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Review

Pentatomic cyclic antagonists and muscarinic receptors: a 30-year review [1]

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Abstract

This work is a sequel to and completes the review, that recently appeared in this journal, of pentatomic cyclic muscarinic agonists. It reports the results of structure–activity relationship (SAR) studies of pentatomic cyclic antagonists on muscarinic receptors and compares these results with some recent advances in molecular biology and quantitative structure–activity relationship (QSAR) studies.

Keywords: Pentatomic cyclic antagonists; Muscarinic receptors

1. Introduction

Twenty years ago, muscarinic receptors were still regarded as a homogeneous population, and no muscarinic selective ligands were really available except the agonist McN-A-343 [2]. The search for selective agonists and antagonists of the muscarinic receptors received a strong impulse after the radioligand binding studies on pirenzepine, demonstrating that this antagonist could discriminate between two different populations (M_1 and M_2) of muscarinic receptors [3] showing higher affinity for the M_1 neuronal type. Direct evidence for multiple muscarinic receptor subtypes came from the functional and binding studies of other antagonists such as methoctramine, himbacine, AF-DX 116 and gallamine, which display high affinity for M_2 cardiac binding or receptor subtypes, and 4-DAMP, hexahydrosiladifenidol (HHSiD) and *p*-fluorohexahydrosiladifenidol (*p*-FHHSiD), with high affinity for the M_3 smooth muscle-glandular sites [4]. The recent discovery of tripitramine, a polymethylene tetraamine which significantly discriminates between M_2 and M_4 sites [5], precisely confirms the pharmacological identification of this fourth muscarinic subtype.

Later, the appearance of molecular cloning studies allowed a further and in-depth investigation of muscarinic receptor heterogeneity, with the identification of five unique gene sequences coding for muscarinic receptors (m_1–m_5) [6]; the comparison between the functional (M_1–M_4) and cloned (m_1–m_4) receptors enabled their identity to be verified.

2. 2-Substituted dioxolanes

The attention given by our group to pentatomic cyclic muscarinic antagonists derives from the papers of Triggle and Belleau [7], Brimblecombe and Inch [8], Brimblecombe et al. [9,10], Chang et al. [11], Fisher et al. [12] and Dahlbom [13]. All these authors ventured on structure–activity relationships (SAR) with cholinergic and anticholinergic 2-substituted dioxolanes (Fig. 1) trying to answer questions regarding the optimal structural requirements for muscarinic activity, the geometry of the binding sites designed with stereoselectivity, the relation of the cholinergic and anticholinergic receptor sites, and the heterogeneity among muscarinic receptors.

The papers produced by these authors gave some answers and raised some doubts. Brimblecombe and Inch [8], for example, pharmacologically compared their results obtained with enantiomeric *cis* and *trans* 2-cyclohexyl-2-phenyl-4-dimethylaminomethyl-1,3-dioxolane methiodides, and came to the conclusion that it is the absolute configuration at the acetal (C-2) carbon (Fig. 1) which contributes most to anticholinergic potency. On the other hand, Brimblecombe et al.

[1] A sequel to Ref. [11].

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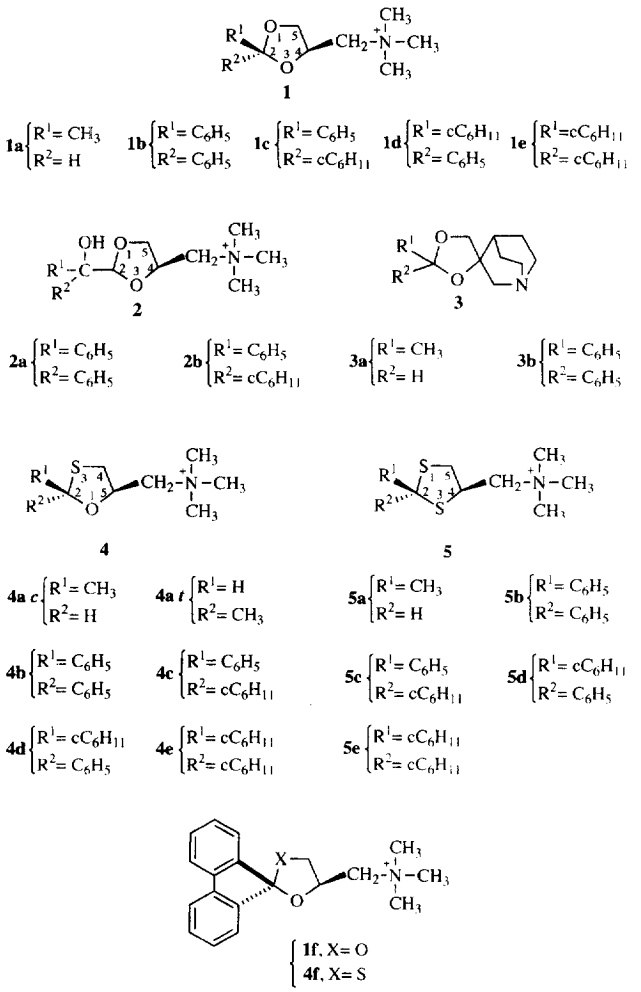


Fig. 1. Structural formulae of the pentatomic cyclic antagonists discussed.

[9], comparing the SAR of a series of agonists and antagonists derived from the 2-substituted 1,3-dioxolanes, deduced that anticholinergic drugs act at sites different from the agonist ones and do not allosterically modify the nature of the cholinergic receptor. These authors, moreover, explained the fact that anticholinergic drugs appear to be competitive antagonists of cholinergic drugs assuming the presence of a large receptor reserve. Brimblecombe et al. [10] synthesized and further investigated some anticholinergic isomers formally derived from 1,3-dioxolane by replacing the 2-methyl substituent with bulkier groups (**1b–e**, **2**, Fig. 1). The stereochemical comparison of these anticholinergic drugs with the related cholinergic ones could provide information about the relationships of the cholinergic and anticholinergic receptor sites. Analysis of the results in Table 1 evidences that, as in the glycollates, the activity of the 8 isomers of anticholinergic 4-trimethylaminomethyl-2-(1-cyclohexyl-1-hydroxy-1-phenyl)-methyl-1,3-dioxolane depends critically on the configuration at the benzylic center (**2**, Fig. 1), and that the absolute configuration of the C-2 and C-4 asymmetric centers is more important than the geometrical relationships between the C-2 and C-4 substituents. Moreover, the compounds with the highest activities have the *S*-configuration at C-2, and the replacement of the 2-methyl substituent in **1a** (Fig. 1) with a 2(1-cyclohexyl-1-hydroxy-1-phenyl)methyl group afforded much more potent compounds than when the C-2 substituents in **1** were replaced by phenyl and cyclohexyl groups.

Chang et al. [11] noted the lack of stereoselectivity of the 2-substituted-4-trimethylaminomethyl-1,3-dioxolane antagonists, in marked contrast to the interaction of the potent isomers of the 2-methyl-substituted agonist **1a** (Fig. 1). The authors concluded their study affirming that it was not possible to state from their results alone whether the agonist or antagonist binding sites are totally distinct or whether they are only partially distinct retaining a common binding area (ammonium binding site).

The problem of the possible heterogeneity of the muscarinic receptors arose with Fisher et al. [12] in 1976, and the rigid dioxolane derivative 2-methyl-spiro-(1,3-dioxolane-4,3′)-quinuclidine (MSDQ) (**3a**) (Fig. 1) played the main role. In fact, this compound behaved as a muscarinic agonist both in vitro (where it behaves as a poor agonist) and in vivo tests, displaying some subtle differences in receptor specificity: however, such differences were not detected when the 2,2-diphenyl analogue (DiPSDQ, a powerful competitive antagonist) **3b** (Fig. 1) was tested (Table 2). These results are conflicting if we assume that the agonists and antagonists of these receptors bind to a common binding site, but they could be explained supposing that agonistic and antagonistic activities may be measures of interaction with the receptor in two different but not directly comparable states.

Table 1
Affinity constant ($-\log K$) of the enantiomers of 4-dimethylaminomethyl-2-(1-cyclohexyl-1-hydroxy-1-phenyl)methyl-1,3-dioxolane on gpi (compound **2b**, Fig. 1) [10]

Configuration Compound **2b**	Benzylic center	C-2	C-4	$-\log K$
L-*cis*	*R*	*S*	*R*	11.09
D-*trans*	*R*	*S*	*S*	9.37
L-*trans*	*R*	*R*	*R*	7.60
D-*cis*	*R*	*R*	*S*	7.28
L-*trans*	*S*	*R*	*R*	6.77
D-*cis*	*S*	*R*	*S*	6.56
L-*cis*	*S*	*S*	*R*	nd
D-*trans*	*S*	*S*	*S*	nd

Table 2
Potency (EC_{50}) and affinity ($-\log K$) of *spiro*-(1,3-dioxolane-4,3′)-quinuclidines **3a** and **3b** (Fig. 1) on gpi [12]

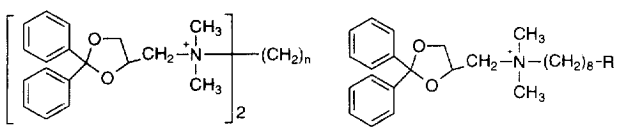
Compound	R^1	R^2	EC_{50} (M)	EPMR [a]	$-\log K$
3a MSDQ	CH_3	H	1.2×10^{-5}	240	nd
3b DiPSDQ	C_6H_5	C_6H_5			9.6

[a] Equipotent molar ratio relative to acetylcholine.

Dahlbom in 1983 [13] reconsidered the role of stereoselectivity in yielding information not only on the geometry of the relevant binding sites but also on the relationships between agonist and antagonist sites. In his paper, he also compared the antimuscarinic activities of drugs containing an asymmetric benzylic carbon atom (**2**, Fig. 1) and showed that the amino residue, the aromatic ring and the hydroxyl group occupy the same relative positions in the most active enantiomers, and that the configuration of the benzylic center is of great importance, the *R*-enantiomers being the most active. In his conclusions, Dahlbom also emphasized that it seems highly improbable that pentatomic cyclic agonists and antagonists bind at the same muscarinic receptor sites.

It is in this scenario that our group began to be involved in the study of pentatomic cyclic muscarinic antagonists.

The bivalent ligands approach was used by Piergentili et al. [14] in the design of antimuscarinic drugs containing two pharmacophores linked through a spacer whose constitution may play an important role in modulating selectivity and potency (Fig. 2). The data in Table 3 suggest that muscarinic bivalent ligands bridge two proximal binding sites and the nearly homogeneous set of results indicates the lack of any clear relationship between spacer length and affinity. One of these might be the pharmacophore binding site and the other



Comp.	n
6	2
7	4
8	6
9	8
10	10
11	12
12	14

13 R = $\overset{+}{N}(CH_3)_3$

14 R = CH_3

Fig. 2.

Table 3
Affinity constants (pA_2 values) of the muscarinic ligands of Fig. 2 on rvd (M_1), gpla (M_2), and gpi (M_3) [14]

Compound	rvd (M_1)	gpla (M_2)	gpi (M_3)
1b	8.36	8.29	7.91
6	<5	5.29	5.38
7	nd	6.34	6.60
8	6.46	6.20	6.38
9	6.31	7.05	7.50
10	6.53	7.20	7.05
11	6.59	6.97	7.12
12	6.82	6.56	6.86
13	6.74	6.78	6.50
14	<5	5.46	5.51

an anionic site which, regardless of spacer length, can accommodate the cationic head of the second pharmacophore. Compound **13**, which displays affinity values similar to those of compounds **8–12** and contains one pharmacophore unit and a second different cationic head, confirms this hypothesis. Compound **14**, a ligand carrying just one cationic head, is almost ineffective.

A series of 2-aryl- and 2-alkyl-aryl-4-[(dimethylamino)methyl]-1,3-dioxolane methiodides and oxalates was synthesized and examined for antimuscarinic activity by Angeli et al. [15] in 1995 (Fig. 3). The results, presented in Table 4, suggest that when two phenyl rings at position 2 are separated by not more than one carbon atom from the dioxolane ring (**15ac**, **15at** and **16ac**, **16at**), an optimal affinity is obtained. In fact, apparently one phenyl ring can still be accommodated in the lipophilic cavity of the receptor binding site; a further separation of phenyl rings from the dioxolane nucleus (**15bc**, **15bt** and **16bc**, **16bt**) no longer allows any possible accommodation in this pocket, thus producing a noticeable decrease in affinity. Compounds carrying a spiro group at position 2 (**15d** and **16d**) show a drop in potency. As far as selectivity is concerned, compound **15ac** evidences a ratio of 10 between M_1 and M_3 and one of 15 between M_1 and M_2. The data in Table 4 show that the tertiary amines are less active (5- to 40-fold) than the corresponding quaternary

15 ac, 15 at, 15 bc, 15 bt, 15 cm, 15 d **16 ac, 16 at, 16 bc, 16 bt, 16 cm, 16 d**

a: R = $(C_6H_5)_2$-CH, R^1 = H; **b**: R = $(C_6H_5)_2$-CH-CH_2, R^1 = H

c: R= $(C_6H_5)_2$-CH-CH_2-CH_2, R^1=H; **d**: R,R^1 = $(C_6H_5)_2$-C(CH_2-CH_2)$_2$

c = *cis*; *t* = *trans*; *m* = *cis*/*trans* mixture

Fig. 3.

