SAR Studies on the Potent and Selective Muscarinic Antagonist 2-Ethylthio-2,2-diphenylacetic Acid *N,N*-Diethylaminoethyl Ester

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***Key Words**: Muscarinic antagonists; Alzheimer's disease; M_2 selectivity*

Summary

Molecular modification of the potent and selective muscarinic antagonist 2-ethylthio-2,2-diphenylacetic acid *N,N*-diethylaminoethyl ester was performed in order to identify M_2 selective antagonists able to cross the blood brain barrier and potentially useful in the treatment of Alzheimer's disease. Modifications included substitution or hydrogenation of one of the phenyl rings as well as their *incorporation* in a tricyclic system. In general the changes introduced were detrimental for both affinity and selectivity. Only a modest M_2 selectivity is present in some compounds that, on the other hand, carry a quaternary ammonium group which precludes their penetration into the brain.

Introduction

Recently we have reported on the synthesis and antimuscarinic properties of a new class of compounds derived from 2-alkylthio-2,2-diphenylacetic acids [1,2] . Some of these compounds are potent and selective muscarinic antagonists toward M_2 and toward M_1 and M_2 subtypes, but their selectivity was apparent only in functional tests and disappeared when tested on CHO-K1 cells expressed human muscarinic receptors [3].

Since their pharmacological behavior was fairly intriguing[4,5] we decided to explore further structure-activity relationships (SAR) in this class of compounds.

We were also encouraged in this extension of the work from the results of an analgesic test (hot-plate) performed on a selected number of such compounds (**I–VI**: Table 1). It was found that some of these compounds are endowed with antinociceptive activity comparable to that of *R*(+) hyoscyamine and probably due to a similar cholinergic mechanism [6,7]. It is interesting that analgesic activity seems to correlate with M_2 selectivity.

M_2 antagonists, by selectively blocking the central presynaptic autoreceptor (thought indeed to be of the M_2 subtype) [8], would enhance the release of ACh and as a consequence show cholinergic analgesia[9] and, what is much more interesting now, increase the central cholinergic tone with beneficial effects on cognitive processes[10] and on neurodegenerative diseases like Alzheimer's disease[11–13]. Therefore, the development of M_2 selective antagonists was the main reason for our efforts.

Another major goal of the research was to identify compounds which, besides being M_2 selective, would be able to cross the blood brain barrier (BBB) since the majority of the compounds shown in Table 1 are ammonium salts that are not expected to penetrate readily into the CNS.

In this paper we report on the synthesis and antimuscarinic activity in functional tests of several derivatives of 2-ethylthio-2,2-diphenylacetic acid *N,N*-diethylaminoethyl ester (**I**) and its methyl iodide (**II**) which both numbered among the most interesting compounds studied in previous work[1,2].

Molecular manipulation of the lead molecules was performed along the following lines:

1 – *Further variations in the sulfur alkyl substituent and aminoalcohol moiety.* The results reported in the previous paper[1] showed that the *n*-butyl derivative **IV**, as well as 2-ethylthio-2,2-diphenylacetic acid esters of 2-(*N*-pirrolidyl)- and 2-(*N*-piperidyl)-ethanol (**III** and **VI**), were the compounds showing the highest M_2 selectivity. This observation prompted us to combine higher alkyl groups (C_4–C_6) with 2-(*N*-pyrrolidyl)- and 2-(*N*-piperidyl)-ethanol moieties into new molecular entities. The resulting compounds are reported in Table 2.

2 – *Phenyl ring substitution and/or hydrogenation.* Substituents into the phenyl ring were introduced to evaluate their influence on affinity and subtype selectivity and, after separation of the enantiomers, the relevance of stereochemistry. Fluoro and methoxy groups were chosen since they were shown to modulate both affinity and efficacy in other groups of antimuscarinic compounds [14,15]. At the same time, one of the phenyl rings was substituted with a cyclohexyl group: a change that has often afforded more potent compounds in several classes of muscarinic antagonists [16–18]. The compounds synthesized according with these strategies are reported in Table 3. The separation of the enantiomers was not performed due to the disappointing pharmacological results obtained.

3 – *Restriction of the conformational freedom of the phenyl rings.* Reduction of the conformational freedom of the phenyl rings of the diphenyl acetic or diphenyl glicolic acid esters with anticolinergic properties has usually afforded potent muscarinic antagonists,[16] although such is not always the case [19]. We have applied this approach to our compounds and the resulting products are reported in Table 4.

