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Synthesis, NMR Spectroscopy Study, and Antimuscarinic Activity of a Series of 2-(Acyloxymethyl)-1,3-dioxolanes

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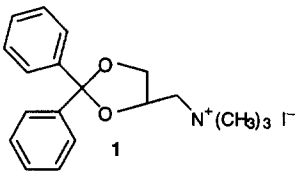
Abstract—A series of 1,3-dioxolane-based ligands, bearing hydroxymethyl or ester functionalities, was synthesized and tested as potential muscarinic antagonists. The compounds display moderate to low affinity for the three receptor subtypes M_1–M_3, with some of them showing a significant selectivity for the M_3 subtype. The configurational and conformational properties were studied using NOE experiments and vicinal coupling constants. The ^1H and ^{13}C NMR chemical shifts show stereochemically dependent trends. Quantitative analysis of conformer populations showed that 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. Copyright © 1996 Elsevier Science Ltd

Introduction

Muscarinic acetylcholine receptors are pharmacologically classified as M_1, M_2, and M_3 subtypes, depending upon the action of selective antagonists.[1,2] This has led to renewed interest in the search for selective ligands, which interfere specifically with one or another of the subtypes, that would represent potential therapeutic agents. Since muscarinic receptor subtypes are variously involved in secretory and cardiovascular functions, smooth muscle control, and in central nervous system transmission, M_2 and M_3 antagonists have been proposed for the treatment of cardiac[3] and gastrointestinal tract[4,5] disorders, respectively. Furthermore, selective lipophilic M_2 antagonists could serve to improve memory and learning in neurodegenerative disorders,[6,7] since there is evidence to support the existence of functional M_2 autoreceptors in the brain.[8] A potential role as selective broncodilators has also been proposed for M_3 or combined M_2 and M_3 antagonists.[9]

Molecular cloning studies have shown that muscarinic receptors consist of five molecular forms (m_1–m_5)[10,11] and that three of them (m_1–m_3) correspond to the pharmacologically defined M_1–M_3 subtypes. The pharmacological characterization of these receptors is thus still incomplete, most likely owing to the lack of an appropriate selective ligand. Hence the urgent need to discover selective antagonists that would make for a more definitive classification and represent potential therapeutic agents.



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With this in mind we have undertook a research project aimed at developing selective antagonists, taking as a lead compound **1**, which has been reported to be a very potent muscarinic antagonist[12–14] but lacking in significant selectivity.[15] We focused our attention on position 2 and while keeping one of the two phenyl rings constant, replaced the other with different functionality that could cause a discriminative interaction with the receptor subtypes. Here we report on the synthesis, structural determination, and pharmacological evaluation of a series of 1,3-dioxolane with an hydroxymethyl group and related esters of varying degrees of bulkiness in position 2.

Chemistry

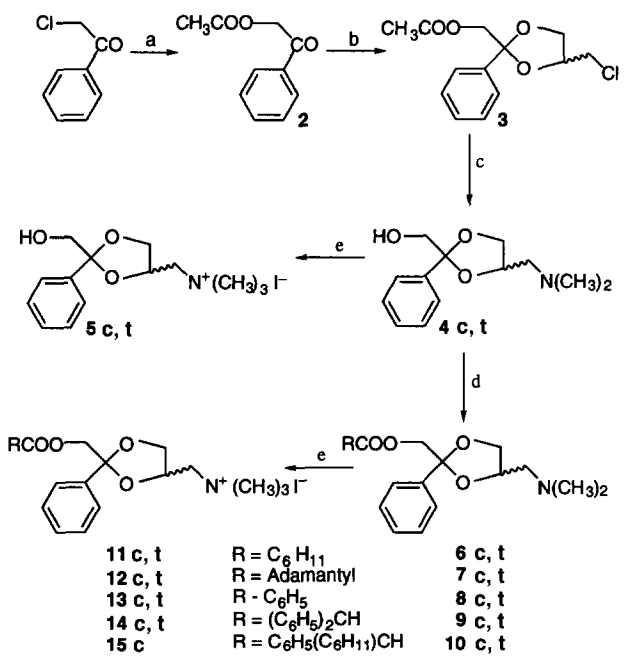
The syntheses of the compounds used in the present study are reported in Schemes 1 and 2. The tertiary amines **6–10**, **23**, **24c,t** were prepared by esterification of the hydroxyamines **4c,t**, **21c,t**, and **22** with the appropriate acylchlorides in dry pyridine.[16] The hydroxyamines were prepared in turn by reacting the chloroesters **3**, **19**, and **20** with an excess of $NH(CH_3)_2$

at 100 °C. Under these conditions both chlorine atom substitution and hydrolysis of ester function take place.[17] The hydroxyamines were obtained as a mixture of diastereoisomers, which are separated by flash chromatography with the exception of **22**. In the latter case, diastereoisomer separation was accomplished after the following esterification step. The chloroester **3** was prepared starting from chloroacetophenone which was reacted with CH_3COOK[18] in dimethylformamide (DMF) in the presence of catalytic amounts of the phase-transfer catalyst dicyclohexyl-18-crown-6, to give **2**, which was converted into **3** by ketalization with 3-chloro-1,2-propanediol in dry CH_3NO_2 and a catalytic amount of trifluoromethanesulfonic acid.[19]

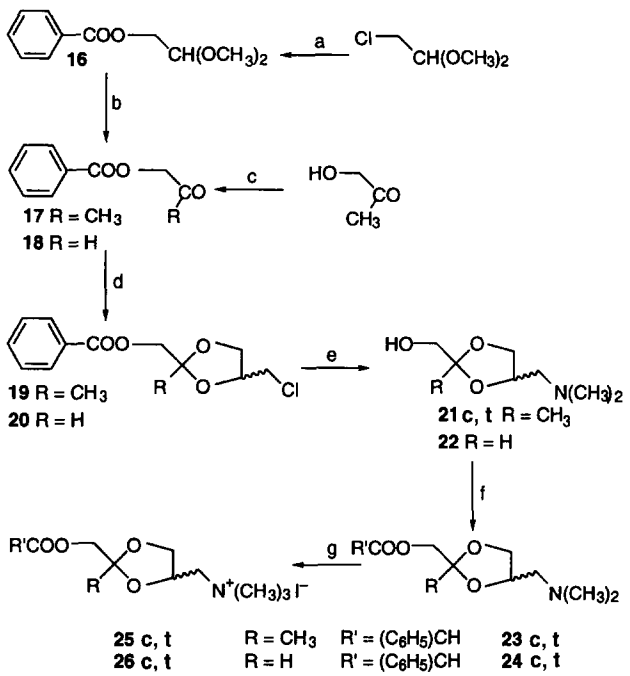
The chloroester **19** was prepared from hydroxyacetone that was esterified with benzoylchloride, followed by ketalization with 3-chloro-1,2-propanediol in dry toluene with *p*-toluensulfonic acid as the catalyst.[20]

The chloroester **20** was prepared from chloroacetaldehyde dimethyl acetal that was converted into **16** by treatment with C_6H_5COOK in DMF with dicyclohexyl-18-crown-6 as the catalyst. The intermediate **16** was then hydrolyzed (2 N H_2SO_4) to the aldehyde **18**, which was cyclized with the same condition used to prepare **19**.