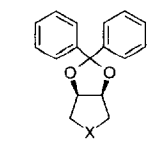
Table 4
Affinity constants (pA_2 values) for the quaternary salts (A) and the tertiary amines (B) of Fig. 3 on rvd (M_1), gpla (M_2), and ri (M_3) [15]

Compound	pA_2			Selectivity ratios		
	rvd (M_1)	gpla (M_2)	ri (M_3)	M_1/M_3	M_1/M_2	M_2/M_3
A						
15ac	8.71	7.54	7.69	10	15	0.7
15at	8.14	7.24	7.46	5	8	0.6
15bc	7.24	6.86	6.56	5	3	2
15bt	6.63	6.10	6.24	3	4	0.7
15cm	5.36	5.93	5.55	0.6	0.3	2
15d	5.23	5.30	5.08	1	0.9	2
1b	8.36	8.29	7.91	3	1	2
B						
16ac	7.31	6.62	6.07	17	5	4
16at	6.90	6.56	6.67	2	2	0.8
16bc	6.82	6.14	6.57	2	5	0.4
16bt	6.85	5.91	6.52	2	9	0.2
16cm	6.90	5.72	5.80	13	15	0.8
16d	6.92	5.83	5.75	15	12	1
1b oxalate	7.15	7.11	6.38	6	1	5

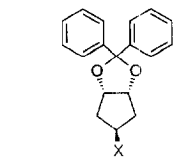
salts; some of them display an interesting selectivity for M_1 muscarinic receptor subtype (more than one order of magnitude). The two series of compounds show a different variation in affinity and suggest a possibly different mode of interaction with the muscarinic receptors. In the series of quaternary salts, the ammonium group very likely strongly binds to the receptor molecule through an ion–ion interaction that drives and influences the subsequent binding of the bulky substituents in position 2 with the lipophilic cavity of the receptor. In this case, an appropriate distance between the cationic head and the lipophilic moiety is very important in order to display good affinity. The ion–ion interaction is evidently somewhat weaker in the tertiary amine series, and the subsequent binding of the diphenyl moiety might be less effected by distance variations.

Piergentili et al. [16] recently synthesized a series of muscarinic antagonists where the hydrophobic and bulky diphenylmethyl moiety is fused in a rigid structure (Fig. 4). These compounds are bridged bicyclic derivatives of the potent muscarinic antagonist 2,2-diphenyl-[1,3]-dioxolan-4-yl-methyl-dimethylamine methiodide. The authors reasoned that the reduced conformational freedom would give selectivity in the interaction with the different muscarinic receptor subtypes. As a matter of fact, it is widely accepted that agonists and antagonists bind in different conformations with receptor subtypes [17]. Oxalates and methiodides were studied in order to verify the different influence on the activity of an ammonium quaternary group with respect to an aminodimethyl protonated group. The influence on the activity of different substituents on nitrogen, the different distance between the active functions (benzhydryl group and nitrogen) and the different stereochemistry of the annulation were also examined. The results, reported in Table 5, suggest that the tertiary amines and the corresponding quaternary salts display, in some cases, different structure–activity relationships and selectivity, suggesting a different binding of this kind of compound to the receptor sites, as previously noted [15]. As far as selectivity is concerned, Table 5 shows that the *cis* form is the only one which displays some selectivity (the tertiary amine **24a** for the M_1 and M_2 subtypes and the quaternary salt **24b** for the M_1 subtype). The different stereochemistry of the annulation has little influence since the reciprocal positions of the aromatic nuclei and the nitrogen atom, which are essential for recognizing the receptorial sites, are not significantly modified in these restricted flexibility compounds. The distance between these two groups seems to be crucial to activity since it shows optimal values when nitrogen is enclosed in the ring (**17**, **18**) or directly bound to the cyclopentane nucleus (**26**). Finally, the drug–receptor complexation process seems to be negatively influenced by nitrogen substituents bulkier than methyl (**19–23**).

A moderate M_3 selectivity is reported in a paper by Malmusi et al. [18], for a series of 1,3-dioxolane-based ligands (Fig. 5). These compounds display, in general, a modest affinity for the muscarinic receptor subtypes M_1–M_3. In the case of the diphenyl-methyl-carboxylates **28** (*cis* and *trans*),



Compound	X
17a	NCH_3 Ox
17b	NCH_3 CH_3I
18a	N iPr Ox
18b	N iPr CH_3I
19a	$NCH_2(CH_2)_6CH_3$ Ox
19b	$NCH_2(CH_2)_6CH_3$ CH_3I
20a	$NCH_2C{\equiv}CH$ Ox
21a	N-cC_6H_{11} Ox
21b	N-cC_6H_{11} CH_3I
22a	$NCH_2C_6H_5$ Ox
22b	$NCH_2C_6H_5$ CH_3I
23a	$NCH_2CH_2C_6H_5$ Ox
23b	$NCH_2CH_2C_6H_5$ CH_3I
24a	$CHN(CH_3)_2$ Ox
24b	$CHN(CH_3)_2$ CH_3I
25b	$CHCH_2N(CH_3)_2$ CH_3I



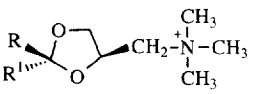
Compound	X
26a	$N(CH_3)_2$ Ox
26b	$N(CH_3)_2$ CH_3I
27a	$CH_2N(CH_3)_2$ Ox
27b	$CH_2N(CH_3)_2$ CH_3I

Fig. 4.

Table 5
Affinity constants (pK values) of bicyclic dioxolanes of Fig. 4 on rvd (M_1), gpla (M_2) and gpi (M_3) [16]

Compound	pK_i			Selectivity ratios		
	rvd (M_1)	gpla (M_2)	gpi (M_3)	M_1/M_3	M_1/M_2	M_2/M_3
1b oxalate	7.15 [a]	7.11 [a]	6.38 [a]	5.9	1.1	5.4
1b	8.36 [a]	8.29 [a]	7.91 [a]	2.8	1.2	2.4
17a	5.92	6.24	6.01	0.8	0.5	1.7
17b	7.62	7.66	7.62	1	0.9	1.1
18a	7.47	6.49	6.14	21	9.5	2.2
18b	7.60	7.09	7.00	4	3.2	1.2
19a	<5	<5	<5			
19b	5.24	5.19	6.34	0.08	1.1	0.07
20a	5.82	5.40	5.78	1.1	2.6	0.4
21a	5.68	5.60	<5		1.2	
21b	<5	<5	5.20			
22a	6.18	5.96	6.14	1.1	1.7	0.7
22b	5.85	5.15	5.28	3.7	5	0.7
23a	<5	5.25	<5			
23b	5.47	5.38	5.83	0.4	1.2	0.4
24a	6.80	6.80	5.90	8	1	8
24b	7.56	6.80	6.90	4.6	5.8	0.8
25b	5.41	5.46	5.39	1	0.9	1.2
26a	6.69	6.74	6.88	0.7	0.9	0.7
26b	7.57	7.01	7.27	2	3.6	0.6
27a	5.77	5.69	5.91	0.7	1.2	0.6
27b	5.64	5.47	5.56	1.2	1.5	0.8

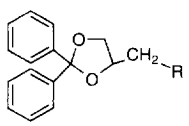
[a] pA_2 values.



28 *cis* $\begin{cases} R = (C_6H_5)_2CHCOOCH_2 \\ R^1 = C_6H_5 \end{cases}$

28 *trans* $\begin{cases} R = C_6H_5 \\ R^1 = (C_6H_5)_2CHCOOCH_2 \end{cases}$

Fig. 5.



1b R = $N(CH_3)_2$

29a, 29b R = N

30a, 30b R = N

31a, 31b R = N

32a, 32b R = N O

33a, 33b R = N

34a, 34b R = N CH_3

35a, 35b R = N CH_3

36a, 36b R = N CH_3

37a, 37b R = N CH_2CH_3

38a, 38b R = N $CH_2N(CH_3)_2$

39a, 39b R = N N CH_3

40a, 40b R = N CH_3 C_2H_5

41a, 41b R = $N(C_2H_5)_2$

42a, 42b R = N $CH_2C_6H_5$ CH_3

43a, 43b R = N $CH_2CH_2C_6H_5$ CH_3

a: oxalates

b: methiodides

Fig. 6.

a modest but clearcut M_3 selectivity is reported ($pK < 5$ at M_1 and M_2 and $pK = 6.15$ at M_3 receptors for **28** *cis*, and $pK = 5.3$ at M_1, $pK < 5$ at M_2 and $pK = 6.19$ at M_3 receptors for **28** *trans*), but low affinity renders such compounds not very interesting for receptor characterization.

In a further attempt to develop muscarinic antagonists selective for the M_3 subtype, a series of analogues of 2,2-diphenyl-[1,3]-dioxolan-4-ylmethyldimethylamine oxalate and methiodide, involving modification of the cationic head, was synthesized and studied by Angeli et al. [19] (Fig. 6). Aliphatic linear and cyclic amines were inserted with the hope of increasing the affinity and selectivity of the 2-substituted dioxolanes. Analysis of data reported in Table 6 suggests that different substituents on the quaternary and tertiary nitrogen affect in different ways the affinity and selectivity of these series of ligands and can modulate the binding of the molecules with receptor subtypes. An ethyl substituent on the cationic head (**40**, **41**) improves the affinity at the three subtypes, and a phenethyl substituent (**43**) seems to induce M_3 selectivity. In particular, compound **43a** displays an interesting range of affinities for the muscarinic subtypes ($pK_b = 4.80$ for M_2; $pA_2 = 5.27$ for M_1; $pA_2 = 5.82$ for M_3 bladder; $pA_2 = 6.97$ for M_3 ileum) studied, suggesting a further investigation of the complete muscarinic profile of this molecule.

3. 2-Substituted oxa- and di-thiolanes

In 1985, Angeli et al. [20] published their results on a series of 2-substituted-5-trimethylaminomethyl-1,3-oxathiolanes (**4b–f**, Fig. 1), to check the influence of the spatial arrangement of the substituent in 2 and to compare the behavior of the oxathiolane nucleus with the dioxolane (**1**) and dithiolane (**5b–e**) ones (Fig. 1). Their results (Table 7) suggested that two bulky substituents in 2 position were necessary for full antagonistic potency and that the resultant hydrophobic interactions needed a suitable steric arrangement of the groups responsible for the interaction in order to be fully operative. Fig. 7 shows, in fact, a model of molecular interactions of this series of antagonists which present a butterfly-like arrangement, with one substituent (cyclohexyl or phenyl ring) lying approximately on the same plane as the pentatomic nucleus and the onium group, and the second substituent (cyclohexyl or phenyl ring), which is roughly perpendicular to this plane, involved in a binding with the lipophilic cavity of the receptor site. This hypothesis was also supported by the drop in potency for the spiro compounds **1f** and **4f**, which present two phenyl rings flattened on the same plane, with a spatial arrangement which hinders an optimal receptor interaction. The affinity values of the compounds in Table 7 allow further deductions, in particular that the dithiolane nucleus displays the highest affinity for the muscarinic receptor, and, among the 2 substituents, the phenyl-cyclohexyl groups lead to antagonists with the highest affinity; on the other hand, controversial indications can be assumed regarding the identity of the binding site for agonists and antagonists. In fact, 2-substitution on the 1,3-dioxolane and 1,3-oxathiolane series has similar pharmacological effects, suggesting that these two series of compounds bind to the same recognition site of the receptor and that the binding sites of agonists and antagonists are largely coincident. Opposite conclusions can be drawn when we compare the results of the dithiolane agonist **5a** (Fig. 1) [1] with those of the corresponding antagonists. In fact, while the dithiolane nucleus displays the highest affinity as antimuscarinic, its agonist potency is some 500 and 200 times lower, respectively, than those of the oxathiolane and dioxolane nuclei, suggesting the possibility of a different binding mode of both agonist and antagonist dithiolane compounds.