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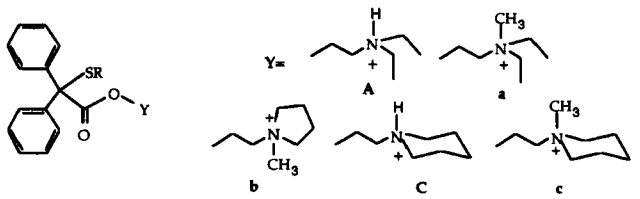
Table 1: Analgesic activity (hot plate test) of some 2-alkylthio-2,2-diphenylacetic acid esters[a].



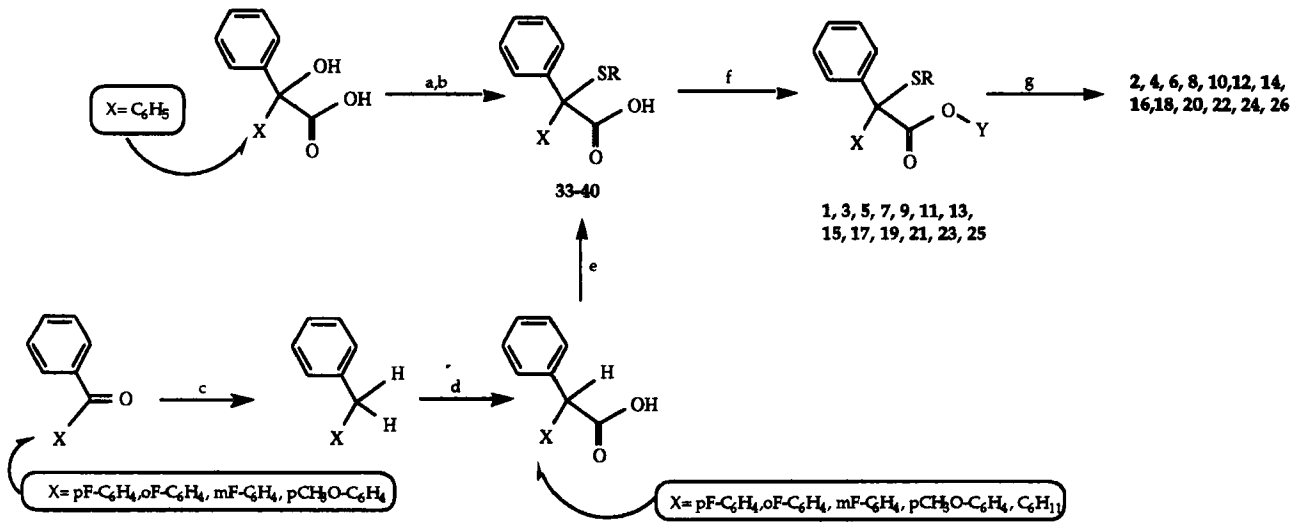
N	R	Y	Anion	M_1/M_2[b]	Analgesic activity[c]			
					ED_{50} i.c.v.[d]	i.p.[d]	Efficacy (Morphine = 100)	
I	C_2H_5	A	Cl^-	0.7	not analgesic		–	
II	C_2H_5	a	I^-	0.7	not analgesic		–	
III	C_2H_5	b	I^-	0.05	1.7 (1.3–2.0)		44	
IV	*n*-C_4H_9	a	I^-	0.02	5.6 (4.8–5.1)		44	
V	C_2H_5	C	oxalate	0.7	6.3 (5.9–6.6)	27 (21.3–36.1)	51	37[f]
VI	C_2H_5	c	I^-	0.02	3.3 (3.1–3.7)		44	
***R*(+)-hyoscyamine**[e]				1	4×10^{-4} ($3.7–4.2)\times10^{-4}$		41	

[a] For further details on muscarinic activity and selectivity of these compounds see ref. [1]. [b] Antilog of the difference between the pK_b values for M_1 and M_2 muscarinic receptor subtypes. For further information see ref. [1]. [c] Determined on mice through the hot plate test; efficacy is referred to morphine 8 mg/kg s.c. taken as reference. For further details see ref. [6,13]. [d] Expressed in mg/kg. for i.p. and µg/kg for i.c.v. Confidence limits in brackets. [e] See ref. [6]. [f] i.p.

