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SYNTHESIS AND BINDING AFFINITY OF NEW MUSCARINIC LIGANDS STRUCTURALLY RELATED TO OXOTREMORINE

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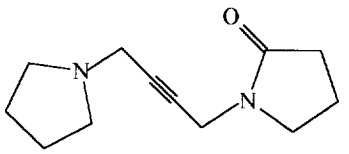
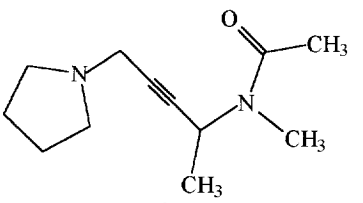
Abstract: The synthesis and radioligand binding assays of a group of muscarinic ligands related to oxotremorine are reported. The new compounds displayed binding affinities comparable to those of the parent molecule with the exception of the trimethylammonium salt **6**, which behaved as a full muscarinic agonist and showed a pronounced selectivity for M_2 versus M_1 muscarinic receptor subtypes. © 1997 Elsevier Science Ltd.

Oxotremorine (**1**, Figure 1) has long been known to act as a potent muscarinic agonist, moderately selective for the central nervous system.[1] The relevant muscarinic potency of **1** is somewhat surprising in view of its structural dissimilarity to other muscarinic agonists. As a matter of fact, oxotremorine bears an acetylenic moiety at a position in the molecule where the most potent muscarinic ligands have an oxygen atom. Detailed structure-activity studies have been carried out on **1** in order to elucidate the role played by the different ligand component parts in affecting muscarinic receptor affinity as well as intrinsic efficacy.

Figure 1



1



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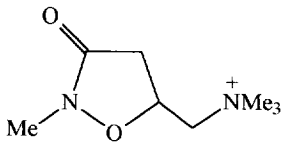

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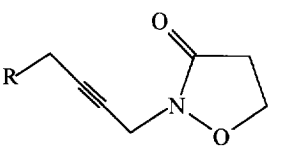

4 : R = 1-Pyrrolidinyl
5 : R = NMe_2
6 : R = $\overset{+}{N}Me_3$

These studies have shown that the presence of both the acetylenic group and the pyrrolidine ring is essential for the potency of **1**.[1] Furthermore, structural modifications of the 2-pyrrolidone moiety gave rise

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mostly to compounds with reduced efficacies, thus behaving as partial agonists or antagonists.[1-3] In this respect, BM 5 (**2**, Figure 1) was reported to act *in vitro* and *in vivo* as a presynaptic antagonist and a postsynaptic agonist at muscarinic receptors.[4,5] With the aim of defining the stereochemical requirements for such a peculiar biological activity of **2**, a series of structural analogs with a reduced conformational flexibility was prepared and thoroughly investigated.[6,7] In the course of further studies, the introduction of a methyl group on one of the seven non equivalent carbon atoms of **1** produced derivatives with sharp differences in the pharmacological profile, ranging from full agonists to competitive antagonists.[1,8,9] Parallel results were essentially obtained with a set of hydroxylated derivatives of oxotremorine.[10]

