Cholinergic Agents Structurally Related to Furtrethonium. 2. Synthesis and Antimuscarinic Activity of a Series of *N*-[5-[(1′-Substituted-acetoxy)methyl]-2-furfuryl]dialkylamines[1]

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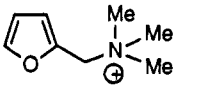
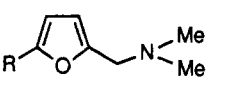
In the first part of this study, devoted to the discovery of selective antimuscarinic agents, (±)-*N*-[5-[(1′-phenyl-1′-cyclohexylacetoxy)methyl]-2-furfuryl]dimethylamine (**5a**) proved to be at least 20 times more potent in the rat ileum and bladder than in guinea pig atria.[1] Several (±)-*N*-[5-[(1′-substituted-acetoxy)methyl]-2-furfuryl]dialkylamine analogs of **5a** were subsequently prepared. This involved exploration of the tertiary nitrogen substituents and modulation of the lipophilic side chain at position 5 of the furan ring, using the Hansch approach. A QSAR study was conducted to correlate activity with physicochemical properties of substituents. The possibility of describing all compounds in a single model indicates that variations of nitrogen and the lipophilic side chain contribute independently to activity. Compounds **5b,c,j**, with bulky lipophilic substituents at the tertiary nitrogen, showed unprecedented selectivity between the two smooth muscle tissues, their antimuscarinic potency being from 10 to 90 times higher in the ileum than in the bladder. It is suggested that their interesting tissue selectivity is probably related to nonspecific phenomena involving the receptor environment, rather than real differences between the muscarinic receptors in the two tissues.

Introduction

During the past decade, more and more laboratories have focused their attention on muscarinic acetylcholine receptors. The main reason for this interest has been the identification of multiple muscarinic receptors, pharmacologically classified into at least four subtypes (M_1–M_4), by the use of suitable selective antagonists and on the basis of functional studies in various tissues preparations.[2,3] Thus, cardiac muscarinic receptors are called M_2-receptors, and those in the glands are M_3-receptors, while smooth muscle receptors are a mixture of M_2 and M_3. This differentiation suggests that it might be possible to discover tissue-selective antimuscarinic agents. To this end we have described, in a recent paper,[1] a structure–activity study involving the introduction of lipophilic and bulky groups into the structure of the well-known muscarinic agonist, furtrethonium, which led to the discovery of antimuscarinic agents endowed with interesting selectivity for ileum and bladder smooth muscle versus atrial cardiac muscle. These compounds, having the general structure **1**, were characterized by the presence of a tertiary nitrogen head and a lipophilic side chain at position 2 and 5 of the furan ring, respectively. The introduction of an ester, as a spacer group, between the furan ring and the lipophilic side chain led to the identification of a compound (**5a**) which proved to be at least 20 times more potent in the rat ileum ($pK_b = 7.3$) and rat bladder ($pK_b = 7.2$) than in guinea pig atria ($pK_b = 5.9$).



Furtrethonium



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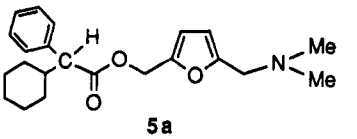
R = alkyl, alkyl-aryl, aryl, hydroxyalkyl, hydroxyaryl, alkenyl, alkynyl, 1′-phenyl-1′-cyclohexyl-acetoxymethyl, 1′-phenyl-1′-cyclohexyl-acetamidomethyl, 2′-phenyl-2′-cyclohexyl-ethoxymethyl.



5a

Against this background, the aim of the present study was to improve both antimuscarinic potency and selectivity of compound **5a** by extensive modification of the substituents on tertiary nitrogen and the lipophilic side chain. Substituents were chosen from the field of alkyl and arylakyl groups to provide both a broad distribution of physicochemical properties (lipophilicity and electronic and steric effects) and minimum correlation among the corresponding parameters which were subsequently used for a QSAR study. Moreover, in order to evaluate the influence of steric hindrance on both the stability of the ester function and selectivity of the lead compound **5a**, a methyl group was introduced at the 1′-position of the C-5 substituent of the furan ring (**11**). Finally, the influence of a chiral center within the ester moiety was also investigated.

Chemistry

Compounds **5b–f,h–j,n–q** (Scheme 1) were obtained, as already described by us,[1] by reaction of compounds

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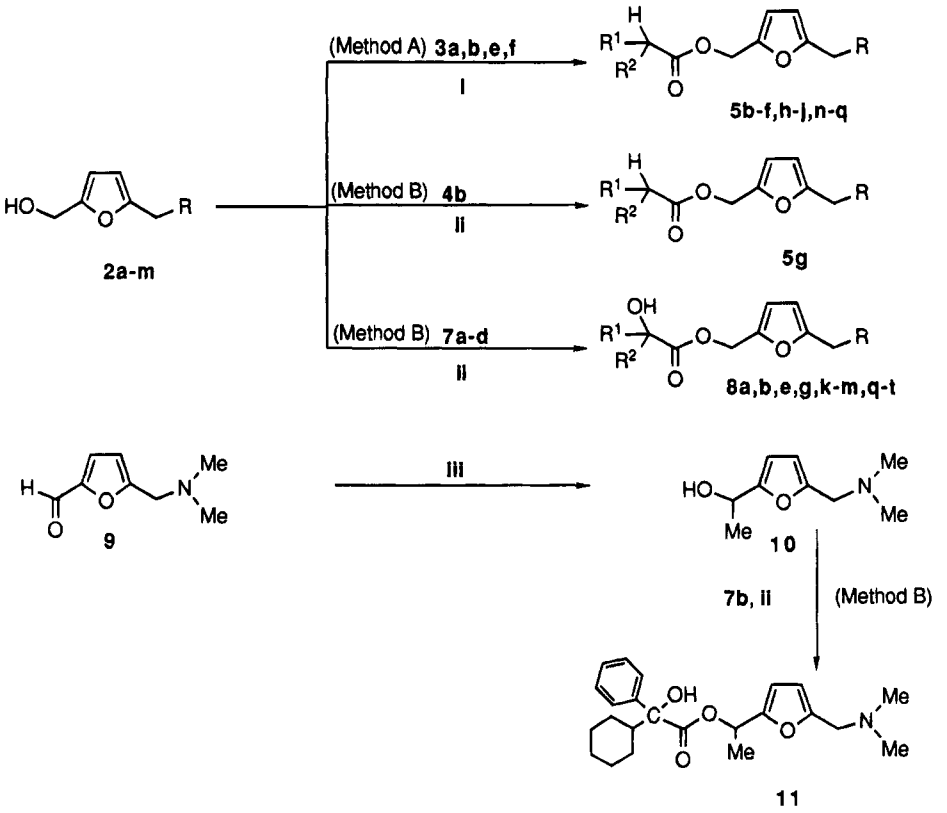
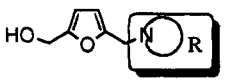
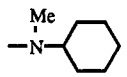
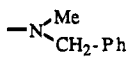
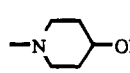
Scheme 1[a]



[a] (i) DCC, DMAP, CH_2Cl_2; (ii) NaOMe, cyclohexane, Δ; (iii) CH_3MgI, Et_2O.

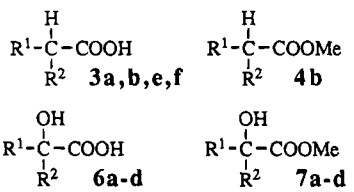
Table 1. 5-(Hydroxymethyl)-2-furfurylamines **2a–m**



COMPOUND	R	COMPOUND	R
2a		**2h**	
2b		**2i**	
2c		**2j**	
2d		**2k**	
2e		**2l**	
2f		**2m**	
2g			

2b,e,f,[4] **h–j** (Table 1) with **3a,e** and **f** (method A). Attempts to extend the same procedure to the preparation of **5g** and **8a,b,e,g,k–m,q–t** resulted in complex mixtures of products. The problem of competition between the two hydroxyl groups led us, after several attempts, to use the transesterification (method B)

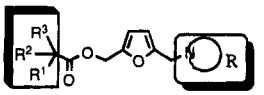
Table 2. α-Substituted Carboxylic Acids and Esters

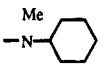
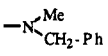
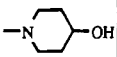
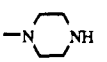
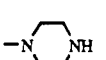
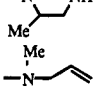


Compound	R1	R2	Compound	R1	R2
a			**d**		
b			**e**		
c			**f**		

between the amino alcohol and the appropriate methyl ester **4b**, **7a–d** (Table 2), with sodium methoxide as the catalyst.[5] This method proved to be sufficiently mild, selective, and satisfactory in terms of yield. The starting methyl ester **4b** was prepared by the standard procedure from the acid **3b**, whereas **7a–c** were prepared by reaction of diazomethane with the corresponding acids (**6a–c**) in good yields. Compound **6b** has been reported, in poor yields (25%),[6] through Grignard reaction of the cyclohexylmagnesium bromide derivative to the ethyl phenylglyoxylate. Thus, **6b,c** were better prepared by direct hydroxylation, following the methodology reported by Adam and Cueto for arylacetic acids.[7] Attempts to apply this procedure to **5a** failed. Preparation of glycolate **7d** was achieved by simple hydrogenation of **6b** at 50 psi in a Parr apparatus, in the presence of Adams' catalyst.