Chemistry

The reaction pathways used to synthesize compounds **1–26** are reported in Scheme 1. *n*-Butyl (**1–4**), *n*-pentyl (**5–10**), and *n*-hexyl (**12–16**) analogs of the lead compounds were obtained from the corresponding thioacids (**33–35**), synthesized according to ref. [1]. The synthesis of the ring-substituted compounds (**17–26**) started from the corresponding ketones that were quantitatively reduced to the corresponding alkanes and then treated with a superbase (LDA/*t*-ButOK) and CO_2 to give the corresponding acids [20]. The reaction with BuLi, using $(EtS)_2$ as electrophile, gave the ethylthioacids (**36–39**) [20]. In the same way 2-cyclohexyl-2-ethylthiophenylacetic acid (**40**) was obtained using cyclohexyl-phenylacetic acid as starting material.

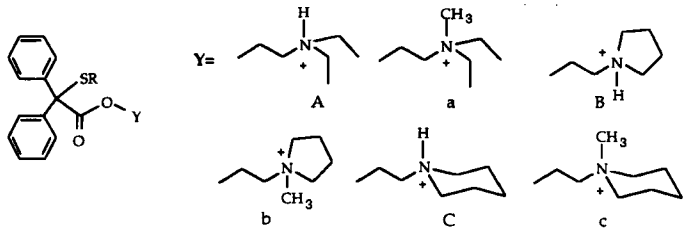
Compounds (**27–32**) shown in Table 4 were synthesized according to Scheme 2. The commercially available xan-



a) $SOCl_2$; b) R-SH : (R= n-C_4H_9, n-C_5H_{11}, n-C_6H_{13}) / $CaCO_3$; c) AlH_3; d) LDA/t-ButOK, CO_2; e) BuLi, $(EtS)_2$; f) $SOCl_2$, Y-OH (for Y see Table II); g) CH_3I

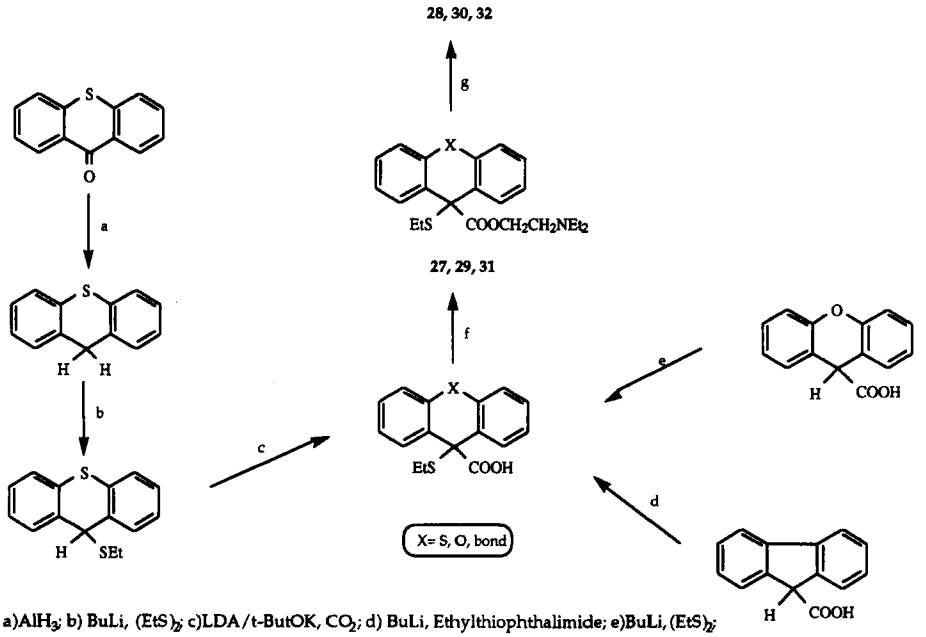
Scheme 1

Table 2: Chemical-physical characteristics, functional activity[a] and subtype selectivity of 2-alkylthio-2,2-diphenylacetic acid esters.



