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Potent anti-muscarinic activity in a novel series of quinuclidine derivatives

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Abstract—The synthesis and biological evaluation of a novel family of M_3 muscarinic antagonists are described. A systematic modification of the substituents to a novel alkyne-quinuclidine scaffold yielded original compounds displaying potent in vitro anticholinergic properties.

Antimuscarinic agents are the most widely used therapy for overactive bladder (OAB)[1–3] despite exerting some adverse effects like dry mouth, blurred vision, and constipation.[4,5]

Although M_2-muscarinic receptors (M_2-R) are predominant in the urinary bladder, it appears that the population of M_3-receptors (M_3-R) is of great importance for mediating direct contraction of the detrusor muscle. Thus, M_3-R antagonism represents the most clinically effective treatment of overactive bladder related disorders. Various anticholinergic agents (Fig. 1) have already proven to be effective in patients suffering from OAB. Nevertheless, due to the side-effect profile displayed by these molecules, there is still a need to develop new safer anti-muscarinic agents for the treatment of OAB.

As part of our continuous efforts to identify potent antimuscarinic molecules displaying a good safety profile,[6] we wish to report preliminary results on a novel quinuclidine containing series.[7] Quinuclidine containing derivatives such as Solifenacin (Fig. 1) have already been reported to show interesting pharmacological anti-muscarinic properties.[8]

The compounds were synthesized by means of standard procedures as outlined in Scheme 1.

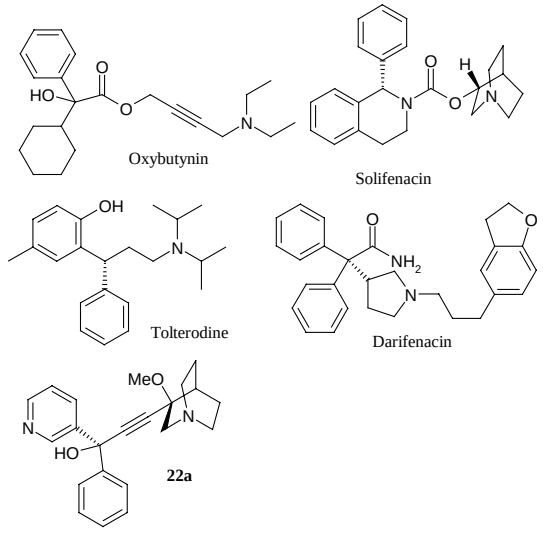

Figure 1. Selected anti-muscarinic agents.

Keywords: Muscarinic receptor(s); Bladder; Urinary incontinence; Quinuclidine; Muscarinic antagonist(s); Overactive bladder.
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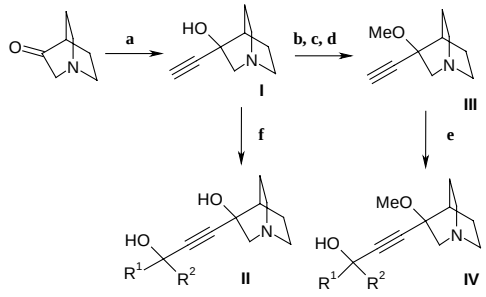


Scheme 1. Reagents and conditions: (a) Li Acetylide.ed cpx, THF; (b) BH_3.THF, THF, −10 °C; (c) NaH, NBu_4I, MeI, THF, rt; (d) 5 M HCl, acetone/Et_2O, rt; (e) BuLi, ketone, THF, −78 °C to rt; (f) 2 equiv BuLi, ketone, THF, −78 °C to rt.

Briefly, a mixture of 3-quinuclidinone (free base) and lithium acetylide (ethylenediamine complex) in THF yielded the acetylenic quinuclidinol **I**. The diol derivatives **II** were obtained by the coupling of **I** with the corresponding ketones at low temperature. The synthesis of **III** was accomplished via the protection of **I** as an amino-borane complex, determinant for the following methylation step.[9] Thus, methylation of the hydroxyl group of **I** using classical conditions (NaH, TBAI catalytic, and MeI) only occurred after protection of the nitrogen via the formation of this borane complex. Addition of methyl iodide to the unprotected molecule resulted in the exclusive formation of the corresponding ammonium salt. The deprotection of the borane complex in a mixture of 5 N HCl/acetone was followed by the deprotonation of the alkyne using BuLi at low temperature. The resulting alkynelide **III** was then reacted with the appropriate ketone[10] yielding compounds **IV** which were isolated as mixtures of diastereomers or as optically pure products.

