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Synthesis and Structure–Activity Relationship Studies in a Series of 2-Substituted 1,3-Dioxolanes Modified at the Cationic Head

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Abstract—To develop ligands that may be useful in exploring muscarinic receptor heterogeneity, we synthesized a series of analogues of 2,2-diphenyl-[1,3]-dioxolan-4-ylmethyl-dimethylamine oxalate and methiodide bearing a modified cationic head. These compounds, when tested on tissues containing the three subtypes M_1, M_2, and M_3, behaved as muscarinic antagonists whose results showed that different substituents on the quaternary and tertiary nitrogen affect affinity and selectivity in different ways. In particular, comparison of the affinities of these ligands with those of the reference compounds points out that compounds bearing an ethyl substituent improve the affinity of the molecule at the three subtypes, while compounds bearing a phenethyl substituent are more selective for the M_2 sites. © 1997 Published by Elsevier Science Ltd.

Introduction

Muscarinic acetylcholine receptors are a family of heterogeneous receptors[1] that can be classified, on a pharmacological basis, into three subtypes (M_1–M_3):[2,3] M_1 (neuronal type) has high affinity for pirenzepine; M_2 (cardiac type) displays high affinity for methoctramine, himbacine, AF-DX 116, gallamine, and the new antagonists tripitramine and C-tripitramine;[4] M_3 (smooth muscle-glandular type) shows high affinity for 4-DAMP and *p*-fluoro-hexahydro-sila-difenidol. Recently, a fourth subtype, M_4, has been pharmacologically characterized and Melchiorre et al.[4] have shown that tripitramine is able to discriminate significantly between M_2 and M_4 receptors.

At the same time, molecular cloning studies have demonstrated the presence of five distinct muscarinic receptor genes (m_1–m_5) in the brain and in peripheral tissues. The pharmacologically identified M_1–M_4 subtypes seem to be identical to the cloned m_1–m_4 receptors.[1] The antagonist ligands are useful tools for characterizing the receptor subpopulations.

The 1,3-dioxolane nucleus has been studied for many years because of its capacity to behave as a potent muscarinic agonist or antagonist, depending on the size of substituents at position 2.[5] With regard to this, in some of our previous papers, we inquired into the distance between the two phenyl substituents in the 2-position and the dioxolane ring,[6] and into the modification of the lipophilic fraction of the molecule by doubling the molecular model,[7] and by contracting the

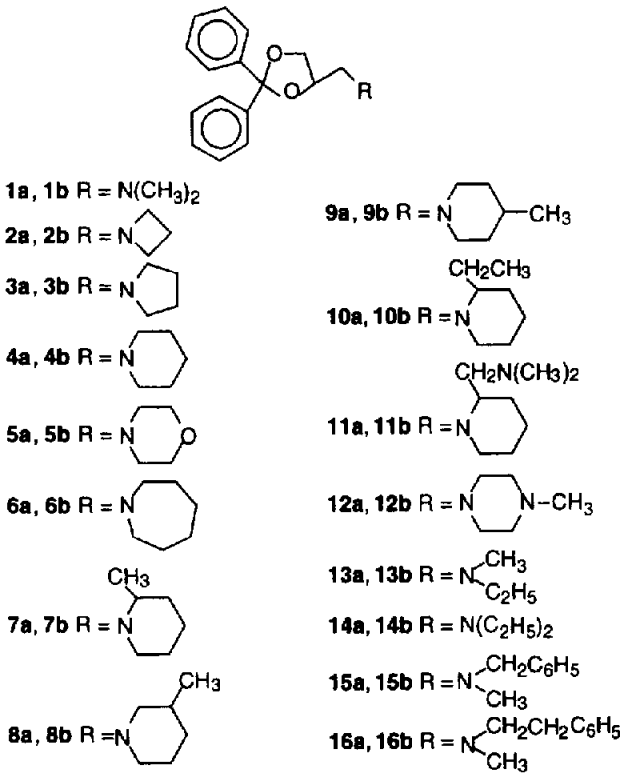


Chart 1.

structure of the molecule by means of fusing the hydrophobic bulky moiety in a rigid structure.[8]