Table 3. In Vitro Muscarinic Antagonist Potencies of Tested Compounds[a]



Compound R3=H	Guinea-pig atria pKb	Rat ileum pKb	Rat bladder pKb	R1	R2	R	Compound R3=OH	Guinea-pig atria pKb	Rat ileum pKb	Rat bladder pKb
5a[c]	5.9±0.1(4)[c]	7.3(7.2-7.5)[d,e]	7.2(7.0-7.4)[d,e]				**8a**	7.2(7.0-7.5)[e]	8.1(8.0-8.3)[e]	8.2(8.0-8.4)[e]
5b	< 4.5[b,c]	6.5±0.1(6)[d]	≤ 5[b,d]				**8b**	6.0±0.2(4)[e]	7.7(7.4-7.9)[e]	7.0(6.8-7.2)[e]
5c	< 5.0[b,c]	6.3±0.2(4)[d]	5.3±0.2(4)[d]							
5d	< 5.0[b,c]	< 5.0[b,c]	< 5.0[b,d]							
5e	5.0±0.2(3)[c]	7.0±0.2(4)[d]	6.7±0.2(4)[d]				**8e**	6.4±0.1(4)	7.5±0.1(6)	7.5±0.1(6)
5f	< 5.0[b,c]	5.5±0.2(3)[d]	5.7±0.2(3)[d]							
5g	< 5.0[b,c]	6.5±0.1(4)[d]	6.0±0.1(4)[d]				**8g**	6.3±0.1(4)[c]	7.0±0.1(4)	6.8±0.1(4)
5h	< 5.0[b,c]	6.7±0.1(4)[d]	6.0±0.2(4)[d]							
5i	< 5.0[b,c]	6.8±0.1(4)[d]	6.0±0.1(4)[d]							
5j	< 5.0[b,c]	6.9±0.2(4)[d]	<5[b,d]							
							**8k**	5.0±0.1(4)[c]	5.5±0.1(4)[d]	5.5±0.1(4)[d]
							**8l**	< 5.0[b,c]	5.8±0.2(3)[d]	5.0±0.2(3)[d]
							**8m**	6.8±0.1(4)	7.5±0.1(6)	7.8±0.1(6)
5n	< 5.0[b,c]	6.0±0.2(4)[d]	6.2±0.4(4)							
							8r	7.2(7.0-7.4)[e]	7.4(7.2-7.6)[e]	7.7(7.6-7.9)[e]
							8s	7.7±0.1(4)	7.8±0.1(6)	7.8±0.1(6)
5o	4.8±0.2(3)[c]	5.8±0.2(3)[d]	5.6±0.1(4)[d]							
5p	6.5±0.1(4)	7.1±0.1(4)	6.6±0.1(4)							
5q	5.9±0.1(4)	6.5±0.1(4)	6.5±0.1(4)				**8q**	6.7±0.1(4)	6.5±0.1(4)	6.7±0.1(4)
							8t	< 5.0[b,c]	7.1(7.0-7.3)[e]	7.2(7.0-7.5)[e]

[a] Values represent mean ± standard error with number of experiments in brackets, where appropriate. [b] No appreciable shift to the right of concentration response curve to the agonist in the presence of concentrations of 10−30 μM of test compound. [c] Appreciable negative and/or chronotropic effects (atropine insensitive) at the concentrations of 10−30 μM; depression of the maximum at concentrations of 10−100 μM. [d] Depression of the maximum at concentrations of 10−100 μM. [e] 95% confidence limits.

The furylcarbinol **10** was best prepared[8] by the reaction of methylmagnesium iodide with the corresponding aldehyde **9**, which was in turn obtained from **2a** through oxidation with manganese(IV) oxide.[9] This avoided side reactions usually occurring during the aminomethylation step of 1′-alkylfuryl carbinols.[1,8] Compound **10** was fully converted into the ester **11**, as described above for **5g** and **8a,b,e,g,k−m,q−t** (method B, Scheme 1).

To further investigate structure−activity relationships, the two enantiomers of compound **5b** were prepared, as previously described for **5a**,[1] starting from the (+)- and (−)-(phenylcyclohexyl)acetic acid and the amine **2b**. The optical purity of enantiomers was

Table 4. Pharmacological and Physicochemical Data

compd	pK_b (bladder)	pK_b (ileum)	pK_b (atrium)	$p(R^1 + R^2)$[d]	$\Sigma\sigma^*$[e]	MR(N)[f]	CLOGP[g]	D[h]
			Subset A					
5a	7.2[a]	7.3[a]	5.9[b]	3.95	0.00	0.00	4.90	0
8a	8.2	8.1	7.2	3.95	0.00	0.00	4.10	1
5o	5.6[b]	5.8[a]	4.8[a]	2.70	0.00	0.00	3.80	0
5p	6.6	7.1	6.5	3.55	0.00	0.00	4.50	0
5q	6.5	6.5	5.9	2.87	0.00	0.00	3.82	0
8s	7.8	7.8	7.7	3.39	0.00	0.00	3.54	1
8q	6.7	6.5	6.7[b]	2.80	0.00	0.00	3.01	1
8t	7.2	7.1	<5.0[b,c]	5.08	0.00	0.00	6.03	1
			Subset B					
5b	<5.0[a,c]	6.5[a]	<4.5[b,c]	3.95	−0.18	2.14	6.93	0
5c	5.3[a]	6.3[a]	<5.0[b,c]	3.95	−0.18	1.21	6.33	0
5e	6.7[b]	7.0[a]	5.0[c]	3.95	−0.19	0.93	5.58	0
5f	5.7[a]	5.5[a]	<5.0[b,c]	3.95	0.67	0.90	4.94	0
5g	6.0[a]	6.5[a]	<5.0[b,c]	3.95	0.17	1.37	4.24	0
5h	6.0[a]	6.7[a]	<5.0[b,c]	3.95	−0.20	0.93	5.80	0
5i	6.0[a]	6.8[a]	<5.0[b,c]	3.95	−0.12	0.93	5.80	0
5j	<5.0[a,c]	6.9[a]	<5.0[b,c]	3.95	−0.23	1.86	6.69	0
8b	7.0	7.7	6.0[b]	3.95	−0.18	2.14	6.13	1
8e	7.5	7.5	6.4	3.95	−0.19	0.93	4.77	1
8g	6.8	7.0	6.3[b]	3.95	0.17	1.37	3.44	1
8k	5.5[a]	5.5[a]	5.0[b]	3.95	1.20	1.12	3.90	1
8l	5.0[a]	5.8[a]	<5.0[b,c]	3.95	1.20	1.58	4.42	1
8m	7.8	7.5	6.8	3.95	0.23	0.90	4.45	1
8r	7.7	7.4	7.2	3.39	−0.19	0.93	4.22	1
5n	6.2	6.0[a]	<5.0[b,c]	2.87	−0.18	1.21	5.25	0

[a] Concomitant depression of the maximum at the concentration of 10−100 μM. [b] Appreciable negative inotropic and/or chronotropic effects (atropine insensitive) at the concentrations of 10−30 μM. [c] No appreciable shift to the right of concentration response curve to the agonist in the presence of concentrations of 10−30 μM of test compound. [d] $p(R^1 + R^2)$ = relative lipophilicity for substituents $R^1 + R^2$, calculated on the CLOGP3 program[20] with $p(R^1 + R^2) = 0$ for $R^1 = R^2 = R^3 = H$. [e] $\Sigma\sigma^*$ = sum value of inductive effects on nitrogen substituents, σ^* = electronic parameter of Taft.[21] [f] MR(N) = overall relative molar refractivity of nitrogen substituents calculated by the program CMR[22] with MR(N) = 0 for R = $N(CH_3)_2$. [g] Global lipophilicity, calculated with the CLOGP3 program.[20] [h] D = dummy (1 = presence, 0 = absence of OH group).

determined by the observation of the ^1H NMR shift of the CH_2N singlet, centered at about 3.50 ppm, induced by the addition of the chiral shift reagent $Eu(Tfc)_3$.

