Presynaptic Cholinergic Modulators as Potent Cognition Enhancers and Analgesic Drugs. 2. 2-Phenoxy-, 2-(Phenylthio)-, and 2-(Phenylamino)alkanoic Acid Esters

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Further modifications of the leads ((*R*)-(+)-hyoscyamine and (*p*-chlorophenyl)propionic acid α-tropanyl ester), which show analgesic and nootropic activities as a consequence of increased central presynaptic ACh release, are reported. 2-Phenoxy- and 2-(phenylthio)alkanoic acid esters showed the best results. Several members of these classes possess analgesic properties which are comparable to that of morphine and at the same time are able to reverse dicyclomine-induced amnesia. Confirmation was found that the mechanism of action is due to an increase in ACh release at central muscarinic synapses and that both auto- and heteroreceptors controlling ACh release are very likely involved. According to the results obtained with (*R*)-(+)-hyoscyamine, analgesic activity is stereochemistry dependent, since the *R*-(+)-enantiomers are always more efficacious than the corresponding *S*-(−)-ones. On the basis of their potency and acute toxicity, compounds (±)-**28** (SM_{21}) and (±)-**42** (SM_{32}) were selected for further study.

Introduction

In the previous paper,[1] we described the synthesis and pharmacological evaluation of a series of tropic and 2-phenylpropionic acid esters endowed with nootropic and analgesic activity. These compounds appear to act by facilitating central cholinergic transmission through presynaptic auto- or heteroreceptor blockade.

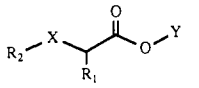
The rationale for their design and for their use in analgesia and cognitive disorders characterized by impaired cholinergic transmission has been already discussed in detail.[1]

In this part of the work, we describe further modulation of the leads (atropine and the α-tropanol ester of 2-phenylpropionic acid) that led to the discovery of a new class of compounds whose structure is reported in Chart 1. These act with the same mechanism and possess high nootropic and analgesic activity.

Since, as reported in the previous paper, analgesic and nootropic activity depend on the same mechanism of action, we followed molecular manipulation of the leads through the simple and relatively inexpensive hot-plate test for analgesia, checking in each case that this effect could be reversed by suitable doses of atropine and the ACh depletor hemicolinium-3 (HC-3), in order to be sure that analgesia was in fact due to a cholinergic mechanism. Under these conditions, we were able to select the most interesting compounds, which indeed on further testing showed parallel, *in vitro* potentiation of cholinergic activity and nootropic activity *in vivo*.

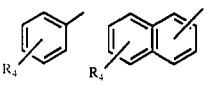
As compounds with general structure A (Chart 1) are chiral and as in the leads the analgesic activity of the enantiomers was quite different,[2] we also synthesized and tested the pure enantiomers of the most potent of our compounds.

Chart 1



A

R_1= H, CH_3, C_2H_5, $CH(CH_3)_2$, C_6H_5, CH_2-C_6H_5

R_2= 

X= O, S, NH, NCH_3, CO

Y= See Table I

Chemistry

The synthetic pathways used to obtain the compounds studied (Tables 1–4) are reported in Schemes 1–3. Most of the acids used as starting material were known.[3-18] Those obtained for the first time are reported in Table 6. The amino alcohols were either commercially available or synthesized according to the literature.[19-23] The methods used in the synthesis and described in Schemes 1–3 are standard and do not require further comment.

Chiral compounds (Table 5) were obtained starting from the corresponding chiral acids and using an esterification procedure that occurs without racemization, provided the hydrochloride of the amino alcohol is used. Use of the free bases in fact always resulted in partial or total racemization.

The chiral acids used are known, and their optical purity has been reported to be higher than 98%.[24-26] However, the enantiomers of 2-(phenylthio)butyric acid ((±)-**72**) have not yet been described, and we attempted resolution of the racemate through the classic diastereomeric salt formation. Unfortunately, to date, only a partial resolution has been achieved, and the two enantiomers ((−)- and (+)-**72**) show an ee of about 40% (evaluated through gas chromatography after chiral derivatization and chiral