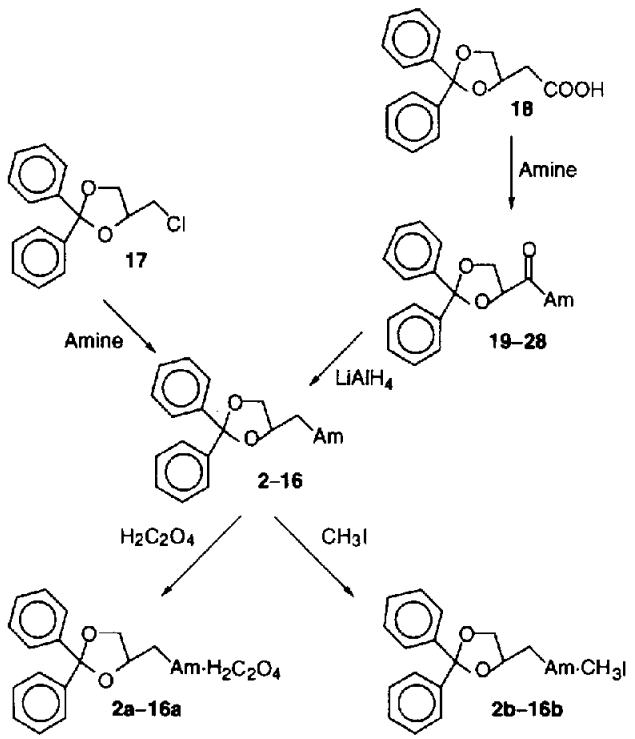
As a continuation of the study of the 1,3-dioxolane nucleus to further investigate muscarinic receptor heterogeneity, a series of analogues of 2,2-diphenyl-[1,3]-dioxolan-4-ylmethyl-dimethylamine oxalate (**1a**) and methiodide (**1b**), involving modification of the cationic head, was synthesized (Chart 1) and examined for antimuscarinic activity in guinea pig ileum (M_3), left atrium (M_2), and rabbit vas deferens (M_1).

It is well known that muscarinic M_3 receptors mediate contraction of most types of smooth muscle[9] and that their selective blockade may be therapeutically useful in the treatment of many disorders (gastrointestinal, urinary tract, and respiratory). The therapeutical utility of tissue-selective M_3 muscarinic antagonists encouraged us to further investigate our compounds on two different M_3 tissues (guinea pig ileum and bladder). Although these tissues are still classified as both belonging to a homogeneous M_3 population, nevertheless some authors proved that differences exist between them.[5,10,11]

Chemistry

The reaction pathways used to synthesize the designed compounds are reported in Scheme 1.

Compounds **2a–16a** and **2b–16b** were prepared by treating 2,2-diphenyl-[1,3]-dioxolane-4-chloromethyl[12] with the appropriate amine or by amidation of 2,2-diphenyl-[1,3]-dioxolane-4-carboxylic acid[13] with appropriate amine followed by reduction with $LiAlH_4$ (Scheme 1).



Scheme 1.

The separation of the diastereomeric mixtures of compounds **7**, **8**, **10**, and **11** was attempted by column chromatography and was unsuccessful.

When treated with an excess of oxalic acid or CH_3I, the amines were transformed into the corresponding oxalate (**2a–16a**) or methiodide (**2b–16b**).

Treating amines **11** and **12** with CH_3I, the corresponding monomethiodides **11b** and **12b** were obtained; of the former, only the nitrogen on the side chain of 2-[(dimethylamino)methyl]piperidine, while of the latter, only the *N*-methyl group of 1-methylpiperazine were methylated (the structures were assigned by ^1H NMR spectra and microanalyses).

Results

All the compounds tested behaved as muscarinic antagonists. Table 1 shows the results obtained on the three subtypes M_1, M_2 and M_3 of the muscarinic receptor. Data are expressed as pK_b, which represents the $-\log K_b$ obtained from the expression $\log(DR-1) = \log[\text{antagonist}] - \log K_b$ at a given concentration of the antagonist.[14] Compounds 2,2-diphenyl-4-[(dimethylamino)methyl]-1,3-dioxolane methiodide (**1b**) and oxalate (**1a**) were used as reference compounds.