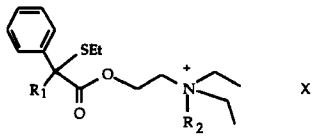
N	R	Y	Anion[b]	Mp °C[c] (Recr. Solv.)	M_1[d]	M_2[e]	M_3[f,g]	M_1/M_3[h]	M_1/M_2[h]	M_2/M_3[h]
III	C_2H_5	b	I^-	i	6.78(0.20)[l]	8.10(0.05)[m]	6.60(0.04)f	1.5	0.05	32
IV	*n*-C_4H_9	a	I^-	i	7.00(0.10)[l]	8.75(0.13)[o]	6.90(0.10)[n]	1	0.02	71
V	C_2H_5	C	oxalate	i	6.21(0.08)[l]	6.34(0.06)[n]	5.81(0.06)[n]	2.5	0.7	3.4
VI	C_2H_5	c	I^-	i	6.17(0.03)[l]	7.79(0.09)[m]	6.29(0.06)[n]	0.8	0.02	32
1	*n*-C_4H_9	B	oxalate	129–131 (A)	–	5.60(0.24)[l]	<5[l]	–	–	–
2	*n*-C_4H_9	b	I^-	149–151 (B)	7.46(0.27)[l]	6.73(0.11)[l]	5.97(0.22)[l]	31	5	6
3	*n*-C_4H_9	C	Cl^-	107–109 (A)	–	6.01(0.19)[l]	<6[l]	–	–	–
4	*n*-C_4H_9	c	I^-	170–171 (B)	5.65(0.15)[l]	6.89(0.16)[m]	<6[l]	–	0.06	–
5	*n*-C_5H_{11}	A	oxalate	98–100 (A)	–	5.81(0.09)[l]	<6[l]	–	–	–
6	*n*-C_5H_{11}	a	I^-	125–127 (B)	7.16(0.16)[l]	7.37(0.24)[l]	6.62(0.05)[m]	4	0.6	6
7	*n*-C_5H_{11}	B	oxalate	120–121 (A)	–	5.94(0.05)[l]	5.39(0.19)[l]	–	–	4
8	*n*-C_5H_{11}	b	I^-	119–120 (B)	–	6.88(0.06)[l]	7.15(0.14)[m]	–	–	0.5
9	*n*-C_5H_{11}	C	oxalate	153–155 (A)	–	6.17(0.22)[l]	5.73(0.27)[l]	–	–	3
10	*n*-C_5H_{11}	c	I^-	158–160 (B)	–	6.48(0.10)[l]	6.63(0.08)[m]	–	–	0.7
11	*n*-C_6H_{13}	A	oxalate	86–88 (A)	–	5.98(0.25)[l]	<6[m]	–	–	–
12	*n*-C_6H_{13}	a	I^-	118–119 (B)	7.16(0.08)[l]	6.98(0.21)[m]	6.19(0.13)[m]	9	2	6
13	*n*-C_6H_{13}	B	oxalate	101–102 (A)	–	<5[l]	<6[m]	–	–	–
14	*n*-C_6H_{13}	b	I^-	102–103 (B)	–	5.61(0.12)[l]	6.57(0.16)[m]	–	–	0.1
15	*n*-C_6H_{13}	C	oxalate	138–140 (A)	–	5.35(0.08)[l]	<6[m]	–	–	–
16	*n*-C_6H_{13}	c	I^-	135–137 (B)	–	6.49(0.18)[l]	6.54(0.10)[m]	–	–	0.9
***S*-Hyoscyamine[p]**			–	–	9.04(00.7)	8.95(0.01)	9.04(0.03)	1	1.3	0.8

a) $-\log K_b$ calculated from the equation $\log(DR-1) = \log[ant] - \log K_b$. 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) On this tissue (g.p. ileum) at doses higher than those used to calculate pK_b, all the compounds of this table give a reduction of the maximum effect of the agonist ranging from –24 to –78%. h) Antilog of the difference between the pK_b (or pA_2) values for M_1, M_2, and M_3 muscarinic receptor subtypes. i) See ref. [1]. l) $-\log K_b$ calculated at the concentration of 10^{-5}M. m) $-\log K_b$ calculated at the concentration of 10^{-6}M. n) $-\log K_b$ calculated at the concentration of 3×10^{-6}M. o) $-\log K_b$ calculated at the concentration of 3×10^{-7}M. p) See reference [26].



Scheme 2

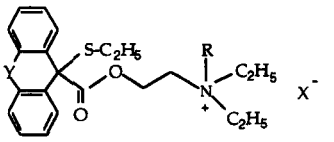
Table 3: Chemical-physical characteristics, functional activity[a] and subtype selectivity of α-substituted-α-ethylthiophenylacetic acid 2-*N,N*-diethylethylamino esters.



