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Dialkylaminoalkyl Esters of 2,2-Diphenyl-2-Alkylthioacetic Acids: A New Class of Potent and Functionally Selective Muscarinic Antagonists

S. Scapecchi,[a] P. Angeli,[b] S. Dei,[a] F. Gualtieri,[a]* G. Marucci,[b] R. Moriconi,[b] F. Paparelli,[b] M. N. Romanelli[a] and E. Teodori[a]

[a]*Dipartimento di Scienze Farmaceutiche, Università di Firenze, Via G. Capponi 9, I-50121 Firenze, Italy*
[b]*Dipartimento di Scienze Chimiche, Università di Camerino, Via S. Agostino 1, I-62032 Camerino (MC), Italy*

Abstract—The synthesis and pharmacological activity as muscarinic antagonists of a number of 2-alkylthio-2,2-diphenylacetic acid esters are reported. The compounds studied are potent muscarinic antagonists and many of them show from moderate to high selectivity toward M_2 or toward M_1 and M_2 receptors when tested on tissues but lack selectivity on five muscarinic human receptors (m1–m5) cloned and expressed in CHO-K1 cells. As a consequence, the compounds behave as functionally selective antagonists. Those showing M_2 selectivity appear to be good drug candidates for the treatment of cognitive disorders connected with central cholinergic deficit.

Introduction

In the cholinergic hypothesis of Alzheimer's disease[1] it has been estabilished that in this pathological state there is a reduced cholinergic activity and a reduction of acetylcholine release.[2]

A number of different strategies have been considered to restore the muscarinic cholinergic transmission within the central cortex, among them: a) the development of muscarinic agonists acting at central postsynaptic receptors (M_1),[3,4] and b) the development of muscarinic antagonists blocking the central presynaptic receptors (M_2) that modulate acetylcholine release.[4] It is important to remember that this approach would also be beneficial in other pathological states related to a cholinergic deficit like age-related cognitive disorders.[5]

The first strategy has been pursued by many industrial and academic groups, but the results have generally been disappointing, possibly because of the consequence of the side effects connected with muscarinic agonists.[6,7]

The second approach seems more physiological even though M_2 receptors are selectively lost in Alzheimer patients.[8] In fact, muscarinic M_2 receptor antagonists might be a further option for the treatment of Alzheimer-type dementia in addition to acetylcholine agonists and acetylcholinesterase inhibitors.[9]

Unfortunately, the muscarinic M_2 antagonists discovered to date, due to their poor penetration of the blood–brain barrier, have no activity in the central nervous system, after peripheral administration, even if they do enhance the release of acetylcholine when injected directly into the brain.[10,11] Only recently, Doods *et al.*[12] have described an M_2 receptor antagonist able to penetrate the brain (BIBN 99) and Gualtieri *et al.* have identified some putative M_2 antagonists able to increase the central level of acetylcholine and to produce analgesic and nootropic effects.[13,14]

In the course of continuing research directed to study the molecular requirements of the muscarinic ligands,[15–17] a program has been started to develop potent, selective and centrally acting ligands by molecular manipulation of classical muscarinic antagonists, usually very potent but lacking subtype selectivity. It was hoped that some M_2 selective antagonists could be identified that would be useful in the treatment of Alzheimer's disease and of the cognitive disorders characterized by a central cholinergic deficit.

We started from the consideration that, in the class of tricyclic muscarinic antagonists related to pirenzepine, several drugs endowed with very interesting subtype selectivity were found, such as pirenzepine itself (M_1), AF-DX 116 and AQ-RA 741 (M_2).[18–20] Although apparently different in molecular structure, these compounds can be considered as restricted-flexibility analogues of classical muscarinic antagonists like adiphenine, benactyzine and 4-DAMP (Chart I). These latter are potent muscarinic antagonists showing only moderate selectivity, if any, among subtypes of muscarinic receptors.[21,22]

Based on these considerations, a series of compounds (**1–43**) with general structure A (Chart II), has been designed and synthesized, where the conformational flexibility of classical muscarinic antagonists is sterically hindered by an increasingly large group that has been inserted on the carbon atom carrying the bulky lipophilic substituents known to drive the interaction with the receptors.[17]

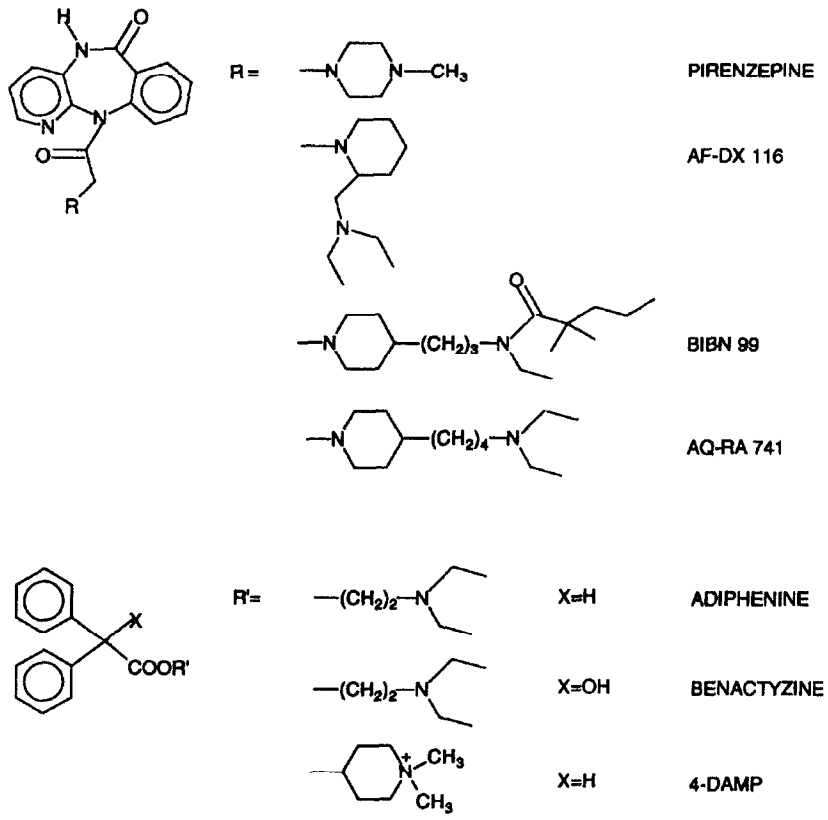


Chart I.

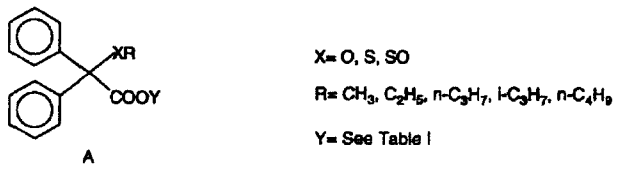


Chart II.