In some cases the affinity values are reported in terms of pA_2 obtained according to Schild[15] and constraining the slope to -1.0 as required by the theory,[16] as shown in Table 1. All the compounds behave as competitive antagonists at the concentrations used to calculate pK_b or pA_2 values. At higher concentrations, as indicated in Table 1, some compounds cause a decrease of the maximum effect of the reference agonist.

Substitution of the *N*-dimethylamino group of **1b** with *N*-methylethylamino or *N*-diethylamino groups yielded compounds **13b** and **14b**, respectively, which both display a higher affinity for the three subtypes. Compound **14b**, the most potent ligand among the tertiary amines and the methiodide series, is five times more active at the M_1 site and three times more at M_2 and M_3 sites than is the reference compound **1b**. The piperidine analogue **4b** shows affinity values at M_1 and M_3 sites similar to those of **1b**, whereas its affinity is slightly lower at the M_2 site. When the piperidine ring is substituted in position two with a methyl group (**7b**), a threefold increase of affinity at the M_1 site is observed. Substitutions at the same position with bulkier groups (**10b** and **11b**) or at 3- and 4-positions with a methyl group (**8b** and **9b**, respectively) are always detrimental to affinity in all the subtypes. Compound **12b** displays the highest decrease (400- to 1880-fold) in affinity among the quaternary salts investigated. A serious drop in activity at the three sites is also observed with compound **15b** carrying a benzyl group on the nitrogen

Table 1. Affinity values and selectivity ratios for the muscarinic antagonists (oxalates **1a–16a** and methiodides **1b–16b**) examined