Methoxylation of the quinuclidine moiety, as shown in **IV**, turned out to be of crucial importance. As a representative example, **IV** (R^1/R^2 = Ph/cyclohexyl (**2**) in Table 1) showed almost a 60-fold increase in affinity for the M_3-R (K_i: 0.2 nM) and the M_2-R (K_i: 2.0 nM) when compared to the corresponding diol analogue **II** (K_i M_3-R = 13 nM, K_i M_2-R = 100 nM).

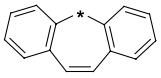
Taking advantage of the presence of methoxy group in **IV**, various chemical modulations were performed to better characterize the hydrophobic part (R^1, R^2) of the molecule (Table 1). The introduction of non-aromatic groups in various anti-muscarinic series has been reported to be well tolerated.[11,12] Similarly, compounds (**2**) and (**3**), respectively, bearing a cyclohexyl and a cyclopentyl moiety had similar affinity to (**1**). Substitution of a single aryl moiety by a cyclobutyl (**4**) or short linear alkyl groups such as *n*-pentyl (**5**) or *n*-butyl (**6**) resulted in a slight decrease in affinity for the M2 muscarinic receptor subtype.

The introduction of a benzyl group at the R^2 position (**7**) or the replacement of both phenyl groups by benzyl moieties (**8**) induced a 100- to 1000-fold decrease in affinity. Along the same lines, while substitution of the *para* position of both aromatic rings by fluorine (**9**) was tolerated, introduction of bulkier groups, such as chlorine (**10**) and methyl (**11**), induced a decrease in affinity. The same observation was made when the *meta* position of the aromatic rings was considered. Thus, fluorine (**12**) substitution was far better tolerated than methyl (**13**) or trifluoromethyl (**14**) replacements. Furthermore, rigidification of the hydrophobic part (R^1, R^2) of the molecule, as exemplified by the suberenyl analogue (**15**), caused almost a 1000-fold decrease in affinity. These results suggest that strong binding to the muscarinic receptors requires the molecules to have their hydrophobic groups in orthogonal orientations.

As seen with compounds (**16**) and (**17**), the 2-thienyl or 3-thienyl moiety could successfully be used as a phenyl bioisostere. On the contrary, replacement of one aromatic group by basic moieties such as 4-piperidyl (**18**) led to a total loss of affinity.

The 2-pyridyl derivatives (**19**, **20**, and **21**) displayed a 30- to 100-fold decreased muscarinic affinity when

Table 1. Affinity for human M_3-R and M_2-R for **IV** analogues

Compound	R^1	R^2	Binding affinity (K_i)	
			M_3	M_2
1*	Ph	Ph	0.13	0.79
2*	Ph	*c*-Hexyl	0.20	2.0
3	Ph	*c*-Pentyl	0.25	3.2
4	Ph	*c*-Butyl	1.0	13
5	Ph	*n*-Pentyl	2.5	40
6	Ph	*n*-Butyl	0.63	25
7	Ph	Bn	40	251
8	Bn	Bn	631	3161
9*	4-F-Ph	4-F-Ph	1.0	20
10*	4-Cl-Ph	4-Cl-Ph	7.9	126
11*	4-Me-Ph	4-Me-Ph	100	501
12*	3-F-Ph	3-F-Ph	0.79	5.0
13*	3-Me-Ph	3-Me-Ph	7.9	50
14*	3-CF_3-Ph	3-CF_3-Ph	1000	#
15*			100	631
16*	2-Thienyl	2-Thienyl	0.05	0.20
17*	3-Thienyl	3-Thienyl	0.08	0.40
18	Ph	4-Piperidyl	#	#
19	2-Pyridyl	*c*-Pentyl	4.0	50
20	2-Pyridyl	*c*-Butyl	20	316
21	Ph	2-Pyridyl	10	79
22	Ph	3-Pyridyl	0.40	1.3
23	Ph	4-Pyridyl	1.3	5.0
24*	3-Pyridyl	3-Pyridyl	50	100
25*	2-Pyridyl	2-Pyridyl	#	#
26	Ph	6-F-3-pyridyl	0.32	4.0
27	Ph	2-F-3-pyridyl	0.79	3.2
28	Ph	3-Pyridazinyl	7.9	40
29	Ph	5-Pyrimidinyl	1.0	2.0
Tolterodine[13]			3.2	4.0
Oxybutynin[14]			1.3	16
Darifenacin[13]			1.3	50