Biology

Rat ileum and bladder were used as target tissues to evaluate the antimuscarinic properties of compounds at muscarinic acetylcholine receptors of the M3 subtype, while the guinea pig atria, endowed with receptors of the M2 subtype, were used to ascertain their selectivity. The antimuscarinic potencies were expressed as pK_b ($-\log K_b$). The pharmacological results are summarized in Table 3. All tested compounds were devoid of agonist activity.

Results and Discussion

Compound **5a**, which proved to be at least 20 times more potent in smooth muscle than in cardiac muscle, was assumed as the lead compound at the beginning of the present study. In derivatives **5b**−**j**, carrying the same lipophilic side chain as **5a**, the substitution of the *N*-dimethylamino function with bulky groups (R) induced an overall decrease in antimuscarinic potency in all three target tissues; this phenomenon was particularly marked in the atria and, for compounds **5b**, **5c**, and **5j**, also in the bladder (Table 3); their potency in the ileum, 10−90 times higher in the bladder, suggests ileum selectivity. In view of the results of the QSAR study (see QSAR section point b), this remarkable selectivity is probably attributable to physicochemical properties [(i.e., high overall lipophilicity and low pK_b values (Table 4)], which might determine phenomena involving the different receptor environments rather than the receptors themselves.

In the derivatives **5o**,**p**,**q**, carrying the same cationic head as **5a**, the variations at the lipophilic side chain led to a reduction of selectivity toward smooth muscle compared with cardiac tissue and to loss of selectivity for ileum versus bladder.

Compounds **8a**,**b**,**e**,**f**,**q**, carrying the OH group (R^3) in the lipophilic side chain, showed increased antimuscarinic potency in all three tissues relative to their dehydroxy homologues. However, this was accompanied by concomitant decreased selectivity (compared with compounds **5a**,**b**,**e**,**g**,**q**) for ileum and bladder versus atria. Among the remaining compounds, only compound **8t** showed greater selectivity than the lead **5a** for smooth versus cardiac muscle (100 versus 20 times). Compounds **8m**,**r**,**s** showed fairly potent antimuscarinic activity, associated with a reduced degree of selectivity for ileum and bladder smooth muscle versus atrial cardiac muscle by comparison with the lead **5a**. The optical isomers of **5a** and **5b** showed no significant improvement in either potency or selectivity over the racemic mixtures,[10] indicating the absence of antimuscarinic enantioselective effects. This is in contrast to the known stereoselective interaction of quinuclidinyl benzylate (QNB) and other muscarinic cholinergic agents.[11]

It is noteworthy that a number of compounds (**5b**−**o** and **8g**,**k**,**l**,**t**), when tested at concentrations from 10 to 100 μM, caused some nonmuscarinic effects (Table 3). In the ileum and bladder, these effects consisted of a reduction of maximal contractile response to the reference agonist and a flattening of the concentration−response curve, not overcome by the addition of even large concentrations of reference agonist. In the atria, negative inotropic and/or chronotropic effects were

Table 5. Correlation Matrix

Equations 1 and 2					
	p(R1+R2)	$p(R1+R2)^2$	D		
p(R1+R2)	1	0.995	0.361		
$p(R1+R2)^2$		1	0.372		
D			1		
Equation 3					
	Σσ*	MR(N)	D		
Σσ*	1	0.999	0.113		
MR(N)		1	0.107		
D			1		
Equation 4					
	Σσ*	MR(N)	D		
Σσ*	1	-0.008	0.403		
MR(N)		1	0.006		
D			1		
Equation 5					
	Σσ*	MR(N)	D		
Σσ*	1	0.202	0.303		
MR(N)		1	0.276		
D			1		
Equation 6					
	Σσ*	MR(N)	D		
Σσ*	1	0.037	0.301		
MR(N)		1	0.405		
D			1		
Equation 7					
	p(R1+R2)	$p(R1+R2)^2$	Σσ*	MR(N)	D
p(R1+R2)	1	0.993	0.141	0.281	0.185
$p(R1+R2)^2$		1	0.127	0.228	0.195
Σσ*			1	0.080	0.303
MR(N)				1	-0.049
D					1

observed when antagonists were allowed to equilibrate for 30 min; these effects were not inhibited by atropine. The nature of these nonmuscarinic effects has not been determined, but might be ascribed to postreceptorial effects. When these concentrations produced significant differences in slope functions and maximal responses in comparison with control curves (see Analysis of Data), the responses were not utilized for the estimation of pK_b values.

For compound **11**, the introduction of a methyl group at 1′-position of the C-5 chain in the furan ring induced considerable reduction of potency by comparison with the parent **8a**: pK_b were <5 (atria), 5.3 (ileum), and 5.4 (bladder). This loss of activity is probably attributable to the steric hindrance at 1′-position; a more rigid analog is probably unable to accommodate particular conformational requirements.

QSAR. A structure–activity study was performed on congeneric series of derivatives. Of these, subset B was obtained by varying the substituents on the nitrogen (R), and subset A by variations to the lipophilic side chain (R^1, R^2, R^3) (Table 4). The compounds comprising the sets were chosen to ensure maximum variance and minimum colinearity between parameters characterizing the physicochemical properties of the substituents (Table 5). Using the Hansch approach, the various substituents were evaluated for electronic and steric effects and lipophilicity.[12–14] As in previous structure–activity studies,[15–19] these effects were considered additive both for nitrogen substituents and for the lipophilic side chain and thus grouped according to individual variables. Lipophilicity (p) of substituents at the lipophilic side chain was calculated from CLOGP,[20] with $p(R^1 + R^2) = 0$ for $R^1 = R^2 = R^3 = H$. Electronic effects of nitrogen substituents were determined by using the Taft electronic parameter (σ^*)[21] and molecular refractivity MR, used as a steric index, was calculated from CMR[22] with MR [$N(CH_3)_2$] = 0.

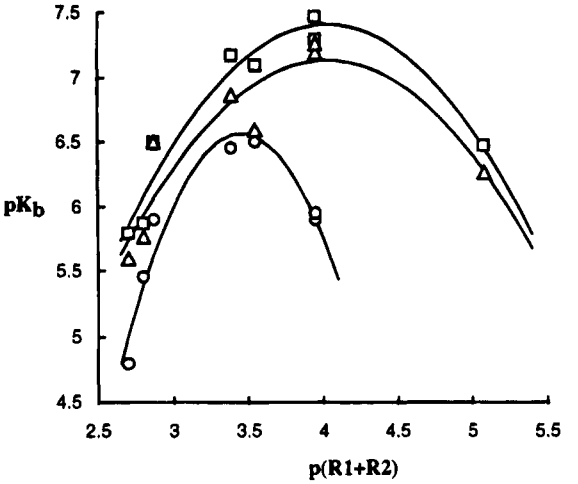


Figure 1. Best fitting of pK_b of compounds reported in Table 4 (subset A) vs lipophilicity of R^1 and R^2. Squares, activities on ileum; triangles, activities on bladder; circles, activities on atrium. For compounds with R^3 = OH, a constant value (regression coefficient of D in eqs 1, 2, and 3) was subtracted from pK_b.