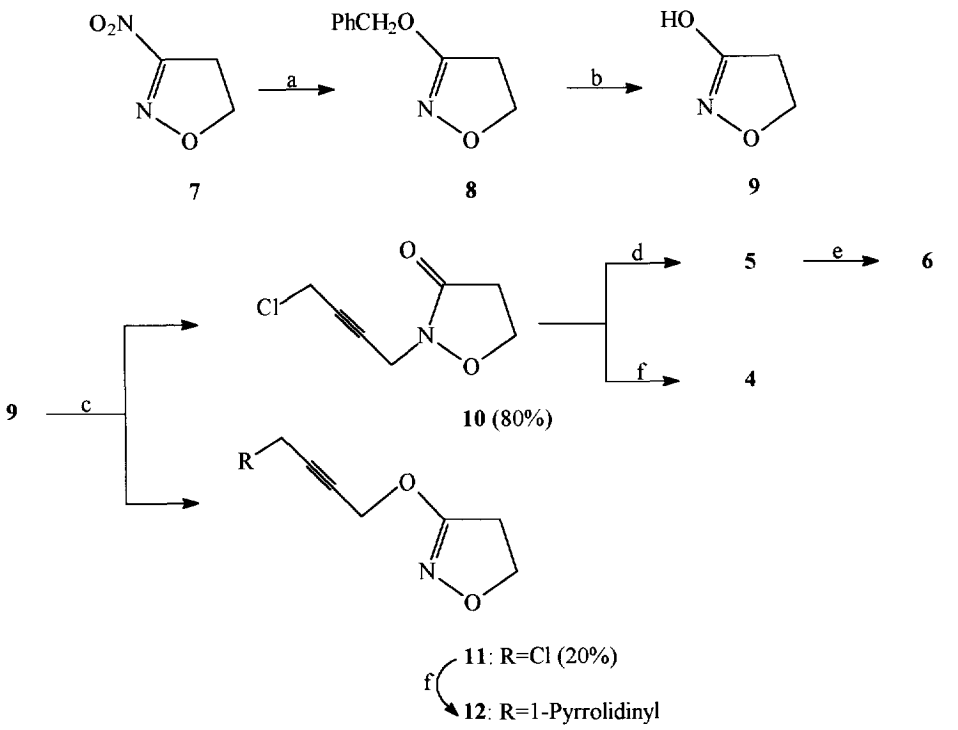
In connection with our studies on the structure–activity relationships of a set of chiral muscarinic ligands,[11-13] we synthesized and tested azamuscarone **3** (Figure 1). Azamuscarone is a high efficacy muscarinic agonist, with potency values comparable to those of natural muscarine.[14,15] Based on this outcome, we planned to incorporate the isoxazolidin-3-one moiety of azamuscarone in the skeleton of oxotremorine. Therefore, by means of classical isosteric replacement of a methylene group with oxygen, we designed compound **4**, the closest analog of **1**, as well as the related dimethylamino (**5**) and trimethylammonium (**6**) derivatives, respectively (Figure 1).

Target compounds were prepared along the sequence reported in Scheme 1. Known 3-nitro-2-isoxazoline **7**[16] was reacted with a DMSO suspension of lithium benzylate at room temperature to give benzyl ether **8** (63% yield) which, in turn, was transformed into 3-hydroxy-2-isoxazoline **9** by catalytic hydrogenation. Electrophilic addition of 1,4-dichloro-2-butyne (K_2CO_3 in refluxing acetone) to **9** resulted in a 4:1 mixture of regioisomers **10** and **11**. The two compounds were easily separated by column chromatography (48% overall yield). Their structure was assigned by comparing the ^{1}H-NMR chemical shift value of methylene groups attached to the heteroatom in the side chain (4.17 δ for **10** and 4.78 δ for **11**). Reaction of intermediate **10** with excess dimethylamine or pyrrolidine produced the corresponding amines **5** and **4**, respectively. Dimethylamino derivative **5** was reacted with iodomethane to afford trimethylammonium salt **6**. Following the same procedure, the O–alkylated isomer **12** was also prepared and tested.

Derivatives **4**, **5** ,**12**[17] and **6**[17] were assessed for binding affinity at muscarinic receptor subtypes in the rat brain and heart (Table I), following a previously described experimental protocol.[18,19] [^{3}H]–quinuclidinyl benzylate (QNB) and [^{3}H]–pirenzepine (PZ) were used as M_2 non-selective and M_1 selective muscarinic antagonists, respectively. [^{3}H]–OXO–M, a muscarinic agonist, has been employed to evaluate the muscarinic agonist or partial agonist character of the compounds under study. The ratio between the K_i values of a ligand determined in QNB (brain) and OXO–M binding assays (see footnote c, Table I) has been utilized as a measure of the muscarinic agonist index of that compound.[18-20] Agonist index values above 1500 are indicative of full agonism, whereas values in the range 20–200 and below reflect the profiles of partial agonists and antagonists at muscarinic receptors. The ratio between K_i values of a derivative determined in PZ (brain) and QNB (heart) binding experiments (see footnote d, Table I) allows to evaluate the selectivity at M_2 receptors (M_1/M_2 index), higher values of this index reflecting higher degrees of M_2 selectivity.