The tertiary amines **4c,t**, **6–9c,t**, **10c**, **23**, and **24c,t** were transformed into the quaternary salts **5c,t**, **11–14c-t**, **15c**, **25**, and **26c,t** with methyl iodide.



Scheme 1. (a) CH_3COOK, DMF, dicyclohexyl 18-crown ether-6, reflux; (b) an. CH_3NO_2, 3-chloro-1,2-propandiol, triflic acid, molecular sieve 4 Å, reflux; (c) $HN(CH_3)_2$, 100 °C; (d) RCOCl, an. pyridine, 0 °C to rt; (e) CH_3I, an. $(C_2H_5)_2O$. When the compound number is followed by **c** (*cis*) and **t** (*trans*), the two isomers have been separated.



Scheme 2. (a) C_6H_5COOK, DMF, dicyclohexyl 18-crown ether-6, reflux; (b) THF, H_2SO_4, reflux; (c) C_6H_5COCl, an. pyridine, −5 °C to ≥rt; (e) $C_6H_5CH_3$, 3-chloro-1,2-propandiol, *p*-toluensulfonic acid, reflux; (f) R'COCl, an. pyridine, 0 °C to ≥rt; (g) CH_3I, $(C_2H_5)_2O$. When the compound number is followed by **c** (*cis*) and **t** (*trans*), the two isomers have been separated.

Results and Discussion

Spectroscopic study

The ^1H chemical shifts and ^nJ(H, H) values of compounds **5c,t**, **11–14c,t**, **15c**, **25**, and **26c,t** are reported in Table 1. The configurational assignment of compounds **5c,t** has already been reported[21] and the shielding effect of the 2-phenyl group on H-4, H-5a, and H-5b has been pointed out. The configurations of compounds **11–14c,t**, **15c**, and **25c,t** are known from those of the corresponding hydroxyamines **4c,t**, which are also precursors of compounds **5c,t** and **21c,t**, the configuration of which has also been reported as methyl iodide salts.

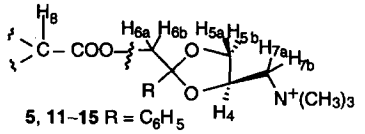
The configurational assignment of the diastereoisomeric pairs of derivatives **26c,t** was obtained by 1-D Nuclear Overhauser Effect (1-D NOE)-difference experiments.[22] Figure 1a displays the 1-D NOE difference spectrum of **26c**, obtained by irradiation of H-4 at 4.64 ppm: a positive NOE at H-2 (5.25 ppm) and at H-5b (4.13 ppm) protons, which are in a *cis* relationship with H-4, is evident. The *gauche* relationship between H-4 and H-7a (3.43 ppm) is also confirmed. In the 1-D NOE difference spectrum of **26t** (Fig. 1b) the only positive NOEs (upon irradiation of H-4 at 4.47 ppm) are at H-5b (4.17 ppm) and at H-7a (3.39 ppm).

The ^1H chemical shifts of compounds **11–25** parallel those previously reported for some closely related 1,3-dioxolanes[21] and display some common trends. The H-5a is always shielded with respect to its geminal partner owing to the shielding effect of the vicinal

ammonium group. The difference $\Delta\delta = \delta$H-5a$-\delta$H-5b, when a 2-phenyl group is present, is greater in the *trans* than in the *cis* derivative, reflecting the remote shielding effect of the aromatic substituent on the protons in a *cis* relationship with it. This effect is also seen, albeit to a lesser extent, on H-4, which is shielded in the *cis* with respect to the *trans* diastereoisomer. Nevertheless, whereas H-4, H-5a, and H5b of compounds **5c,t** and **11–13c,t** show very similar chemical shifts, the three protons of compounds **14c,t** are shielded with respect to them. This shielding is more effective on H-5a in the *cis* form, and on H-4 and H-5b in the *trans* form. This observation suggests a predominant long-distance shielding effect of the bulky diphenylmethine ester group and, probably, a change in the 2-phenyl group conformation. These two effects also seem to be operative on the methylene ammonium methyl protons, which are identified as H-7a and H-7b on the basis of their coupling constant with H-4: lower for H-7a which is *gauche*, and higher for H-7b which is in an *anti* relationship with respect to H-4. The conformational behavior of this group is similar to that already described.[21] As can be observed from Table 1, the presence of an ester group linked to C-6 (derivatives **11–13**) deshields H-7a by about 0.1 ppm, shields H-7b by about 0.3 ppm in the *cis* forms with respect to the derivative **5c**, and leaves these two protons almost unaffected in the *trans* forms. In the case of the derivative **14c**, a strong shielding effect on both H-7s is present, especially on H-7b. Since this proton is approximately direct towards the centre of the dioxolane ring, the shielding must be due not only to the ester group (which shields by about 0.3 ppm) but also to the diphenylmethine group. This can also explain the strong shielding of H-4 and H-5b in the *trans* form **14t**. The other lower shielding, on H-4 and H-5b in the *cis* form, on H-5a in the *trans* form and on $N(CH_3)_3$ protons in both diastereoisomers, are probably due to a different conformation of the 2-phenyl group. The ^{1}H chemical shifts of compounds **25** and **26c,t** that do not have a phenyl group on C-2, confirm the remote shielding effect of the diphenylmethine ester on the protons in a *cis* relationship with it.

Conformational preference of the 1,3-dioxolane nucleus can be obtained by exploiting the vicinal H–H coupling constants. The Haasnoot et al. equation[23] utilized in previous work, enables us to confirm the

Table 1. ^{1}H chemical shifts (ppm relative to TMS), and coupling constants, J (Hz), of compounds **5**, **11–15**, **25**, and **26**[a]