The choice of the sulfur atom to connect the quaternary carbon atom with the alkyl chain was made on the basis of previous work that had shown the utility of the presence of the sulfur atom in cholinergic ligands. As a matter of fact, when the 1-oxygen of the 1,3-dioxolane cholinergic ligands was substituted with a sulfur atom to give the corresponding 1,3-oxathiolanes, compounds with comparable or improved activity were found.[15] Moreover, oxidation of the sulfide group to a sulfoxide function would introduce into the molecule a potent hydrogen bonding group, equivalent to a C–OH group,[16] that has a role on the potency of the benactyzine-like antagonists.[23,24] The alkoxy derivatives (X = O) were synthesized for comparative purpose, while it was not necessary to prepare the corresponding alkyl derivatives (X = CH_2) as they had already been prepared and studied.[24]

In this paper the results of the research which has led to the discovery of a new class of potent muscarinic antagonists and to the identification of several compounds with strong selectivity for M_2 vs M_1 and M_3 or for M_2 and M_1 vs M_3 receptors are reported. Some of the results have already been disclosed in preliminary communications.[25,26]

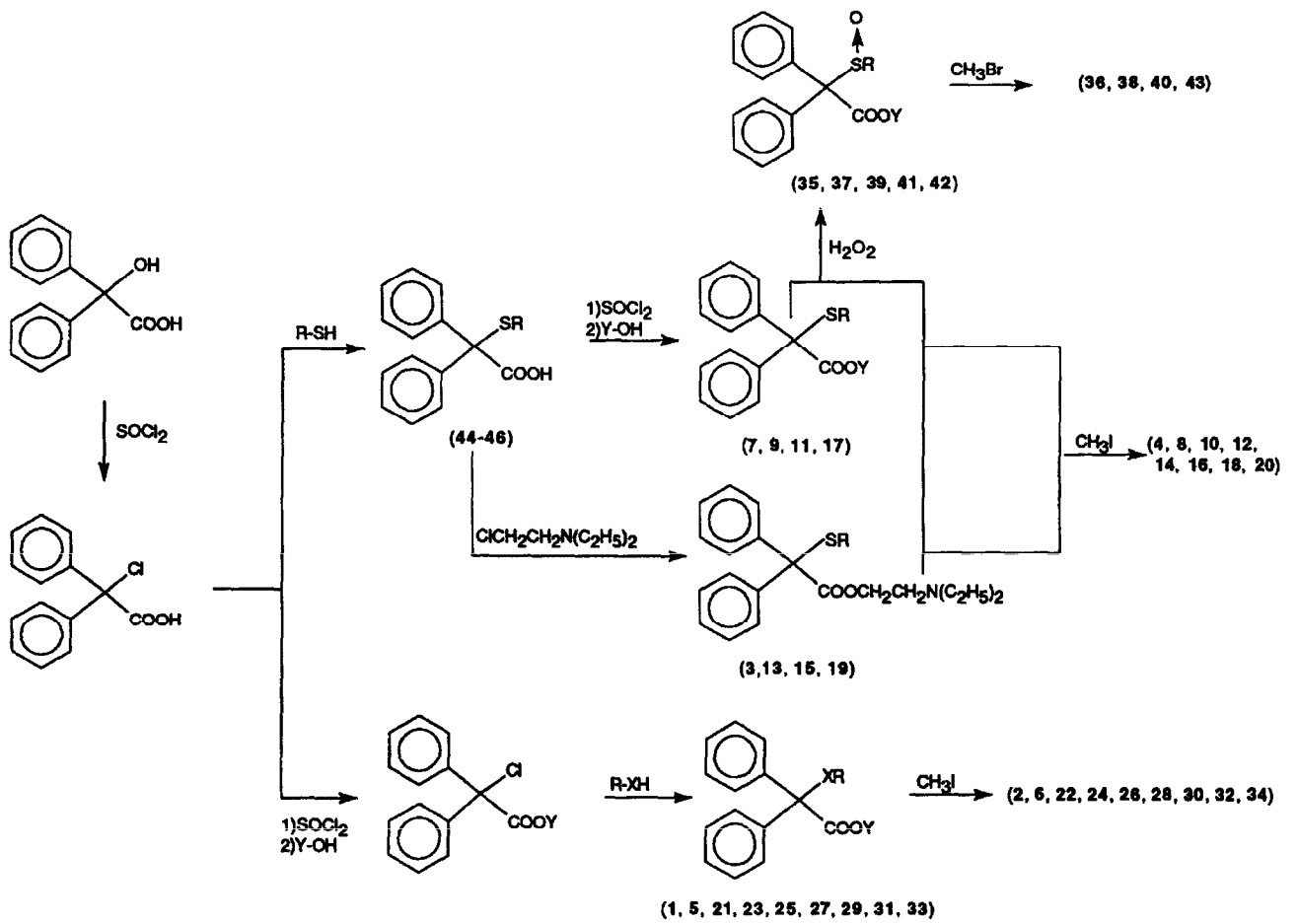
Chemistry

The reaction pathways used to synthesize the designed compounds are reported in Scheme I. Although the reactions used are standard, few comments are necessary to understand the rationale of the protocol followed.

2-Alkylthio and 2-alkoxy-2,2-diphenylacetic acids are quite hindered molecules and their esterification gives problems, especially with secondary alcohols like *N*-methyl-4-piperidinol and α-tropanol.

The commercially available 2,2-diphenyl-2-ethylthioacetic acid and its *n*-propyl (**44**), isopropyl (**45**) and *n*-butyl (**46**) analogs (already known but obtained with a new procedure), reacted smoothly with *N*,*N*-diethylaminoethylchloride to give the corresponding *N*,*N*-diethylaminoethyl esters (**3**, **13**, **15**, **19**). Other derivatives were obtained through the acylchlorides and the appropriate alcohol (**7**, **9**, **11**, **17**), but this method failed to give esters of *N*-methyl-4-piperidinol and α-tropanol.

To overcome this problem, solvolysis of 2,2-diphenyl-2-chloroacetic acid esters was performed. This approach easily yielded the alkoxy derivatives **21**, **23**, **25**, **27**, **29**, **31** and **33** among which are a few products of esterification with secondary alcohols (**23**, **27**, **29**, **33**). The method failed when mercaptans were used. In fact, only the ester of 2,2-diphenyl-2-ethylthioacetic acid with *N*-methyl-4-piperidinol (**5**) was obtained and not the corresponding esters with α-tropanol, an aminoalcohol moiety that proved very useful in another series of muscarinic antagonists.[13,14]



Scheme I.

On the other hand, solvolysis with methyl and ethyl mercaptans was the more convenient way to obtain compounds **1** and **5** respectively.

The reaction of the alkylthio derivatives with hydrogen peroxide went smoothly but the products were unstable as free bases and had to be quickly transformed into the salts reported in Table 3.

Quaternization with CH_3I gave no problems with alkylthio- and alkoxy- esters but afforded unworkable brown oily products when sulfoxide derivatives were used, probably because of a reduction of the sulfoxide function by the iodide. For this reason, CH_3Br was used and the ammonium salts reported in Table 3 are therefore methyl bromides.

Results

The functional antimuscarinic activity of the compounds on three receptor models M_1 (rabbit vas deferens), M_2 (guinea-pig atria, force), M_3 (guinea-pig ileum) is reported in Tables 1–3.

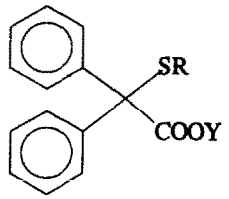
In general, the data are expressed as pK_b which represents the $-\log K_b$ obtained from the expression log(DR-1) = log[ant]–log K_b at a given concentration of the antagonist. For the compounds that were more interesting due to their properties or for comparative reasons, the value reported is the pA_2 obtained according to Schild. In this case, the data carry the supplementary information that (at least at the concentration values spanned by the dose used) the compounds behave as competitive antagonists. The Schild plots of such compounds (**3–6** and **25–28**) on the tissues used are reported in Figures 1a and 1b.