Compd	Rabbit vas deferens (M_1)[a]	Guinea pig atria (M_2)[a]	Guinea pig ileum (M_3)[a]	Guinea pig bladder (M_3)[a]	Selectivity ratio[b]			
					M_1/M_2	M_1/M_3	M_2/M_3	M_3 ileum/ M_3 bladder
1a	7.15 ± 0.12[c]	7.11 ± 0.03[c]	6.38 ± 0.08[c]	6.32 ± 0.27[c]	1	6	5	1
1b	8.36 ± 0.07[c]	8.29 ± 0.06[c]	7.91 ± 0.07[c]	7.67 ± 0.24[c]	1	3	2	2
2a	7.02 ± 0.29	7.01 ± 0.04[c]	6.41 ± 0.09[c]	6.67 ± 0.09	1	4	4	0.6
2b	7.11 ± 0.21	6.81 ± 0.04[c]	6.29 ± 0.14[c]	6.56 ± 0.20	2	7	3	0.5
3a	6.63 ± 0.21	6.58 ± 0.08	6.12 ± 0.09	6.52 ± 0.07	1	3	3	0.4
3b	6.86 ± 0.07	6.64 ± 0.11	6.13 ± 0.06	6.86 ± 0.12	2	5	3	0.2
4a	6.88 ± 0.08	6.65 ± 0.06	6.85 ± 0.06	6.69 ± 0.05	2	1	0.6	2
4b	8.53 ± 0.16	7.94 ± 0.07	7.84 ± 0.09	7.73 ± 0.02	4	5	1	1
5a	5.45 ± 0.11	<6	<5.52	<5.52	-	-	-	-
5b	7.36 ± 0.13	7.21 ± 0.07	6.99 ± 0.04	6.69 ± 0.05	1	2	2	2
6a	6.68 ± 0.20	6.40 ± 0.08	6.13 ± 0.25[d]	5.19 ± 0.17	2	4	2	9
6b	6.98 ± 0.01	6.00 ± 0.29	6.54 ± 0.09	6.47 ± 0.10	10	3	0.3	1
7a	7.73 ± 0.13	7.08 ± 0.05	6.52 ± 0.04	6.22 ± 0.10	5	16	4	2
7b	8.83 ± 0.17	8.25 ± 0.08	7.82 ± 0.02	7.98 ± 0.01	4	10	3	0.7
8a	5.71 ± 0.14	5.59 ± 0.09	5.92 ± 0.15[d]	5.88 ± 0.10	1	0.6	0.5	1
8b	6.90 ± 0.14	6.40 ± 0.18	6.15 ± 0.15	6.44 ± 0.04	3	6	2	0.5
9a	5.58 ± 0.10	5.30 ± 0.12	<5[d]	5.15 ± 0.34	2	–	–	–
9b	7.05 ± 0.12	6.41 ± 0.17	6.42 ± 0.04	6.31 ± 0.01	4	4	1	1
10a	6.29 ± 0.16	6.30 ± 0.11	6.24 ± 0.19[d]	5.95 ± 0.14	1	1	1	2
10b	6.82 ± 0.16	7.01 ± 0.17	6.48 ± 0.16	6.37 ± 0.19	0.6	2	3	1
11a	7.86 ± 0.11[c,e]	6.74 ± 0.08[c]	6.40 ± 0.04[e]	6.51 ± 0.01	13	29	2	0.8
11b	6.36 ± 0.03	6.23 ± 0.04	6.14 ± 0.18	5.38 ± 0.09	1	2	1	6
12a	6.30 ± 0.08	6.05 ± 0.09	5.62 ± 0.04	5.88 ± 0.10	2	5	3	0.6
12b	5.10 ± 0.20	5.22 ± 0.08	5.28 ± 0.06	<5.52	0.8	0.7	1	–
13a	7.88 ± 0.08	7.38 ± 0.10	6.85 ± 0.16[c]	7.39 ± 0.05[c]	3	11	3	0.3
13b	8.79 ± 0.13	8.64 ± 0.06	8.13 ± 0.15	8.26 ± 0.06	1	5	3	0.7
14a	7.73 ± 0.13	7.49 ± 0.05	6.52 ± 0.09	7.10 ± 0.07	2	16	9	0.3
14b	9.07 ± 0.04	8.71 ± 0.12	8.33 ± 0.07	7.89 ± 0.17	2	6	2	3
15a	5.17 ± 0.11	4.84 ± 0.20	6.38 ± 0.21[c]	5.70 ± 0.30	2	0.06	0.03	5
15b	5.73 ± 0.19	5.55 ± 0.13	5.79 ± 0.20	5.92 ± 0.10	2	1	0.6	0.7
16a	5.27 ± 0.08[c]	4.80 ± 0.13	6.97 ± 0.06[c,d]	5.82 ± 0.19[c,d]	3	0.02	0.007	14
16b	6.45 ± 0.01[c]	6.02 ± 0.09[c]	7.24 ± 0.06[c,d]	6.70 ± 0.07[c,d]	3	0.2	0.06	4

[a] Affinity constants (pK_b ± SEM) calculated from the equation log (DR − 1) = log[ant.] − logK_b for a single concentration (0.1–30 μM) of the antagonist according to van Rossum are reported.
[b] Antilog of the difference between the pK_b or pA_2 values for M_1, M_2, and M_3 muscarinic receptor subtypes.
[c] pA_2 values.
[d] A decrease of the maximum effect of the reference agonist was present at 3×10^{-5} M.
[e] A noncompetitive antagonism was observed at concentrations higher than 1×10^{-5} M.

atom. On the contrary, the phenethyl analogue **16b** shows a fivefold lower affinity than that of compound **1b** for the M_3 subtype and a remarkable decrease (80- to 190-fold) at M_1 and M_2 sites, respectively. Comparison between compound **1b** and its derivative **5b** carrying the morpholine ring shows a 10-fold decrease in affinity for the latter, while when the substituents are azetidine (**2b**), pyrrolidine (**3b**), and homopiperidine (**6b**) a more pronounced decrease is observed.