Results are expressed as K_i (nM) or # (when <50% inhibition of radioligand specific binding by 10 μM of compounds). The compounds have been tested as optically pure *R* isomers* or as a mixture of diastereomers bearing the *S*/*R*, *R*/*R* configurations.

compared to their corresponding cycloalkyl (**5**, **6**) and phenyl analogues (**1**). Interestingly, compounds bearing a 3-pyridyl (**22**) or a 4-pyridyl (**23**) moiety as potential phenyl isosteres were highly active on both subtypes of muscarinic receptors. However, the bis-pyridyl derivatives (**24**, **25**) were less potent. Similar to what was observed with the phenyl analogues, fluorination at the *para* position (**26**) or the *ortho* (**27**) position of the 3-pyridyl moeity is well tolerated. Among the various nitrogen containing heterocycles that have been investigated (pyridyl, **21**–**23** and **26**–**27**; pyridazinyl, **28**; pyrimidinyl, **29**), those bearing the Ph/3-pyridyl moieties in R^1/R^2 showed the highest affinity for the M_3-R and M_2-R. It should be noted that compound **22** (R^1/R^2 = Ph/3-pyridyl) has almost the same affinity as the one displayed by **1**, highlighting the fact that the nitrogen located at the *meta* position of the pyridyl ring does not affect the affinity for either muscarinic receptor subtype.

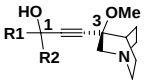
In order to characterize the biologically active isomers, enantiomerically pure compounds were prepared[15] and their absolute configuration was established via X-ray crystallography.

As shown in Table 2, the (*R*)-enantiomer **1a** displayed a greater affinity than that of the corresponding (*S*)-isomer **1b**. The same trend was observed when testing the diastereomers **2a/2b** (R^1/R^2 = Ph/cyclohexyl). Thus, the molecules bearing the (*R*,*R*) configuration exhibited affinity for muscarinic receptors higher than those in the (*S*,*R*) configuration. These data are in line with those reported for other structurally close muscarinic antagonists described in the literature (e.g., trihexylphenidyl, oxybutynin, and *p*-fluoro-HHSiD).[16]

The anti-muscarinic potency of several representative molecules was next evaluated in isolated guinea-pig bladder (M_3-R) and atria (M_2-R) (Table 3).

All these compounds behaved as competitive antagonists of the carbachol-induced response. Their pA_2 values were consistently similar in both organs confirming the compounds were non-selective M_3-R and M_2-R antagonists. The pA_2 values as well as the pK_i values of the most potent compounds were superior to those documented for darifenacin, tolterodine, and oxybutynin, indicating that these compounds are very potent

Table 2. In vitro M_3/M_2 binding affinity of optically pure compounds



Compound	R^1/R^2	Configuration	Binding (K_i, nM)	
			M_3	M_2
1a	Ph/Ph	(3*R*)	0.13	0.79
1b	Ph/Ph	(3*S*)	63	251
2a	Ph/*c*-hexyl	(1*R*,3*R*)	0.20	2.0
2b	Ph/*c*-hexyl	(1*S*,3*R*)	3.2	20