In Figures 2 and 3 are graphically reported the variation of the antagonist potency (pK_b or pA_2) for iodomethylate (a) and tertiary bases (b) as a function of the size of the alkyl group (Fig. 2) or as a function of the variation of the aminoalkyl moiety (Fig. 3).

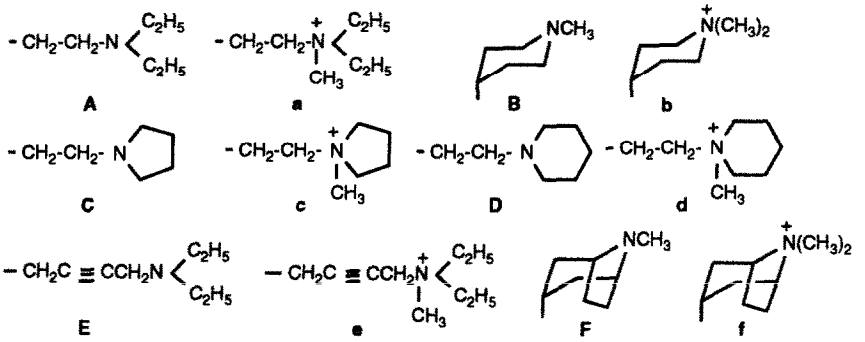
Structure–Activity relationships

The amount of the data collected and the number of variations considered defy a complete and meaningful analysis. For this purpose, QSAR methods such as PLS and cluster analysis seem more useful and work is in progress to examine the whole set of data by these methods.

Table 1. Chemical–physical characteristics, functional pharmacological activity[a] and subtype selectivity of 2,2-diphenyl-2-alkylthioacetic acid esters



Y=

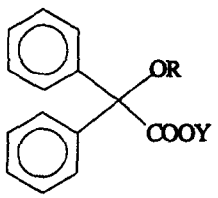


N	R	Y	Salt/Anion [b]	M p °C [c] (Rec. Solv.)	M_1 [d]	M_2 [e]	M_3 [f]	M_1/M_3 [g]	M_1/M_2 [g]	M_2/M_3 [g]
1	CH_3	A	Hydrochloride	102-103 (B)	-	7.54(0.08)	7.07(0.14) [h]	-	-	3.0
2	CH_3	a	I^-	120-122 (A)	7.66(0.10)	7.11(0.04) [i]	7.04(0.08)	1.2	3.6	4.2
3	C_2H_5	A	Hydrochloride	107-110 (B)	7.79(0.01) [i]	7.93(0.03) [i]	5.88(0.09) [m]	81	0.7	112
4	C_2H_5	a	I^-	134-135 (A)	8.97(0.06) [i]	9.12(0.03) [i]	6.50(0.07) [i]	295	0.7	417
5	C_2H_5	B	Hydrochloride	217-219 (B)	8.82(0.03) [i]	8.56(0.01) [i]	6.36(0.05) [i]	288	2.0	158
6	C_2H_5	b	I^-	190-191 (A)	9.30(0.04) [i]	9.62(0.05) [i]	7.92(0.03) [i]	24	0.5	50
7	C_2H_5	C	Oxalate	163-164 (B)	6.46(0.10) [n]	7.31(0.03) [i]	6.20(0.02) [h]	1.8	0.14	13
8	C_2H_5	c	I^-	192-194 (A)	6.78(0.20) [m]	8.10(0.05)	6.60(0.04) [h]	1.5	0.05	32
9	C_2H_5	D	Oxalate	120-123 (B)	-	6.34(0.06) [h]	5.81(0.06) [h]	-	-	5
10	C_2H_5	d	I^-	160-161 (A)	6.17(0.03) [m]	7.79(0.09)	6.29(0.06) [h]	0.8	0.02	37
11	C_2H_5	E	Oxalate	91-95 (B)	6.40(0.12)	6.36(0.08)	< 6	-	1.0	-
12	C_2H_5	e	I^-	155-157 (A)	< 6	6.84(0.14)	6.08(0.02) [m]	-	-	6.0
13	$(CH_2)_2CH_3$	A	Oxalate	116-118 (B)	7.24(0.22)	7.59(0.17) [i]	6.47(0.10) [h]	6.0	0.4	13
14	$(CH_2)_2CH_3$	a	I^-	120-122 (A)	7.76(0.11) [m]	8.53(0.15)	7.69(0.06) [h]	1.2	0.2	7.0
15	$CH(CH_3)_2$	A	Hydrochloride	110-112 (B)	6.59(0.11)	7.70(0.07) [i]	5.70(0.03) [h]	7.8	0.08	100

N	R	Y	Salt/Anion [b]	M p °C [c] (Rec. Solv.)	M_1 [d]	M_2 [e]	M_3 [f]	M_1/M_3 [g]	M_1/M_2 [g]	M_2/M_3 [g]
16	$CH(CH_3)_2$	a	I^-	160-161 (A)	8.22(0.08)	8.24(0.07) [l]	6.69(0.08) [h]	34	1.0	35
17	$CH(CH_3)_2$	E	Hydrochloride	98-100 (B)	7.20(0.11)	7.33(0.16)	6.59(0.16)	4.0	0.7	6.0
18	$CH(CH_3)_2$	e	I^-	119-121 (A)	8.05(0.12)	7.61(0.01)	6.77(0.04)	19	3.0	8.0
19	$(CH_2)_3CH_3$	A	Hydrochloride	89-91 (B)	6.48(0.20) [h]	6.92(0.29)	6.14(0.15) [h]	2.0	0.3	6.0
20	$(CH_2)_3CH_3$	a	I^-	106-108 (A)	7.00(0.10) [m]	8.75(0.13) [i]	6.90(0.10) [h]	1.3	0.02	71

a) -log K_b calculated from the equation log(DR-1) = log[ant] - logK_b at the concentration of 10^{-6}M unless otherwise stated. Number of replications from 5 to 7. SEM in brackets.
b) All compounds were analyzed for C, H, N and the results are within 0.4 % of the theoretical values. IR and NMR spectra are in accord with the proposed structure.
c) Recrystallization solvents: A-abs. ethanol; B=abs. ethanol+anhydrous ether.
d) Rabbit vas deferens.
e) Guinea pig atria (force).
f) Guinea pig ileum.
g) Antilog of the difference between the pK_b (or pA_2) values for M_1, M_2 and M_3 muscarinic receptor subtypes.
h) -log K_b calculated at the concentration of 3×10^{-6}M.
i) -log K_b calculated at the concentration of 3×10^{-7}M.
l) pA_2 calculated from the Schild correlation constrained to n=1. Number of replications from 5 to 7.
m) -log K_b calculated at the concentration of 10^{-5}M.
n)-log K_b calculated at the concentration of 10^{-7}M.
*) At this concentration the compound gives a reduction of the maximum effect of the agonist.