Because of the small number of compounds for each set, it was not always possible to obtain statistically rigorous quantitative relationships. The equations nevertheless confirmed the results reported in the literature.[15–19]

(a) Variations on the Lipophilic Side Chain [(R^1, R^2, R^3) = Variable; R = $N(CH_3)_2$]. Compounds in subset A (Table 4) were considered. Comparison of pharmacological data on the ileum, bladder, and atria shows that the various substituents produce comparable effects on antimuscarinic activity for the ileum and bladder, while activity on smooth muscle is almost always greater than on the atria. It was also noted that the compounds with the R^3 = OH group are always more active than those for which this group is absent. MRA (multiple regression analysis) on activity data for the ileum does not, however, give any obvious correlation with any of the physicochemical parameters considered, including lipophilicity of the lipophilic side chain: $p(R^1 + R^2 + R^3)$. It was thus postulated that the OH group might contribute to activity by another type of interaction (i.e., electrostatic, hydrogen bonding, ...).[23] Accordingly, we considered the contribution of the OH constant, irrespective of R^1 and R^2, and separated it from the overall lipophilicity of the substituents ($R^1 + R^2 + R^3$). It was then accounted for with a dummy variable (D = 1 presence and D = 0 absence). In this way, antimuscarinic activity on the ileum may fit a parabolic (or bilinear) trend with lipophilicity, in agreement with the results reported by other authors[15–17,19,24] (eq 1, Figure 1).

$$pK_b(\text{ileum}) = 7.06(\pm0.93)p(R^1 + R^2) - 0.87(\pm0.12)p(R^1 + R^2)^2 + 0.62(\pm0.15)D - 6.82(\pm1.73) \quad (1)$$

$$n = 8 \quad R = 0.98 \quad s = 0.20 \quad F = 30 \quad p < 0.01$$

where $p(R^1 + R^2)$ = relative lipophilicity for substituents $R^1 + R^2$, calculated on the CLOGP program[20] with $p(R^1 + R^2) = 0$ for $R^1 = R^2 = R^3 = H$; D = dummy.

The optimal lipophilicity value, $p(R^1 + R^2)_o = 4.04$, is comparable to that obtained by other authors.[15-17,19,24] The correlation matrix is given in Table 5.

A similar equation was obtained for the bladder (eq 2, Figure 1). The coefficients of this equation are statistically not distinguishable from those for the ileum (significance of differentiation between the two evaluated by t-test, $p < 0.05$).

$$pK_b(\text{bladder}) = 6.35(\pm1.42)p(R^1 + R^2) - 0.79(\pm0.19)p(R^1 + R^2)^2 + 0.93(\pm0.24)D - 5.68(\pm2.65) \quad (2)$$

$$n = 8 \quad R = 0.96 \quad s = 0.31 \quad F = 15 \quad p < 0.025$$

Optimal lipophilicity value: $p(R^1 + R^2)_o = 4.04$. The correlation matrix is given in Table 5.

In the same way, a parabolic (or bilinear) correlation was obtained for the atrium.

$$pK_b(\text{atrium}) = 18.78(\pm3.55)\sum\sigma^* - 2.72(\pm0.53)\text{MR(N)} + 1.25(\pm0.16)D - 25.88(\pm5.80) \quad (3)$$

$$n = 7 \quad R = 0.99 \quad s = 0.21 \quad F = 40 \quad p < 0.001$$

Optimal lipophilicity value: $p(R^1 + R^2)_o = 3.46$. The correlation matrix is given in Table 5.

This equation, though statistically significant, has less observables than those obtained previously ($n = 7$) and covers a more restricted range of lipophilicity. Regression coefficient values are thus subject to even greater fluctuation, particularly for lipophilicity. Figure 1 shows the trend for antimuscarinic activity on the atrium as a function of lipophilicity, as obtained by the regression analysis; for comparison, activity on the ileum is also shown. Difference in activity on the two tissues (i.e., selectivity) can be clearly seen to depend on their differing lipophilicity trends (optimal lipophilicity being about 3.5 for the atrium and 4.0 for the ileum), as well as on the differing contribution of the OH group (dummy coefficients for the two equations were 0.62 for the ileum and about 1.2 for the atrium).

Thus, ileum selectivity increases with lipophilicity and in the absence of the OH group, with concomitant decreases of activity in both ileum and atria.

(b) Variations of Tertiary Nitrogen (R = Variable, R^1 = Phenyl, R^2 = Cyclohexyl, R^3 = H/OH). Compounds considered were those in subset B, together with compounds **5a** and **8a** of subset A (see Table 4).

Comparison of pharmacological data on the ileum, bladder, and atrium (Figures 2 and 3) shows that substitution of nitrogen leads to similar effects on the ileum and bladder for compounds with pK_b greater than about 5.5, while for low activity values there is tissue selectivity toward the ileum. Activity on the ileum remains systematically higher than on the atrium.

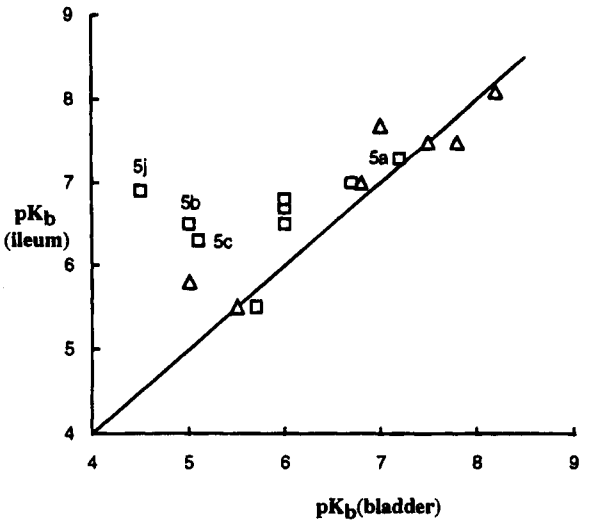


Figure 2. Comparison of the antimuscarinic activity on the ileum and bladder of compounds reported in Table 4 (subset B and compounds **5a** and **8a**). Squares, R^3 = H; triangles, R^3 = OH. The line represents equal pK_b values in the ileum and bladder. Compounds **5a**, **5b**, **5c**, and **5j** are indicated.

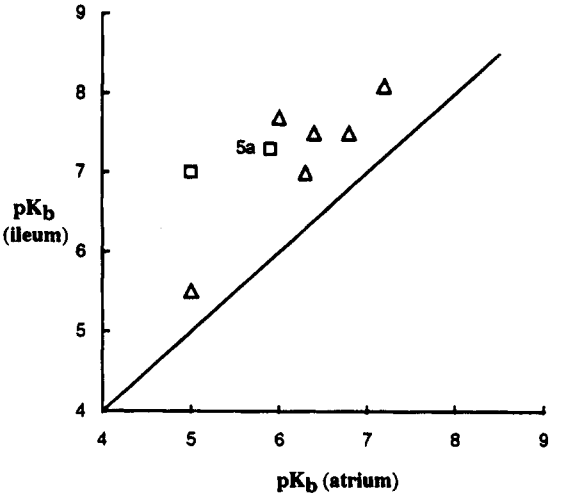


Figure 3. Comparison of the antimuscarinic activity on the ileum and atrium of compounds reported in Table 4 (subset B and compounds **5a** and **8a**). Squares, R^3 = H; triangles, R^3 = OH. The line represents equal pK_b values in the ileum and atrium. Compound **5a** is indicated.

Linear MRA of data for the ileum gives the following equation (eq 4):

$$pK_b(\text{ileum}) = -1.49(\pm0.18)\sum\sigma^* - 0.34(\pm0.12)\text{MR(N)} + 0.97(\pm0.16)D + 6.96(\pm0.17) \quad (4)$$

$$n = 16 \quad R = 0.94 \quad s = 0.29 \quad F = 21 \quad p < 0.001$$

where $\sum\sigma^*$ = sum value of inductive effects on nitrogen substituents: Taft's electronic parameter,[20] MR(N) = overall relative molar refractivity of nitrogen substituents, calculated by the program CMR[21] with MR(N) = 0 for R = $N(CH_3)_2$, and D = dummy.

From eq 4, it can be seen that antimuscarinic activity on the ileum increases as $\sum\sigma^*$ decreases, i.e., as the electron donor inductive effect of the nitrogen substituents increases and their steric hindrance, given as MR(N), decreases. The dummy variable makes it possible to bring together in a single set of compounds molecules both with and without the hydroxyl on the lipophilic side

chain. The OH group leads to an increase in activity, as already seen at point a; the coefficient of D is not statistically different from that obtained in eq 1 (t-test with $p < 0.05$).

The overall inductive effect of the substituents on the nitrogen is important in modulating antimuscarinic activity, as seen from the high value of the coefficient of regression. A similar but less marked electronic effect observed in quaternary ammonium compounds has been ascribed to the influence of the nitrogen substituents on dispersion of the positive charge of the cationic head.[16,18,19,25,26] In the present case, since there is evidence that the active form is the protonated compound,[27] this effect can be considered responsible for an increase in the basicity of the molecule. The contribution of MR(N) is less important for antimuscarinic activity, though statistically significant. Contrary to reports by other authors,[23] there does not seem to be any significant correlation between antimuscarinic activity on the ileum and the lipophilicity of the nitrogen substituents.