Table 3. Functional antagonism in isolated organs

Compound	R^1/R^2	Guinea pig (pA_2)	
		Bladder (M_2)	Atria (M_3)
1	Ph/Ph	9.3	9.1
2	Ph/*c*-hexyl	9.1	8.8
4	Ph/*c*-butyl	8.5	8.0
16	2-Thienyl/2-thienyl	9.3	8.6
21	Ph/2-pyridyl	7.5	7.5
22	Ph/3-pyridyl	9.1	8.9
23	Ph/4-pyridyl	8.2	8.4
27	Ph/2-F-3-pyridyl	8.7	8.6
Tolterodine[17]		7.8	7.7
Oxybutynin[18]		7.4	7.1
Darifenacin[14]		7.8	7.3

Values are means of at least three experiments. Antagonism was measured by the ability of compounds to shift to the right (pA_2)[19] the concentration-response curve to carbachol on isolated guinea-pig urinary bladder and left atria.[13,20]

M_3-R antagonists and bladder anticontractile agent in vitro.

During evaluation of the aqueous chemical stability of this novel series, we demonstrated that compounds bearing two aromatic rings (e.g., **1**) or two thienyl groups (e.g., **16**) were unstable at acidic pH while remaining stable at neutral pH. We succeeded in isolating and characterizing the rearrangement product of **1** under acidic conditions as being the corresponding α,β-unsaturated ketone, formed via a classical Meyer–Schuster reaction (Fig. 3).[21]

On the contrary, the 2-pyridyl compound **21** was shown to be stable at pH 1, whereas it was surprisingly unstable at pH 7.4. Interestingly, the 3-pyridyl (**22**) and the 4-pyridyl (**23**) derivatives were stable under acidic as well as neutral conditions.

Furthermore, as illustrated in Figure 4, within that series of molecules, a positive correlation between in vitro metabolic clearance (human liver microsomes) and lipophilicity (K'_{IAM}) has been established.

On scaffold IV (Scheme 1) R^1 = indicated groups, R^2 = Ph. Exception: when R^2 = 2-thiophen then R^1 = 2-thiophen. Parent drug consumption was measured following incubation (up to 90 min) of test compounds (0.25 μM) with NADPH-fortified human liver microsomes (0.1 mg/ml). Metabolic clearance (Clint) was calculated with the following equation: Clint = dose/AUC.[22] $LogK'_{IAM}$ was measured using acetonitrile/phosphate buffer mobile phase.[23]

Overall, there was a trend toward improving the metabolic clearance profile when a less hydrophobic group

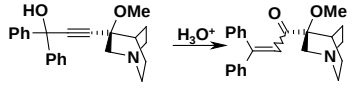


Figure 3. Rearrangement of **1** under acidic conditions.

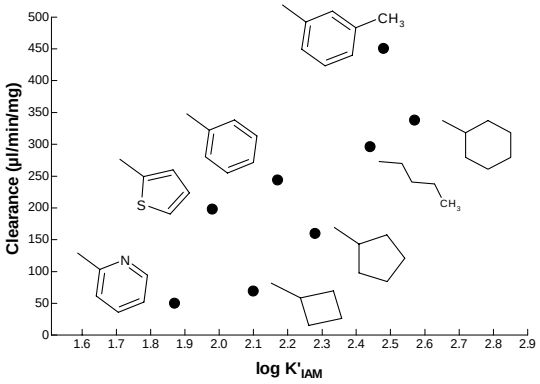


Figure 4. Correlation between metabolic clearance and lipophilicity.

(e.g., pyridyl moieties) was introduced into the core structure of **IV**. Thus, the 2-pyridyl ($\mathrm{Log}K'_{\mathrm{IAM}} = 1.87$), 3-pyridyl ($\mathrm{Log}K'_{\mathrm{IAM}} = 1.40$), and 4-pyridyl ($\mathrm{Log}K'_{\mathrm{IAM}} = 1.41$) derivatives were less rapidly metabolized than the corresponding Ph/Ph analogue **1** ($\mathrm{Log}K'_{\mathrm{IAM}} = 2.17$).

