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Synthesis and In Vitro Binding Affinities of 1-Azabicyclic Compounds as Muscarinic Ligands

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Abstract—Two series of compounds, **2** and **3**, were synthesized and their binding affinities were evaluated for the human recombinant muscarinic M_1 receptor subtype expressed in CHO cells. Comparing their binding affinities for the NMS binding sites and the Oxo-M binding sites, they were assumed as agonists. In particular, compound **2e** was a good ligand for the agonist binding sites with an IC_{50} of 23 nM, which represents over 1585 times stronger binding than for the antagonist binding sites. © 2001 Elsevier Science Ltd. All rights reserved.

Recently, we have reported that 3-(3-phenylisoxazol-5-yl)methylidene-1-azabicyclic compounds **1** showed characteristic binding to acetylcholine receptors according to the substituent on the phenyl group.[1] Generally they exhibited preferential binding to muscarinic receptors over nicotinic receptors. Thereafter we have focused on ligands that preferentially bind to muscarinic receptors and modified the structure of the lead compound accordingly. The methylidene group of **1** was replaced by oxyimino group and oxyiminomethyl group to give the compounds **2** and **3**, respectively (Fig. 1).

We wish to report the synthesis and binding affinities of the 1-azabicyclic compounds **2** and **3** to the muscarinic M_1 receptor in this paper.

Chemistry

The 1-azabicyclic compounds **2** and **3** were prepared by the condensation reaction of isoxazol-5-ylmethoxyamines with the corresponding carbonyl compounds, that is **4** and **5**, respectively (Scheme 1).[2] Analysis of ^1H NMR spectra showed that they were obtained as 1:1 mixtures of *syn* and *anti* isomers. When $n=1$, the reactants (**4** and **5**) and the products (**2** and **3**) are all racemates. All the final compounds were subjected to receptor binding study without further separation.

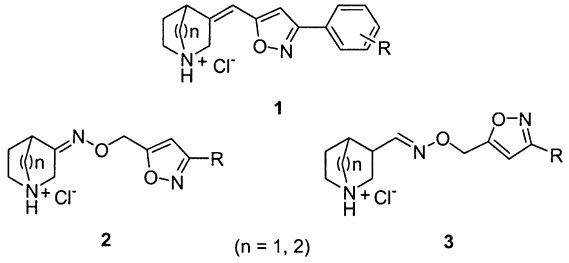
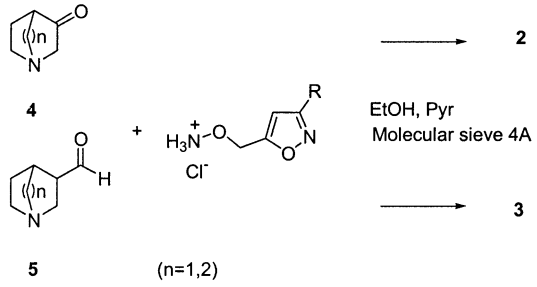


Figure 1. Structures of 1-azabicyclic compounds.



Scheme 1. Synthesis of 1-azabicyclic compounds.

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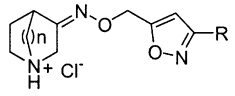
Table 1. In vitro binding affinities of **2** to the muscarinic M_1 receptor[a]

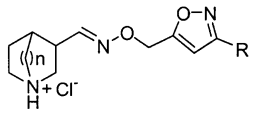


2

Compd	*n*	R	IC_{50} (μM)[b]		NMS/Oxo-M ratio
			[^{3}H]-NMS	[^{3}H]-Oxo-M	
2a	1	OCH_3	40.54±8.9	0.131±0.010	309.47
2b	2		35.6±9.1	0.362±0.110	98.38
2c	1	CN	32.5±7.2	0.082±0.016	396.34
2d	2		8.7±2.0	0.081±0.008	106.97
2e	1	Cl	36.3±4.9	0.023±0.006	1585.13
2f	2		13.8±1.9	0.057±0.002	243.38
2g	1	Br	48.8±7.4	0.079±0.008	614.34
2h	2		11.5±1.3	0.072±0.002	158.79
Oxotremorine-M			27.1±7.7	0.0023±0.0013	11,645.95
Arecoline			115.5±19.5	0.115±0.019	1001.62

[a]See refs 3–9.
[b]IC_{50} (μM) = mean±SE, $n = 3$.

Table 2. In vitro binding affinities of **3** to the muscarinic M_1 receptor[a]



3

Compd	*n*	R	IC_{50} (μM)[b]		NMS/Oxo-M ratio
			[^{3}H]-NMS	[^{3}H]-Oxo-M	
3a	1	OCH_3	16.7±2.9	0.287±0.034	58.11
3b	2		29.5±6.2	1.641±0.194	17.97
3c	1	CN	189.5±38.7	1.860±0.500	101.9
3d	2		132.7±30.6	0.341±0.137	388.9
3e	1	Cl	14.9±2.9	0.044±0.017	342.18
3f	2		23.5±3.7	0.795±0.080	29.5
3g	1	Br	17.7±3.7	0.385±0.143	46
3h	2		8.4±2.1	1.217±0.186	6.87
Oxotremorine-M			27.1±7.7	0.0023±0.0013	11,645.95
Arecoline			115.5±19.5	0.115±0.019	1001.62

[a,b]See the corresponding footnotes in Table 1.