Regression analysis of bladder activity data did not give any statistically significant equation. As can be seen in Figure 2, activity on the bladder corresponds to that on the ileum for all products except **5b,c,j**: these are the most lipophilic of the whole series and have a very low pK_b (Table 4), which is borderline in terms of the appropriate pharmacological detectability threshold. Excluding these products, the resulting equation (eq 5) is similar (with statistically distinguishable coefficients for the t-test with $p < 0.05$) to that for the ileum (eq 4), though less satisfactory:

$$pK_b(\text{bladder}) = -1.40(\pm 0.24)\sum\sigma^* - 0.80(\pm 0.21)\text{MR(N)} + 1.22(\pm 0.24)D + 7.02(\pm 0.24) \quad (5)$$

$$n = 13 \quad R = 0.94 \quad s = 0.39 \quad F = 21 \quad p < 0.001$$

The correlation matrix for this equation is given in Table 5.

It is thus plausible that the differing behavior of products **5b,c,j** is due not to phenomena closely related to the interaction with the receptor binding site, but to nonmuscarinic effects attributable to the high overall lipophilicity of these molecules. This hypothesis is consistent with a receptor model providing for a "specific" portion (identifiable with the protein) and an "annexed" portion (identifiable with a lipid pool, known as the proximal lipid, with is closely linked to the protein portion).[28-32] If the receptor affinity constant (pK_b) of a compound competes with its partition coefficient (log $P_{o/w}$, which is used as a model for the partitioning in a biological lipophilic phase), as may be the case for highly lipophilic compounds with low activity (i.e. **5b**, **5c**, and **5j**; see Table 4), the molecule may accumulate in the proximal lipid and alter the structure of the binding site. This interaction could impair accurate determination of the antimuscarinic activity and may give rise to a nonparallel dose–response curve with depression of the maximum. There could be a differing extent of this phenomenon in various tissues, related to the differences in the proximal lipid, which may also lead to a nonhomogeneous significance for pK_b. Accordingly, since compounds **5b,c,j** can readily be accommodated in the equation for the ileum, it is reasonable to postulate a certain difference between the two receptor complexes, in terms not so much of the binding site as of the receptor environment.

For the activity of this series of derivatives on the atrium, an equation (eq 6) whose regression coefficients $\sum\sigma^*$ and MR(N) do not differ statistically from those for the ileum and bladder (eqs 4 and 5) has been obtained.

$$pK_b(\text{atrium}) = -1.06(\pm 0.31)\sum\sigma^* - 0.72(\pm 0.21)\text{MR(N)} + 1.59(\pm 0.34)D + 5.68(\pm 0.27) \quad (6)$$

$$n = 8 \quad R = 0.94 \quad s = 0.36 \quad F = 10 \quad p < 0.025$$

The correlation matrix for this equation is given in Table 5.

In addition to coefficient D—as expected, higher in the atrium (see point a)—the intercept value (lower for atrium) also differs significantly for eq 6 by comparison with eqs 4 and 5. Figure 3 shows that atrial activity is systematically lower than that on the ileum (and thus on the bladder). This could be because the interaction of the lipophilic side chain with the receptors differs for cardiac and smooth muscle, as seen at point a. The difference in the intercept values is comparable with the difference in activity for compund **1** in the two types of tissue.

It can thus be concluded that the selectivity of these compounds for smooth muscle is related essentially to the interaction of the lipophilic side chain.

(c) Simultaneous Variations on the Lipophilic Side Chain and Tertiary Nitrogen (R^1, R^2, R^3, and R = Variable). Analysis of the entire set of derivatives (Table 4), including all compounds so far considered and two products (**8r** and **5j**) not belonging to the previous series, gave a single equation (eq 7).

$$pK_b(\text{ileum}) = 5.84(\pm 1.12)p(R^1 + R^2) - 0.73(\pm 0.15)p(R^1 + R^2)^2 - 1.44(\pm 0.16)\sum\sigma^* - 0.39(\pm 0.10)\text{MR(N)} + 0.84(\pm 0.12)D - 4.60(\pm 2.04) \quad (7)$$

$$n = 24 \quad R = 0.94 \quad s = 0.28 \quad F = 26 \quad p < 0.001$$

The correlation matrix for this equation is given in Table 5.

It can thus be reasonably concluded that variations on the lipophilic side chain and tertiary nitrogen independently affect antimuscarinic activity. A similar equation was obtained for the bladder (excluding compounds **5b,c,j**; see point b). No equation was obtained for the atrium, because of insufficient data.

Conclusions

Optimization of the pharmacological profile of compound **5a**, in terms of antimuscarinic potency and selectivity, was achieved by systematic exploration of tertiary nitrogen substituents and modulation of the lipophilic side chain. Moreover, the introduction of a methyl group at the 1′-position of the C-5 chain in the furan ring and enantioselectivity were also investigated.

From the data presented here, the following considerations can be drawn:

(1) Antimuscarinic activity on smooth and cardiac muscle seems to follow a parabolic trend relative to the lipophilicity of the lipophilic side chain. Peak activity

for the ileum and bladder corresponds to a relative lipophilicity of about 4.04 (as in the literature),[14-16,18,23] while lipophilicity for peak activity in the atrium is lower (about 3.5).

(2) Antimuscarinic activity increases together with basicity of the tertiary nitrogen and decreases in all tissues as the steric hindrance of the substituents in this position becomes greater.

(3) The presence of an OH on the lipophilic side chain increases antimuscarinic activity. This increase, not related to the hydrophilicity of the OH group, is greater in the atrium than in smooth muscle. It seems independent of the substituents to the lipophilic side chain and the tertiary nitrogen.

(4) The tissue selectivity between the ileum and the bladder for products **5b,c,j** is probably related to nonspecific phenomena which may involve the receptor environment, rather than real differences between the receptors themselves.

(5) Selectivity between smooth and cardiac muscle is probably related to the differing requirements of the site which interacts with the lipophilic side chain in the two types of tissue, not to the interaction of the tertiary nitrogen.

(6) Smooth muscle selectivity can be enhanced by increasing the lipophilicity of groups R^1 and R^2 and with R^3 = H. This implies, however, a decrease in the potency of these derivatives for all tissues.

(7) Finally, no increase of potency or selectivity was observed for the optical isomers of **5a** and **5b**, whereas the introduction of a methyl group at the 1′-position of the C-5 chain in the furan ring reduced potency and selectivity.

In conclusion, in the attempt to improve the selectivity for smooth toward cardiac muscle of the lead **5a**, the sequential modification of the substituents on the lipophilic side chain and tertiary nitrogen has led to the discovery of ileum selective antimuscarinic agents. This result is particularly intriguing in view of the fact that the pharmacological profile of compounds **5b,c,j** is the best example of selectivity between ileum and bladder in the literature. A recent report has identified marked bladder selectivity for some propanone derivatives, though only *in vivo*.[33] Taken together, these data should prove useful in drawing attention to the functional importance of different environments or different accessory binding areas of receptors in different tissues.[34]

Experimental Protocols

Chemistry. Materials and Methods. Reaction courses and product mixture were routinely monitored by thin-layer chromatography (TLC) on silica gel precoated F_{254} Merck plates. Infrared spectra (IR) were measured on a Perkin-Elmer 257 instrument. Nuclear magnetic resonance (^{1}H-NMR) spectra were obtained in $CDCl_3$ or $CDCl_3$–DMSO-d_6 with Bruker WP 80 and AC 200 spectrometers, and peak positions are given in parts per million (δ), downfield from tetramethylsilane as the internal standard. Melting points were obtained in open capillary tubes and are uncorrected. Purification by ball tube distillation were performed on a Buchi GKR-50 instrument. Column chromatographies were performed with Merck 60–200 mesh silica gel. All final compounds were submitted to the pharmacological test as monooxalic acid salts. All the compounds reported showed IR and ^{1}H NMR spectra in agreement with their assigned structures. Ambient temperature was 24–27 °C. Microanalyses, unless indicated, were in agreement with calculated values within ±0.4%.