5, 11–15 R = C_6H_5
25 R = CH_3
26 R = H

	5c[b]	5t[b]	11c	11t	12c	12t	13c	13t	14c	14t	15c	25c	25t	26c	26t
H-4	4.68	4.95	4.64	4.90	4.62	4.89	4.67	4.93	4.53	4.57	4.53	4.63	4.48	4.64	4.47
H-5a	3.91	3.52	3.88	3.58	3.88	3.58	3.92	3.62	3.66	3.48	3.54	3.50	3.61	3.63	3.55
H-5b	4.09	4.48	4.15	4.48	4.17	4.48	4.19	4.52	4.04	4.31	4.00	4.21	4.16	4.13	4.17
H-6a	3.69	3.68	4.30	4.26	4.29	4.22	4.60	4.57	4.31	4.33	4.24	4.17	4.09	4.24	4.23
H-6b	3.71	3.68	4.42	4.44	4.39	4.43	4.65	4.64	4.59	4.55	4.49	4.30	4.32	4.45	4.32
H-7a	3.58	3.60	3.73	3.62	3.74	3.56	3.70	3.59	3.27	3.52	3.48	3.38	3.46	3.43	3.39
H-7b	3.89	3.30	3.59	3.35	3.59	3.34	3.55	3.36	3.00	3.26	3.11	3.03	3.41	3.05	3.46
$N(CH_3)_3$	3.30	3.29	3.28	3.24	3.28	3.22	3.23	3.21	3.07	3.11	3.18	3.08	3.06	3.09	3.11
H-*ortho* ϕ	7.55	7.55	7.57	7.58	7.58	7.58	7.66	7.65	7.51	7.49	[f]	—	—	—	—
H-*meta* ϕ	7.45	7.45	7.46	7.47	7.47	7.47	7.48	7.48	[f]	[f]	[f]	—	—	—	—
H-*para* ϕ	7.45	7.45	7.46	7.47	7.47	7.47	7.48	7.48	[f]	[f]	[f]	—	—	—	—
H-8	—	—	—	—	—	—	—	—	5.21	5.19	3.35	5.28	5.24	5.26	5.22
H-2	—	—	—	—	—	—	—	—	—	—	—	—	—	5.25	5.34
2-CH_3	—	—	—	—	—	—	—	—	—	—	—	1.36	1.40	—	—
J(H-4, H-5a)	5.40	8.19	6.07	8.02	6.30	8.04	6.03	8.09	5.78	8.14	5.97	7.40	6.88	5.47	6.63
J(H-4, H-5b)	6.95	6.36	6.97	6.37	6.96	6.38	6.97	6.32	7.08	6.33	7.16	6.50	6.68	6.98	6.42
J(H-4, H-7a)	1.18	1.29	~2[d]	~2[d]	~2[d]	1.35	~2[d]	~2[d]	1.38	1.30	~2	1.27	2.19	1.60	2.13
J(H-4, H-7b)	9.76	9.72	9.68	9.62	9.63	9.62	9.61	9.59	9.96	9.60	9.9d	9.68	9.10	9.99	9.32
J(H-5a, H-5b)	−8.63	−8.45	−8.75	−8.52	−8.71	−8.50	−8.78	−8.52	−8.79	−8.49	−8.70	−8.62	−8.52	−8.78	−8.67
J(H-6a, H-6b)	−12.7	[c]	−11.97	−11.95	−11.84	−11.92	−11.97	−11.91	−12.18	−12.02	−12.03	−11.88	−11.80	−12.27	−12.09
J(H-7a, H-7b)	−13.64	−13.90	−13.76	−14.01	−13.85	−13.98	−13.83	14.02	−13.75	−14.00	−13.9[d]	−13.80	−13.89	−13.84	−14.08
J(H-8, H-1cy[e])	—	—	—	—	—	—	—	—	—	—	10.56	—	—	—	—
J(H-2, H-6a,b)	—	—	—	—	—	—	—	—	—	—	—	—	—	3.51	3.05

[a]Spectra were obtained in CD_3CN.
[b]Data from ref. 17.
[c]A_2 spin system.
[d]Broad signals.
[e]cy = Cyclohexyl.
[f]Overlapped signals.

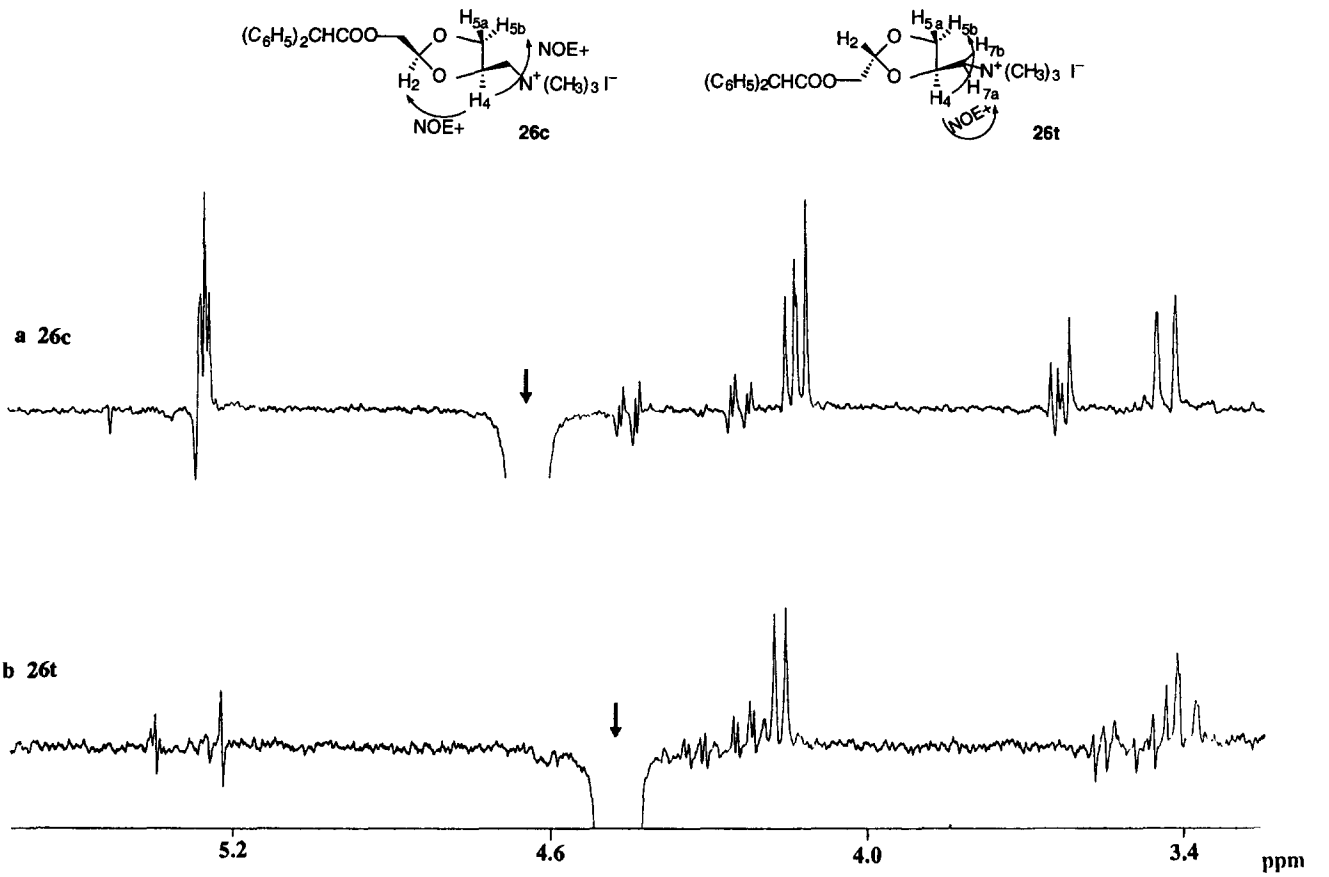
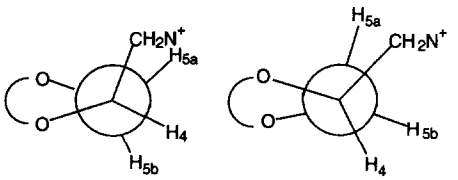