Table 2. Chemical–physical characteristics, functional pharmacological activity[a] and subtype selectivity of 2,2-diphenyl-2-alkoxy acetic acid esters

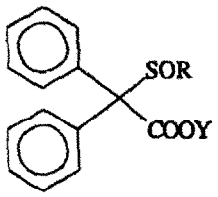


N	R	Y	Salt/Anion [b]	M p °C [c] (Rec. Solv.)	M_1 [d]	M_2 [e]	M_3 [f]	M_1/M_3 [g]	M_1/M_2 [g]	M_2/M_3 [g]
21	CH_3	A	Hydrochloride	117-119 (B)	6.41(0.07)	6.82(0.04)	6.21(0.11)	1.6	0.4	4.1
22	CH_3	a	I^-	109-111 (A)	7.13(0.21)	7.32(0.10)	6.90(0.02)	1.7	0.6	2.6
23	CH_3	B	Hydrochloride	216-220 (B)	7.73(0.18)	7.59(0.11)	7.09(0.09)	4.3	0.7	3.1
24	CH_3	b	I^-	106-208 (A)	8.53(0.18)	7.78(0.17)	8.06(0.06)	3.0	6.0	0.5
25	C_2H_5	A	Hydrochloride	152-154 (B)	6.34(0.06) [l]	6.57(0.08) [l]	5.87(0.09) [m*]	3.0	0.6	5.0
26	C_2H_5	a	I^-	141-143 (A)	7.41(0.01) [l]	6.79(0.02) [l]	7.20(0.06) [l]	2.0	4.0	0.4
27	C_2H_5	B	Hydrochloride	168-172 (B)	7.64(0.03) [l]	7.03(0.10) [l]	6.96(0.04) [l]	5.0	4.0	1.0
28	C_2H_5	b	I^-	239-241d (A)	7.21(0.10) [l*]	7.29(0.04) [l]	7.44(0.04) [l]	0.6	0.8	0.7

N	R	Y	Salt/Anion [b]	M p °C [c] (Rec. Solv.)	M_1 [d]	M_2 [e]	M_3 [f]	M_1/M_3 [g]	M_1/M_2 [g]	M_2/M_3 [g]
29	C_2H_5	F	Hydrochloride	203-205 (B)	7.75(0.18)	7.68(0.09)	6.76(0.17)	10	5.0	8.3
30	C_2H_5	f	I^-	217-219d (A)	9.53(0.19) [m*]	8.59(0.07)	8.61(0.09)	8.0	9.0	0.6
31	$CH(CH_3)_2$	A	Hydrochloride	158-160 (B)	6.93(0.20)	7.15(0.09)	6.94(0.20)	1.0	0.6	1.6
32	$CH(CH_3)_2$	a	I^-	198d (A)	7.76(0.20)	7.96(0.03)	7.60(0.20)	1.5	0.6	2.3
33	$CH(CH_3)_2$	B	Oxalate	77-80 (B)	8.00(0.20)	6.59(0.08)	6.66(0.07)	22	25	0.2
34	$CH(CH_3)_2$	b	I^-	228-230 (A)	8.53(0.30)	8.04(0.06)	7.86(0.10)	5.0	3.0	1.5

[a-n] See the corresponding footnotes in Table 1. For the meaning of Y see Table 1.

Table 3. Chemical–physical characteristics, functional pharmacological activity[a] and subtype selectivity of 2,2-diphenyl-2-alkylsulphinil acetic acid esters



N	R	Y	Salt/Anion [b]	M p °C [c]	M_1 [d]	M_2 [e]	M_3 [f]	M_1/M_3 [g]	M_1/M_2 [g]	M_2/M_3 [g]
35	CH_3	A	Citrate	65-66	6.92(0.07) [m]	6.94(0.04)	6.64(0.10) [h]	1.9	1.0	2.0
36	CH_3	a	Br^-	52-54	N.T.	8.62(0.09)	8.42(0.02) [i]	-	-	2.0
37	C_2H_5	A	Citrate	58-62d	N.T.	7.29(0.07)	6.70(0.15) [h]	-	-	4.0
38	C_2H_5	a	Br^-	51-53	N.T.	8.64(0.06)	8.51(0.09)	-	-	1.3
39	C_2H_5	D	Dibenzoyltartrate	104-106	6.53(0.07) [m]	6.52(0.11)	6.48(0.06) [m, h]	1.1	1.0	1.1
40	C_2H_5	d	Br^-	55-56	N.T.	7.47(0.07)	6.56(0.08)	-	-	8.0
41	C_2H_5	C	Dibenzoyltartrate	100-101	6.59(0.05) [m]	6.97(0.12)	6.48(0.10) [h]	1.3	0.4	7.0
42	$CH(CH_3)_2$	A	Dibenzoyltartrate	65-67	8.01(0.04)	8.09(0.07)	7.34(0.06) [h]	4.7	0.8	6.0
43	$CH(CH_3)_2$	a	Br	51-53	N.T.	7.94(0.06)	7.23(0.03) [h]	-	-	5.0

[a-m] See the corresponding footnotes in Table 1. N.T.: not tested. For the meaning of Y see Table 1.

In the following sections, the most evident relationships that can be deduced from the data by a qualitative point of view are discussed. Although far from being exhaustive they nevertheless allow some useful preliminary conclusions.

Variation of the heteroatom (X)

The alkoxy compounds (Table 2) show low potency and no remarkable selectivity on all three receptor models examined. In contrast, the alkylthio compounds (Table 1),

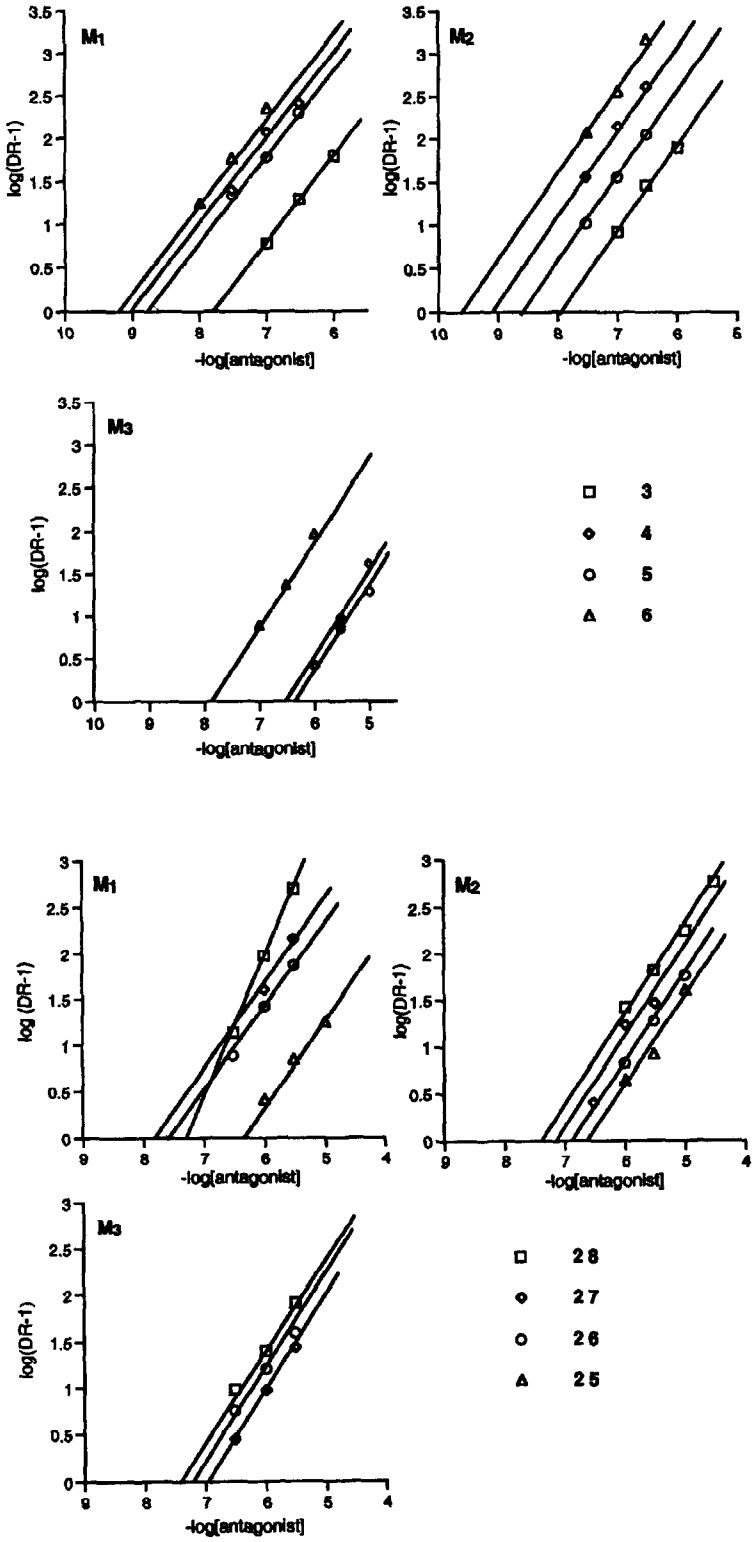
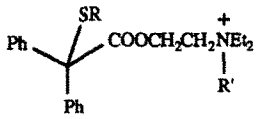