N	R_1	R_2	X[b]	Mp °C[c] (Recr. Solv.)	M_1d	M_2e	M_3f	M_1/M_3h	M_1/M_2h	M_2/M_3h
I	C_6H_5	H	Cl^-	i	7.79(0.01)^	7.93(0.03)^	5.88(0.09)^	81	0.7	112
II	C_6H_5	CH_3	I^-	i	8.97(0.06)^	9.12(0.03)^	6.50(0.07)^	295	0.7	417
17	C_6H_{11}	H	Cl^-	163–164 (B)	7.15(0.05)[o]	6.45(0.06)[l]	6.51(0.07)[n]	4	5	0.9
18	C_6H_{11}	CH_3	I^-	120–121 (A)	7.00(0.01)[o]	7.20(0.05)[o]	6.90(0.02)[l]	1	0.6	2
19	*p*-F-C_6H_4	H	Cl^-	88–89 (B)	6.50(0.08)[n]	7.15(0.20)[m]	5.28(0.03)[l]	17	0.2	74
20	*p*-F-C_6H_4	CH_3	I^-	122–124 (A)	6.04(0.11)[n]	7.15(0.18)[m]	6.29(0.17)[m]	0.6	0.08	7
21	*m*-F-C_6H_4	H	Cl^-	143–145 (B)	6.80(0.11)[l]	6.73(0.13)[l]	6.21(0.11)[n]	4	1	3
22	*m*-F-C_6H_4	CH_3	I^-	124–126 (A)	7.35(0.03)[l]	6.74(0.22)[l]	7.15(0.25)[l]	2	4	0.4
23	*o*-F-C_6H_4	H	Cl^-	160–162 (B)	6.01(0.18)[l]	6.24(0.23)[l]	<5[g]	–	0.6	–
24	*o*-F-C_6H_4	CH_3	I^-	133–135 (A)	7.40(0.16)[l]	6.80(0.12)[l]	6.50(0.20)[n]	8	4	2
25	*p*-OMe-C_6H_4	H	Cl^-	120–121 (B)	6.82(0.12)[n]	6.96(0.01)[n]	6.08(0.05)[l]	6	0.7	8
26	*p*-OMe-C_6H_4	CH_3	I^-	78–80 (A)	7.09(0.19)[n]	7.23(0.12)[n]	6.94(0.14)[l]	1	0.7	2

[a–o] See the corresponding footnotes of Table 2. ^) pA_2 calculated from the Schild correlation constrained to $n = 1$. See references [1,2].

Table 4: Chemical-physical characteristics, functional activity[a] and subtype selectivity of tricyclic analogues of 2-ethylthio-2,2-diphenylacetic acid 2-*N*,*N*-diethylethylamino esters



N	Y	R_2	X[b]	Mp °C[c] (Recr. Solv.)	M_1[d]	M_2[e]	M_3[f]	M_1/M_3[h]	M_1/M_2[h]	M_2/M_3[h]
27	bond	H	Cl^-	146–148 (B)	7.15(0.02)[l]	6.92(0.20)[m]	7.18(0.06)[m]	1	2	0.6
28	bond	CH_3	I^-	80–82 (A)	7.55(0.15)[m]	7.47(0.03)[m]	7.20(0.20)[m]	2	1	2
29	O	H	Cl^-	178–179 (B)	7.05(0.07)[l]	6.93(0.20)[m]	5.35(0.23)[l]	50	1	38
30	O	CH_3	I^-	175–177 (A)	7.26(0.16)[l]	7.93(0.24)[m]	6.77(0.08)[l]	3	0.2	14
31	S	H	Cl^-	167–170 (B)	6.41(0.25)[l]	5.80(0.16)[l]	5.81(0.06)[l]	4	4	1
32	S	CH_3	I^-	60–62 (A)	6.59(0.23)[l]	7.03(0.16)[l]	6.91(0.19)[l]	0.5	0.4	1

[a–m] See the corresponding footnotes of Table 2.

thene-9-carboxylic acid smoothly reacted with BuLi and $(EtS)_2$ to give the corresponding 9-ethylthioacid[21]. To obtain fluorene-9-carboxylic acid it was necessary to use the more potent electrophile, ethylthiophthalimide. The synthesis of the thioxanthene derivative started from the corresponding ketone, following the path reported for the ring-substituted compounds.

Pharmacology

The analgesic activity of compounds (**I–VI**) was tested on mice with the hot plate test,[22] using the previously reported protocols[6,13]. The results are reported in Table 1.