The dimensional and steric requirements of the lipophilic pocket involved in the interaction of the muscarinic receptor with 2-substituted 1,3-oxathiolane antagonists (**4b**, **4f**, Fig. 1) was the goal of a paper by Angeli et al. in 1988 [21]. In particular, these authors confirmed that two bulky substituents in position 2 are necessary for antagonistic activity, and

Table 6
Affinity values (pA_2) and pK, for the muscarinic antagonists of Fig. 6 on rvd (M_1), gpla (M_2), gpi and gpb (M_3) [19]

Compound	rvd (M_1)	gpla (M_2)	gpi (M_3)	gpb (M_3)	Selectivity ratios			
					M_1/M_2	M_1/M_3	M_2/M_3	M_3i/M_3b
1b [a]	(7.15)	(7.11)	(6.38)	(6.32)	1	6	5	1
1b	(8.36)	(8.29)	(7.91)	(7.67)	1	3	2	2
29a	7.02	(7.01)	(6.41)	6.67	1	4	4	0.6
29b	7.11	(6.81)	(6.29)	6.56	2	7	3	0.5
30a	6.63	6.58	6.12	6.52	1	3	3	0.4
30b	6.86	6.64	6.13	6.8	2	5	3	0.2
31a	6.88	6.65	6.85	6.69	2	1	0.6	2
31b	8.53	7.94	7.84	7.73	4	5	1	1
32a	5.45	<6	<5.52	<5.52				
32b	7.36	7.21	6.99	6.69	1	2	2	2
33a	6.68	6.40	6.13	5.19	2	4	2	9
33b	6.98	6.00	6.54	6.47	10	3	0.3	1
34a	7.73	7.08	6.52	6.22	5	16	4	2
34b	8.83	8.25	7.82	7.98	4	10	3	0.7
35a	5.71	5.59	5.92	5.88	1	0.6	0.5	1
35b	6.90	6.40	6.15	6.44	3	6	2	0.5
36a	5.58	5.30	<5	5.15	2			
36b	7.05	6.41	6.42	6.31	4	4	1	1
37a	6.29	6.30	6.24	5.95	1	1	1	2
37b	6.82	7.01	6.48	6.37	0.6	2	3	1
38a	(7.86)	(6.74)	6.40	6.51	13	29	2	0.8
38b	6.36	6.23	6.14	5.38	1	2	1	6
39a	6.30	6.05	5.62	5.88	2	5	3	0.6
39b	5.10	5.22	5.28	<5.52	0.8	0.7	1	
40a	7.88	7.38	(6.85)	(7.39)	3	11	3	0.3
40b	8.79	8.64	8.13	8.26	1	5	3	0.7
41a	7.73	7.49	6.52	7.10	2	16	9	0.3
41b	9.07	8.71	8.33	7.89	2	6	2	3
42a	5.17	4.84	6.38	5.70	2	0.06	0.03	5
42b	5.73	5.55	5.79	5.92	2	1	0.6	0.7
43a	(5.27)	4.80	(6.97)	(5.82)	3	0.02	0.007	14
43b	(6.45)	(6.02)	(7.24)	(6.70)	3	0.2	0.06	4

[a] Oxalate.

Table 7
pA_2 values of pentatomic cyclic antagonists of Fig. 1 on rj or gpi (M_3 subtype) [20]

Compound	M_3	Compound	M_3
1b	7.66	**4b**	7.53
1c	8.11	**4c**	7.86
1d	7.88	**4d**	8.26
1e	6.44	**4e**	7.01
1f	4.45	**4f**	5.52
5b	8.31		

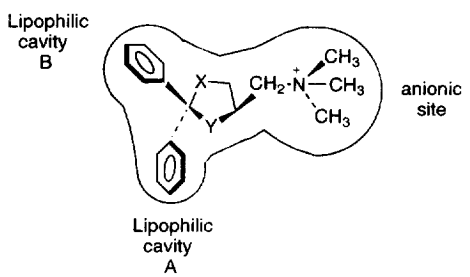
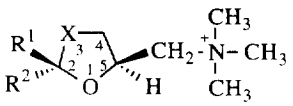


Fig. 7. From Ref. [20].

that a spirofluorene group is detrimental to antimuscarinic potency. Moreover, they hypothesized the change in the lipophilicity of the sulfoxide derivatives (Table 8) to explain the reduced potency of these molecules compared with that of the corresponding oxathiolanes.

As already emphasized in the previous review [1], the study of the enantioselectivity of drugs can be determinant for understanding the mode of interaction of ligands and for characterization of receptor subgroups. In fact, a suitable approach for studying the relationship between the binding sites of agonists and competitive antagonists is the comparison of the chirality of strictly-related compounds. When these compounds show identical stereoselectivity they are likely to interact with a common binding site. In the eighties only ligands related to oxotremorine provided some evidence that muscarinic agonists and antagonists could interact with a common binding site. On this topic, in the period 1988–1990, Gualtieri and co-workers produced six papers [22–27] comparing the stereoisomery of agonist and antagonist interac-

Table 8
Muscarinic affinity (pK) of 1,3-oxathiolane agonists and antagonists on gpi, gpla and rb [22–27]



Compound	X[a]	R^1	R^2	Absolute stereochemistry			gpi pK	E.I.[b]	gpla pK	E.I.[b]	rb pK	E.I.[b]
(+)**4ac**	S	CH_3	H	2R		5R	6.87		5.59		5.73	
(−)**4ac**				2S		5S	4.62	2.25	4.20	1.39	3.68	2.05
(−)**4at**	S	H	CH_3	2S		5R	4.35		4.29		4.18	
(+)**4at**				2R		5S	4.37	0.02	4.28	0.01	4.04	0.14
(−)**4gt**	S→O	CH_3	H	2R,	3R,	5R	5.38		5.22		4.88	
(+)**4gt**				2S,	3S,	5S	3.66	1.72	4.30	0.92	3.70	1.18
(−)**4gc**	S→O	CH_3	H	2S,	3R,	5S	3.24		3.49		3.12	
(+)**4gc**				2R,	3S,	5R	2.65	0.59	3.41	0.08	3.16	0.04
(+)**4h**	SO_2	CH_3	H	2S		5S	3.39		4.21		3.61	
(−)**4h**				2R		5R	3.14	0.25	3.60	0.61	3.52	0.09
(+)**4e**	S	cC_6H_{11}	cC_6H_{11}			5R	6.98		6.37		6.28	
(−)**4e**						5S	6.92	0.06	6.02	0.35	6.34	0.06
(+)**4d**	S	cC_6H_{11}	C_6H_5	2R		5S	8.57		8.10		8.29	
(−)**4d**				2S		5R	7.86	0.71	8.07	0.03	7.61	0.68
(+)**4c**	S	C_6H_5	cC_6H_{11}	2R		5R	8.79		8.47		8.58	
(−)**4c**				2S		5S	7.86	0.93	8.16	0.31	7.45	1.13
(−)**4it**	S→O	cC_6H_{11}	cC_6H_{11}		3R,	5R	6.69		6.79		6.36	
(+)**4it**					3S,	5S	5.81	0.88	5.55	1.24	5.51	0.85
(+)**4ic**	S→O	cC_6H_{11}	cC_6H_{11}		3R,	5S	6.32		6.45		6.27	
(−)**4ic**					3S,	5R	5.92	0.40	5.61	0.84	5.48	0.79
(+)**4jt**	S→O	cC_6H_{11}	C_6H_5	2R,	3S,	5S	6.07		5.54		5.94	
(−)**4jt**				2S,	3R,	5R	5.75	0.32	6.24	0.70	5.88	0.06
(+)**4jc**	S→O	cC_6H_{11}	C_6H_5	2R,	3R,	5S	7.19		6.80		7.19	
(−)**4jc**				2S,	3S,	5R	5.55	1.64	5.48	1.32	5.52	1.67
(+)**4kt**	S→O	C_6H_5	cC_6H_{11}	2R,	3R,	5R	7.84		7.69		7.73	
(−)**4kt**				2S,	3S,	5S	5.52	2.32	5.37	2.32	5.49	2.24
(−)**4kc**	S→O	C_6H_5	cC_6H_{11}	2S,	3R,	5S	6.17		6.01		6.07	
(+)**4kc**				2R,	3S,	5R	5.96	0.21	5.55	0.57	5.56	0.51

[a] Stereochemistry referred to the 5 side chain.
[b] Eudismic index = $pK^{eutomer} - pK^{distomer}$.

tions in different muscarinic tissues. They synthesized and studied a series of 2-substituted 1,3-oxathiolane and 1,3-oxathiolane 3-oxide methiodides (Table 8) whose absolute configurations were attributed by synthesis, X-ray crystallography, and circular dichroism. The advantage of 1,3-oxathiolanes over the isosteric and well-known 1,3-dioxolanes is that the sulfur atom in **3** can be oxidized to introduce into the molecule up to three chiral centers, so increasing the amount and value of the information provided by a study of their enantioselectivity. Careful examination of the data reported in Table 8 reveals that the stereochemical requirements for optimum binding of both agonists and antagonists of the 1,3-oxathiolane series are identical, thus indicating a common binding site on the muscarinic receptor. The sharp drop in affinity in both series when the optimal *R* configuration of the sulfoxide function is inverted suggests that both agonists and antagonists share a common binding site and that their sulfoxide functions identify one identical subsite. The enantioselectivity of the antagonists is usually low unless a sulfoxide function is present. In the latter case, this parameter is of the same order as the agonist one. This difference can be explained by the fact that antagonist affinity depends very largely on hydrophobic forces, that is on the interaction of the bulky groups in position 2, with a low contribution of the ammonium head. As a consequence, the close chiral center is quite uninfluential, as is shown by compound **4e** which lacks any enantioselectivity. On the other hand, the low enantioselectivity displayed by compounds **4a** and **4c** suggests a slight interdependence of hydrophobic interactions and chirality. Compound **4kt**, which shows lower affinities but a definite increase in enantioselectivity confirmed in in vivo studies as well [28], suggested that when a 3-sulfoxide function is present, hydrophobic forces are no longer the only ones responsible for affinity and must compete with electrostatic forces, connected with this function, in fitting the receptor molecule. All these observations allowed Gualtieri to

propose the wedge-like model [26] which seems to satisfy completely the interaction of this class of muscarinic agonists and competitive antagonists.

Angeli and co-workers [29] have compared the influence of the pentatomic cycle (dioxolane, oxathiolane, dithiolane) and of stereoisomery on the affinity of compounds **1** (**c** and **d**), **4** (**c** and **d**) and **5** (2-phenyl-2-cyclohexyl-4-[(dimethylamino)methyl]-1,3-dithiolane methiodide, **c** and **d**, Fig. 1) on the three different muscarinic receptor subtypes M_1, M_2 and M_3. Table 9 shows that all the compounds were potent muscarinic antagonists and that *cis* and *trans* isomers bind with similar affinities to the receptor site in the three different subtypes. This fact confirmed Angeli et al.'s previous hypothesis [20] whereby a lipophilic pocket in the binding site of the receptor can accommodate in the same way a phenyl ring or an equivalent lipophilic group. Moreover, the dithiolane derivative **5c** proved to be the most M_1 selective muscarinic antagonist of the series.

Among the numerous attempts made to develop compounds useful in the treatment of memory and cognitive disorders, can be found the 1,3-oxathiolane derivatives bearing an amidine function, prepared and studied in 1994 by Teodori et al. [30] (Fig. 8). These authors, in fact, reasoned that substitution of the classical ammonium head with amidine moieties with the relative loss of a permanent charge on the basic center, would improve brain penetration. The results obtained on peripheral and central models of muscarinic receptors show that, unlike other classical antimuscarinic ligands, 1,3-oxathiolane antagonists carrying amidine moieties instead of classical cationic heads, definitely lose their affinity without any remarkable induction of subtype selectivity (M_2 selectivity is required for antagonists to be useful in this statement of Alzheimer disease). The lack of bioisosterism between amidines and ammonium function in this series of compounds was explained on the basis of the geometric variation introduced by the substitution in the shape of the cationic head of the molecule, since amidine cations are planar while the ammonium head has a tetrahedral arrangement. The rigidity of the 1,3-oxathiolane molecule probably does not allow a suitable binding between the planar amidine cation and the anionic site of the receptor.