Figure 1. 1-D NOE difference spectra of compounds **26c** (trace a) and **26t** (trace b). The region between 3.3 and 5.5 ppm is shown.

preferred orientation of the exocyclic methylene ammonium group and to describe the ring conformation in the light of two conformers, pseudoaxial and pseudoequatorial. Table 2 reports the molar fraction of the conformer 1 [$CH_2N^+(CH_3)_3$ pseudoaxial]. In the *cis* derivatives, conformer 1 is preferred, whereas in the *trans* derivatives, in which the phenyl group on C-2 forces the 4-exocyclic $CH_2N^+(CH_3)_3$ group to a pseudoequatorial position, conformer 2 [$CH_2N^+(CH_3)_3$ pseudoequatorial] is predominant. Compounds **25** and **26c,t** show a slightly different behavior, owing to the absence of the phenyl ring on C-2.

The ^{13}C chemical shifts, reported in Table 3, were obtained by direct acquisition experiments with proton decoupling, and their assignment, when not trivial, was based on the value of the coupling constant between carbons and the nitrogen atom of the $CH_2N^+(CH_3)_3$ group. In fact, C-5, C-7, and $N(CH_3)_3$ carbons display a $^3J(^{13}C, ^{14}N)$ coupling constant of the order of 1–1.5 Hz and $^1J(^{13}C, ^{14}N)$ coupling constant of the order of 3–4 Hz. The presence of these coupling constants enable us to distinguish between C-5, C-6, and C-7, which fall in a very narrow range. The detection of the $J(^{13}C, ^{14}N)$ coupling constant is due to the presence of the quaternary nitrogen.[24–26] Instead, broadening of the H-4 signal, owing to an unresolved $^3J(^1H, ^{14}N)$ coupling constant, is observed and this behavior is characteristic of all the compounds examined.

Table 2. Molecular fractions of the pseudoaxial conformer calculated by Haasnoot et al.'s equation[23]



Compound	Molecular fraction of the pseudoaxial conformer
5c	0.66
5t	0.33
11c	0.60
11t	0.34
12c	0.56
12t	0.36
13c	0.60
13t	0.32
14c	0.64
14t	0.33
15c	0.63
25c	0.42
25t	0.47
26c	0.50
26t	0.67

The analysis of the ^{13}C chemical shifts enables small but nevertheless significant differences between *cis* and *trans* isomers to be observed in the presence of a phenyl group in the 2-position. C-4 is always shielded (~1 ppm), C-7 deshielded (~0.7 ppm) and C-1′ϕ shielded in the *cis* with respect to the *trans* isomers.

The behavior of compounds **25c,t** and **26c,t** reflects the variation of the substituent on C-2.

Antimuscarinic activity

All the newly synthesized compounds were tested on three different preparations such as rabbit vas deferens, guinea pig heart, and ileum for M_1, M_2, and M_3 antimuscarinic activity, respectively. Compound **1**, was included in the study for comparison. The results reported in Table 4 show that the hydroxymethyl derivatives **5** display lower affinity than those of compound **1**, for the three muscarinic receptor subtypes. The different affinity values between the *cis* and *trans* isomers indicate a certain degree of diastereoselectivity in the binding process. The presence of a hydroxy group in compounds **4c,t** offers the opportunity for preparing a series of ester derivatives with different sizes of the acyl moiety. The first esters to be prepared were the cyclohexylcarboxylates **11c,t**. They have moderate affinity, lower than that of the hydroxymethyl derivatives **5c,t**, and conserve diastereospecificity for M_1 and M_3 subtypes, the *trans* isomer being more active than the *cis* isomer. The introduction of a bulkier group, as in the case of adamantyl derivatives **12c,t**, determines a reduction in affinity and a parallel loss of diastereoselectivity. Replacement of the cyclohexyl group in **11c,t** by a phenyl group to give the benzoates **13c,t** has a different effect on the two isomers. In fact, in the case of the *trans* isomer no variation in affinity is noted, while in that of the *cis* isomer a significant increase for the subtype M_3, with a consequential loss of diastereoisomeric preference, is observed. A further increase in the substituent size, as in the case of compounds **14c,t** and **15c**, results in a complete lost of affinity for M_1 and M_2 receptor subtypes. At the M_3 site, however, compound **15c** is inactive while compounds **14c,t** maintain a moderate affinty, thus showing a significant selectivity for this receptor subtype.

It is worth noting that the diastereoisomeric preference, when present, is always in favor of the *trans* isomer. An attempt to correlate these results with the ones obtained in the conformational preference study, suggests that a pseudo-equatorial orientation of the $CH_2N^+(CH_3)_3$ group might be an important factor for affinity. Since the diastereoisomeric preference seems to be associated with the compounds with the highest affinity, a larger number of high affinity derivatives is needed in order to support this hypothesis.

Earlier work,[27,28] on furane-based ligands, had shown that bulky groups like cyclohexyl-phenyl-methane or diphenylmethane were important structural features on account of their antimuscarinic activity. The inactivity or low activity found with compounds **14** and **15** was therefore quite surprising and was thought perhaps to be due to the presence of a phenyl ring in position 2 of the 1,3-dioxolane ring that might constitute a steric hindrance and so prevent an optimal interaction of the bulky moiety with a corresponding receptor subsite. However, when the phenyl ring was replaced with a methyl group (**25c,t**) or hydrogen atom (**26c,t**) no improvement in activity was observed; on the contrary, at the M_3 site there was a moderate decrease. These results indicate that the low activities of **14c,t** are not due to the presence of a phenyl group in the 2-position of the 1,3-dioxolane ring, but most probably to a different spacial orientation with respect to the cationic head as a consequence of a different conformation of the 1,3-dioxolane ring from that of the furane ring.