Inspection of the results gathered in Table I suggests that the oxotremorine analog **4** may be a partial agonist, with a quite similar behaviour to that shown by the parent molecule (agonist index equal to 284 and 162, respectively). Moreover, both the ligands are unable to discriminate between M_1 and M_2 muscarinic receptor subtypes (M_1/M_2 index equal to 2.9 and 9.5, respectively). A similar lack of selectivity has been previously observed on **4** in the course of a study carried out using different muscarinic test preparations.[21]

Scheme 1



a: $C_6H_5CH_2OLi$, DMSO; b: H_2–Pd/C, 5%; c: 1,4-Dichloro-2-butyne; d: $NHMe_2$, DMSO, t.a.; e: CH_3I, Et_2O; f: Pyrrolidine, DMSO, –20°C.

Table I. Binding affinity of derivatives **4-6** and **12** for M_1 and M_2 muscarinic receptor subtypes.[a]

Compd.	Receptor binding, IC_{50}, (μM)				Calculated Indexes	
	[^{3}H] PZ[b] brain	[^{3}H] QNB[b] brain	[^{3}H] QNB[b] heart	[^{3}H] OXO-M[b] brain	Agonist[c] index	M_1/M_2[d] index
Oxotremorine	0.1	2.0	0.084	0.002	162	9.5
4 . $C_2H_2O_4$	0.078	2.8	0.215	0.0016	284	2.9
5 . $C_2H_2O_4$	7.1	101	1.38	0.023	711	41.2
6	1.3	17.4	0.074	0.0008	3524	140.5
12 . $C_2H_2O_4$	0.412	8.6	0.233	0.004	348	14.2

[a] Data represent the mean of three independent experiments, and SEMs were less than 10%.
[b] Radioligand concentration : [^{3}H] PZ, 1.0 nM; [^{3}H] QNB, 0.12 nM; [^{3}H] OXO-M, 0.2 nM.
[c] Agonist index = K_i $(QNB)_{brain}$/ K_i $(OXO\text{-}M)_{brain}$= $IC_{50}(QNB)_{brain}$/ IC_{50} $(OXO\text{-}M)_{brain}$ x 0.162.[19]
[d] M_1/M_2 index = K_i $(PZ)_{brain}$/ K_i $(QNB)_{heart}$= $IC_{50}(PZ)_{brain}$/ IC_{50} $(QNB)_{heart}$ x 8.[19]

The same pattern is observed for compound **12**, although the IC_{50} values are lower and the agonist index is higher. Therefore, the replacement of the 2-pyrrolidone moiety of **1** with an isoxazolidin-3-one or 3-hydroxy-2-isoxazoline ring induces marginal changes in the binding affinity profile.

Conversely, the binding data on dimethylamino derivative **5**, and especially trimethylammonium salt **6**, reveal a remarkable selectivity for M_2 receptor subtype (M_1/M_2 index equal to 41.2 and 140.5, respectively), mainly due to a reduced ability of the compounds to displace [^{3}H]–PZ from the rat brain preparation (IC_{50} values equal to 7.1 and 1.3 μM, respectively). Interestingly, derivative **6** behaves as a full muscarinic agonist (agonist index equal to 3524) and displaces [^{3}H]–OXO–M with an IC_{50} equal to 0.8 nM.

In conclusion, the results of the present study indicate that introduction of both the isoxazolidin-3-one ring and the trimethylammonium group in the structure of oxotremorine brings forth a potent muscarinic agonist with a remarkable selectivity for cardiac M_2 muscarinic receptor subtype. The pharmacological profile of **6** will be studied in detail and the results reported in due course.

Acknowledgment

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17. Elemental analyses (C,H,N) of final derivatives agreed with theoretical values ± 0.3%. All the salts were crystallized as colorless prisms from 2-propanol: **4** oxalate, m.p. 120-121°C; **5** oxalate, 127-128°C; **6**, m.p. 186-187°C; **12** oxalate, m.p.106-107°C. ^{1}H-NMR (200 MHz, DMSO-d_6) of **6** : δ 2.81 (t, 2, H-4, J=7.9 Hz); 3.14 (s, 9, NMe_3); 3.38 (bd, 2, CH_2N–C=O); 4.37 (t, 2, H-5, J=7.9 Hz); 4,41 (bd, 2, CH_2N^+).
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