4. Benactyzine analogues

Benactyzine and like compounds have obvious structural similarities with 2,2-disubstituted pentatomic cyclic compounds. For this reason they are being considered in the present review. Conformationally restricted analogues of benactyzine were synthesized and studied as anticholinergic by Flavin et al. in 1987 [31]. These authors proposed two different conformational models for the structurally flexible benactyzine in its binding with the muscarinic receptor molecule (Fig. 9), in which an intramolecular hydrogen bond may exist between the hydroxyl group and the carbonyl oxygen (conformation I, Fig. 9) or the ether oxygen (confor-

Table 9
Affinity constants (pA_2 values) of dioxolanes (**1c**, **1d**), oxathiolanes (**4c**, **4d**), and dithiolanes (**5c**, **5d**) (Fig. 1) on rvd (M_1), gpla (M_2), and gpi (M_3) [29]

Compound	pA_2			Selectivity ratios		
	rvd (M_1)	gpla (M_2)	gpi (M_3)	M_1/M_3	M_1/M_2	M_2/M_3
1c	8.99	8.16	8.29	5	7	0.7
1d	8.72	8.11	8.06	5	4	1
4c	8.46	8.13	8.38	1	2	0.6
4d	8.57	8.11	8.26	2	3	0.7
5c	9.00	8.28	7.99	10	5	2
5d	8.67	8.11	8.04	4	4	1

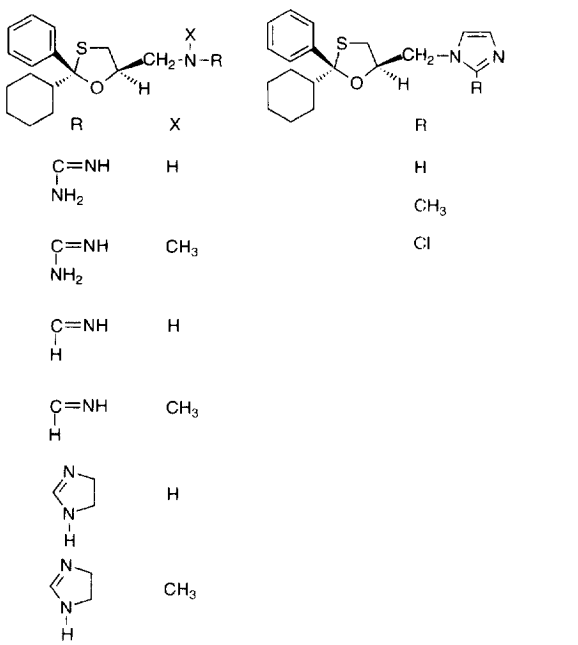
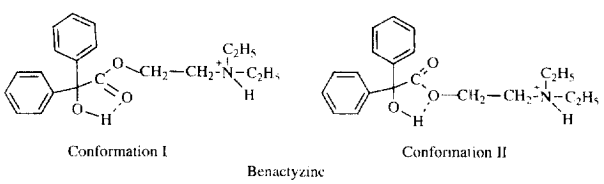
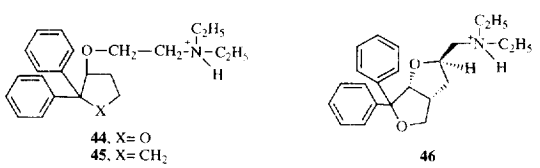


Fig. 8.





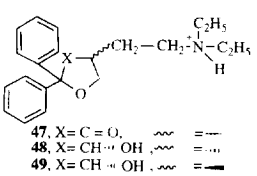


Fig. 9.

mation II, Fig. 9) of the ester group. The in vitro study of the benactyzine analogues **44–49**, which approximate the two conformations of Fig. 9, gave useful suggestions regarding the topography of the muscarinic receptor and on the preference of the receptor-bound conformation for benactyzine. In fact, while compounds **44**, **45** and **46**, which approximate to conformation I, behaved as non-competitive antagonists on rat ileum, compounds **47**, **48** and **49**, which approximate to conformer II, exhibited competitive antagonism on the same tissue, similarly to benactyzine. These results suggested that the receptor-bound conformation II for benactyzine is preferred to conformation I. Moreover, the great difference in affinity between the *cis* amino alcohol **48** ($pA_2 = 6.19$) and the *trans* one **49** ($pA_2 = 4.70$) also evidences the importance of the stereochemical relationship between the hydroxyl group and the side chain and confirms Gualtieri et al.'s receptor model [32] in which an esteratic site was characterized by a strictly oriented dipole.

Among many useful applications of selective compounds, ligands with M_3 receptor selectivity could be therapeutically useful in the treatment of urinary incontinence. This was the goal of Kaiser and co-workers [33], when, in 1992, they prepared a series of N,N-disubstituted 5-(aminomethyl)-, 5-(1-imidazolylmethyl)-, 5-(1*H*-pyrazol-1-ylmethyl)-, and 5-(1,2,4-triazol-1-ylmethyl)-4,5-dihydro-3,3-diphenyl-2(3*H*)-furanones and related bridged bicyclic quaternary salts (Fig. 10). Their results, obtained evaluating the antimuscarinic potency at M_1, M_2 and M_3 muscarinic receptor subtypes, showed that incorporation of the N-substituent into an imidazole ring and introduction of appropriate substituents into position 2 of this ring strikingly affected antimuscarinic activity, in particular causing M_3 selectivity. The pyrazoles paralleled the imidazole series in so far that substitution of position 2 increased potency, whereas substitutions at other positions of the ring had little effect. This fact is consistent with the location of an M_3 receptor binding site complementary to this substitution. A clinical candidate for the treatment of bladder dysfunction, (*R*)-[(2-isopropyl-1*H*-imidazol-1-yl)methyl]-4,5-dihydro-3,3-diphenyl-2(3*H*)-furanone (**50**) was identified.

Continuing in their goal to find new muscarinic antagonists active in treating urinary incontinence associated with bladder muscle instability, Kaiser et al. [34] in 1993 prepared a series of substituted 1-phenyl-3-piperazinyl-2-propanones which was examined for M_1, M_2, and M_3 muscarinic receptor selectivity in in vitro and in vivo assays (Fig. 11). The piperazinylpropanones, in fact, might present a hydrogen-bond-constrained conformation resembling the one preferred by the prototypical muscarinic antagonist benactyzine (Fig. 9), while the distal piperazine nitrogen might assume the role of the cationic head binding to the anionic site of the receptor. Table 10, in which only the most meaningful compounds are reported, indicates that considerable bulk is tolerated on the cationic head at least in some subpopulations of muscarinic receptors. Among the series of 1-cyclobutyl-1-hydroxy-1-phenyl-3-piperazinyl-2-propanones, selectivity towards M_3 and M_1 versus M_2 receptors was generally noted. The pharmacological profile of the N-benzylated derivative **51** met the objectives of the study; this compound, in fact, showed a modest M_3 selectivity in vitro (Table 10), but in vivo an 11-fold and a 37-fold separation between bladder function on the one hand, and mydriasis and salivation responses, on the

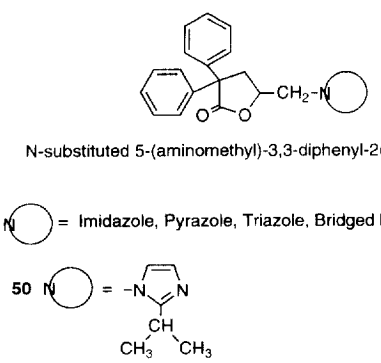
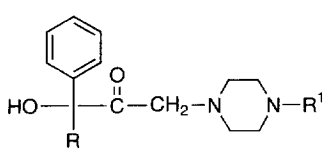


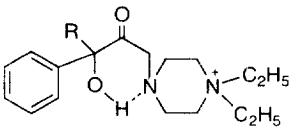
Fig. 10.

Table 10
Affinity constants ($-\log K$ values) of substituted 1-phenyl-3-piperazinyl-2-propanones (Fig. 11) on rvd (M_1), gpra (M_2), and gpi (M_3) [34]

Compound	pA_2			Selectivity ratios		
	rvd (M_1)	gpra (M_2)	gpi (M_3)	M_3/M_1	M_1/M_2	M_3/M_2
51	7.60	7.22	7.82	1.7	2.4	4.0
52	<5	<5	7.25	>178	1	>178
53	6.96	5.84	8.22	18	13	240



1-cycloalkyl-1-hydroxy-1-phenyl-3-piperazinyl-2-propanones



piperazinylpropanones

51 $\begin{cases} R = c\text{-}C_4H_7 \\ R^1 = CH_2C_6H_5 \end{cases}$ **52** $\begin{cases} R = c\text{-}C_4H_7 \\ R^1 = CH_2C_6H_4\text{-}2\text{-}Cl \end{cases}$ **53** $\begin{cases} R = C_6H_5 \\ R^1 = CH_2C_6H_5 \end{cases}$

Fig. 11.

other. Several substituted benzyl derivatives were prepared and studied, and while *meta* and *para* substitution had relatively little effect on activity, *ortho* substitution markedly decreased M_1 and M_2 antagonist activity while retaining the M_3 one. Compound **52**, in fact, was a highly selective M_3 antagonist being more than 170-fold selective for M_3 versus M_1 and M_2 muscarinic receptors. Another antagonist, 3-(4-benzylpiperazinyl)-1,1-diphenyl-1-hydroxy-2-propanone (**53**) showed an 18- and 240-fold selectivity for M_3 versus M_1 and for M_3 versus M_2 receptors, respectively. In vivo this compound displayed 20- and 41-fold separations between bladder function on the one hand, and mydriasis and salivation effects, on the other. In conclusion, in this series, appropriate terminal nitrogen substitution provided compounds with muscarinic receptor subtype selectivity.

5. Procyclidine and analogues

Structurally flexible antimuscarinic compounds that can be considered analogues of 2-substituted dioxolanes have been prepared and studied by many authors. Among them, Waelbroeck et al. [35] gave their contribution to the study of the stereoselectivity of procyclidine (Fig. 12) trying to identify the interactions responsible for muscarinic receptor stereoselectivity. To do that, besides (*R*)- and (*S*)-procyclidine, they extended this binding analysis on M_1, M_2 and M_4 muscarinic receptors to two achiral compounds, pyrrinol and hexahydro-procyclidine, the respective diphenyl and dicyclohexyl derivatives of the two enantiomers (Fig. 12). Their results showed that procyclidine binding was highly stereoselective and this led them to suppose that at least three groups surrounding the chiral carbon atom can contribute to overall drug binding affinity (Fig. 12). From free energy calculations these authors derived that (*R*)-procyclidine binds to muscarinic receptors by an optimal interaction of its hydrophobic phenyl ring with receptor site 1, of the cyclohexyl group with receptor site 2, of the hydroxy group with receptor site 3 and of the protonated amino group with receptor site 4 (Fig. 12). Moreover, this enantiomer binds preferentially to M_1 and M_4 muscarinic receptors; its lower affinity for M_2 sites was apparently due to a poorer fit of the cyclohexyl group in receptor subsite 2. Finally, muscarinic M_1, M_2 and M_4 receptors clearly discriminated between the two procyclidine enantiomers showing their preference for (*R*)-procyclidine.

In 1992, Waelbroeck et al. [36] came back to the stereoselectivity of procyclidine and related antagonists (Fig. 12). They compared the muscarinic affinity and stereoselectivity at M_1, M_2, M_3 and M_4 receptor subtypes of chiral antagonists possessing a hydroxy, phenyl (or *para*-fluorophenyl), and cyclohexyl group bound to the center of chirality; these differ in the structure of the amino group and the nature of the chain connecting the carbinol carbon atom and the cationic head. The (*R*)-enantiomers (eutomers) of the studied compounds displayed higher affinities for M_1–M_4 receptors than did the (*S*)-isomers (distomers), and the eudismic indexes varied

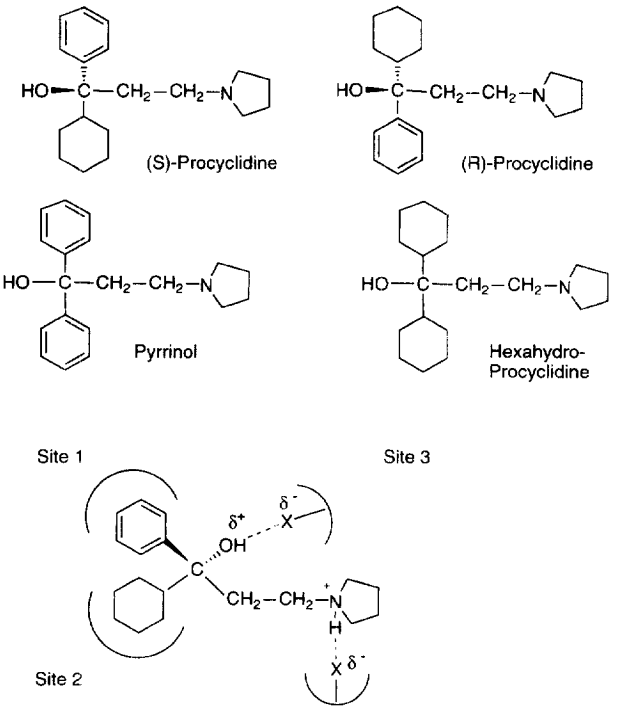


Fig. 12. From Ref. [35].