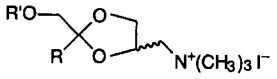
In conclusion, the compounds reported in this paper show low to moderate affinity for the three muscarinic receptors subtypes M_1–M_3. Some of them display a

Table 3. ^{13}C chemical shifts (ppm relative to TMS) of compounds **5**, **11**–**15**, **25**, and **26**[a]

	5c[b]	5t[b]	11c	11t	12c	12t	13c	13t	14c	14t	15c	25c	25t	26c	26t
C-2	112.3	112.3	110.18	110.04	110.16	110.10	110.24	110.08	110.21	109.92	110.17	109.83	109.70	102.81	101.94
C-4	70.5	71.6	70.43	71.63	70.36	71.33	70.52	71.52	79.26	71.42	70.25	70.15	70.65	70.38	70.20
C-5	68.2	67.8	67.82	67.73	67.84	67.75	67.91	67.83	67.71	67.68	67.52	67.71[d]	67.58	67.98	67.86
C-6	65.8	66.6	65.86	65.54	66.15	65.66	66.62	66.59	66.34	66.59	65.93	66.72	66.03	63.51	63.73
C-7	68.2	68.1	68.21	67.52	68.18	67.50	68.28	67.51	68.02	67.34	67.95	67.71[d]	67.95	68.05	66.97
$N(CH_3)_3$	54.3	54.2	54.35	54.21	54.35	54.21	54.28	54.18	54.22	54.09	54.29	54.12	54.04	54.16	54.06
C-1′ ϕ	139.6	140.1	138.57	139.06	138.62	139.10	138.52	138.06	[c]	138.89	138.34	—	—	—	—
C-*ortho* ϕ	126.0	125.9	126.08	125.90	126.15	125.92	126.09	125.90	125.97	125.83	126.02	—	—	—	—
C-*meta* ϕ	128.3	128.3	128.28	128.26	128.23	128.22	128.38	128.34	128.38	128.32	128.34	—	—	—	—
C-*para* ϕ	128.6	128.7	128.95	128.85	128.90	128.85	129.07	128.96	129.11	128.94	128.99	—	—	—	—
C=O	—	—	174.72	174.91	176.22	176.44	165.49	165.43	[c]	171.67	172.53	171.78	171.90	171.85	171.88
C-8	—	—	—	—	—	—	—	—	56.51	56.55	58.35	56.46	56.55	56.36	56.46
2-CH_3	—	—	—	—	—	—	—	—	—	—	—	21.48	21.86	—	—

[a]Spectra were obtained in CD_3CN.
[b]Data from ref. 17.
[c]Not detected.
[d]Overlapped signals.

Table 4. In vitro antimuscarinic activity of the tested compounds



Compound	R	R′	pK_b		
			M_1	M_2	M_3
1			8.36 ± 0.07	8.29 ± 0.06	7.91 ± 0.07
5c	C_6H_5	H	6.35 ± 0.12	6.01 ± 0.15	5.97 ± 0.21
5t	C_6H_5	H	7.13 ± 0.10	6.49 ± 0.21	7.01 ± 0.09
11c	C_6H_5	$C_6H_{11}CO$	5.61 ± 0.10	5.74 ± 0.09	5.59 ± 0.19
11t	C_6H_5	$C_6H_{11}CO$	6.71 ± 0.09	5.96 ± 0.12	6.37 ± 0.11
12c	C_6H_5	AdamCO	5.14 ± 0.21	5.47 ± 0.22	5.93 ± 0.08
12t	C_6H_5	AdamCO	5.86 ± 0.23	5.40 ± 0.16	5.77 ± 0.21
13c	C_6H_5	C_6H_5CO	5.84 ± 0.15	5.37 ± 0.24	6.52 ± 0.21
13t	C_6H_5	C_6H_5CO	6.73 ± 0.19	5.84 ± 0.16	6.49 ± 0.18
14c	C_6H_5	$(C_6H_5)_2CHCO$	< 5	< 5	6.15 ± 0.23
14t	C_6H_5	$(C_6H_5)_2CHCO$	5.3 ± 0.22	< 5	6.19 ± 0.13
15c	C_6H_5	$C_6H_5(C_6H_{11})CHCO$	NC[a]	< 5	NC
25c	CH_3	$(C_6H_5)_2CHCO$	NC	< 5	5.30 ± 0.24
25t	CH_3	$(C_6H_5)_2CHCO$	NC	< 5	5.30 ± 0.25
26c	H	$(C_6H_5)_2CHCO$	< 5	< 5	5.20 ± 0.11
26t	H	$(C_6H_5)_2CHCO$	< 5	5.2 ± 0.22	5.68 ± 0.18

[a]NC: Not competitive; the compound at 30 μM causes a decrease of agonist maximum response.

significant selectivity for the M_3 subtype. These results, though not striking, show that it is possible to achieve selectivity within 1,3-dioxolane-based ligands, and that appropriate structural modification could improve potency as well as selectivity. Work along this line is in progress.

Experimental

Chemistry

Melting points were taken in glass capillary tubes on a Buchi apparatus and are uncorrected. IR spectra were measured on a Perkin–Elmer 1600 instrument. NMR (^1H and ^{13}C) spectra were obtained in $CDCl_3$ or CD_3CN, with Varian XL-200 and Bruker AMX-400 WB spectrometers, and peak positions are given in parts per million (δ), downfield from tetramethylsilane as the internal standard. The typical resolution for ^1H NMR spectra was 0.05 Hz per point. Differential steady-state NOE experiments were performed, acquiring 128 + 128 transients in groups of eight, alternately irradiating on- and off-resonance, with a presaturation time of 10 s. Fully proton-decoupled ^{13}C NMR spectra were obtained with standard pulse sequence, and the resolution being 0.05 ppm.

The microanalyses were performed on a Carlo Erba 1106 Analyzer in the Microanalytical Laboratory of our department; where indicated by symbols, the results are within ±0.4% of the theoretical values. Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.040–0.063 mm, Merck) by flash chromatography. Reaction courses and product mixture were routinely monitored by thin-layer chromatography (TLC) on silica gel-precoated F254 Merck plates.

Acetic acid 2-oxo-2-phenyl-ethyl ester (2). 9 g (57.6 mmol) of chloroacetophenone, 8 g (81.4 mmol) of CH_3COOK and 60 mg of dicyclohexyl-18-crown-6 in 600 mL of DMF were refluxed for 90 min, under vigorous stirring. After cooling to room temperature (rt) the solid was filtered off and the solvent eliminated by vacuum distillation. The residue was purified by chromatography on silica gel column using cyclohexane:EtOAc (9:1) as the eluant to give product **2**; ^1H NMR ($CDCl_3$): δ 2.16 (s, 3H), 5.28 (s, 2H), 7.49 (m, 3H), 7.86 (m, 2H).

Acetic acid 4-chloromethyl-2-phenyl-[1,3]-dioxolan-2-yl-methyl ester (3). To a soln of 8.4 g (47.1 mmol) of **2** and 6 mL (71.7 mmol) of 3-chloro-1,2-propanediol in 350 mL of anhydrous CH_3NO_2, cooled at 0 °C, were slowly added 0.4 mL of trifloromethanesulfonic acid (triflic acid) and molecular sieve 4 Å. The reaction was heated at reflux under nitrogen for 36 h. After cooling to rt, Et_2O was added and the organic phase extracted with a satd soln of $NaHCO_3$ (3 × 50 mL) and dried over Na_2SO_4. The solvent was evapd and the residue was purified on a silica gel column (cyclohexane:EtOAc, 95:5), to give product **3** (6.45 g, 23.8 mmol, 50.5%); ^1H NMR ($CDCl_3$): δ 2.06, 2.07 (s, s, 3H), 3.22, 3.50 (dd, dd, 1H), 3.56, 3.60 (dd, dd, 1H), 3.81, 3.91 (dd, dd, 1H), 4.25, 4.35 (q, q, 2H), 4.37, 4.22 (dd, dd, 1H), 4.53, 4.36 (m, m, 1H), 7.38 (m, 3H), 7.35 (m, 2H).