Figure 1. Schild plots obtained for the 2-ethoxy derivatives (25–28); (a) and the 2-ethylthio derivatives (3–6); (b) on rabbit vas deferens and guinea pig heart and ileum. The points are the means of five to seven experiments. S.E.M.s are not reported for clarity and are less than 10 %. Ordinate: log(DR-1) where DR = EC_{50} ratio measured from the displacement of the agonist concentration–response curves by the antagonist. Abscissa: -log[antagonist]. The correlation concerning compounds **3** and **25** on M_3 receptor are not reported since, as specified in Table 1, on this tissue the compounds reduce the maximum effect of the agonist.

while maintaining a low potency on M_3 receptor, have, in general, a much higher potency on M_1 and M_2 receptors. As a consequence, the alkylthio derivatives show an increased, and in some cases remarkable, subtype selectivity. This is particularly evident when the 2-ethylthio (**3–6**) and the 2-ethoxy derivatives of 2,2-



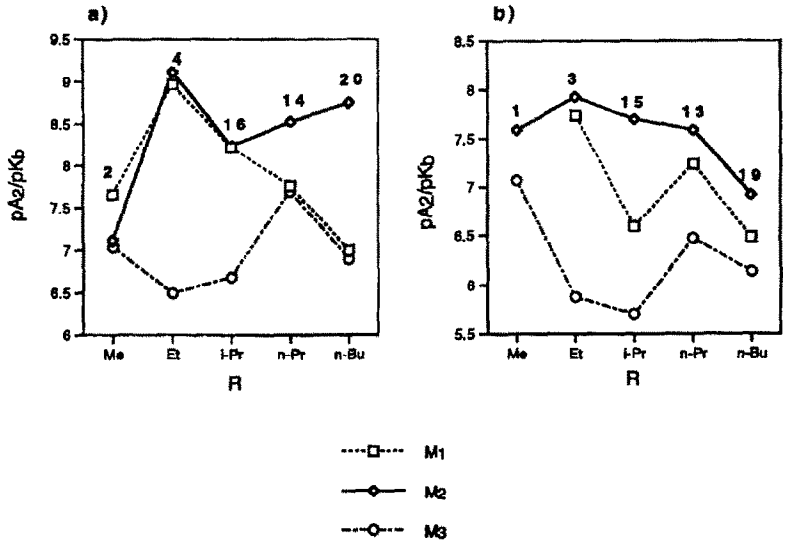
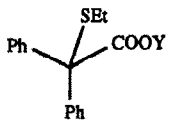


Figure 2. Variation of the potency (pA_2 or pK_b) of *N,N*-diethylaminoethyl esters of 2,2-diphenyl-2-alkylthioacetic acid with the size of the alkyl group. a) Methiodides (R' = Me). b) Tertiary bases (R' = H).



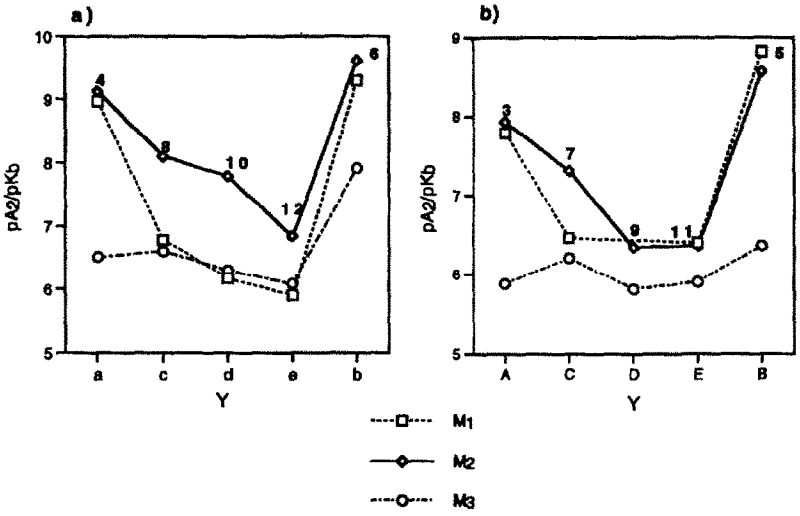


Figure 3. Variation of the potency (pA_2 or pK_b) of esters of 2,2-diphenyl-2-ethylthioacetic acid with the aminoalcohol moiety. a) Methiodides. b) Tertiary bases (for the meaning of Y, see Table 1).

diphenylacetic acid (**25–28**) are considered.[25] The sulfur compounds show selectivities toward M_1 and M_2 receptors that in the case of compounds **4** and **5** are as high as 417 (M_2/M_3) and 288 (M_1/M_3) respectively.

Variation of the alkyl chain (R)

The variation of the alkyl chain in the alkoxy derivatives has modest consequences both in terms of potency and

selectivity which, with the exception of compounds **30** for potency and **33** for selectivity, remain quite low.

On the contrary, in the sulfur derivatives, as shown in Figure 2, both potency and selectivity vary considerably with the size of the alkyl group. So, when quaternary compounds are considered (Fig. 2a), compounds **4** (R = C_2H_5) and **16** (R = *i*-C_3H_7) show high affinity and high selectivity for M_1 and M_2 receptors, while compound **20** (R = *n*-C_4H_9) shows higher affinity for M_2 with respect to M_1 and M_3 receptors. In the tertiary base series (Fig. 2b), compound **3** (R = C_2H_5) is selective for M_1 and M_2 receptors while compound **15** (R = *i*-C_3H_7) seems able to discriminate M_1, M_2 and M_3 subtypes. As expected, the ammonium salts show generally higher potency with respect to the corresponding tertiary bases.[27] As a general trend, alkyl groups larger than methyl seem to impair proper fit to the M_3 receptor.

Variation of the aminoalcohol moiety (Y)

The variations of the potency with respect to the variation of the aminoalcohol, in the series of 2,2-diphenyl-2-ethylthioacetic acid esters are reported in Figures 3a and 3b.