Many of the observations we made in the quaternary salt series also apply in the tertiary amines one. In fact, compounds **13a** and **14a** display affinity values in all the subtypes, which are among the highest in this series, analogously to the corresponding methiodide **13b** and **14b**. The 2-methylpiperidine ring improves, once again, the affinity of compound **7a** (fourfold) at the M_1 site while it has no influence at the M_2 and M_3 ones. Generally, a drop or no variation in activity is observed with all the other substitutions, as also observed in the other series, with the exception of compound **11a** at the M_1 site, and **4a** and **16a** at the M_3 site, where they show an eight-, three-, and fourfold increase, respectively. Moreover, compound **16a** displays a significant decrease (80- to 200-fold) at M_1 and M_2 sites when compared with the reference compound **1a**. The same trend is observed with compound **15a**, confirming the feature of the benzyl substituent in increasing the selectivity of the molecule towards the M_3 subtype.[10]

Discussion

Substitutions carried out on the cationic head of potent antimuscarinic ligands (**1**) were designed with aliphatic linear and ringed groups in order to increase affinity and selectivity.

Analysis of these data suggests that when nitrogen is enclosed in a ring, the affinity values of the relative ligands are generally lower for all the subtypes,

whatever the sizes. The only exceptions are observed with the piperidine (**4b**), and particularly 2-methylpiperidine (**7a** and **7b**) derivatives at the M_1 site where an opposite trend occurs. On the other hand, the phenethyl derivatives **16a** and **16b** show that this group is still well tolerated in the binding of the ligand with the M_3 subtype but is detrimental to the M_1 and M_2 ones. Compounds **13a**, **13b**, **14a**, and **14b**, clearly show that nitrogen carrying ethyl groups produces the best improvement in affinity, and that this improvement is slightly higher for the M_1 subtype.

The dramatic fall in activity observed with compound **12b** and the 100-fold decrease in the same parameter for compound **11b** could be attributed to the fact that the quaternary *N*-methyl and *N*-trimethyl nitrogens, which bind to the anionic site of the receptor, do not allow subsequent optimal hydrophobic interactions between the 2-substituents and the lipophilic cavity of the receptor binding sites.

Although it seems difficult to find a general, clear relationship between the quaternization effect (**b/a**) and affinity for these series of ligands, nevertheless some considerations sound reasonable. Starting from the observation that the quaternization of the reference compound (**1b/1a**) causes an increase in affinity of around 15- (for M_1 and M_2 sites) and 30-fold (for the M_3 site), it is evident that when nitrogen is enclosed in a 4- or 5-membered ring (compounds **2** and **3**, respectively) or in a 7-membered ring (compound **6**), no, or a very small, difference in affinity is observed between the tertiary amines and the corresponding quaternary salts. On the other hand, significant differences in affinity are shown when the tertiary amines **4a** and **5a**, carrying a piperidine and morpholine ring, respectively, are compared with the corresponding quaternary salts **4b** and **5b**. The results in Table 2 clearly indicate that the quaternization effect is particularly evident for the 6-membered ring derivatives, suggesting a possibly different approach in the interactions of the latter compounds with the muscarinic receptors. It can be assumed that the piperidine (with and without substituents) and morpholine methiodide derivatives possess the appropriate steric and electronic requirements for a stronger binding through an ion–ion interaction between the cationic head and the anionic cavity of the receptor site. This fact explains the difference in affinity (which is notable in some cases) with the corresponding tertiary amines, which are less influenced by this ion–ion approach, as we already supposed with other series of 2-substituted dioxolanes.[6,8] This trend is also maintained in the linear nitrogen compounds, while it is inverted for compounds **11** and **12**, which display a higher affinity as tertiary amines. This behavior could depend on the fact that only the nitrogen on the side chain (compound **11**) and only the *N*-methyl group (compound **12**) are methylated. In both these cases, the position of the cationic nitrogen is different with respect to that of the other compounds.

Table 2. Potency ratios[a] for the methiodide/oxalate pair of the antagonists examined

Pair	Receptor subtype		
	M_1	M_2	M_3
1b/1a	16	15	34
2b/2a	1	0.6	0.8
3b/3a	1.7	1	1
4b/4a	45	20	10
5b/5a	81	>16	>29.5
6b/6a	2	0.4	2.6
7b/7a	12.6	14.8	20
8b/8a	15.5	6.5	1.7
9b/9a	29.5	12.9	>26
10b/10a	3.4	5.1	1.7
11b/11a	0.03	0.3	0.6
12b/12a	0.06	0.2	0.5
13b/13a	8	18	19
14b/14a	21.9	16.6	64.6
15b/15a	3.6	5	0.3
16b/16a	15.1	16.6	1.9

[a]Antilog of the difference between the pK_b or pA_2 values of methiodide and oxalate pairs at M_1, M_2, and M_3 receptor subtypes.