***cis*-(4c) and *trans*-(4-Dimethylaminomethyl-2-phenyl-[1,3]dioxolan-2-yl)-methanol (4t).** 4.0 g (14.8 mmol) of **3** and 10 mL of $NH(CH_3)_2$ in a steel bomb were heated at 100 °C for 48 h. After evapn of the excess

$NH(CH_3)_2$ the residue was chromatographed on a silica gel column (EtOAc:$NH(C_2H_5)_2$, 95:5) to give the less polar isomer **4c** (1.92 g, 8.1 mmol, 54.7%); ^{1}H NMR ($CDCl_3$): δ 2.28 (s, 6H), 2.60 (dd, 1H), 3.27 (dd, 1H), 3.68 (q, 2H), 3.80 (dd, 1H), 3,89 (dd, 1H), 4.18 (m, 1H), 7.35 (m, 3H), 7.46 (m, 2H); and the more polar isomer **4t** (1.57 g, 6.6 mmol, 44.6%); ^{1}H NMR ($CDCl_3$): δ 2.17 (dd, 1H), 2.24 (s, 6H), 2.39 (dd, 1H), 3.75 (m, 2H), 3.82 (t, 1H), 4.26 (dd, 1H), 4.93 (m, 1H), 7.31 (m, 3H), 7.49 (m, 2H).

Benzoic acid 2,2-dimethoxy-ethyl ester (16). To a soln of 1.0 g (8.0 mmol) of chloroacetaldehyde dimethyl acetal in 60 mL of DMF were added 30 mg of dicyclohexyl-18-crown-6 and 1.4 g (8.74 mmol) of C_6H_5COOK, and the reaction was heated at reflux for 20 h. After cooling to rt, the solid was filtered off and the solvent was evapd by vacuum distillation. The residue was purified on silica gel column (cyclohexane:EtOAc, 75:25) to give 1.25 g (5.95 mmol, 74.0%) of product **16**; ^{1}H NMR ($CDCl_3$): δ 3.43 (s, 6H), 4.33 (d, 2H), 4.70 (t, 1H), 7.41–7.54 (m, 3H), 8.10 (m, 2H).

Benzoic acid 2-oxo-propyl ester (17). Benzoyl chloride (8.5 mL, 73.2 mmol) was added dropwise, over a 45 min period, to 5 mL (65.7 mmol) of hydroxyacetone (90%) in 50 mL of anhydrous pyridine, maintained at −5 °C. The reaction was stirred at rt for 12 h, cooled to 0 °C, diluted with 50 mL of H_2O and extracted with Et_2O (3 × 15 mL). The ethereal extracts were combined and dried over anhydrous Na_2SO_4. After evapn of the solvent, the residue was purified on a silica gel column (cyclohexane:EtOAc, 7:3), to give product **17** (8.96 g, 39.6 mmol, 76.5%); ^{1}H NMR ($CDCl_3$): δ 2.19 (s, 3H), 4.85 (s, 2H), 7.48 (m, 3H), 8.10 (m, 2H).

Benzoic acid 2-oxo-ethyl ester (18). To a soln of 1.0 g (4.76 mmol) of **16** in 50 mL of anhydrous THF, 10 mL of H_2SO_4 1 M was added and the reaction was heated at 100 °C for 12 h. After cooling to rt the reaction mixture was washed with 1 M NaOH (3 × 15 mL) and dried over $MgSO_4$. Evaporation of the solvent and purification of the residue by chromatography (cyclohexane:EtOAc, 8:2) gave 0.45 g (2.74 mmol, 58%) of product **18**; ^{1}H NMR ($CDCl_3$): δ 4.88 (d, 2H), 7.36–7.58 (m, 3H), 8.10 (m, 2H), 9.71 (t, 3H).

Benzoic acid 4-chloromethyl-2-methyl-[1,3]dioxolan-2-ylmethyl ester (19). To 1.0 g (5.61 mmol) of **17** in 80 mL of toluene were added 0.5 mL (5.98 mmol) of 3-chloro-1,2-propanediol and 25 mg of *p*-toluensulfonic acid (PTSA). The reaction was heated at reflux for 48 h with the use of a Dean–Stark apparatus. After cooling to rt the reaction mixture was washed with a satd soln of $NaHCO_3$ (3 × 20 mL) and brine (3 × 15 mL) and dried over Na_2SO_4. Evaporation of the solvent gave a residue that was purified on a silica gel column (cyclohexane:EtOAc, 9:1), to give 1.1 g (4.06 mmol, 72.4%) of product **19**; ^{1}H NMR ($CDCl_3$): δ 1.46, 1.53 (s, s, 3H), 3.49, 3.47 (dd, dd, 1H), 3.59 (dd, 1H) 3.95, 3.91 (dd, dd, 1H), 4.21, 4.18 (dd, dd, 1H), 4.32, 4.28 (q, q, 2H), 4.43, 4.37 (m, m, 1H), 7.43 (m, 2H), 7.45 (m, 1H), 8.05 (m, 2H).

Benzoic acid 4-chloromethyl-[1,3]dioxolan-2-ylmethyl ester (20). To a soln of 5.0 g (30.5 mmol) of **18** in 200 mL of toluene, 3.5 mL (4.19 mmol) of 3-chloro-1,2-propanediol and 100 mg of PTSA were added. The reaction was heated at reflux for 15 h with the use of a Dean–Stark apparatus. After cooling to rt the mixture was extracted with a satd soln of $NaHCO_3$ (3 × 30 mL) and brine (3 × 30 mL) and dried over Na_2SO_4. Evaporation of the solvent afforded a residue that was purified on silica gel column (cyclohexane:EtOAc, 8:2) to give compound **20** (5.86 g, 22.8 mmol, 75%); ^{1}H NMR ($CDCl_3$): δ 3.49, 3.43 (dd, dd, 1H), 3.60, 3.55 (dd, dd, 1H), 3.84, 3.98 (dd, d, 1H), 4.20, 3.98 (dd, d, 1H), 4.29, 4.31 (m, m, 1H), 4.32, 4.40 (d, d, 2H), 5.41, 5.24 (t, t, 1H), 7.39 (m, 2H), 7.49 (m, 1H), 8.05 (m, 2H).