Starting Materials. Optically acitve (phenylcyclohexyl)-acetic acids **3b**-(+) and **3b**-(−) were prepared as reported.[1] α,α-Diphenylhydroxyacetic acid (**6a**) was commercially available, while α-phenyl-α-cyclohexyl- and -α-cyclopentylhydroxyacetic acids (**6b,c**) were prepared from **3b,c** by the procedure described by Adam and Cueto.[7] The residue solids were crystallized from hexane after the workup: **6b** mp 156–158 °C (lit.[6] mp 161–162 °C), yield 90%; **6c** mp 150–151 °C, yield 85%. The system was very sensitive to reaction conditions and required particular attention, in the presence of excess *n*-BuLi (equivalent ratio 0.9:1), to the exposure of the base to the bubbling oxygen. The methyl ester **4b** was prepared by the standard procedure heating at reflux condition a solution of **3b** in benzene in the presence of methanol and a catalytic amount of 4-toluenesulfonic acid (*p*-TsOH). Methyl glycolates **7a–c** were prepared by adding a slight excess of ethereal diazomethane to an ether solution of the acid and then evaporating the solution until it dried.[5] The crude products were used without additional purification. α,α-Dicyclohexylhydroxyacetic acid methyl ester (**7d**) was prepared by hydrogenation in AcOH of **7b** over PtO_2, at 50 psi for 12 h, in a Parr apparatus. After the usual workup and concentration in vacuum, the crude **7d** was used without any further purification in the next step.

Preparation of 5-(Hydroxymethyl)-2-*N*,*N*-dialkylfurfurylamine Derivatives 2b–m. Derivatives **2b–m** were prepared, by the procedure reported for **2a**,[1] heating a mixture of the appropriate amine, furfuryl alcohol and 40% formaldehyde/H_2O in anhydrous acetic acid at 100 °C for 3 h.[34] After workup, the crude oils were purified by column chromatography (EtOAc/MeOH). **2b**: oil, yield 65%. **2c**: oil, yield 72%. **2d**: oil, yield 85%. **2e**: oil, yield 56%. **2f**:[4] oil, yield 52%. **2g**: oil, yield 47%. **2h**: oil, yield 78%. **2i**: oil, yield 70%. **2j**: oil, yield 63%. **2k**: mp 112–113 °C, yield 74%.[35] **2l**: oil, yield 75%.[35] **2m**: oil, yield 30%. A sample of pure material gave analytical data consistent with the assigned structures. Anal. C, H, N. **2g** C: calcd, 62.54; found, 63.02. **2m** C: calcd, 66.27; found, 66.69; N: calcd, 7.73; found, 8.28.

General Procedure for the Preparation of (±)-*N*-[5-[(1′-Substituted-acetoxy)methyl]-2-furfuryl]dialkylamines 5b–f,h–j,n–q. Compounds **5b–f,h–j,n–q** were prepared by the procedure described for **5a**,[1] reacting the appropriate acetic acid derivatives **3a,e,f** with the corresponding amino alcohols **2b–f,m–j**. After workup and evaporation in vacuum, the residue oil was purified by column chromatography on silica gel (EtOAc/MeOH) and converted into the corresponding oxalates (Table 6). The spectroscopic data of compound **5b** are reported as an example. **5b**: IR (film, cm^{-1}) 3100–2740, 1740, 1640, 1600, 1550; ^{1}H-NMR ($CDCl_3$) δ 7.40–7.20 (m, 5 H), 6.25 (d, 1 H, J = 3 Hz), 6.13 (d, 1 H, J = 3 Hz), 5.10 (d, 1 H, J = 13 Hz), 4.93 (d, 1 H, J = 13 Hz), 3.50 (s, 2 H), 3.23 (d, 1 H, J = 10 Hz), 2.4, 0 (s, 3 H), 2.35–1.00 (m, 22 H).

General Procedure for the Preparation of (±)-*N*-[5-[(1′-substituted- and 1′-hydroxy-1′-substituted-acetoxy)-methyl]-2-furfuryl]dialkylamines 5g, 8a,b,e,g,k–m,q–t, and 11. To a solution of amino alcohol (**2a,c,f,h–m** and **10**) (3.4 mmol) and the equimolar amount of the appropriate ester (**4b** and **7a–d**) in anhydrous cyclohexane (100 mL) was added sodium methoxide (0.1 g, 1.8 mmol). The resulting mixture was heated at reflux for 24 h and then cooled, diluted with EtOAc (100 mL), and washed with water (3 × 100 mL). The organic phase was dried over anhydrous Na_2SO_4. After removal of the solvent, the residue oil was purified by column chromatography on silica gel (EtOAc/MeOH) (Table 6).

Compounds **5g**, **8d,l,m**, and **11** were recoverd in low yields, due to the formation of mixtures of products. The spectroscopic data of compound **8a** and **11** are reported as examples.

8a: oil; yield 65%; IR (film, cm^{-1}) 3500, 3000–2800, 1740; ^{1}H-NMR ($CDCl_3$) δ 7.80–7.60 (m, 2 H), 7.40–7.20 (m, 3 H), 6.31 (d, 1 H, J = 3 Hz), 6.15 (d, 1 H, J = 3 Hz), 5.20 (d, 1 H, J = 13 Hz), 5.00 (d, 1 H, J = 13 Hz), 3.67 (s, 1 H), 3.57 (s, 2 H), 2.25 (s, 6 H), 2.00–1.10 (m, 11 H).

11: oil; yield 50%; IR (film, cm^{-1}) 3500, 3000–2800, 1740; ^{1}H-NMR ($CDCl_3$) δ 7.80–7.60 (m, 2 H), 7.40–7.20 (m, 3 H),

Table 6. Physical Data for Compounds **5b-j,n-q**, **8a,b,e,k-m,q-t**, and **11**

compd	method	mp, °C (oxalate)[b]	formula	yield, %[a]
5b[c]	A	148	$C_{27}H_{37}NO_3 \cdot H_2C_2O_4$	72
5c	A	172–174	$C_{25}H_{33}NO_3 \cdot H_2C_2O_4$	79
5d	A	135–136	$C_{28}H_{33}NO_3 \cdot H_2C_2O_4$	71
5e	A	119–121	$C_{24}H_{33}NO_3 \cdot H_2C_2O_4$	64
5f	A	151–152	$C_{24}H_{31}NO_4 \cdot H_2C_2O_4$	90
5g	B	155–157	$C_{25}H_{33}NO_4 \cdot H_2C_2O_4$	50
5h	A	123–125	$C_{24}H_{33}NO_3 \cdot H_2C_2O_4$	76
5i	A	115–119	$C_{24}H_{33}NO_3 \cdot H_2C_2O_4$	70
5j	A	175–178	$C_{26}H_{37}NO_3 \cdot H_2C_2O_4$	65
5n	A	158–160	$C_{25}H_{27}NO_3 \cdot H_2C_2O_4$	70
5o	A	139–141	$C_{22}H_{21}NO_3 \cdot H_2C_2O_4$	55
5p	A	153–155	$C_{22}H_{21}NO_4 \cdot H_2C_2O_4$	58
5q	A	120–123	$C_{22}H_{23}NO_3 \cdot H_2C_2O_4$	75
8a	B	151–153	$C_{22}H_{29}NO_4 \cdot H_2C_2O_4$	65
8b	B	145–147	$C_{27}H_{37}NO_4 \cdot H_2C_2O_4$	65
8e	B	130–132	$C_{24}H_{33}NO_4 \cdot H_2C_2O_4$	60
8g	B	165	$C_{25}H_{33}NO_5 \cdot H_2C_2O_4$	45
8k	B	>200	$C_{24}H_{32}N_2O_4 \cdot H_2C_2O_4$	70
8l	B	99–100	$C_{25}H_{34}N_2O_4 \cdot H_2C_2O_4$	50
8m	B	95–96	$C_{24}H_{31}NO_4 \cdot H_2C_2O_4$	50
8q	B	110–111	$C_{22}H_{23}NO_4 \cdot H_2C_2O_4$	75
8r	B	91–92	$C_{23}H_{31}NO_4 \cdot H_2C_2O_4$	62
8s	B	80–82	$C_{21}H_{27}NO_4 \cdot H_2C_2O_4$	60
8t	B	126–127	$C_{22}H_{35}NO_4 \cdot H_2C_2O_4$	60
11	B	85–88	$C_{23}H_{31}NO_4 \cdot H_2C_2O_4$	50

[a] Oils. [b] Ethanol. [c] (−)-**5b** (oxalate): α^{20}_D −2.98 (*c* 4, ethanol); ee > 98; (+)-**5b** (oxalate): α^{20}_D +3.00 (*c* 4, ethanol); ee > 98.

6.31 (d, 1 H, J = 3 Hz), 6.15 (d, 1 H, J = 3 Hz), 5.89 (q, 1 H, J = 7.5 Hz), 3.67 (s, 1 H), 3.57 (s, 2 H), 2.25 (s, 6 H), 2.00–0.90 (m, 14 H).