For both the quaternary (3a) and tertiary (3b) series, the *N,N*-diethylaminoethanol and *N*-methyl-4-piperidinol esters show the highest affinity and selectivity toward M_1 and M_2 receptors (compounds **3, 4, 6** and **5**, respectively).

Quite interestingly, the ammonium salts of 2-(*N*-pirrolidinyl) and 2-(*N*-piperidinyl)ethyl esters (3a; **8** and **10**), although less potent than **4** and **6**, display higher affinity for M_2 compared to M_1 and M_3 receptors, a feature that is only partially present in the tertiary base **7** and absent in **9** (3b).

Sulfoxide derivatives (X = SO)

The oxidation of sulfur to the corresponding sulfoxide does not give interesting results. The trend towards increasing potency with the size of the alkyl group shown by the tertiary bases **35**, **37** and **42** is contradicted by the corresponding ammonium salts **36**, **38** and **43**. However, the differences are small except for the case of **36** which is much more potent than the tertiary base **35**.

The oxidized compounds do not maintain the high selectivity shown by the corresponding sulfides (compare **3** with **37** and **4** with **38**); this seems due to the fact that sulfoxides, compared to the corresponding sulfides, have larger affinities for the M_3 receptors.

Discussion

The most interesting result obtained from this research is the high subtype selectivity of some of the compounds studied. Therefore, compounds **3–6** and **16** are able to discriminate M_1 and M_2 receptors from the M_3 type, with ratios that range from 24 to 417. Compound **15** is able to discriminate among M_1, M_2 and M_3 receptors with ratios of 7.8 (M_1/M_3), 12.5 (M_2/M_1) and 100 (M_2/M_3), respectively (see Table 1 and Fig. 2b). More interestingly, compounds **8**, **10**, **20** and to a lesser extent **7**, are selective for M_2 receptors, a property that, as already discussed, seems useful for the treatment of Alzheimer's and Alzheimer's-like pathologies.

However it must be acknowledged that while **7**, which is a tertiary base, can be expected to readily cross the blood–brain barrier (BBB), compounds **8**, **10** and **20** carry an ammonium function that usually prevents CNS penetration. Although there are indications that their lipophilicity is unusually high[28] for ammonium compounds (e.g. they are readly soluble in $CHCl_3$), further studies which are in progress will be necessary to check if this is sufficient for brain penetration.

All the discriminating compounds belong to the 2-alkylthio series of 2,2-diphenylacetic acid esters. The 2-alkoxy and the 2-sulfinyl derivatives show no selectivity and 2-alkyl derivatives[29] are also devoid of any subtype selectivity.

Therefore, our hypothesis that the steric lock imposed on the molecules by the 2-substituents would be responsible for their subtype selectivity is no longer tenable. This emphasizes the role of the sulfur atom in giving high selectivity towards M_1 and M_2 receptors to this series of muscarinic antagonists. Theoretical calculations of the properties conferred by sulfur to the molecules seem to offer an explanation of the properties of these compounds; in fact sulfur seems to interact more favourably than oxygen with aromatic amino acid residues, which could explain at least the higher activity of the alkylthio compounds compared to the alkoxy ones. The results of this work will be referred to in a forthcoming paper.[30]

In order to further study the pharmacological profile of the series, the affinity with respect to five cloned human muscarinic receptors (m1–m5) of a few selected compounds was studied on receptors stably expressed in Chinese hamster ovary cells (CHO-K1); affinity estimates were derived from labelled *N*-methylscopolamine ([^{3}H] NMS) displacement experiments, while specific binding was determinated as that displaced by 1 μM atropine (Table 4).[26] Disappointingly, it was found that the high selectivity shown by **3–6** on functional models completely disappears on cloned receptors. Moreover, the inhibition curves of some of the muscarinic receptors tested were not consistent with a competitive antagonism.

There are several things that can explain the discrepancy observed between functional and binding studies. For example, the compounds may not be completely competitive antagonists. The curves presented in Fig. 1 show that all compounds examined behave as competitive antagonists in the concentration range studied (with the exception of compounds **3** and **25** at M_3 and compound **28** at M_1 subtypes). However, the range of concentrations used is quite narrow (about 1 log unit) and therefore a further and higher concentration has been tested. In Figures 4 and 5 are reported the results obtained with compound **4** (one of the most discriminating drugs studied) on vas

deferens (M_1) and heart (M_2) tissues when a concentration of 3×10^{-6} M is added to the Schild correlations. It is apparent that in both cases (Figs 4b and 5b) the Schild correlations, based on four points, deviate from linearity suggesting a noncompetitive behaviour of the drug when concentrations higher than 10^{-6} M are considered. These results are confirmed by the analysis of the agonist–dose response curves of Figures 4a and 5a according to Pöch[31] (data not shown). There is a significant reduction of I_{max} of the antagonist dose–response curves derived from the curves of 4a and 5a respectively, by vertical evaluation at a fixed dose ratio (DR).

These experiments suggest a competitive type of interaction at low concentrations and a noncompetitive interaction (possibly allosteric) at high concentrations that might be responsible for the different results obtained with functional and binding experiments. It may be worthwhile

Table 4. Binding parameters[a] of selected compounds (3, 4, 5, 6) at five human muscarinic receptor subtypes expressed in CHO-K1 cells

N	m1	m2	m3	m4	m5
3	6.94(0.92)	6.24(0.94)	6.27(1.07)	6.47(0.80)	6.30(1.03)
4	7.31(0.83)	6.80(0.75)[b]	6.69(0.95)	6.72(0.82)	6.59(0.96)
5	7.86(0.95)	6.77(1.02)	7.54(1.04)	7.34(0.90)	7.57(0.96)
6	8.59(0.66)[b]	7.43(0.88)	8.28(0.95)	8.03(0.86)	8.07(0.97)

[a]The affinity estimates were derived from [^{3}H]NMS desplacement experiments and represent the mean for the negative logarithm of the K_i. Hill coefficients are given in parentheses.
[b]Hill coefficient significantly different from unity ($p<0.05$). For statistical analyses and more details see ref. 26.

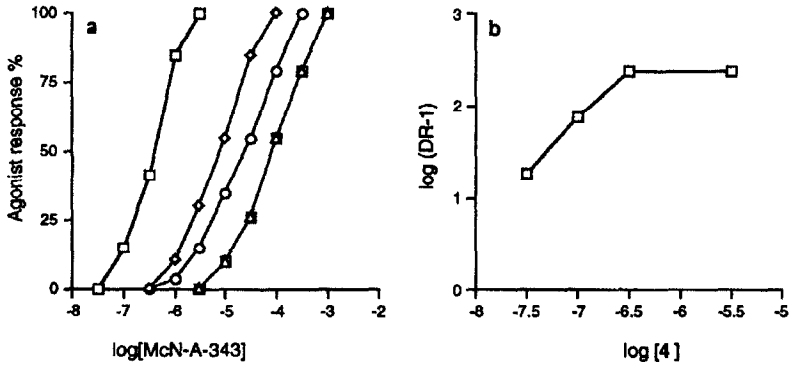


Figure 4. a) Experimental dose–response curves (DRCs) of McN-A-343 in the absence (❑) and presence of 0.03 (◆), 0.1 (○), 0.3 (△) and 3 (▦) μM compound 4 . The last two curves (0.3 and 3 μM) are perfectly superimposable. b) Schild-plot derived from the agonist DRCs in (a).