Results

The abilities of the compounds **2** and **3** to displace [^{3}H]-*N*-methylscopolamine (NMS), a muscarinic receptor antagonist, and [^{3}H]-oxotremorine-M (Oxo-M), a muscarinic receptor agonist, from the muscarinic M_1 receptor were determined by methods described previously with some modification.[3–9] Test results for the compounds **2** are summarized in Table 1.

Binding affinities of arecoline and oxotremorine-M, muscarinic receptor agonists, were also measured for comparison. The affinities of the compounds for the receptors labeled by NMS were considered to be the compounds' affinities for the antagonist binding sites while affinities for the receptors labeled by Oxo-M were considered to be the compounds' affinities for the agonist binding sites. Generally, the compounds showed exclusive displacement of Oxo-M implying that they might be functionally muscarinic receptor agonists. Almost all of the compounds were stronger ligands than arecoline for both binding sites of the M_1 receptor. In particular, the compound **2e** was the best ligand of the compounds tested for the agonist binding sites. It showed an IC_{50} of 23 nM for the Oxo-M binding sites and an IC_{50} of 36,300 nM for the NMS binding sites, giving an IC_{50} ratio of over 1585. The quinuclidine compounds ($n = 2$) bound to the antagonist binding sites more strongly than the 1-azabicyclo[2.2.1]heptane compounds ($n = 1$). When R = CN or Br, the two types of compounds showed similar binding affinities for the agonist binding sites.

Binding affinities of the compounds in series **3**, one-carbon homologues of the compounds in series **2**, are summarized in Table 2.

Insertion of a carbon unit between the nitrogen atom and the azabicycle of **2** gave unfavorable effects on binding. All the compounds **3** exhibited weaker binding affinities than the compounds **2** to the agonist binding sites of the M_1 receptor. Their putatively agonistic activities apparently decreased. Except when R = CN, 1-azabicyclo[2.2.1]heptane compounds ($n = 1$) showed much stronger binding affinities than the quinuclidine compounds ($n = 2$) to the agonist binding sites. The compound **3e**, which corresponds to **2e** of the former series, however, was still the strongest ligand of this series.

In the previous report,[1] the compound **1** showed considerable binding affinity for the NMS binding sites when R was 4-*p*-methoxybenzyloxy group ($IC_{50} < 1$ μM), but the compounds **2** and **3** with the same R showed almost no binding affinities for the same sites even at 1 μM concentration. Thus the increase in size of molecules seemed not good for binding.

In summary, both the compounds **2** and **3** seemed to be agonists of the M1 receptor, and the compounds **2** were better ligands than the compounds **3** for the agonist binding sites. Generally 1-azabicyclo[2.2.1]heptane system ($n = 1$) was favorable for the agonist binding sites, and the quinuclidine system ($n = 2$) for the antagonist binding sites.

Acknowledgements

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References and Notes

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2. A typical synthetic procedure is as follows: to a solution of 3-quinuclidinone (10 mg, 0.18 mmol) in ethanol (0.5 mL) were added (3-chloroisoxazol-5-yl)methyloxyamine hydrochloride (22 mg, 0.12 mmol), pyridine (19 μL, 0.24 mmol) and molecular sieve 4 Å at 23 °C. After stirring for 24 h, the reaction solution was filtered, and the filtrate was poured into a mixture of ethyl acetate and water (1 mL/1 mL), and the solution was extracted with ethyl acetate (1 mL×3). The collected organic extract was washed with water (3 mL×2) and brine. The solution was dried over anhydrous $MgSO_4$, filtered, evaporated and purified by flash chromatography (EtOAc/MeOH/43% NH_4OH: 3:1:2%, v/v) to give 1-azabicyclo[2.2.2]octan-3-one *O*-(3-chloroisoxazol-5-ylmethyl)oxime (**2f**) (19.6 mg, 98%) as oil. ^1H NMR ($CDCl_3$, 300 MHz) δ 6.30 (s, 1H, isoxazole-H), 5.07 (s, 2H, –NOCH_2CH$_3$), 3.64 (s, 2H, –NCH_2CH=), 3.01–2.88 (m, 4H, –N(CH_2)$_2$–), 2.62–2.60 (m, 1H, –C*H*CH=), 1.86–1.75 (m, 4H, –N(CH$_2$CH_2)$_2$–). ^{13}C NMR ($CDCl_3$, 75MHz) δ 171.76, 165.71, 153.70, 104.53, 66.36, 53.70, 52.44, 47.50, 28.84, 26.19, 24.73; HRMS [CI, M^+], Anal. calcd for $C_{11}H_{14}ClN_3O_2$ 255.0775, found 255.0780.

3. Competitive muscarinic receptor binding assay was performed by measuring the abilities of compounds to inhibit specific binding of 1 nM [^{3}H]*N*-methylscopolamine or 5 nM [^{3}H]oxotremorine, and nonspecific binding was determined in the presence of 1 μM atropine. Receptor source was the human recombinant muscarinic receptor subtype M_1 expressed in CHO cells (Biosignal). The reaction mixture containing 2 μg of receptor (100 μL suspension) was incubated at 27 °C for 1 h, and analyzed by a conventional filtration assay method.

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