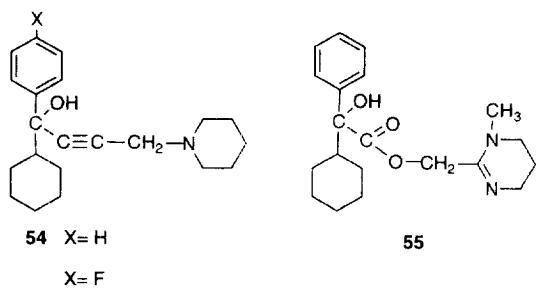
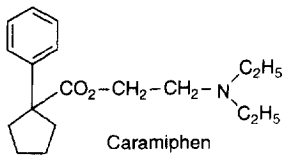


Fig. 13.

by more than two orders of magnitude. The authors, who did not observe any general correlation between the potency of the high-affinity enantiomer and the affinity ratio (eudismic ratio) of the two enantiomers, suggested that the position and conformation of each compound within the receptor could be adjusted to achieve an optimal overall free energy change. Since in their previous paper [35] they had suggested that the phenyl rings of the (*R*)- and (*S*)-enantiomers recognized different sites in the muscarinic receptor, each new *para*-fluoro substituted enantiomer could presumably be affected differently in its binding properties. Finally, they suggested that the protonated amino group of the enantiomers of hexbutinol (**54**) and oxyphencyclimine (**55**) (Fig. 13) formed ionic bonds with an aspartate residue of the receptor.

6. Caramiphen analogues

Caramiphen (Fig. 14) is an antimuscarinic agent which shows high affinity for muscarinic receptors with a 26-fold

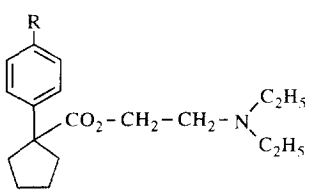


Caramiphen

Fig. 14.

preference for M_1 over M_2 subtype. The study proposed by Hudkins et al. [37] was addressed to designing *para*-substituted analogues of caramiphen in order to look for a possible relationship in binding affinity or receptor subtype selectivity with aromatic substituent parameters such as Hammett's σ or Hansch's π values, and to explore whether the muscarinic receptor subtypes could accommodate additional steric bulk in the *para* position, or the phenyl pocket be only large enough for the phenyl ring itself. In their approach, the authors used the amino, 1-tetrazolyl, 1-pyrrolidinyl, cyano and iodo derivatives, compounds carrying substituents which were selected for their extreme value for σ and for π in a positive or negative direction, in all combinations. The results presented in Table 11 show that the phenyl pocket can accommodate additional volume from the drug molecule and that the contribution which the substituent imparts to binding can drastically affect affinity and selectivity. A loss in affinity results at both M_1 and M_2 receptor subtypes when *para*-substituted compounds are examined, although this loss of affinity is more pronounced at the M_2 site with $+\sigma$ substituents with proper steric bulk. Consequently, derivatives with electron-withdrawing substituents ($+\sigma$) showed M_1 subtype selectivity equal to or greater than that of caramiphen. Nitro- and iodo-caramiphen bind with high affinity for M_1 sites and show a great separation in binding selectivity of M_1 versus M_2 receptors.

Table 11
Affinity constants ($-\log K$) of *para*-substituted caramiphen analogues on rc (M_1) and rh (M_2) [37]



Compound (R)	σ [a]	π [a]	$-\log K$		Selectivity ratio
			rc (M_1)	rh (M_2)	M_1/M_2
caramiphen (H)			8.92	7.50	26
(NO_2)	+0.78	−0.28	8.26	6.41	71
(NH_2)	−0.66	−1.23	7.70	6.94	6
(cNC_4H_8)	−0.90	+1.18	6.82	6.26	4
(1-tetrazolyl)	+0.50	−1.04	7.84	6.37	30
(I)	+0.18	+1.12	8.68	6.91	58
(CN)	+0.66	−0.57	8.09	6.67	26

[a] Electronic (σ) and lipophilic (π) substituent constants.

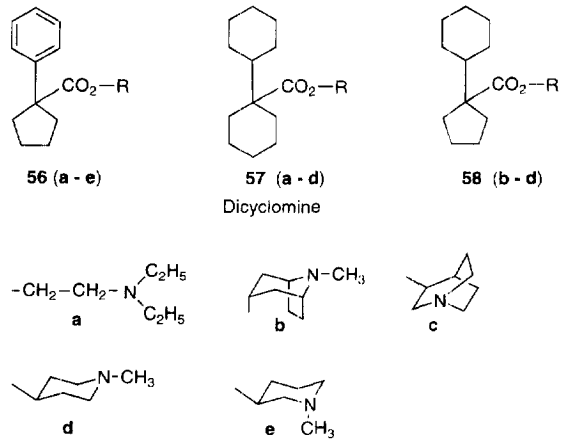


Fig. 15. From Ref. [38].

Stubbins et al. in 1992 [38] and Hudkins et al. in 1993 [39] came back to caramiphen and dicyclomine, two muscarinic antagonists selective for the M_1 subtype. Their papers, in fact, described analogs of caramiphen (**56a–56e**), dicyclomine (**57a–57d**) and the reduced caramiphen derivative 1-cyclohexylcyclopentanecarboxylate (**58b–58d**, Fig. 15) containing conformationally restricted amine groups, to evaluate the structure–activity relationships for M_1 subtype affinity and selectivity. The 3-tropinyl (**56b**), 3-quinuclidinyl (**56c**), *N*-methyl-4-piperidinyl (**56d**) and *N*-methyl-3-piperidinyl (**56e**) derivatives of caramiphen were prepared and evaluated for binding affinity for the muscarinic M_1 and M_2 subtypes. This study revealed that many of the novel derivatives showed improved potency in the binding assays, although the degree of M_1 selectivity was reduced compared to the parent 2-(diethylamino)ethyl compounds caramiphen and dicyclomine (data not reported). Moreover, it was clear that the flexible 2-(diethylamino)ethyl portion is important for controlling the M binding selectivity of these ester-type muscarinic antagonists since incorporation of the flexible amino side chain into a conformationally restricted group decreases M_1 subtype selectivity.

7. Tropate derivatives

Lu et al. in 1991 [40] proposed the presence of an intramolecular hydrogen bond between the hydroxy group and the ester carbonyl oxygen in tropate derivatives such as atropine (Fig. 16), which are muscarinic receptor antagonists. The double role of such a hydrogen bond would be to reduce the free rotation of the ligand and to orient the essential phenyl group towards a specific region of the receptor for optimal binding producing stereospecificity as shown in Table 12. Table 12 also confirms the known structural differences for ligand binding between the muscarinic receptors of ileum and left atrium of guinea pig. Also evident, in fact, is the ileal selectivity seen with isomer **59** and its corresponding quaternary salt (**60**), in contrast to the lack of selectivity seen with isomer **61**. The high affinity ratio (155) displayed by isomer **59** made this compound one of the most ileal selective M_3

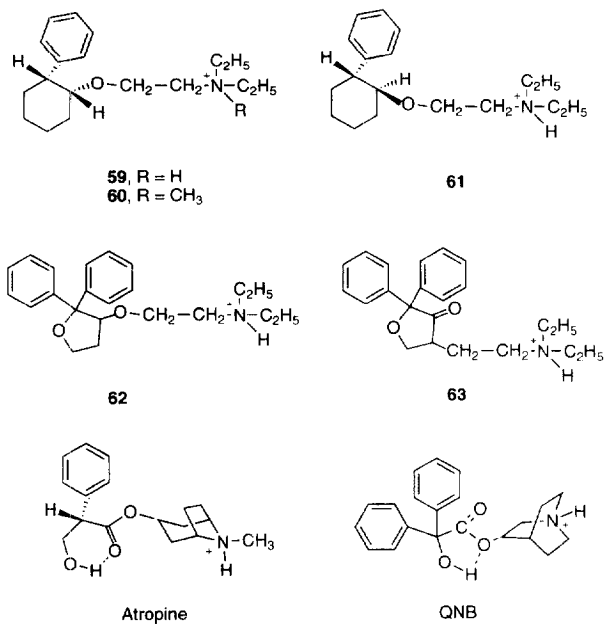


Fig. 16. From Ref. [40].

Table 12
Affinity constants (− log K) of tropate- and benzylate-derived muscarinic antagonists (Fig. 16) on rla (M_2) and ri (M_3) [40]

Compound	− log K		Selectivity ratio
	rla (M_2)	ri (M_3)	M_3/M_2
59	4.96	7.15	154.9
60	5.54	7.31	58.9
61	5.65	6.38	5.4
62 [a]			
63	5.56	6.35	7.8
Atropine	8.28	8.73	2.8

[a] Non-competitive antagonist.

muscarinic receptor antagonists. Conformationally-restricted analogues of benactyzine such as **62** and **63** were also examined. Compound **63** behaved as a competitive antagonist at both atrium and ileum with little ileal selectivity, whereas a non-competitive inhibition at these tissues was observed for compound **62**. These results suggested a different receptor-bound conformation for the benzilate-derived muscarinic antagonists and, differently from tropate derivatives, a possible intramolecular hydrogen bonding between the hydroxy group and the ether oxygen of the ester function (Fig. 16).

8. 4-DAMP derivatives

In the period 1990–1992, Melchiorre and co-workers [41–43] synthesized and tested at M_2 and M_3 receptor subtypes a series of phenyl substituted derivatives of 4-DAMP and the analogues spiro-DAMP and hydroxy-DAMP (Fig. 17). With this study these authors tried to clarify the structural requirements determining selectivity to one muscarinic receptor subtype rather than to another. Muscarinic binding site, in fact, involves quite similar amino acids for different subtypes and this results in equivalent binding of most agonists and antagonists. So, very small differences in the binding pockets are responsible for selective binding of antagonists that bind to the same (or largely overlapping) site of agonists; this makes selectivity difficult to achieve. In their research, the acyl portion of 4-DAMP was incorporated into an oxodioxolane ring (spiro-DAMP) in which the two aromatic rings are forced to assume a spatial arrangement at a defined distance from the cationic nitrogen when comparison is made with the flexible structure of 4-DAMP. It is well known, in fact, that the most critical determinant for antimuscarinic activity is the presence of two large hydrophobic groups at an appropriate distance from a positively charged nitrogen of the ligand. The role of the hydroxy group on affinity was also investigated comparing 4-DAMP and spiro-DAMP with hydroxy-DAMP, which is an open homologue of spiro-DAMP and a hydroxy-bearing analogue of 4-DAMP. Phenyl substituents were also studied in order to investigate the size and the lipophilicity of the receptor pocket. The results shown in Table 13 suggested that spiro-DAMP, the cyclic analogue obtained by restricting the conformational freedom of the acyl moiety of 4-DAMP, is a very active but not selective antagonist at muscarinic receptors, while hydroxy-DAMP is significantly more potent than 4-DAMP and spiro-DAMP at both muscarinic receptor subtypes. In the case of spiro-DAMP, this means that the additional ether oxygen is well tolerated in receptor binding and that the receptor-bound conformation of 4-DAMP may approach that frozen in spiro-DAMP itself. Moreover, the ether oxygen of spiro-DAMP and the sulfur of the thio analogue may not be involved in receptor binding and their role would be only that of keeping the key groups for activity at a suitable distance from each other for the interaction with the receptor binding site. On the other hand, the same authors [43] supposed that the hydroxy

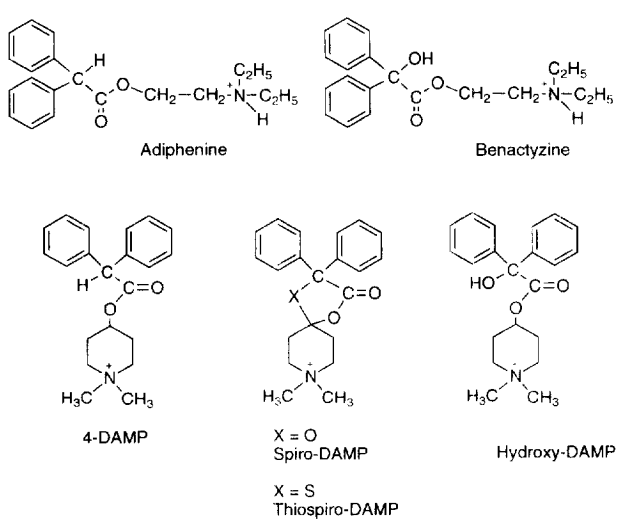
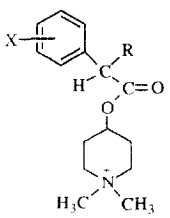
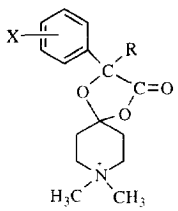


Fig. 17.