***cis*-(21c) and *trans*-(4-Dimethylaminomethyl-2-methyl-[1,3]dioxolan-2-yl)-methanol (21t).** Following the same procedure as adopted for the preparation of **4c** and **t**, 0.85 g (3.14 mmol) of **19**, after chromatography [EtOAc:$NH(C_2H_5)_2$, 95:5], gave the less polar isomer **21c** (0.32 g, 1.83 mmol, 58.3%); IR (KBr, cm^{-1}) 3405 (OH); ^{1}H NMR ($CDCl_3$): δ 1.32 (s, 3H), 2.32 (s, 6H), 2.44 (dd, 1H), 2.55 (dd, 1H), 3.62–3.65 (q, 2H), 3.79 (dd, 1H), 4.11 (dd, 1H), 4.29 (m, 1H); and the more polar isomer **21t** (0.20 g, 1.14 mmol, 36.3%); IR (KBr, cm^{-1}) 3405 (OH); ^{1}H NMR ($CDCl_3$): δ 1.38 (s, 3H), 2.29 (s, 6H), 2.38 (dd, 1H), 2.54 (dd, 1H), 3.51 (s, 2H), 3.64 (dd, 1H), 4.14 (dd, 1H), 4.31 (m, 1H).

(4-Dimethylaminomethyl-[1,3]dioxolan-2-yl)-methanol (22). 6.0 g (23.4 mmol) of **20** was subjected to the same procedure as adopted for the preparation of **4c,t**, followed by chromatography [EtOAc:CH_3OH:$N(CH_3)_3$, 90:5:5], to give compound **22** (3.40 g, 21.1 mmol, 90%); ^{1}H NMR ($CDCl_3$): δ 2.21, 2.22 (s, s, 6H), 2.26, 2.29 (dd, dd, 1H), 2.46, 2.49 (dd, dd, 1H), 3.45, 3.62 (dd, dd, 1H), 3.56, 3.59 (d, d, 2H), 4.09, 3.92 (dd, dd, 1H), 4.22, 4.17 (m, m, 1H), 4.66–4.69 (br s, exchangeable, 1H), 5.01, 4.94 (t, t, 1H).

General procedure for the preparation of compounds 6–9c,t 10c, 23 and 24 c,t

To 200–600 mg of amino alcohols **4c,t**, **21c,t**, or **22** dissolved in 5–15 mL of dry pyridine, at 0 °C, were added dropwise a slight excess of commercially available acyl chloride. The reaction was stirred at rt for 12–24 h, then cooled to 0 °C, diluted with 5–10 mL of H_2O and extracted with Et_2O (3 × 10 mL). The extracts were combined and dried over Na_2SO_4. Evaporation of the solvent gave a residue that was purified by column chromatography, using as eluant EtOAc:NH_4OH [or $NH(C_2H_5)_2$] (98–96:2–4). For the synthesis of **10c**, the acyl chloride was freshly prepared: 0.85 g (3.74 mmol) of cyclohexyl-phenyl-acetic acid (96%) were dissolved in 20 mL of dry benzene; 2 mL of $SOCl_2$ were added and the mixture was refluxed for 24 h. After removal of

the solvent, the acyl chloride was used without further purification. For the preparation of **24c,t** the starting amino alcohol was used as a diastereoisomeric mixture. The separation of the two isomers was accomplished after esterification by column chromatography (EtOAc:NH_4OH 30%, 99:1).

***cis*-Cyclohexancarboxylic acid 4-dimethylaminomethyl-2-phenyl-[1,3]dioxolan-2-ylmethyl ester (6c)**. Yield 95%; ^1H NMR ($CDCl_3$): δ 1.13–1.42 (m, 6H), 1.54–1.86 (m, 5H), 2.24 (s, 6H), 2.43 (dd, 1H), 2.58 (dd, 1H), 3.73 (dd, 1H), 3.94 (dd, 1H), 4.12 (m, 1H), 4.24 (q, 2H), 7.31 (m, 3H), 7.49 (m, 3H).

***trans*-Cyclohexancarboxylic acid 4-dimethylaminomethyl-2-phenyl-[1,3]dioxolan-2-ylmethyl ester (6t)**. Yield 94%; ^1H NMR ($CDCl_3$): δ 1.15–1.45 (m, 6H), 1.60–2.00 (m, 5H), 2.29 (s, 6H), 2.34 (dd, 1H), 2.44 (dd, 1H), 3.51 (t, 1H), 4.25 (q, 2H), 4.28 (dd, 1H), 4.47 (m, 1H), 7.34 (m, 3H), 7.53 (m, 2H).

***cis*-Adamantane-1-carboxylic acid 4-dimethylaminomethyl-2-phenyl-[1,3]dioxolan-2-ylmethyl ester (7c)**. Yield 86%; ^1H NMR ($CDCl_3$): δ 1.60–1.71 (m, 6H), 1.78–1.83 (m, 6H), 1.93–1.98 (m, 3H), 2.23 (s, 6H), 2.47 (dd, 1H), 2.61 (dd, 1H), 3.73 (dd, 1H), 3.97 (dd, 1H), 4.13 (m, 1H), 4.21 (q, 2H), 7.25 (m, 3H), 7.43 (m, 2H).

***trans*-Adamantane-1-carboxylic acid 4-dimethylaminomethyl-2-phenyl-[1,3]dioxolan-2-ylmethyl ester (7t)**. Yield 86%; ^1H NMR ($CDCl_3$): δ 1.60–1.72 (m, 6H), 1.78, 1.83 (m, 6H), 1.93–2.00 (m, 3H), 2.30 (s, 6H), 2.35 (dd, 1H), 2.45 (dd, 1H), 3.53 (t, 1H), 4.29 (dd, 1H), 4.24 (q, 2H), 7.49 (m, 1H), 7.35 (m, 1H), 7.56 (m, 2H).

***cis* - Benzoic acid 4 - dimethylaminomethyl - 2 - phenyl [1,3] dioxolan-2-ylmethyl ester (8c)**. Yield 88%; ^1H NMR ($CDCl_3$): δ 2.73 (s, 6H), 2.60 (dd, 1H), 2.68 (dd, 1H), 3.77 (dd, 1H), 3.99 (dd, 1H), 4.28 (m, 1H), 4.49 (q, 1H), 7.45–7.30 (m, 6H), 7.55, (m, 2H), 7.96 (m, 2H).

***trans* - Benzoic acid 4 - dimethylaminomethyl - 2 - phenyl [1,3] dioxolan-2-ylmethyl ester (8t)**. Yield 93%; ^1H NMR ($CDCl_3$): δ 2.37 (s, 6H), 2.49 (dd, 1H), 2.57 (dd, 1H), 3.52 (t, 1H), 4.31 (dd, 1H), 4.46 (q, 2H), 4.58 (m, 1H), 7.32–7.50 (m, 6H), 7.56 (m, 2H), 7.97 (m, 2H).

***cis*-Diphenyl-acetic acid 4-dimethylaminomethyl-2-phenyl-[1,3]dioxolan-2-ylmethyl ester (9c)**. Yield 98%; ^1H NMR ($CDCl_3$): δ 2.29 (dd, 1H), 2.31 (s, 6H), 2.47 (dd, 1H), 3.45 (dd, 1H), 3.84 (dd, 1H), 4.21 (m, 1H), 5.04 (s, 1H), 4.36 (q, 2H), 7.20–7.34 (m, 13H), 7.43 (m, 2H).