Tissue Selectivity

Some of our compounds display an improvement of the modest or nonexistent selectivity shown by compounds **1a** and **1b**, respectively. Compound **11a**, for example, shows a 13- and fivefold increase in M_1 selectivity in comparison with the reference compound **1a**. The same trend is observed with compounds **7a** and **7b**, although their increases are smaller (five- and around threefold for the former, and four- and threefold for the latter). A small improvement of the M_1/M_3 ratio is also observed for compounds **13a** and **14a** while, interestingly, an opposite trend for selectivity is observed mainly with compounds **15a**, **16a**, and **16b**, which display a better affinity at the M_3 site. With regard to this, it is interesting to note that compound **16a** shows an affinity value at the M_3 site which is 50- and 150-fold higher than those reported at M_1 and M_2 sites, respectively, although this compound and its corresponding methiodide **16b** interact in a noncompetitive manner at high concentrations with the M_3 receptors.

Taken together, these results suggest that different substituents on the quaternary and tertiary nitrogen affect in different ways the affinity and selectivity of these series of antimuscarinic ligands, playing an important role in the binding of the molecule with receptor subtypes.

In conclusion, an ethyl substituent on the cationic head improves the affinity of the reference molecules at the three muscarinic receptor subtypes, and a phenethyl substituent seems to lead to M_3 selectivity.

As far as the M_3 population is concerned, our results (Table 1) show that a significantly higher affinity is displayed by compounds **6a**, **11b**, **15a**, **16a**, and **16b** for ileum, and by compounds **3b**, **13a**, and **14a** for bladder.

Among them, compound **6a**, bearing a nitrogen enclosed in a 7-membered ring, and compound **16a**, bearing a phenethyl substituent, display a 10-fold selectivity for ileum. In particular, compound **16a** displays an interesting range of affinities for the muscarinic subtypes (pK_b = 4.80 for M_2; pA_2 = 5.27 for M_1; pA_2 = 5.82 for M_3 bladder; pA_2 = 6.97 for M_3 ileum) studied; this suggests that a further investigation of the complete muscarinic profile of this molecule on native and cloned binding studies should be made.

Experimental

Melting points were taken in glass capillary tubes on a Büchi SMP-20 apparatus and are uncorrected (Table 3). ^{1}H NMR spectra were recorded on a Varian Gemini 200 (200 MHz) spectrometer. Chemical shifts of compouds **2** and **15** are reported in parts per million (ppm) relative to tetramethylsilane (TMS). The NMR spectra data were obtained for all the other compounds reported and were consistent with the assigned structures. They are not included because they are very similar to the **2** and **15** spectra. The microanalyses of the final compounds were performed by the microanalytical laboratory of our department. The elemental compositions of the compounds agreed to within ± 0.4% of the calculated value and were carried out on a Fisons 1108 Elemental Analyzer. Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.040-0.063, Merck) by flash chromatography.

All the starting amines are commercially available except for 2-[(dimethylamino)methyl]piperidine which was synthesized according to the procedure described.[17] Analogously, amines **3** and **12**,[18] **4**, **5**, and **14**,[19] and **6**[20] were synthesized as previously reported.

General procedure for the preparation of the amines 2, 3, 5, 13, and 14. The procedure adopted for the synthesis of **2** is described. 2,2-Diphenyl[1,3]-dioxolane-4-chloromethyl[12] (2 g, 7.28 mmol) was added at 0 °C to a solution of azetidine (0.5 mL) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.42 g, 2.75 mmol) in anhydrous ether (50 mL). The reaction vessel was sealed and the mixture was left at 110 °C for 48 h. After filtration, the solution was concentrated in vacuum. The oily residue was purified on a silica gel flash chromatography eluting with $CHCl_3$:MeOH (98%:2%) to give **2** (0.4 g, 19% yield). ^{1}H NMR ($CDCl_3$) δ 7.27–7.54 (10H, m, Ar), 4.13 (1H, m, H_4), 4.10 (1H, t, H_{5a}), 3.78 (1H, t, H_{5b}), 3.28 (4H, t, NCH_2), 2.70 (1H, dd, CH_2N), 2.58 (1H, dd, CH_2N), 2.09 (2H, m, $CH_2CH_2CH_2$).