Muscarinic antagonism was evaluated on rabbit vas deferent (M_1), guinea pig heart (force) (M_2) and guinea pig ileum (M_3) tissues using McN-A-343 and carbachol as agonists, respectively.

The results are expressed as pK_b ($-\log K_b$) obtained from the Van Rossum equation $\log(DR - 1) = \log[ant] - \log K_b$[23] at a given concentration with replications of 5-7 times. The results are reported in Tables 2–4.

Results and Discussion

The antimuscarinic activity and subtype selectivity of compounds **1–32** are reported in Tables 2–4 together with those of compounds **I–VI** used as references.

The following is a brief discussion of the results obtained that for sake of clarity has been divided according to the modifications introduced into the lead molecules.

1 – *2-Alkylthio-2,2-diphenylacetic acid esters* (Table 2): The trend indicating that higher alkyl substituents on sulfur would show improved M_2 selectivity[1] has not been confirmed.

In fact, with respect to compound **II** (Table 3), the homologous *n*-pentyl (**6**) and *n*-hexyl (**12**) derivatives showed lower affinity for the M_2 subtype, and as a consequence their M_2 selectivity was practically zero. The corresponding tertiary bases (**5** and **11**) were definitely less potent with both M_2 and M_3 subtypes and showed no selectivity either.

In contrast, an increasing alkyl size seems to induce a modest M_1/M_3 selectivity (compare compounds **IV**, **6**, and **12**).

Even esterification with 2-(*N*-pyrrolidinyl)ethanol (**2, 8, 14**) and 2-(*N*-piperidinyl)-ethanol (**4, 10, 16**) that gave M_2 selective compounds in the case of 2-ethylthio-2,2-diphenylacetic acid (**III, VI**) afforded compounds with poor affinity and no subtype selectivity.

2 – *Ring substituted and ring reduced compounds* (Table 3): The two *N,N*-diethylaminoethanol esters of 2-ethylthio-2,2-diphenylacetic acid **I** and **II** were higly selective for M_1 and M_2 with respect to M_3 subtypes but did not discriminate between M_1 and M_2.

Para substitution of one phenyl ring with a fluorine atom changed this pharmacological profile and compounds **19** and **20** did indeed show a modest M_2 selectivity toward both M_1 and M_3 receptor subtypes. In this respect compound **20** was the most effective ($M_1/M_2 = 0.08$; $M_2/M_3 = 7$) but being a quaternary salt it does not satisfy our needs for a CNS-penetrating drug.

The other substitutions did not give any interesting results either, as both affinity and subtype selectivity were impaired. This resembled the findings of Tumiatti and coworkers[24] who substituted one of the phenyl ring of 4-diphenylacetoxy-*N*-methylpiperidine methiodide (4-DAMP), obtaining similar results.

The effects of reducing one of the phenyl rings of **I** and **II** (compounds **17** and **18**) were unexpected. In fact the increase in affinity that has been reported following a similar modification in other antimuscarinic compounds[16–18] occurred in one case only for the M_3 subtype, while for M_1 and M_2 receptors there was a sharp drop in affinity. As a consequence the discriminating properties of **I** and **II** are completely lost in **17** and **18**.

3 – *Tricyclic derivatives* (Table 4): Reduction of the conformational freedom of the phenyl rings of **I** and **II** has different effects on the receptor subtypes studied. The expected[16] increase in affinity was apparent only for the M_3 subtype while affinity for M_1 and mostly for the M_2 subtypes had an unexpected drop. As a consequence, the subtype selectivity of the parent compound was severely affected. Thus compounds **27**, **28**, **31**, and **32** do not show any remarkable selectivity while compound **29**, although maintaining a moderate M_1/M_3 and M_2/M_3 selectivity, is less potent than the corresponding parent compound **I**.

The only compound of this series that shows a modest M_2 selectivity (**30**; $M_1/M_2 = 0.2$; $M_2/M_3 = 14$) is again a quaternary base and thus useless for our purpose.

Very recently Melchiorre and coworkers[25] applied the same approach to rigid and semirigid analogs of 4-DAMP and observed no selectivity, as well as a large drop in affinity in the more rigid analogs. These results, as those obtained by us in the present research, might suggest that fairly rigid molecules as those studied by Tumiatti and, as regards the lipophilic head, compounds **27–32**, cannot adapt easily to the receptor binding site nor detect the subtle differences among different sites thus loosing both affinity and subtype selectivity.