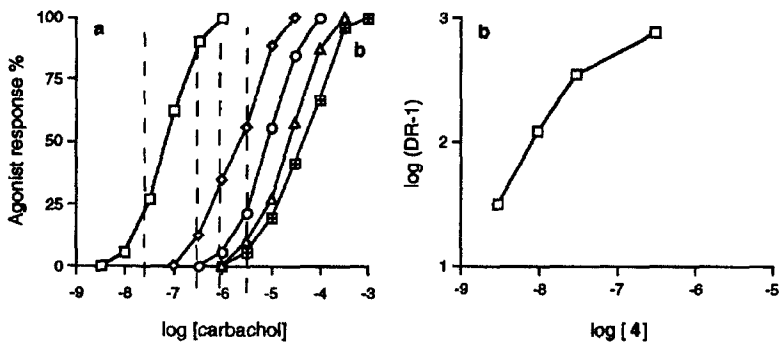


Figure 5. a) Experimental dose–response curves (DRCs) of carbachol in the absence (❑) and in presence of 0.03 (◆), 0.1 (○), 0.3 (△) and 3 (▦) μM compound 4. b) Schild-plot derived from the agonist DRCs in (a).

to remember that a similar behaviour is typical of several muscarinic antagonists,[32] even if in many cases the allosteric component does not seem to influence the subtype selectivity in binding studies.

Another possible explanation of the discrepancy observed might be due to the ability of the compounds to inhibit components which are involved in the response to receptor activation, other than the receptor itself, with the component linked to M_2 and M_3 receptors being different (e.g. the compounds might block a potassium channel linked to M_2 but not to M_3 receptor). The nature of such a component might also be tissue dipendent. Work is in progress to verify this hypothesis.[33]

In conclusion, a new series of compounds has been developed that show high subtype selectivity in functional studies but lack it in binding studies on cloned receptors. These compounds may be more properly defined as functionally selective compounds. As a consequence, in order to develop them as central acting compounds, it will be necessary to prove that they can be delivered into the CNS and that their selectivity can be verified in central tissues.

Experimental Section

All melting points were taken on a Büchi apparatus and are uncorrected. Infrared spectra were recorded with a Perkin-Elmer 337 spectrophotometer in KBr discoid or in Nujol mull for solid or neat for liquids. NMR Spectra were recorded on a Varian GEMINIZOO. Chromatographic separations were performed on silica gel column (Kieselgel 4, 0.063–0.200 mm, Merck). The analytical results are within ± 0.4 % of the theoretical values.

2,2-Diphenyl-2-propylthioacetic acid (44)

2,2-Diphenyl-2-chloroacetic acid, obtained according to Stollé[34] (0.7 g; 2.84 mmol) and $CaCO_3$ (0.28 g; 2.84 mmol) were mixed with an excess of propyl mercaptan (15 mL) and refluxed for 30 h. After cooling, $CHCl_3$ was added, the $CaCO_3$ filtered away and the solvent evaporated to give the acid as a white solid. Yield 95 %. Mp 107–110 °C. IR (neat) ν = 1740 (CO), 3100 (OH) cm^{-1}. ^1H NMR($CDCl_3$) δ 0.91 (t, 3H, S-CH_2-CH_2-CH_3); 1.44–1.56 (m, 2H, S-CH_2-CH_2-CH_3); 2.41 (t, 2H, S-CH_2-CH_2-CH_3); 7.26–7.47 (m,10H, aromatics) ppm. Anal. ($C_{17}H_{18}O_2S$) C, H, N.

The isopropyl (**45**)[35] and *n*-butyl (**46**)[36] analogs were obtained in the same way. Their characteristics correspond to those reported in the literature.

2,2-Diphenyl-2-ethylthioacetic acid N,N*-diethylaminoethyl ester (3)*

2,2-Diphenyl-2-ethylthioacetic acid (0.5 g; 1.8 mmol) was refluxed with *N,N*-diethylaminoethyl chloride (0.42 g; 3.6 mmol) in anhydrous $CHCl_3$ (15 mL) for 15 h. The solution was then added with 10 % Na_2CO_3 and extracted with $CHCl_3$. Evaporation of the solvent gave the product as a thick oil. Yield 86 %. IR (neat) ν = 1740 (CO) cm^{-1}. ^1H NMR($CDCl_3$) δ: 0.94 (t, 6H, N(CH_2-CH_3)$_2$); 1.10 (t, 3H, S-CH_2-CH_3); 2.37 (q, 2H, S-CH_2-CH_3); 2.46 (q, 4H, N(CH_2-CH_3)$_2$); 2.66 (t, 2H, O-CH_2-CH_2-N); 4.26 (t, 2H, O-CH_2-CH_2-N); 7.23–7.48 (m,10H, aromatics) ppm.

The oily product was transformed into the hydrochloride that was recrystallized from abs. ethanol and anhydrous ether. Mp 107–110 °C. Anal. ($C_{22}H_{30}ClNO_2S$) C, H, N.

Compounds **13**, **15** and **19** were obtained in the same way and their characteristics are reported in Table 1.

*2,2-Diphenyl-2-ethylthioacetic acid 2-(*N*-pyrrolidinyl)ethyl ester (7)*

2,2-Diphenyl-2-ethylthioacetic acid (1 g; 3.4 mmol) was converted into the corresponding acetylchloride using $SOCl_2$ (0.3 mL) in 15 mL of anhydrous benzene at 80 °C for 6 h. After removal of the solvent the raw acetylchloride was refluxed with 2-(*N*-pyrrolidinyl)ethanol (0.78 g; 6.8 mmol) in anhydrous CH_2Cl_2 (20 mL) for 24 h. After cooling the solution was treated with 10 % Na_2CO_3 and the organic layer anhydrified and evaporated under vacuum to give an oil that was purified by flash chromatography, using $CHCl_3$:petrol ether:abs. ethanol:conc. NH_4OH 341:60:65:8 as eluent. Yield 80 %. IR (neat) ν = 1740 (CO) cm^{-1}. ^1H NMR($CDCl_3$) δ: 1.10 (t, 3H, S-CH_2-CH_3); 1.65–1.75 (m, 4H, 2 NCH_2-CH_2); 2.30–2.45 (m, 6H, S-CH_2-CH_3; 2 NCH_2-CH_2); 2.66 (t, 2H, O-CH_2-CH_2-N); 4.30 (t, 2H, O-CH_2-CH_2-N); 7.23–7.48 (m,10H, aromatics) ppm.

The oily product was transformed into the oxalate that was recrystallized from abs. ethanol and anhydrous ether. Mp 163–164 °C. Anal ($C_{21}H_{25}NO_2S$) C, H, N.

Compounds **9**, **11**, **17** were obtained in the same way and their characteristics are reported in Table 1.

General procedure for synthesis of 2,2-diphenyl-2-chloroacetic acid esters

A solution of the appropriate alcohol (15 mmol) in anhydrous benzene (20 mL) was added to a solution of 2,2-diphenyl-2-chloroacetyl chloride (commercially available) (7.5 mmol) and the mixture left at rt for 12 h. The solid formed was filtered away and the solvent evaporated to dryness to give the ester as an oily compound, that was used in the following reaction (see Scheme I), without further purification.