We also investigated in vitro (human microsomes) the capacity of the compounds to inhibit marker activities for CYP3A4 and CYP2D6 (Table 4).

It is known that oxybutynin[25] is metabolized by CYP3A4, and tolterodine and darifenacin also by CYP2D6[26,27] and are inhibitors of CYP 3A4 or/and CY2D6 isoenzymes in vitro.[28,29] The 2- and 3-pyridyl derivatives (**21**, **22**, and **24**) exhibited poor to moderate inhibition of CYP3A4 and CYP2D6 activities contrarily to the Ph/cyclohexyl (**2**) and the Ph/4-pyridyl analogues (**23**) which inhibit CYP2D6 activity.

Moreover, representative compounds **1**, **2**, **6**, **16**, **21**, **22**, and **23** did not compete (results not shown) with ligands, selective for adenosine A_1, A_2, adrenergic α_1, α_2, and β, serotoninergic 5-HT_{1-7}, dopaminergic D_1, D_2, and histaminergic H_1, H_2, and H_3 receptors at 10 μM. This preliminary counterscreen suggests that these compounds might possess high selectivity for muscarinic receptors.

Taken together, these results emphasize that the 3-pyridyl compound **22** was the most interesting. This molecule is chemically stable, has high affinity for the M3-R and M2-R and is less metabolized, and has a better in vitro CYP interaction profile than other compounds within this series.

Table 4. CYP interaction studies

Compound	R^1/R^2	CYP enzyme	
		3A4	2D6
2	Ph/*c*-hexyl	74%	28%
21	Ph/2-pyridyl	6%	8%
22	Ph/3-pyridyl	41%	6%
23	Ph/4-pyridyl	58%	71%
24	Bis(3-pyridyl)	18%	4%

Specific substrates were incubated with NADPH-fortified human liver microsomes in the presence of 50 μM of the test compound. Results are expressed as percentage inhibition of midazolam 1′-hydroxylation (CYP3A4) and of dextromethorphan O-demethylation (CYP2D6).[24]

Table 5. In vitro properties of diastereomers **23a** and **23b**

Compounds	K_i (nM)[a]		pA_2[b]	CYP enzyme[c]	
	M_3	M_2	Bladder	3A4	2D6
22a (1*R*,3*R*)	0.25	0.79	9.1	51%	2%
22b (1*S*,3*R*)	13	50	7.4	18%	4%

[a] Results are expressed as K_i (nM).
[b] pA_2 (Antagonism of carbachol induced guinea-pig bladder contraction[13]).
[c] Percentage of inhibition of midazolam 1′-hydroxylation (CYP3A4) and of dextromethorphan O-demethylation (CYP2D6) as measured using NADPH-fortified human liver microsomes[24] in the presence of 50 μM of the test compound.

Chiral chromatography separation of this compound led to the two optically pure diastereomers **22a** (*R*/*R*) and **22b** (*S*/*R*). As shown in Table 5, **22a** did not affect CYP2D6 activity (<5%), displaying slightly less favorable profile toward CYP3A4 inhibition than **22b** but was almost ~50-fold more potent than **22b** in terms of M_3-R affinity and bladder anticontractile potency in vitro.

In conclusion, these studies describe the structure–activity relationships regarding the affinity for the M_3-R and M_2-R of a new alkyne-quinuclidine scaffold. The core structure of the most active compounds bears functional groups (hydrogen-acceptor site, basic nitrogen, and two lipophilic moieties) similar to those found in other muscarinic M_3-R antagonists. The methoxy group attached to the quinuclidine moeity constitutes an additional and original interaction point with the muscarinic receptor. Chemical modulations around this scaffold gave compounds displaying higher affinities for M_3-R and M_2-R as well as more potent in vitro antagonism in isolated tissues than the known reference drugs. Lead optimization involving the introduction of nitrogen heteroatoms in the lipophilic part of the scaffold led to compounds with lower lipophilicity, less metabolism, advantageous drug interaction profiles whilst at the same time solving the problem of aqueous chemical instability.

Acknowledgments

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