***trans*-Diphenyl-acetic acid 4-dimethylaminomethyl-2-phenyl-[1,3]dioxolan-2-ylmethyl ester (9t)**. Yield 96%; ^1H NMR ($CDCl_3$): δ 2.28 (s, 6H), 2.38 (dd, 1H), 2.44 (dd, 1H), 3.37 (t, 1H), 3.98 (dd, 1H), 5.03 (s, 1H), 4.17 (m, 1H), 4.34 (q, 2H), 7.18–7.39 (m, 13H), 7.44 (m, 2H).

***cis*-Cyclohexyl-phenyl-acetic acid 4-dimethylaminomethyl-2-phenyl-[1,3]dioxolan-2-ylmethyl ester (10c)**. Yield 94%; ^1H NMR ($CDCl_3$): δ 0.92–1.40 (m, 6H), 1.54–1.90 (m, 5H), 2.27, 2.24 (s, s, 6H), 2.33, 2.29 (dd, dd, 1H), 2.48, 2.39 (1H), 3.24, 3.22 (s, s, 1H), 3.44, 3.63 (dd, dd, 1H), 3.87, 3.95 (dd, dd, 1H), 4.23, 4.08 (m, m, 1H), 4.28, 4.26 (q, q, 2H), 7.22–7.34 (m, 8H), 7.43 (m, 2H).

***cis*-Diphenyl-acetic acid 4-dimethylaminomethyl-2-methyl-[1,3]dioxolan-2-ylmethyl ester (23c)**. Yield 77%; ^1H NMR ($CDCl_3$): δ 1.28 (s, 3H), 2.22 (s, 6H), 2.24 (dd, 1H), 2.32 (dd, 1H), 3.39 (dd, 1H), 4.02 (dd, 1H), 5.05 (s, 1H), 4.20 (m, 1H), 4.14 (q, 2H), 7.22–7.34 (m, 10H).

***trans*-Diphenyl-acetic acid 4-dimethylaminomethyl-2-methyl-[1,3]dioxolan-2-ylmethyl ester (23t)**. Yield 80%; ^1H NMR ($CDCl_3$): δ 1.35 (s, 3H), 2.33 (dd, 1H), 2.41 (s, 6H), 2.46 (dd, 1H), 3.54 (dd, 1H), 3.93 (dd, 1H), 4.10 (m, 1H), 4.13 (q, 2H), 5.06 (s, 1H), 7.31–7.38 (m, 10H).

***cis* - Diphenyl - acetic acid 4 - dimethylaminomethyl[1,3] dioxolan-2-ylmethyl ester (24t)**. Yield 42%; ^1H NMR ($CDCl_3$): δ 2.24 (s, 6H), 2.29 (dd, 1H), 2.37 (dd, 1H), 3.49 (dd, 1H), 3.96 (dd, 1H), 4.16 (m, 1H), 4.26 (m, 2H), 5.07 (s, 1H), 5.13 (t, 1H), 7.30–7.38 (m, 10H).

***trans* - Diphenyl - acetic acid 4 - dimethylaminomethyl[1,3] dioxolan-2-ylmethyl ester (24c)**. Yield 54%; ^1H NMR ($CDCl_3$) δ 2.24 (s, 6H), 2.28 (dd, 1H), 2.46 (dd, 1H), 3.50 (dd, 1H), 3.98 (dd, 1H), 4.11 (m, 1H), 4.21 (q, 2H), 5.24 (t, 1H), 5.08 (s, 1H), 7.30–7.38 (m, 10H).

General procedure for the preparation of compounds 11–15, 25, 25c,t

100–200 mg of amine was dissolved in 10–15 mL of dry Et_2O; an excess of MeI was added and the reaction left at rt for 12 h. After filtration (or evaporation of the solvent) the solid (or residue) was recrystallized from EtOH:Et_2O (acetone:petroleum ether in the case of compound **14t**).

Pharmacology

General considerations. Male guinea pigs (200–300 g) and male New Zealand white rabbits (3.0–3.5 kg) were killed by cervical dislocation. The organs required were set up rapidly under 1 g of tension in 20 mL organ baths containing physiological salt solution (PSS) maintained at an appropriate temperature (see below) and aerated with 5% CO_2–95% O_2. Dose–response curves were constructed by cumulative addition of agonist. The concentration of agonist in the organ bath was increased approximately threefold at each step, with each addition being made only after the response to the previous addition had attained a maximal level and remained steady. Following 30 min of washing, tissues were incubated with the antagonist for 30 min, and a new dose–response curve to the agonist was

obtained. Contractions were recorded by means of a force transducer connected to a two-channel Gemini polygraph. In all cases, parallel experiments in which tissues did not receive any antagonist were run in order to check any variation in sensitivity.

Guinea pig ileum.[29,30] Two-centimeter-long portions of terminal ileum were taken, about 5 cm from the ileum–cecum junction, and mounted in PSS at 37 °C. The composition of PSS was as follows (mM): NaCl (118), $NaHCO_3$ (23.8), KCl (4.7), $MgSO_4 \cdot 7H_2O$ (1.18), KH_2PO_4 (1.18), $CaCl_2$ (2.52), and glucose (11.7). Tension changes were recorded isotonically. Tissues were equilibrated for 30 min, and dose–response curves to carbachol were obtained at 30-min intervals, the first one being discarded and the second one being taken as the control.

Guinea pig stimulated left atria.[29] The heart was rapidly removed and the right and left atria were separately excised. Left atria were mounted in PSS (the same as used for ileum) at 30 °C and stimulated through platinum electrodes by square-wave pulses (1 ms, 2 Hz, 10–15 V). Inotropic activity was recorded isometrically. Tissues were equilibrated for 2 h and a cumulative dose–response curve to carbachol was constructed.

Rabbit stimulated vas deferens.[29,31] Vasa deferentia were carefully dissected free of surrounding tissue and divided into four segments, two prostatic portions of 1 cm and two epididymal portions approximately 1.5 cm in length. The four segments were mounted in PSS with the following composition (mM): NaCl (118.4), KCl (4.7), $CaCl_2$ (2.52), $MgCl_2$ (0.6), KH_2PO_4 (1.18), $NaHCO_3$ (25), and glucose (11.1); 1 μM yohimbine was included to block α_2-adrenoceptors. The soln was maintained at 30 °C and tissues were stimulated through platinum electrodes by square-wave pulses (0.1 ms, 2 Hz, 10–15 V). Contractions were measured isometrically after tissues were equilibrated for 1 h, then a cumulative dose–response curve to McN-A-343 was constructed.

Determination of antagonist potency. To quantify antagonist potency, pK_b values were calculated from the equation $pKb = \log(DR-1) - \log [B]$, where DR is the ratio of ED_{50} values of agonist after and before treatment with antagonist concentration [B]. Values are given as mean ± standard error of four or five independent observations.

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