In conclusion, the various modifications introduced into the lead molecules gave disappointing results. It has to be accepted that both affinity and subtype selectivity were severely affected and the original goal of identifying M_2 selective antagonists for which BBB permeability could be expected was missed. In fact, the only compound that shows modest M_2 selectivity (**30**) is a quaternary salt that can hardly be expected to cross the BBB. For this reason it was considered useless to test it as analgesic.

Experimental

Chemistry

All melting points were taken on a Büchi apparatus and are uncorrected. Infrared spectra were recorded with a Perkin-Elmer 337 spectrophotometer in a KBr pellet in Nujol mull for solid or neat for liquids. NMR spectra were recorded on a GEMINI 200. Chromatographic separations were performed on silica gel column (Kieselgel 4, 0.063–0.200 mm, Merck). The compounds were analyzed after vacuum drying for 5 hours at a temperature below the melting point. The analytical results are within ±0.4% of the theoretical values.

*2,2-Diphenyl-2-pentylthioacetic acid (**34**)*

2,2-Diphenyl-2-chloroacetic acid, obtained according to Stollè,[1] (0.7 g; 2.84 mmol) and $CaCO_3$ (0.28 g; 2.84 mmol) were mixed with an excess of pentyl mercaptan (15 ml) and refluxed for 30 h. After cooling, $CHCl_3$ was added, the $CaCO_3$ filtered off, and the solvent evaporated to give the acid as a white solid. Yield 80%. Mp 84–86 °C. IR (neat) ν = 1740 (CO), 3100 (OH) cm^{-1}. 1H NMR($CDCl_3$) δ = 0.83 (t, 3H, S-$(CH_2)_4$-CH_3); 1.18–1.28 (m, 4H, S-$(CH_2)_2$-$(CH_2)_2$-CH_3); 1.41–1.52 (m, 2H, S-CH_2-CH_2-$(CH_2)_2$-CH_3); 2.39 (t, 2H, S-CH_2-$(CH_2)_3$-CH_3); 7.26–7.48 (m, 10H, aromatics) ppm. Anal. ($C_{19}H_{22}O_2S$) C, H, N.

The *n*-butyl (**33**)[1] and *n*-hexyl (**35**) analogs were obtained in the same way. Their IR and NMR data are in accord with the structure.

*Ring-substituted ethylthioacetic acids (**36–39**)*

The synthesis of these compounds was performed as reported in a previous paper[20].

*Tricyclic analogs of 2-ethylthio-2,2-diphenylacetic acid (**41–43**)*

The synthesis of these compounds was performed as reported in a previous paper[21].

*2-Cyclohexyl-2-ethylthiophenylacetic acid (**40**)*

Commercially available 2-cyclohexyl-2-phenylacetic acid (0.2 g; 1.25 mmol) was dissolved in 10 ml of anhydrous THF and cooled to 0 °C, then 4.7 ml (7.5 mmol) of a 1.6 M solution of BuLi in hexane was added and the mixture was left at 0 °C for 30 min. When 0.95 g (7.5 mmol) of diethyl disulfide were added, the mixture was allowed to warm to r.t., treated with a saturated solution of NH_4Cl, with 6N HCl, and extracted with diethyl ether. The organic layer was rendered anhydrous and evaporated in vacuum to give the acid as a white solid. Yield 70%. Mp 125–127 °C. IR (neat) ν = 1740 (CO), 3100 (OH) cm^{-1}. 1H NMR($CDCl_3$) δ = 0.61–2.59 (m, 16 H, S-CH_2-CH_3 + cyclohexyl protons); 7.21–7.39 (m, 3H, aromatics); 7.48 (d, 2H, aromatics) ppm. Anal. ($C_{15}H_{22}O_2S$) C, H, N.