Similarly, **3**, **5**, **13**, and **14** were obtained in 18–25% yield starting from the appropriate amine. The free bases were transformed into the corresponding oxalate salts **2a**, **3a**, **5a**, **13a**, and **14a** by treating an ether solution with oxalic acid, and into methiodide **2b**, **3b**, **5b**, **13b**, and **14b** by treating an acetone solution with an excess of MeI.

General procedure for the preparation of the amine 4, 6-12, 15, and 16. The procedure for the synthesis of the amine **15** is described. Ethylchloroformate (0.51 g, 4.7 mmol) was added dropwise to a stirred and cooled (0 °C) solution of 2,2-diphenyl[1,3]-dioxolane-4-carboxylic acid[13] (1.3 g, 4.7 mmol) and triethylamine (0.49 g, 4.7 mmol) in dry $CHCl_3$ (100 mL) followed, after 30 min, by addition of a solution of the amine (0.6 g, 4.7 mmol), in dry $CHCl_3$ (10 mL). The resulting reaction mixture was stirred overnight at room temperature and then washed with NaOH 2 N (50 mL), and finally with water. Removal of dried solvent gave an oil that was purified by column chromatography. Eluting with cyclohexane:AcOEt (50%:50%) afforded **27** (0.6 g). ^{1}H NMR ($CDCl_3$) δ 7.17–7.52 (15H, m, Ar), 4.81 (1H, m, H_4), 4.70 (1H, m, H_{5a}), 4.39 (1H, m, H_{5b}), 4.17 (2H, m, NCH_2), 3.12 (3H, s, CH_3).

A solution of amide **27** (0.6 g, 1.6 mmol) in dry ether (15 mL) was added dropwise to a suspension of $LiAlH_4$ (0.3 g, 7.9 mmol) in dry ether (20 mL), and the reaction mixture was heated at reflux for 6 h. After cooling (0 °C), 0.3 mL of H_2O, 0.3 mL of NaOH 5 N, and again 1.5 mL of H_2O were sequentially added. After filtration, the organic solution was dried over Na_2SO_4. Removal of solvent gave an oil which was purified by column chromatography. Eluting with cyclohexane:AcOEt (80%:20%) afforded **15** (0.4 g, 23%). ^{1}H NMR ($CDCl_3$)