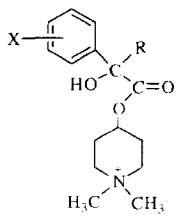
Table 13
Affinity constants (pA_2) for spiro and hydroxy compounds related to 4-DAMP (Fig. 17) on gpla (M_2) and gpi (M_3) [41–43]



4-DAMP
64a - 64c



Spiro-DAMP
65a - 65e

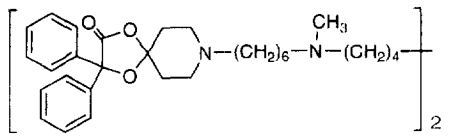


Hydroxy-DAMP
66a - 66c

Compound	X	R	pA_2		Selectivity ratio
			gpla (M_2)	gpi (M_3)	M_3/M_2
4-DAMP	H	C_6H_5	8.53	9.19	4.2
64a	4-F	C_6H_5	8.20	8.70	3.2
64b	4-OCH_3	C_6H_5	7.55	7.64	1.2
64c	H	c-C_6H_{11}	8.81	9.34	3.4
S-DAMP	H	C_6H_5	8.93	8.86	0.8
65a	4-F	C_6H_5	8.01	8.90	7.8
65b	4-OCH_3	C_6H_5	7.19	7.85	4.6
65c	4-F	4-FC_6H_4	7.45	8.05	4.0
65d	4-OCH_3	4-$OCH_3C_6H_4$	6.52	6.12	0.4
65e	H	c-C_6H_{11}	9.40	9.97	3.7
H-DAMP	H	C_6H_5	9.71	9.89	1.5
66a	4-F	C_6H_5	9.36	9.30	0.9
66b	4-OCH_3	C_6H_5	8.59	8.52	0.8
66c	H	c-C_6H_{11}	9.89	9.61	0.5

group which is present in atropine and benactyzine does not play a direct role in receptor binding, but rather it stabilizes an optimal conformation by way of an intramolecular hydrogen bond between the hydroxy group itself and the ether oxygen of the ester function, as already noted in Fig. 9 and in Fig. 16 for QNB. A different role could be attributed to the hydroxy group of hydroxy-DAMP through the formation of a hydrogen bond with a suitable group of the receptor site [44]. While cyclohexyl analogues in the three series (**64c**, **65e** and **66c**, Table 13) showed quite a high affinity at both M_2 and M_3 subtypes, phenyl substituents did not appear to affect selectivity markedly [45]. In both 4-DAMP and spiro-DAMP series the 4-F (**64a** and **65a**, Table 13) and the 4-OCH_3 analogues (**64b** and **65b**, Table 13) were relatively selective for the M_3 receptor. Finally, substitution on both phenyl rings (**65c** and **65d**, Table 13) was not well tolerated even in the case of very small substituents, which indicates a very strict spatial demand for one of the rings and for the whole molecule.

The well-known tetraamine backbone was used by Melchiorre et al. [5,46] as a spacer linking the structural features of 4-DAMP and spiro-DAMP [41–43] pharmacophores. The most interesting compound obtained by these authors was spirotramine (Fig. 18) which displays an inverse selectivity profile in comparison to well-known M_2 selective muscarinic antagonists such as methoctramine and tripitramine, because



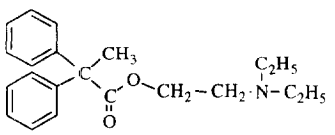
Spirotramine
Fig. 18.

of a higher affinity for M_1 muscarinic receptors ($pK_b = 7.88$) and a significantly lower affinity for all the other subtypes investigated ($pK_b = 6.20$ at M_2, $pK_b = 5.81$ at M_3, $pK_b = 6.27$ at M_4). This compound, in fact, has a significantly better affinity profile than pirenzepine, classified as a highly M_1-selective antagonist, and is able to discriminate between M_1 and M_4 subtypes with a selectivity ratio of 41.

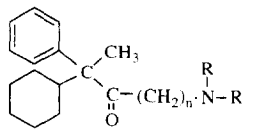
9. Aprophen analogues

The role and location of the functional groups required in antimuscarinic compounds such as aprophen analogues was the subject of a paper by Leader et al. in 1992 [47]. These authors synthesized a series of cyclohexyl-substituted aprophen analogues, called cylexphenes, with some alterations in the chain length of the amino portion of the ester, the alkyl groups on the amino alcohol, and just one cyclohexyl group replacement for one of the phenyl rings. Ileum and pancreas (smooth muscle/glandular, respectively) muscarinic receptor subtypes were investigated. In fact, both these subtypes were supposed to belong to the M_3 subtype although their molecular characterization was not yet completed. In addition, these compounds were also evaluated on the m_1, M_2, m_3 and M_4 muscarinic receptor subtypes. The results in Table 14 suggested that substitution of one phenyl group in aprophen by a cyclohexyl group resulted in about a 13-fold decrease in antimuscarinic activity for the ileum contraction assay. No relevant change for the pancreas assay was noted, and this fact supported a difference between smooth muscle (ileum) and glandular (pancreas) muscarinic receptor subtypes. In fact, this substitution increased the selectivity of all the analogues for the pancreatic subtype over the ileum one, by more than 10-fold. The whole result, pharmacological and binding assays, showed that compound **68** is the analogue exhibiting the greatest selectivity (30-fold) for the pancreas subtype, and in a substantial subtype selectivity showing about 14- to 18-fold higher affinity for the m_1, m_3, and M_4 subtypes than for the M_2 one (data not reported). Compounds with a longer amino alcohol (butyl analogues **70** and **71**) showed significant decrease in potency and affinity in both the pharmacological and binding assays, while the size of the N-alkyl groups (dimethyl or diethyl) was more important in the latter. The authors concluded by proposing that the rigidity or flexibility of an antagonist could determine whether the required conformation can interact with muscarinic receptor subtypes. With regard to this, compound **68** was apparently

Table 14
Affinity constants ($-\log K$ and $-\log IC_{50}$) of aprophen and cylexphenes on ileum contraction and pancreas α-amylase release [47]



Aprophen



Cylexphenes **67** - **72**

Compound	*n*	R	Ileum contraction $-\log K$	Pancreas α-amylase $-\log IC_{50}$	Selectivity [a] pancr./ileum
aprophen			8.51	7.34	1.5
67	2	C_2H_5	7.40	7.28	16.7
68	3	CH_3	7.85	8.00	30.8
69	3	C_2H_5	7.74	7.68	19.1
70	4	CH_3	6.82	6.72	17.4
71	4	C_2H_5	6.68	6.42	12.0
72	5	C_2H_5	5.85	5.57	11.5

[a] Selectivity ratio relative to atropine.

the only cylexphene that could best fit into the antagonist binding site of the m_1, m_3 and M_4 muscarinic receptor subtypes, but not into the M_2 one. In fact, the greater flexibility of the cyclohexyl group compared with a phenyl ring would probably allow the compound to fit better into the hydrophobic binding region of the m_1, m_3 and M_4 muscarinic receptor subtypes than into that of the M_2 one.

10. Antagonists structurally related to furtrethonium

In 1994, two related papers were published by Manfredini et al. [48] and Feriani et al. [49] on the search for selective muscarinic antagonists structurally related to furtrethonium. Manfredini et al. prepared a series of 5-substituted-2-(dimethylaminomethyl)-furyl derivatives starting from furtrethonium and gradually transforming this muscarinic agonist into an antagonist by introduction of lipophilic and bulky groups in position 5 of this molecule (Fig. 19). Rat ileum and bladder were used as target tissues to evaluate the antimuscarinic properties of the compounds at M_3 receptors, while guinea-pig atria were used to evaluate their M_2 potency. In particular, introduction of the α-hydroxy-α-cyclohexyl-benzyl moiety, a lipophilic group characteristic of known antimuscarinic agents, caused an appreciable increase of antagonist potency although no selectivity was observed (Table 15). Attempts were made to induce tissue selectivity by introducing a spacer group between the furan ring and some of the bulky lipophilic moieties, such as the lipophilic tail. The introduction of an ester group bearing the cyclohexylphenyl-methyl moiety led to synthesis of the non-quaternary compound **73** which displays a potency at M_3 receptors at least 20 times greater than at M_2 ones, while its quaternary analogue **74** proved to be 10 times less potent in the ileum and bladder and equally active in the atria. Replacing the ester function of **73** with the ether (**75**) and amide (**76**) groups reduced antagonist activity, while some selectivity towards intestinal and bladder smooth muscle was maintained for compound **76**. Some of these antagonists, however, caused some non-muscarinic-related effects when tested at high concentrations. In conclusion, two classes of compounds could be identified. The first class includes antagonists with the lipophilic bulky moiety located just beyond the furan ring in a position corresponding to that of the N-methyl groups in acetylcholine. These compounds

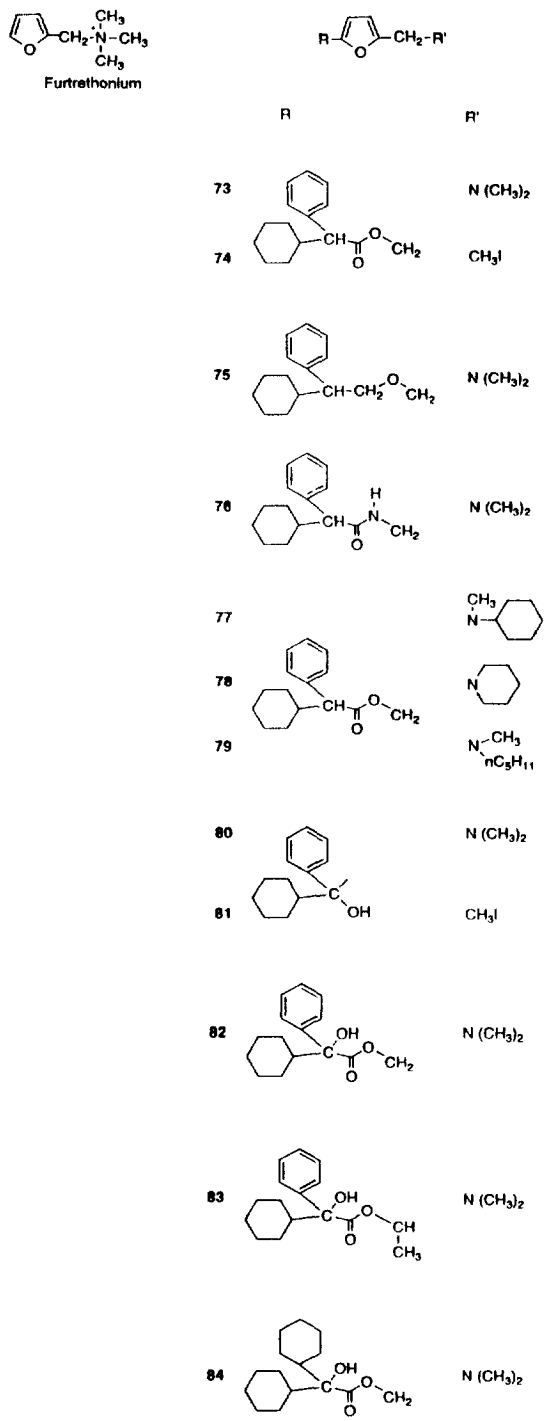


Fig. 19.

Table 15
Affinity constants ($-\log K$ values) of 5-substituted-2-(dimethylaminomethyl)-furyl derivatives (Fig. 19) on ri and rb (M_3) and gpla (M_2) [48,49]

Compound	$-\log K$			Selectivity ratios		
	ri	rb	gpla	ri/rb	ri/gpla	rb/gpla
73	7.3	7.2	5.9	1.3	25	20
74	6.4	6.2	5.5	1.6	7.9	5.0
75	6.0	5.9	5.7	1.3	2.0	1.6
76	5.8	5.9	5.1	0.8	5.0	6.3
77	6.5	<5.0	<4.5	>32	>100	3
78	6.3	5.3	<5.0	10	>20	>2
79	6.9	<5.0	<5.0	>79	>79	1
80	8.0	7.9	7.8	1.3	1.6	1.3
81	7.7	7.5	7.5	1.6	1.6	1.0
82	8.1	8.2	7.2	0.8	7.9	10
83	5.3	5.4	<5.0	0.8	>2	>2.5
84	7.1	7.2	<5.0	0.8	>126	>159

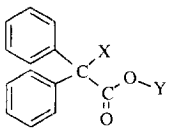
proved to be non-selective. The antagonists of the second class have the lipophilic anchoring moiety located further away from the charged nitrogen and display some selectivity towards smooth muscle preparations. Finally, the –CO– moiety adjacent to the lipophilic tail and the oxygen adjacent to the carbonyl in the ester function seem to be not essential for potency; quaternarization of the amine moiety in compound **74** produced a decrease in activity.