2,2-Diphenyl-2-methoxyacetic acid N,N*-diethylaminoethyl ester (21)*

The 2,2-diphenyl-2-chloroacetic acid *N,N*-diethylamino-ethyl ester was refluxed in methanol for 48 h. The solvent was then evaporated to dryness to give the hydrochloride as a solid that was recrystallized from abs. ethanol and anhydrous ether. Yield 76 %. Mp 117–119 °C. IR (nujol)

ν = 1740 (CO) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.18 (t, 6H, NCH_2-CH_3); 2.71–2.89 (m, 4H, NCH_2-CH_3); 3.15 (s, 3H, OCH_3); 3.12–3.23 (m, 2H, OCH_2-CH_2N); 4.68–4.79 (m, 2H, OCH_2-CH_2N); 7.30–7.45 (m, 10H, aromatics) ppm. Anal. ($C_{21}H_{28}ClNO_3$) C, H, N.

Compounds **23**, **25**, **27**, **29**, **31** and **33** were obtained by the same procedure and their characteristics are reported in Table 2.

2,2-Diphenyl-2-methylthioacetic acid N,N-*diethylaminoethyl ester (1)*

2,2-Diphenyl-2-chloroacetic acid *N,N*-diethylaminoethyl ester (5.3 g) (0.015 mol) and 4.5 g (0.045 mol) of $CaCO_3$ were added to 15 mL of methylmercaptan in a steel bomb and kept at 80 °C for 5 days. After cooling, the excess of the mercaptan was carefully removed and the residue was purified by flash chromatography, using petrol ether:diethyl ether:dichloromethane:abs. ethanol:conc. NH_4OH 10:4:4:2:0.11 as eluent to give an oil that was transformed into the hydrochloride and recrystallized from abs. ethanol and anhydrous ether. Yield 44 %. Mp 102–103 °C. IR (nujol) ν = 1740 (CO) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.92 (t, 6H, $N(CH_2$-$CH_3)_2$); 1.90 (s, 3H, S-CH_3); 2.45 (q, 4H, $N(CH_2$-$CH_3)_2$); 2.65 (t, 2H, O-CH_2-CH_2-N); 4.25 (t, 2H, O-CH_2-CH_2-N), 7.27–7.45 (m,10H, aromatics), ppm. Anal. ($C_{21}H_{28}ClNO_2S$) C, H, N.

Compound **5** was obtained in the same way (Table 1).

2,2-Diphenyl-2-methylthioacetic acid N,N-*diethylaminoethyl ester sulfoxide (35)*

2,2-Diphenyl-2-methylthioacetic acid *N,N*-diethylaminoethyl ester (**1**) (0.33 g; 0.93 mmol) was dissolved in CH_3COOH (5 mL) and 0.15 mL of 30 % H_2O_2 (1.17 mmol) was added at –20 °C. The mixture was left stirring at rt for 12 h. Then the mixture reaction was cooled at 0 °C, made alkaline with 10 % NH_4OH solution and extracted with $CHCl_3$. The solvent was removed under vacuum, without heating, to give the corresponding sulfoxide as an oily compound. Yield 78 %. IR (neat) ν = 1740 (CO), 1060 (SO) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.98 (t, 6H, $N(CH_2$-$CH_3)_2$); 2.20 (s, 3H, S-CH_3); 2.40 (q, 4H, $N(CH_2$-$CH_3)_2$); 2.62 (t, 2H, O-CH_2-CH_2-N); 4.27 (t, 2H, O-CH_2-CH_2-N); 7.30–7.56 (m,10H, aromatics), ppm.

The product is quite unstable and must be quickly transformed into the citrate. Mp 65–67 °C. For the same reason the salt was not recrystallized. Anal. ($C_{21}H_{27}NO_3S$) C, H, N.

Compounds **37**, **39**, **41**, **42** were obtained in the same way. Their characteristics are reported in Table 3.

2,2-Diphenyl-2-methoxyacetic acid N,N-*diethylaminoethyl ester methyl iodide (22)*

Compound **21** (0.2 g) was dissolved in 20 mL of anhydrous ether, treated with an excess of CH_3I and left overnight at rt in the dark. The white crystals were collected and recrystallized from abs. ethanol. Mp 109–111 °C. Yield 90 %. IR (nujol) ν = 1740 (CO) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.20 (t, 6H, $N(CH_2$-$CH_3)_2$); 3.03 (s, 3H, NCH_3); 3.16 (s, 3H, OCH_3); 3.22 (q, 4H, $N(CH_2$-$CH_3)_2$; 3.82–3.98 (m, 2H, OCH_2-CH_2N); 4.63–4.77 (m, 2H, OCH_2-CH_2N); 7.30-7.43 (m, 10H, aromatics) ppm. Anal. ($C_{22}H_{30}INO_3$) C, H, N.

Compounds **2**, **4**, **6**, **8**, **10**, **12**, **14**, **16**, **18**, **20**, **24**, **26**, **28**, **30**, **32**, **34** were obtained in the same way. Their characteristics are reported in Tables 1 and 2.

2,2-Diphenyl-2-methylthioacetic acid N,N-*diethylaminoethyl ester sulfoxide methyl bromide (36)*

Compound **35** (0.2 g) was dissolved in 20 mL of anhydrous ether, added with an excess of CH_3Br and left overnight at rt. The slightly yellow crystals were collected, washed with anhydrous ether and used without recrystallization. Mp 52–54 °C (dec). Anal. ($C_{22}H_{30}BrNO_3S$) C, H, N.

Compounds **38**, **40** and **43** were obtained in the same way and their characteristics are reported in Table 3.

Pharmacology

Compounds **1–43** were tested for antimuscarinic activity on rabbit vas deferens (M_1), guinea-pig heart (M_2) and ileum (M_3) preparations.

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 appoximately 3-fold 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 2-channels 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. Portions of terminal ileum (2 cm long) were taken at about 5 cm from the ileum–caecum 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), $MgSO_4 \cdot 7\ H_2O$ (1.18), KH_2PO_4 (1.18), $CaCl_2$ (2.52), 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 a control.

Guinea-pig stimulated atria. The heart was rapidly removed and the right and left atria were separately excised. Left atria were mounted in PSS (the same used for ileum) at 30 °C and stimulated through platinum electrodes by square-wave pulses (1 ms, 1 Hz, 5–10 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. This preparation was set up according to Eltze.[37] Vasa deferentia were carefully dissected free of surrounding tissue and were divided into four segments, two prostatic portions of 1 cm and two epididymal portions, 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 alpha$_2$-adrenoceptors. The solution was maintained at 30 °C and tissue was stimulated through platinum electrodes by square-wave pulse (2 ms, 0.1 Hz, 10–15 V). Conctractions 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 dissociation constants expressed as $-\log K_b$ were calculated from the equation $\log(DR-1) = \log[ant] - \log K_b$. Dose ratios (DR; ratio of ED_{50} values of agonist after and before antagonist treatment) were calculated at the concentration indicated in the tables. The number of replications varied from 5 to 7.

Determination of pA_2. Dose ratios were calculated at three to four antagonist concentrations and each concentration was tested from five to seven times. pA_2 Values were extimated by Schild analysis[38] constraining the slope to –1 as required by the theory[39] (Fig. 1). When this method was applied, it was always verified that the experimental data generated a line whose derived slope was not significantly different from unity ($P > 0.05$).

Statistical analysis. The results are expressed as the means ± S.E.M. Student's t test was used to assess the statistical significance of the difference between two means.

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