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

2,2-Diphenyl-2-pentylthioacetic acid (0.56 g; 1.78 mmol) was converted into the corresponding acyl chloride 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 acyl chloride was refluxed with 2-(N-pyrrolidinyl)ethanol (0.41 g; 3.56 mmol) in anhydrous CH_2Cl_2 (20 ml) for 24 h. After cooling the solution was treated with a 10% solution of Na_2CO_3 and the organic layer dried and evaporated under vacuum to give an oil that was purified by flash chromatography, using $CHCl_3$:methanol 9:1 as eluent. Yield 50%. IR (neat) ν = 1740 (CO) cm^{-1}. ^1H NMR($CDCl_3$) δ = 0.82 (t, 3H, S-$(CH_2)_4$-CH_3); 1.18–1.51 (m, 6H, S-CH_2-$(CH_2)_3$-CH_3); 1.66–1.75 (m, 4H, 2 NCH_2-CH_2); 2.30–2.48 (m, 6H, S-CH_2-$(CH_2)_3$-CH_3; 2 NCH_2-CH_2); 2.75 (t, 2H, O-CH_2-CH_2-N); 4.34 (t, 2H, O-CH_2-CH_2-N); 7.19–7.43 (m, 10H, aromatics) ppm.

The oily product was transformed into the oxalate that was recrystallized from abs. ethanol and anhydrous ether. Mp 120–121 °C. Anal. ($C_{27}H_{35}NO_6S$) C, H, N.

All other esters were obtained in the same way; their characteristics are reported in Tables 2, 3, and 4 and their ^1H NMR data are consistent with the proposed structures.

*2,2-Diphenyl-2-pentylthioacetic acid 2-(N-pyrrolidinyl)ethyl ester methyl iodide (**8**)*

0.2 g of **7** were dissolved in 20 ml of anhydrous ether, treated with an excess of CH_3I and left overnight at r.t. in the dark. The white crystals were collected and recrystallized from abs. ethanol. Mp 119–120 °C. Yield 90%. IR (nujol) ν =1740 (CO) cm^{-1}. ^1H NMR ($CDCl_3$) δ = 0.88 (t, 3H, S-$(CH_2)_4$-CH_3); 1.21-1.55 (m, 6H, S-CH_2-$(CH_2)_3$-CH_3); 1.76-1.87 (m, 4H, 2 NCH_2-CH_2); 2.30-2.48 (m, 2H, S-CH_2-$(CH_2)_3$-CH_3); 2.82-2.98 (m, 4H, 2 NCH_2-CH_2); 3.03 (s, 3H, N-CH_3); 3.35 (t, 2H, O-CH_2-CH_2-N); 4.34 (t, 2H, O-CH_2-CH_2-N); 7.19–7.43 (m,10H, aromatics) ppm. Anal. ($C_{26}H_{33}NO_2SI$) C, H, N.

Compounds **2**, **4**, **6**, **10**, **12**, **14**, **16**, **18**, **20**, **22**, **24**, **26**, **28**, **30**, **32** were obtained in the same way. Their characteristics are reported in Tables 2 and 3 and 4.

Pharmacology

Analgesic activity

Analgesic activity was evaluated using the hot-plate method according to Woolfe.[22] The plate temperature was fixed at 52.5 ± 0.1 °C. An arbitrary cut-off time of 45 s was adopted. The number of mice treated in each test varied from 8 to 20.

The analgesic potency of the compounds is reported as the ED_{50}. This potency does not however indicate the level of analgesia reached. To evaluate this parameter, the analgesic effect of the new products injected at their maximal non-toxic dose was compared to that of morphine, taken as the reference compound and injected at 8 mg/kg s.c., a dose that does not alter animal behavior.

Calculations were performed using the following formula:

Analgesic efficacy of (X) expressed as % of that of morphine·HCl (8 mg/kg s.c.)

$$= \frac{\text{maximum reaction time of (X)} - \text{pretest reaction of (X)}}{\text{max. react. time of morphine} - \text{pretest react. time of morphine}} \times 100$$

The maximal non-toxic dose is the highest dose of (X) which does not cause any visible change in animal behavior, i.e. such that the researchers who were unaware of the treatment received by the animals were unable to distinguish between treated and non-treated mice.

Standard errors on the values expressed as percentage were not evaluated. Original data however have been statistically processed by employing Dunnett's two tailed test in order to verify the significance of the differences between the means shown by treated mice at the maximum reaction time and pre-test reaction time. Differences were considered statistically significant when $P \leq 0.05$. Percent values were calculated only for those differences that resulted statistically significant; in the other case drugs were considered inactive. Since the reaction times were measured with an accuracy of 15%, the error on the % values calculated through the formula reported above should be in the same range.

Functional tests

Muscarinic antagonism was evaluated with standard procedures[26] on rabbit vas deferent (M_1), guinea pig heart (force) (M_2) and guinea pig ileum (M_3) tissues using McN-A-343 and carbachol as agonists, respectively.

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