Feriani et al. [49] tried to improve the potency and selectivity of compound **73** modifying both the substituents on tertiary nitrogen and the lipophilic side chain. Several (±)-*N*-[5-[(1′-substituted-acetoxy)methyl]-2-furfuryl]dialkylamine analogues of **73** were then prepared (Fig. 19) using the Hansch approach, and a QSAR study was adopted to correlate activity with physicochemical properties of substituents. Table 15 shows that substitution of the *N*-dimethylamino function (**73**) with bulky groups (**77**, **78** and **79**) induced an overall decrease in antimuscarinic potency in all three target tissues, and that compounds **77–79** displayed a greater drop in potency in the atria and bladder. As a consequence, their potency in the ileum was 10–90 times higher than in the bladder; moreover, the QSAR results suggested that this selectivity may be attributable to physicochemical properties determining phenomena which involve the different receptor environments rather than the receptors themselves. Among the compounds carrying an OH group in the lipophilic side chain (**80–84**), showing increased antimuscarinic potency in all three tissues, only compound **84** showed greater selectivity than the lead **73** for M_3 versus M_2 subtypes (100 versus 20). The introduction of a methyl group at 1′-position of the C-5 chain in the furan ring produced compound **83** which showed a loss of activity in all the tissues. This reduced potency could be produced by the steric hindrance at 1′-position which would prevent the molecule from assuming the requested conformation. The influence of a chiral center within the ester moiety was also investigated: the optical isomers of **73** and **77** showed no significant improvement in potency or selectivity when compared with the racemic mixtures. In conclusion, these authors created an interesting new class of ileum selective antimuscarinic compounds.

11. Restricted flexibility analogues of adiphenine and 4-DAMP

In the period 1993–1994, Scapecchi and co-workers [50–52] acting in coordination with Gualtieri produced three papers on a number of esters of 2,2-diphenyl-2-alkylthioacetic and 2,2-diphenyl-2-alkoxyacetic acids, a new class of potent and functionally selective muscarinic antagonists which can be considered as restricted flexibility analogues of adiphenine and 4-DAMP (Fig. 17). These authors, in fact, were looking for M_2 selective antagonists as candidates for the treatment of cognitive disorders connected with central cholinergic deficit. Molecular manipulation of classical muscarinic antagonists such as adiphenine, benactyzine and 4-DAMP produced compounds where the conformational flexibility is sterically hindered by an increasingly large group inserted on the carbon atom already carrying the bulky lipophilic substituents known to drive the interaction with the receptors. Moreover, the choice of the sulfur atom connecting the quaternary carbon atom with the alkyl chain derived from the observation that 1,3-oxathiolanes are equipotent or even more potent compounds than 1,3-dioxolanes, while a sulfoxide function would introduce into the molecule a potent hydrogen bonding group similar to the C–OH group of the benactyzine-like antagonists. In this way alkoxy, sulfur and sulfoxide derivatives were obtained: variations in the alkyl chain, aminoalcohol moiety, and tertiary bases and ammonium salts were evaluated in terms of potency and selectivity at M_1, M_2 and M_3 muscarinic receptor subtypes (Table 16). Some of the compounds studied showed a high subtype selectivity, e.g. **86**, **88**, **90**, **92** and **97**; they were able to discriminate M_1 and M_2 receptors from the M_3 type, with ratios ranging from 24 to 417. Compound **96** was able to discriminate among M_1, M_2 and M_3 receptors ($M_1/M_3 = 7.8$, $M_2/M_1 = 12.5$, $M_2/M_3 = 100$). Compounds **94**, **95**, **98** and **93** were selective for M_2 receptors and could be useful tools for the treatment of Alzheimer's and Alzheimer-like pathologies. Interestingly, this last compound is a tertiary base and can be expected to readily cross the blood–brain barrier. Table 16 shows that all the discriminating compounds belong to the 2-alkylthio series of 2,2-diphenylacetic acid esters, while the 2-alkoxy and 2-sulfinyl derivatives show no selectivity. This fact, while contradicting the hypothesis that the steric lock imposed on the molecules by the 2-substituents would be responsible for their selectivity, emphasizes the role of the sulfur atom in giving this series of muscarinic antagonists high selectivity towards M_1 and M_2 receptors. Sulfur, in fact, seems to interact more favorably than oxygen with aromatic amino acid residues and this could explain at least the higher activity of the alkylthio compounds compared to the alkoxy ones [53]. The pharmacological profile of the most interest-

Table 16
Affinity constants (pA_2 and pK values) of 2,2-diphenyl-2-(alkylthio, alkoxy, alkylsulphinyl) acetic acid esters on rvd (M_1), gpla (M_2) and gpi (M_3) [50–52]



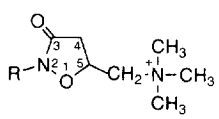
C	X	Y	pA_2 (pK)			Selectivity ratios		
			rvd (M_1)	gpla (M_2)	gpi (M_3)	M_1/M_3	M_1/M_2	M_2/M_3
85	OC_2H_5	*N,N*-diethylaminoethyl·HCl	6.34	6.57	(5.87)	3.0	0.6	5.0
86	SC_2H_5	*N,N*-diethylaminoethyl·HCl	7.79	7.93	(5.88)	81	0.7	112
87	OC_2H_5	*N,N*-diethylaminoethyl·CH_3I	7.41	6.79	7.20	2.0	4.0	0.4
88	SC_2H_5	*N,N*-diethylaminoethyl·CH_3I	8.97	9.12	6.50	295	0.7	417
89	OC_2H_5	*N*-methyl-4-piperidinyl·HCl	7.64	7.03	6.96	5.0	4.0	1.0
90	SC_2H_5	*N*-methyl-4-piperidinyl·HCl	8.82	8.56	6.36	288	2.0	158
91	OC_2H_5	*N*-methyl-4-piperidinyl·CH_3I	7.21	7.29	7.44	0.6	0.8	0.7
92	SC_2H_5	*N*-methyl-4-piperidinyl·CH_3I	9.30	9.62	7.92	24	0.5	50
93	SC_2H_5	1-pyrrolidineethyl·$(COOH)_2$	(6.46)	7.31	(6.20)	1.8	0.14	13
94	SC_2H_5	1-pyrrolidineethyl·CH_3I	(6.78)	(8.10)	(6.60)	1.5	0.05	32
95	SC_2H_5	1-piperidineethyl·CH_3I	(6.17)	(7.79)	(6.29)	0.8	0.02	37
96	$SCH(CH_3)_2$	*N,N*-diethylaminoethyl·HCl	(6.59)	7.70	(5.70)	7.8	0.08	100
97	$SCH(CH_3)_2$	*N,N*-diethylaminoethyl·CH_3I	(8.22)	8.24	(6.69)	34	1.0	35
98	$S(CH_2)_3CH_3$	*N,N*-diethylaminoethyl·CH_3I	(7.00)	8.75	(6.90)	1.3	0.02	71
99	$SOCH_3$	*N,N*-diethylaminoethyl citrate	(6.92)	(6.94)	(6.64)	1.9	1.0	2.0
100	SOC_2H_5	1-piperidineethyl [a]	(6.53)	(6.52)	(6.48)	1.1	1.0	1.1
101	SOC_2H_5	1-pyrrolidineethyl [a]	(6.59)	(6.97)	(6.48)	1.3	0.4	3.0
102	$SOCH(CH_3)_2$	*N,N*-diethylaminoethyl [a]	(8.01)	(8.09)	(7.34)	4.7	0.8	6.0

[a] Dibenzoyltartrate.

ing sulfur derivatives (**86**, **88**, **90** and **92**) was studied on the five cloned human muscarinic receptors (m_1–m_5) and it was found that the high selectivity shown on functional models by these compounds completely disappeared on cloned receptors. The discrepancy observed between functional and binding studies could be explained supposing that the compounds were not completely competitive antagonists; a deeper pharmacological study, in fact, demonstrated a competitive type of interaction at low concentrations and a non-competitive interaction (possibly allosteric) at high concentrations as a suitable reason for the different results obtained with functional and binding experiments [54]. The ability of the compounds to inhibit components involved in the response to receptor activation, with the component linked to M_2 and M_3 receptors being different, could be another explanation. For these reasons, these antagonists may be defined as functionally selective compounds.

12. Structurally related analogues of muscarone

Carnielli et al. [55] prepared and tested the racemic and chiral forms of isoxazolidin-3-ones **104** and **105** (Fig. 20), with the aim of clarifying the reasons for the low eudismic ratio (ER) shown by the enantiomers of the agonist azamuscarone **103** in a variety of muscarinic essays. This low value



103 R = CH_3

104 R = cC_6H_{11}

105 R = C_6H_5

Fig. 20.

(ER = 2.5–10.4), in fact, is in contrast with those shown by other muscarinic agonists which display ratios of at least two orders of magnitude (muscarone displays values ranging from 282 to 436). The data reported in Table 17 show that the eutomers (−)-**104** and (−)-**105** share the same stereochemistry (5*R*) as the most active form of azamuscarone (−)-**103** (5*R*) but display a reversed enantioselectivity in comparison to muscarone. The authors explained the difference in eudismic ratios and the reversed enantioselectivity with the lack of a chiral center at the 2-position in isoxazolidin-3-ones **103**–**105**. In fact, this position is of the utmost importance in the interaction of ligands with all the muscarinic receptor subtypes.

13. Uncharged antagonists

The possibility that uncharged compounds may bind to muscarinic receptors only via hydrophobic interactions has

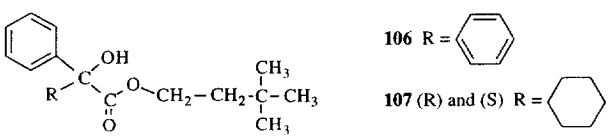
Table 17
Affinity constants (pA_2 values) of the enantiomers of the antagonists of Fig. 20 on gpla (M_2) and rj (M_3) [55]

Compound	gpla pA_2	E.R. [a]	rj pA_2	E.R. [a]
(*R*)-(−)-**103**	8.09 [b]	10.4	7.27 [b]	3.3
(*S*)-(+)-**103**	7.07 [b]		6.75 [b]	
(*R*)-(−)-**104**	5.92	4.2	5.67	4.8
(*S*)-(+)-**104**	5.30		4.99	
(*R*)-(−)-**105**	6.15	1.9	5.81	2.0
(*S*)-(+)-**105**	5.87		5.51	

[a] Eudismic ratio = antilog of the difference between the pA_2 values of the eutomer and distomer.
[b] $-\log EC_{50}$.

been assumed by Waelbroeck et al. (1996) [56]. These authors, in fact, analyzed the binding and functional affinities of the ester of 3,3-dimethylbutan-1-ol with diphenylglycolic acid (compound **106**) and of the chiral derivatives of (*R*)- and (*S*)-cyclohexylphenylglycolic acid (compounds (*R*)-**107** and (*S*)-**107**, Table 18). The data in Table 18 show that uncharged compounds still behave as muscarinic receptor antagonists with a range of affinity values which is only 1–2 log units lower than that of the corresponding charged esters. This fact led the authors to suggest that the cationic head group of the classical muscarinic antagonists does not come in very close contact with the aspartate residue of transmembrane region 3 and, consequently, the formation of an ionic bond between the ligand and the receptor active site is not essential for interaction. These are, indeed, the conclusions that had been reached by Gualtieri in 1990 [27] and much earlier by Ariëns [57] who postulated the overwhelming importance of hydrophobic over ionic interactions in the binding of muscarinic antagonists.

Table 18
Binding affinities (p*K*) (a) and pA_2 values (b) of antimuscarinic compounds **106**, (*R*)-**107** and (*S*)-**107** [56]



106 R =

107 (R) and (S) R =

Compound	NB-OK-1 cells (M_1)	rh (M_2)	Recombinant (Hm3)	rs (M_4)
(a)				
106	7.15	6.78	7.20	7.31
(*R*)-**107**	7.20	6.91	7.70	7.31
(*S*)-**107**	6.23	5.88	6.07	6.92
(b)				
	rvd (M_1)	gpla (M_2)	gpi (M_3)	
106	7.47	6.67	7.55	
(*R*)-**107**	7.55	6.74	7.46	
(*S*)-**107**	5.91	<4.52	5.56	

14. Molecular biology

Molecular biology studies with muscarinic receptors have stressed the longstanding problem that the binding mode of agonists could be different from that of antagonists. Mutagenesis studies suggest a common ion–ion interaction between the cationic head of agonists and antagonists and an aspartic acid residue located in TM (transmembrane domains) III of the receptor proteins (Asp147 in the rat m_3 receptor sequence). On the other hand, many studies with chimeric muscarinic receptors indicate that sequences within the third cytoplasmic loop are responsible for G protein recognition and activation [58].

Since the beginning of the nineties, Wess et al. have been very active in studies with chimeric muscarinic receptors probing the mutated receptors both with agonists and antagonists [58–60]. In fact, this author and his co-workers identified a series of threonine and tyrosine residues in the rat m_3 muscarinic receptor which are critical for the binding of muscarinic agonists but not for antagonists, suggesting a different molecular mechanism for the two kinds of ligands in binding to their target receptor. Again, studies with mutant receptors prompted these authors to conclude that different receptor subsites are involved in the binding of muscarinic agonists and antagonists, although the receptor domains involved in the binding of antagonists are only poorly defined.