Preparation of 5-Formyl-2-*N*,*N*-dimethylfurfurylamine 9.[9] To a solution of **1a**[1] (1.55 g, 10 mmol) in CH_2Cl_2 was added manganese(IV) oxide (8.69 g, 100 mmol), and the suspension was stirred overnight at ambient temperature. The mixture was then filtered, and the residue cake was washed several times with CH_2Cl_2. The filtrate was concentrated under reduced pressure, and the oily residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 9/1). Evaporation of appropriate fractions gave 1.25 g of a pale yellow oil (yield 82%): IR (film, cm^{-1}) 3000, 1720, 1600; ^{1}H-NMR ($CDCl_3$) δ 9.72 (s, 1 H), 7.10 (d, 1 H, J = 2.2 Hz), 6.15 (d, 1 H, J = 2.2 Hz), 3.57 (s, 2 H), 2.25 (s, 6 H). Anal. ($C_8H_{11}NO_2$) C, H, N.

Preparation of (±)-5-(1′-Hydroxyethyl)-2-*N*,*N*-dimethylfurfurylamine (10).[8] Compound **9** (2.5 g, 13.34 mmol) in Et_2O (5 mL) was added to methylmagnesium iodide (24.5 mmol from 0.6 g of magnesium turnings and 3.46 g, 1.52 mL, of methyl iodide) in Et_2O (50 mL) at 0 °C with vigorous stirring. After complete addition, the mixture was left to stir at ambient temperature overnight and then carefully neutralized with NH_4Cl/H_2O (sat.). The product was extracted with Et_2O (5 × 50 mL), washed with water (3 × 50 mL), dried over anhydrous Na_2SO_4, and evaporated. The residue oil was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 9/1): yield 65%; IR (film, cm^{-1}) 3100–2800, 1600, 1560; ^{1}H-NMR ($CDCl_3$) δ 6.35 (d, 1 H, J = 3 Hz), 6.15 (d, 1 H, J = 3 Hz), 5.92 (q, 1 H, J = 7.5 Hz), 4.05 (s, 1 H), 3.57 (s, 2 H), 2.25 (s, 6 H), 1.82 (d, 1 H, J = 7.5 Hz). Anal. ($C_9H_{15}NO_2$) C, H, N.

Biological. Materials. Male Wistar Morini rats (170–220 g) and male guinea pigs (500–600 g) of a local strain (Bettinardi, Dunkin-Hartley) were used. Carbachol chloride and atropine sulfate were obtained from Sigma (St. Louis, MO). Furtrethonium was synthesized in the Glaxo S.p.A Research Laboratories (Verona, Italy). All test drug solutions were freshly prepared prior to each experiment.

Tissue Preparations. For the experiments, rats and guinea pigs were fasted overnight and killed by cervical dislocation. The ileum and urinary bladder were quickly removed from rats and the atria from guinea pigs. Samples prepared for the study were longitudinal segments of ileum about 2 cm in length, strips of extratrigonal portion of urinary detrusor muscle 1 cm long and 2 mm wide, and both atria. These tissues were placed in a 10 or 20 mL organ bath containing Krebs-Henseleit solution (composition in mmol/L: NaCl, 118; KCl, 5.6; $CaCl_2$, 2.5; $MgSO_4$, 1.19; NaH_2PO_4, 1.3; $NaHCO_3$, 25; and glucose, 10), gassed with 5% CO_2 in O_2 (pH 7.4), and heated to 37 °C (ileum and detrusor strips) or to 29 °C (atria).

Experimental Protocol. The preparations were left to equilibrate for 30–45 min, during which time the bathing solution was changed every 10–15 min. Contractile responses of ileum and detrusor strips were recorded isotonically on a LNI recorder through a Basile transducer, the resting tension being 0.5 and 1 g, respectively. Atrial force was recorded with a Statham force transducer connected to a Battaglia-Rangoni polygraphy, resting tension being 1 g. Furtrethonium (ileum and bladder) and carbachol (atria) were used as agonists, and cumulative administration was used. Agonist concentrations were added to the organic bath in 0.5 (ileum and bladder) or 0.25 (atria) unit multiple increments. Increasing concentrations were added after attainment of a steady-state response to the previous concentration. The concentrations were increased until the maximum response was achieved.

Two or, exceptionally, three cumulative concentration–response curves for appropriate reference agonists were constructed. Once two consecutive similar curves were obtained, a final cumulative concentration response curve was repeated after the tissues had been allowed to equilibrate for at least 30 min with the compound under examination.

Curve Fitting Method. Data reported in Table 4 were analyzed with the statistical package RS-1 (BBN Soft. Prod. Corp., Cambridge); multiple regression analysis was performed with the least squares method.

Analysis of Data. Negative inotropic responses to carbachol were expressed as a percentage of the basal twitch contraction, while contractile responses in ileum and bladder to furtrethonium were expressed as a percentage of their own maximum response. The concentration response curve data were analyzed by a four-parameter logistic equation[36]

$$\text{response} = [a + E_{max}[A]^n]/[[A]^n + [E_{50}]^n]$$

where a and E_{max} are the minimum and maximal asymptotes, respectively, [A] the agonist concentration, n the slope factor, and [E_{50}] the concentration of the agonist that induces 50% of the maximal effect. Concentration–response curves in the presence of antagonists were tested for significant differences in slope functions and maximal responses in comparison with the control curves by the above reported equation. In the absence of significant difference, the analysis of antagonist effects was performed. To quantify antagonist potency pK_b values were calculated from the equation

$$pK_b = \log (DR - 1) - \log [B]$$

where DR was the ratio of agonist E_{50} values in the presence and absence of the antagonist concentration [B]. Values of pK_b were given as mean ± standard error. For compounds where three different concentrations were tested in the ileum and bladder (**8a**,**b**,**r**,**l**) and atria (**8a**,**r**), the Schild regression analysis was performed. Since the Schild[37] plot slope was not significantly different from the unit, pK_b values and 95% confidence limits could be estimated.

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Supplementary Material Available: Spectroscopic data (IR and ^{1}H-NMR) of compounds **5c–j,n–q** and **8b,e,g,k–m,q–t** (3 pages). Ordering information is given on any current masthead page.

References

(1) Manfredini, S.; Guarneri, M.; Simoni, D.; Barbieri, A.; Grana, E.; Zonta, F.; Feriani, A.; Gaviraghi, G.; Toson, G. Cholinergic Agents Structurally Related to Furtrethonium. 1. *Eur. J. Med. Chem.* **1994**, *29*, 153–161. Improved ee of (−)-**5a** were now achieved (α^{20}_D −1.98, *c* 2 ethanol/chloroform, 1/1, ee > 98%) *via* activation of the (−)-phenylcyclohexylacetic acid as hydrosuccinimidester.

(2) Hulme, E. C.; Birdsall, N. J. M.; Buckley, N. J. Muscarinic receptor subtypes. *Annu. Rev. Pharmacol. Toxicol.* **1990**, *30*, 633–673.

(3) Nomenclature Committee. Muscarinic acetylcholine receptors. *Trends Pharmacol. Sci. (Suppl.)* **1993**, 25.

(4) Holdren, R. F. Reaction of some furan derivatives with formaldehyde and amine hydrochlorides. *J. Am. Chem. Soc.* **1947**, *69*, 464–465.

(5) (a) Pfister, J. R.; Wymann, W. E.; Weissberg, R. M.; Strosberg, A. M. Synthesis and broncodilation activity of endo-2-2-cyclopentyl-2-hydroxy-2-phenylacetoxy-7-methyl-7-azabicyclo-2.2.1-heptane methobromide a potent and long-acting anticholinergic agent. *J. Pharm. Sci.* **1985**, *74*, 208–210. (b) Atkinson, E. R.; McRitchie, D. D.; Shoer, L. F.; Harris, L. S.; Archer, S.; Aceto, M. D.; Pearl, J.; Luduena, F. P. Parasympatholytic anticholinergic esters of the isomeric 2 tropanols. Part 1 glycolates. *J. Med. Chem.* **1977**, *20*, 1612–1617.

(6) Hoffmann, K.; Schellenberg, H. Preparation of basic esters. *Helv. Chim. Acta* **1947**, *30*, 292–295.

(7) (a) Adam, W.; Cueto, O. Cyclic peroxide. 44. A convenient and efficient preparation of aromatic alpha-hydroperoxy acids via oxygenation of alpha-lithio enolates, prepared by direct alpha-lithiation of arylacetic acids. *J. Org. Chem.* **1977**, *42*, 38–40. (b) Moersch, G. W.; Zwiesler, M. L. Synthesis of alpha-hydroxycarboxylic acids by aeration of lithiated carboxylic acids in tetrahydrofuran solution. *Synthesis* **1971**, 647–648.