Table 3. Chemical-physical characteristics of the compounds examined

Compd	mp °C	Recrytn solvent	Formula[a]
2a	170–171	EtOH	$C_{19}H_{21}NO_2.H_2C_2O_4$
2b	129–130	2-PrOH	$C_{19}H_{21}NO_2.CH_3I$
3a	209–211	EtOH	$C_{20}H_{23}NO_2.H_2C_2O_4$
3b	195–198	2-PrOH	$C_{20}H_{23}NO_2.CH_3I$
4a	204–206	EtOH	$C_{21}H_{25}NO_2.H_2C_2O_4$
4b	158–160	2-PrOH	$C_{21}H_{25}NO_2.CH_3I$
5a	208–210	2-PrOH/Etere	$C_{20}H_{23}NO_3.H_2C_2O_4$
5b	194–197	2-PrOH/Etere	$C_{20}H_{23}NO_3.CH_3I$
6a	173–174	2-PrOH	$C_{22}H_{27}NO_2.H_2C_2O_4$
6b	185–186	2-PrOH	$C_{22}H_{27}NO_2.CH_3I$
7a	141–143	2-PrOH	$C_{22}H_{27}NO_2.H_2C_2O_4$
7b	182–185	2-PrOH	$C_{22}H_{27}NO_2.CH_3I$
8a	150–152	2-PrOH	$C_{22}H_{27}NO_2.H_2C_2O_4$
8b	184–186	2-PrOH	$C_{22}H_{27}NO_2.CH_3I$
9a	197–198	EtOH	$C_{22}H_{27}NO_2.H_2C_2O_4$
9b	186–188	2-PrOH	$C_{22}H_{27}NO_2.CH_3I$
10a	158–160	2-PrOH	$C_{23}H_{29}NO_2.H_2C_2O_4$
10b	143–145	2-PrOH	$C_{23}H_{29}NO_2.CH_3I$
11a	100–103	EtOH	$C_{24}H_{32}N_2O_2.2H_2C_2O_4$
11b	194–197	2-PrOH	$C_{24}H_{32}N_2O_2.CH_3I$
12a	215–218	EtOH	$C_{21}H_{26}N_2O_2.H_2C_2O_4$
12b	213–216	EtOH	$C_{21}H_{26}N_2O_2.CH_3I$
13a	148–150	EtOH	$C_{19}H_{23}NO_2.H_2C_2O_4$
13b	163–165	2-PrOH	$C_{19}H_{23}NO_2.CH_3I$
14a	158–160	2-PrOH	$C_{20}H_{25}NO_2.H_2C_2O_4$
14b	121–124	2-PrOH	$C_{20}H_{25}NO_2.CH_3I$
15a	137–138	EtOH	$C_{24}H_{25}NO_2.H_2C_2O_4$
15b	172–173	EtOH	$C_{24}H_{25}NO_2.CH_3I$
16a	160–162	EtOH	$C_{25}H_{27}NO_2.H_2C_2O_4$
16b	227–228	EtOH	$C_{25}H_{27}NO_2.CH_3I$

[a]Analyses for C, H, N were within ±0.4 of the theoretical values.

δ 7.23–7.58 (15H, m, Ar), 4.39 (1H, m, H_4), 4.12 (1H, m, H_{5a}), 3.80 (1H, m, H_{5b}), 3.59 (2H, q, NCH_2), 2.74 (1H, dd, CH_2N), 2.55 (1H, dd, CH_2N), 2.29 (3H, s, CH_3).

Similarly, **4**, **6–12**, and **16** were obtained in 20–44% yields starting from the appropriate amine. The free bases were transformed into the corresponding oxalate salts **4a**, **6a–12a**, **15a**, and **16a** by treating an ether solution with oxalic acid, and into methiodide **4b**, **6b–12b**, **15b**, and **16b** by treating an acetone solution with an excess of MeI.

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 and the organs required were set up rapidly under 1 g of tension in 20-mL organ baths containing physiological salt solution (PSS) kept 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. Two-centimeter portions of terminal ileum were taken at 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), $MgSO_4.7H_2O$ (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, discarding the first and taking the second as control.

Guinea pig bladder. A 2-mm-wide longitudinal strip of bladder from urethra to the apex of the bladder was cut, excluding the portion under the urethra orifice, and mounted in PSS (the same used for ileum) at 37 °C. Contractions were recorded isometrically. Tissues were equilibrated for 30 min (see procedure for ileum).

Guinea pig stimulated left atrium. The right and left atria were separately excised. Left atria were mounted in PSS (the same used for ileum) at 30 °C in a 20-mL organ bath and stimulated through platinum electrodes by square-wave pulses (1 ms, 1 Hz, 4–7 V). Tissues were equilibrated for 1 h and inotropic activity was recorded isometrically (see procedure for ileum).

Rabbit stimulated vas deferens. This preparation was set up according to Eltze.[21] Vasa deferentia were carefully dissected free of surrounding tissue and were divided into four segments, two prostatic portions of 1 cm and two epididymal ones of approximately 1.5 cm 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), glucose (11.1); 10^{-6} M yohimbine was included to block α_2-adrenoceptors. The solution was maintained at 30 °C and tissues were stimulated through platinum electrodes by square-wave pulses (2 ms, 0.1 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 dissociation constants. The results reported in Table 1 are expressed as $-\log K_b$ or pA_2 (see Results).

Statistical analysis. The results are expressed as mean ± SEM. Student's *t*-test was used to assess the statistical significance of the difference between two means.

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