Very recently [61], Wess published a stimulating review on the molecular biology of muscarinic acetylcholine receptors stressing that reliable structural models of antagonist/receptor complexes cannot be established at present, because of the structural complexity and diversity of muscarinic antagonists. This author also says that it is not clear which subsites interact with the bulky hydrophobic ring systems or side chains present in the most potent muscarinic antagonists. Nevertheless, mutational modification studies showed that two aromatic residues (rat m_3 Trp143 and Tyr529) probably play a role in stabilizing the ion pair formed between the TM III Asp residue and the positively charged amino/ammonium group present in virtually all muscarinic antagonists. Again, two residues, rat m_3 Ser120 and Asn507, seem to be required for high-affinity antagonist binding, while the subtype selectivity of structurally different antagonists involves several receptor regions including different TM domains. For instance, the receptor regions important for the binding selectivities of tricyclic antagonists (pirenzepine, AF-DX 116, himbacine) are different from those critical for high-affinity binding of HHSiD, 4-DAMP and like compounds.

At the same time, other authors came to the conclusion, by kinetic studies on ligand–receptor interaction, that agonists and antagonists occupy different binding sites on the muscarinic receptor, and the two-site receptor model attempts to explain the discrimination between agonists and antagonists by means of quantitative differences in the interactions of ligands with the receptor [62].

Unlike agonists, in the case of antagonists the efforts to establish sound structure–activity relationships are frustrated

by the uncertainty on the site of interaction, that can vary with the structure of the ligand. Actually, the binding mode of a molecule is dictated by the force-field experienced and very likely, since antagonist structures vary considerably, also the mode and the site of interaction are different. Of course, pentatomic cyclic antagonists and the bio-isosteric analogues are relatively homogeneous classes. However, even in this case, the simple introduction of a hydroxy group may change the binding mode; it is very difficult to foresee the way of the changes. As a consequence, in the literature there are many reports that are difficult to bring into a common scheme. Fig. 21 represents a tentative sketch in this direction.

Studies with enantiomers gave an important contribution to the knowledge of the geometry of muscarinic binding sites. The researchers occupied in this fascinating field seem to agree on the fact that the absolute configuration of the C-2 and C-4 asymmetric centers of 2-substituted 1,3-dioxolanes and their analogues (Fig. 1) is more important than the geometrical relations between the C-2 and C-4 substituents; the *S*-configuration at C-2 gives the highest activities [8–10]. On the other hand, the *R*-configuration of the benzylic center of compound **2** (Fig. 1) is important for activity [10], while the *R* enantiomer of procyclidine (Fig. 12) binds preferentially to M_1 and M_4 muscarinic receptors [35].

Pentatomic cyclic muscarinic antagonists and their related conformational analogues, such as benactyzine, procyclidine, caramiphen, dicyclomine, QNB, adiphenine, 4-DAMP, aprophen, reasonably interact with the same site as the muscarinic receptors, as many indications seem to suggest. These binding sites are characterized by an anionic site which is probably shared with the pentatomic cyclic agonists [1], two lipophilic sites at the opposite end and two middle 'heteroatom' sites. As far as the anionic site is concerned, it has been noted that appropriate terminal nitrogen substitution may induce subtype selectivity [34] while, when nitrogen is incorporated into an imidazole or pyrazole ring, appropriate substituents in position 2 of the ring increase M_3 selectivity [33]. An ethyl substituent on the cationic head improves the affinity of the molecule [19] and a phenethyl one leads to M_3 selectivity [19]. The flexible 2-(diethylamino)ethyl portion is important for controlling the M_1 binding selectivity of the ester-type antimuscarinics [38], while bulky lipophilic substituents at the tertiary nitrogen induce selectivity between smooth muscle tissues [49].

The two lipophilic sites can accommodate phenyl, cyclohexyl and pentyl groups; as far as substitution on the phenyl ring is concerned, electron-withdrawing groups give the ligand a high affinity for the M_1 site [37] while substitution on both possible phenyl rings is not well tolerated [43]. On the other hand, it seems that fluoro and methoxy derivatives show a high affinity for the M_3 site [43] while ligands with a cyclohexyl instead of a phenyl group do not fit properly into the M_2 subtype [43,38]. Appropriate distance between the hydrophobic groups in 2 position and the cationic nitrogen is crucial to activity [15]: ligands with the lipophilic bulky

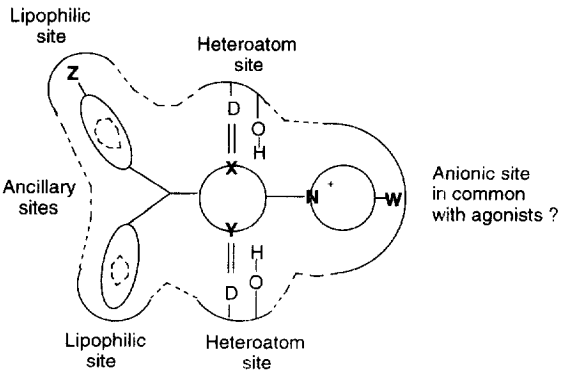


Fig. 21. Schematic representation of the possible mode of interaction of the pentatomic cyclic antagonists and their bioisosters.

moiety located farther away from the charged nitrogen display some selectivity towards the M_3 subtype [41–43,48].

The two 'heteroatom' sites are responsible for hydrogen bond and dipole–dipole interactions with functions of the ligand such as ester, thioester, amide, ether, thioether and alcohol, so that intramolecular hydrogen bonds can stabilize the most suitable molecule conformation [40]. The presence of a hydroxy group in 2 position increases the ligand affinity for all the subtypes [10,13,34,48,49], while compounds with 2-alkylthio substituents display a functional selectivity; M_1 and M_2 selectivity seems to be correlated to the role of the sulfur atom [50–52].

15. Quantitative structure–activity relationships

Quantitative structure–activity relationship (QSAR) models are consistent both with molecular biology and SAR studies.

In the period 1989–1996, several authors [63–72] worked on the definition of the topography of the muscarinic receptor antagonist binding sites, trying to correlate the pharmacological activities and the physicochemical properties of a series of ligands such as 2,2-diphenylpropionates, 4-DAMP and related compounds, 1,3-disubstituted pentatomic cyclic antagonists, aprophen and related compounds, and quinuclidine-based ligands.

Gordon et al. [63] observed that the antimuscarinic activities of 2,2-diphenylpropionates were significantly related to the distances between the carbonyl oxygen and the protonated nitrogen and that the maximum antimuscarinic potency was observed with a calculated bond distance of about 5.2 Å.

As far as 4-DAMP and related compounds are concerned, Barlow et al. [64] noted that the distance between the onium and acetyl groups in the crystal of 4-DAMP is 6.5 Å, and the relative positions of the two lipophilic sites (claw-like structure) are important for a good interaction between ligand and receptor subsite. As we have seen, the binding of the onium group involves an aspartate residue in transmembrane helix 3; tryptophan and tyrosine residues could accordingly be involved in the binding of phenyl groups and alike. On the

same topic, Recanatini et al. [65] proposed that two different pharmacophoric frames could account for the differences in the affinity values shown by a series of compounds structurally related to 4-DAMP (Fig. 22). In fact, an *endo*- or *exo*-like orientation of the benzylic substituents with respect to the piperidine ring is possible in these derivatives. Among the R substituents of the *exo* orientation, the methyl group shows the best interaction with the lipophilic pocket also occupied by the two phenyl rings because of its small size, while the OMe and OH groups in the *endo* orientation probably occupy the 'heteroatom' site also occupied by the etheral O atom of the spiro derivatives or by the S atom of thiospiro-DAMP (Fig. 17). A very strong interaction with the sites of the muscarinic receptor can explain the very high affinity of hydroxy-DAMP, while a hydrogen bond formation with a donor residue on the receptor can be supposed.

According to Pratesi et al. [66], the transformation of the compounds with the general structure shown in Fig. 23 from agonists to antagonists is probably due not only to steric factors but also to an over-increase in lipophilia [20]. Substituents in 2-position can be accommodated beyond the hydrophobic pocket of the agonist interaction site contacting hydrophobic regions surrounding this portion of the receptor. As far as the ammonium head is concerned, small groups on this ending are necessary for stimulant activity, while inductive effects of onium substituents and their bulkiness rather than their hydrophobicity play a major role in determining the inhibitory activity. Accordingly, Banerjee and Lien [67] noted that in a series of 1,3-dioxolanes, replacement of the $-N^+(CH_3)_3$ head by the $CH_3N^+(C_2H_5)_2$ produces more potent compounds. Moreover, for maximum cholinergic potency, the 2-substituents can be either phenyl or cyclohexyl but, when both are cyclohexyl groups, the ligand potency decreases. Malmusi et al. [18] reported the results of the structural determination of the already cited 1,3-dioxolanes of Fig. 5, where the exocyclic $CH_2N^+(CH_3)_3$ group is prevalently in a pseudo-axial orientation in the *cis* isomers and in a pseudo-equatorial orientation in the *trans* isomers.

Triggle et al. [68] and Karle et al. [69] tried to correlate the three-dimensional structure of aprophen, azaprophen and thiodeacylaprophen (Fig. 24) to their antimuscarinic activity. In particular, Triggle et al. demonstrated that the azabicyclo ring system in azaprophen shows a significant enantio- and stereoselectivity, so confirming the steric requirements of the muscarinic antagonists binding sites. The ether oxygen in aprophen and related compounds is buried and cannot interact with the receptor, while the carbonyl oxygen atom is exposed and is readily accessible for hydrogen bonding or for non-bonded interactions with the receptor. Karle et al. supposed that the weaker antimuscarinic activity of thiodeacylaprophen, with respect to aprophen, may be due to its short $S-N^+$ distance, the more shielded nature of its S atom relative to the carbonyl O atom, the differing position of the N^+-H group, and the greater hydrophobicity of a thioether group versus an ester group.

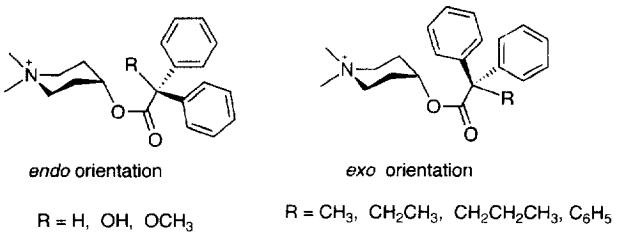


Fig. 22. From Ref. [65].

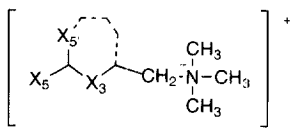
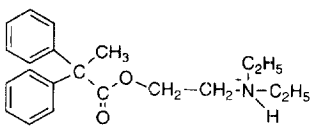


Fig. 23. From Ref. [66].



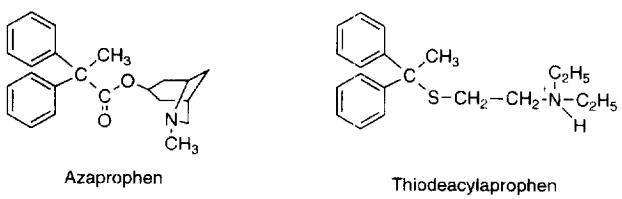


Fig. 24.

A QSAR analysis of quinuclidine-based muscarinic cholinergic receptor ligands was performed by Fanelli et al. [70–72]. They developed models supporting the pharmacophoric interacting model suggested by Saunders et al. [73]: first, the quinuclidine cationic headgroup recognizes and docks a common protophilic site in the receptor, then different mechanisms of interaction are involved in order to express the different pharmacological role of the ligands. Agonists, in fact, are characterized by two H-bonding interactions involving the nitrogen atoms of the oxadiazole ring, while antagonists are characterized by lipophilic interactions, and the partial agonist interaction mechanism shows an intermediate behavior. A model with different but overlapping binding sites for the agonists and the antagonists seems to these authors to be more realistic than a single-site model. In particular, the positively charged nitrogen atom of the antagonists interacts with an Asp residue (Asp308 in the human M_1-muscarinic receptor sequence), while the other molecular moieties occupy the binding pocket mainly formed by the amino acid side chains of helices 2 and 7 (Ser216, Tyr220, Glu708, Tyr711, Trp712, Tyr715).

16. Conclusions

Although the results obtained in the last 30 years have brought more valuable information on the problem of SAR

in the class of muscarinic ligands, it seems that the suggestions coming from all these analyses cannot be set in a single plan, because the different structures, and the different mode of interaction of the various ligands here reported, make a common solution unlikely.

17. Abbreviations used in Tables

Guinea pig ileum	gpi
Guinea pig left atrium (force)	gpla
Guinea pig right atrium (rate)	gpra
Guinea pig bladder	gpb
Rabbit vas deferens	rvd
Rat ileum	ri
Rat jejunum	rj
Rat bladder	rb
Rat cortex	rc
Rat heart	rh
Rat left atrium	rla
Rat striatum	rs
Not determined	nd
Selectivity ratios	antilog of the difference between the affinity values (pK or pA_2) for the muscarinic receptor subtypes studied

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