(8) Vereshchagin, L. I.; Katkevich, R. I.; Korshunov, S. P. Aminomethylation of some furan derivatives. *Khim. Geterotsikl. Soedin.* **1967**, *6*, 990–992; *Chem. Abstr.* **1968**, *69*, 59003f. Compound **10** was prepared by these authors by aminomethylation of 1′-methylfuryl carbinol.

(9) Vereshchagin, L. I.; Katkevich, R. I.; Korshunov, S. P. Synthesis of Dialkylaminomethyl Furfurals. *Khim. Geterotsikl. Soedin.* **1967**, *1*, 12–13; *Chem. Abstr.* **1967**, *67*, 64140m.

(10) Antimuscarinic activity of compounds (+)- and (−)-**5a,b**: (+)-**5a** rat ileum (pK_b = 7.2 ± 0.3), rat bladder (pK_b = 7.1 ± 0.3) and guinea pig atria (pK_b = 5.7 ± 0.1); (−)-**5a** rat ileum (pK_b = 7.3 ± 0.3), rat bladder (pK_b = 7.2 ± 0.2) and guinea pig atria (pK_b = 5.9 ± 0.1); (+)-**5b** rat ileum (pK_b = 6.3 ± 0.1), rat bladder (pK_b = 4.5 ± 0.3) and guinea pig atria (pK_b < 5); (−)-**5b** rat ileum (pK_b = 6.5 ± 0.2), rat bladder (pK_b = 5 ± 0.4) and guinea pig atria (pK_b < 5).

(11) (a) De Amici, M.; Dallanoce, C.; De Micheli, C.; Grana, E.; Barbieri, A.; Landinsky, H.; Schiavi, G. B.; Zonta, F. Synthesis and Pharmacological Investigation of the Enantiomers of Muscarone and Allomuscarone. *J. Med. Chem.* **1992**, *35*, 1915–1920. (b) Dahlbom, R. Stereoselectivity of Cholinergic and Anticholinergic Agents. *In Stereochemistry and Biological Activity of Drugs*; Ariens, E. J., Ed.; Blackwell Scientific: Oxford, 1983; pp 127–142.

(12) Hansch, C.; Fujita, T. Rho-sigma-p Analysis: A Method for the Correlation of Biological Activity and Chemical Structure. *J. Am. Chem. Soc.* **1964**, *86*, 1616–1626.

(13) Hansch, C. On the Structure of Medicinal Chemistry. *J. Med. Chem.* **1976**, *19*, 1–6.

(14) Hansch, C.; Leo, A. J. *Substituent constants for correlation analysis in chemistry and biology*; Wiley Interscience: New York, 1979.

(15) Bowden, K.; Young, R. C. Structure-Activity Relations I. A Series of Antagonists of Acetylcholine and Histamine at the postganglionic Receptors. *J. Med. Chem.* **1970**, *13*, 225–230.

(16) Cappello, B.; Silipo, C.; Vittoria, A. Quantitative Structure – Activity Relationships in a Set of Antimuscarinic Agents. *Il Farmaco Ed. Sci.* **1984**, *39*, 991–1007.

(17) Banerjee, S.; Lien, E. J. Quantitative Correlations and Reexamination of the Importance of Hydrophobic and Steric Factors in Anticholinergic drug Receptor Interactions. *Pharm. Res.* **1990**, *7*, 746–750.

(18) Caliendo, G.; Perissutti, E.; Santagata, V.; Silipo, G.; Vittoria, A.; Di Carlo, R.; Meli, R.; Muccioli, G. Application of QSAR Strategies in the Design of Antimuscarinic Benzotriazole Derivatives. In *QSAR: Rational Approach to the Design of Bioactive Compounds*; Silipo, C., Vittoria, A., Eds.; Elsevier: Amsterdam, 1991; pp 439–442.

(19) Pratesi, P.; Caliendo, G.; Silipo, C.; Vittoria, A. A QSAR Approach to the Study of Structural Requirements of Muscarinic Receptor Ligands – Part II: Antagonists. *QSAR* **1992**, *11* (2), 151–161.

(20) Medicinal Chemistry Project, Pomona College, CLOGP – 3.54.

(21) Taft, R. W. *Steric Effects in Organic Chemistry*; Wiley Interscience: New York, 1956; pp 556–675.

(22) Medicinal Chemistry Project, Pomona College, CMR – 3.54.

(23) Lien, E. J.; Ariens, E. J.; Beld, A. J. Quantitative Correlation between Chemical Structure and Affinity on Acetylcholine Receptors. *Eur. J. Pharmacol.* **1976**, *35*, 245–250.

(24) Pratesi, P.; Villa, L.; Ferri, V.; Grana, E.; Sossi, D. Molecular Properties and Anti-Muscarinic Activity in Some Series of Quaternary Ammonium Salts. *Il Farmaco Ed. Sci.* **1969**, *24*, 313–328.

(25) Pratesi, P.; Villa, L.; Grana, E. Proprieta' molecolari e attivita' anticolinergica in composti a funzione amminica terziaria e ammonica quaternaria. (Molecular properties and anti-muscarinic activity in compounds bearing a tertiary and quaternary aminic functions.) *Il Farmaco Ed. Sci.* **1963**, *18*, 3–19.

(26) Pullman, B.; Courrère, P. H.; Coubeils, J. L. Quantum Mechanical Study of the Conformational and Electronic Properties of Acetylcholine and its Agonists Muscarine and Nicotine. *Mol. Pharmacol.* **1971**, *7*, 397–405.

(27) Ehlert, F.J.; Jenden, D. J. Comparison of the Muscarinic Receptor Binding Activity of Some Tertiary Amines and Their Quaternary Ammonium Analogues. *Mol. Pharmacol.* **1984**, *25*, 46–50.

(28) Ariens, E. J.; Simonis, A. M.; Van Rossum, J. M. The Relations Between Stimulus and Effect. In *Molecular Pharmacology*; Ariens, E. J., Eds.; Academic Press: New York, 1964; Vol. 1, pp 394–462.

(29) Brittain, R. T.; Dean, C. M.; Jack, D. Sympathomimetic Bronchodilator Drugs. *Pharm. Ther. B.* **1976**, *2*, 423–462.

(30) Wilson, G.; Fox, C. F. Biogenesis of Microbial Transport System: Evidence for Coupled Incorporation of Newly Synthesized Lipids and Proteins into Membrane. *J. Mol. Biol.* **1971**, *55*, 49–60.

(31) Kenakin, T. Drugs and Receptors: An Overview of the Current State of Knowledge. *Drugs* **1990**, *40*, 666–687.

(32) Frye, L. D.; Edidin, M. The Rapid Intermixing of Cell Surface After Formation of Mouse-Human Heterokaryons. *J. Cell. Sci.* **1970**, *7*, 319–335.

(33) Kaiser, C.; Audia, V. H.; Carter, J. P.; McPherson, D. W.; Waid, P. P.; Lowe, V.; Noronha-Blob, L. Synthesis and Antimuscarinic activity of some 1-cycloalkyl-1-hydroxy-1-phenyl-3-(4-substituted piperazinyl)-2-propanones and related compounds. *J. Med. Chem.* **1993**, *36*, 610–616.

(34) Ariens, E. J.; Rodriguez De Miranda, J. F. The receptor concept: recent experimental and theoretical developments. In *Recent Advances in Receptor Chemistry*; Gualtieri, M., Giannella, A., Melchiorre, C., Eds.; Elsevier: Amsterdam, 1979; pp 1–36.

(35) For the preparation of compounds **2k,l**, prior protection of piperazine N-4 was required in order to avoid side reaction. The protection was achieved by reaction in standard conditions with ethylchloroformate. After reaction with furfuryl alcohol, the final **2k,l** were obtained by deprotection with methanolic potassium hydroxyde at reflux conditions for 3 h.

(36) De Lean, A.; Munson, P. J.; Rodbard, D. Simultaneous analysis of families of sigmoidal curves: application yo bioassay, radioligand assay, and physiological dose-response curves. *Am. J. Physiol.* **1988**, *235*, 97–102.

(37) Arunlakshana, O.; Shild, H. O. Some quantitative uses of drug antagonists. *Br. J. Pharmacol. Chermother.* **1959**, *14*, 48–58.