cyclohexyl-Substituted Aprophen Analogues)

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A series of aprophen [(*N*,*N*-diethylamino)ethyl 2,2-diphenylpropionate] analogues, called cylexphenes, were synthesized with alterations in (1) the chain length of the amine portion of the ester, (2) the alkyl groups on the amino alcohol, and (3) a cyclohexyl group replacing one of the phenyl rings. The antimuscarinic activities of these analogues were assessed in two pharmacological assays: the inhibition of acetylcholine-induced contraction of guinea pig ileum, and the blocking of carbachol-stimulated release of α-amylase from rat pancreatic acinar cells. These two tissues represent the M_3(ileum) and M_3(pancreas) muscarinic receptor subtypes. In addition, the analogues were also evaluated for their competitive inhibition of the binding of [^{3}H]NMS to selected cell membranes, each containing only one of the m_1, M_2, m_3, or M_4 muscarinic receptor subtypes. The m_1 and m_3 receptors were stably transfected into A9 L cells. The replacement of one phenyl group of aprophen with a cyclohexyl group increased the selectivity of all the analogues for the pancreatic acinar muscarinic receptor subtype over the ileum subtype by more than 10-fold, with the (*N*,*N*-dimethylamino)propyl analogue exhibiting the greatest selectivity for the pancreas receptor subtype, over 30-fold. The cylexphenes also showed a decrease in potency in comparison to the parent compound when examined for the binding of [^{3}H]NMS to the M_2 subtype. In agreement with the pharmacological data obtained from the pancreas, the (*N*,*N*-dimethylamino)propyl cylexphene **3** demonstrated the greatest selectivity for the m_3 subtype, and additionally showed a preference for the m_1 and M_4 receptor subtypes over the M_2 receptor subtype in the binding assay. Thus, this compound showed a potent selectivity according to the pharmacological and binding assays between the muscarinic receptor subtypes of the pancreas and ileum. In both the pharmacological and binding assays, the potency of the analogues decreased markedly when the chain length and the bond distance between the carbonyl oxygen and protonated nitrogen were increased beyond three methylene groups. When the structures of these analogues were analyzed using a molecular modeling program, the bond distance between the carbonyl oxygen and protonated nitrogen was deduced to be more important for the antagonist activity than subtype specificity.

Introduction

Aprophen [(*N*,*N*-diethylamino)ethyl 2,2-diphenylpropionate] is a potent anticholinergic and antispasmodic agent possessing a wide number of distinct pharmacological actions, including both antimuscarinic and noncompetitive nicotinic antagonist activities.[1-8] The potent antimuscarinic and, to a lesser extent, the antinicotinic effects of aprophen make it a potential drug of choice in the therapy of poisoning by organophosphate agents.[4,9] Although muscarinic receptors have been shown recently to exist in five subtypes,[10-18] the subtype specificity of aprophen and its analogues has not been determined.

Several functional groups are required in a molecule to achieve potent antimuscarinic properties.[19,20] First, a protonated nitrogen atom near one end of the molecule acts as the cationic site. Second, the center of the compound contains an electronegative ester group which is part of the anionic site. Lastly, a relatively bulky hydrophobic

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