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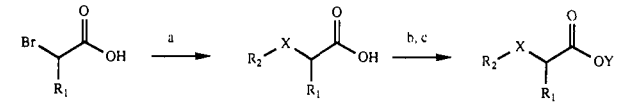
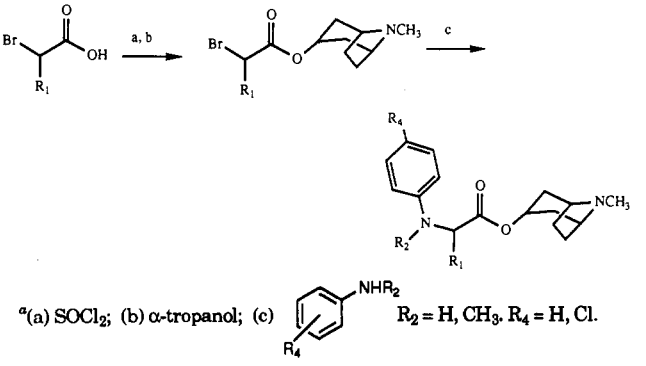
Scheme 1[a]



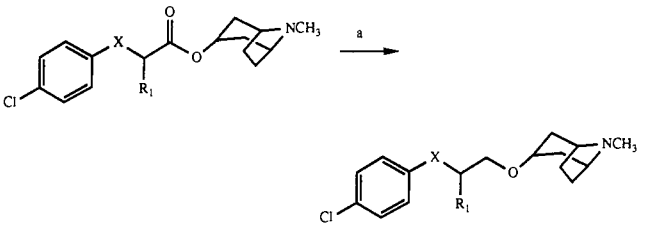
[a] (a) NaOH, R_2-OH (or R_2-SH); (b) $SOCl_2$; (c) Y-OH. R_1 and R_2 are as reported in Tables 1–3.

Scheme 2[a]



[a](a) $SOCl_2$; (b) α-tropanol; (c) R_2 = H, CH_3. R_4 = H, Cl.

Scheme 3[a]



[a] (a) $NaBH_4$, $BF_3{\cdot}O(C_2H_5)_2$. R_1 = CH_3, C_2H_5. X = O, S.

HPLC, see the Experimental Section). As a consequence, compounds (+)- and (−)-**42** have a similarly low optical purity.

Results

The introduction of a phenoxyacyl group was attempted in the search for an alternative way of modifying the atropine acyl moiety other than elimination of the troublesome hydroxy group.[1] In this way, an oxygen atom and its two lone pairs that might have a role in binding to the receptor were conserved inside the molecule but in an arrangement that would not compromise its stability.

While the α-tropanol esters of phenoxyacetic and 2-phenoxypropionic acids were inactive (**1** and **2**), the corresponding ester with 2-(4-chlorophenoxy)propionic acid ((±)-**4**) was a potent analgesic with an efficacy which was comparable to that of morphine.

This unexpected, somehow contradictory, result gave us a new lead that was extensively manipulated toward optimization; the results obtained can be summarized as follows.

1. In this new series of compounds, only esters with α-tropanol maintained activity. Some activity was shown by *N*-ethyl-α-nortropanol esters **6** and **29**, although with a reduced efficacy. Esterification with the isomeric β-tropanol **12** gave a definitely less active compound. Other amino alcohols resulted in inactive compounds.

2. *p*-Halogen substitution was very effective in giving compounds with high efficacy (**3**, (±)-**4**, **15**). The 2-chloro isomer **13** was inactive, while the 3-chloro derivative **14** was less potent and efficacious than the 4-chloro analogue (±)-**4**. All other substituents tested were detrimental for activity, to differing extents.

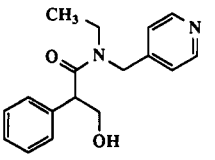
3. Homologation of the propionic acid moiety was beneficial, and indeed, the butyric acid derivative (±)-**28** was one of the most potent and efficacious compounds obtained. Higher homologues (**33–35**) were inactive. Compound **36**, designed to stress the similarity with atropine, was only feebly active. Double substitution of the α-carbon of alkanoic acid **65** was detrimental for efficacy.

4. Isosteric substitution of oxygen with sulfur was accepted by the molecule, and compound (±)-**42** was the most efficacious and potent of the compounds studied. Activity was abolished when sulfur was oxidized to the corresponding sulfoxide **45**.

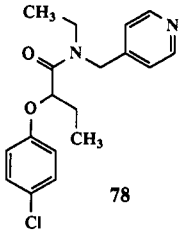
Substitution with NH or NCH_3 still gave efficacious compounds (i.e., **48**); instead, substitution of oxygen with a carbonyl (**58**) or methylene group (see previous paper) resulted in poorly efficacious or inactive compounds.

5. Shifting the phenoxy group in β to the acyl moiety resulted in a compound (**59**) of reduced efficacy.

6. Reduction of the ester to an ether function gave negative results, leading to inactive compounds (i.e., **62**) or to compounds with reduced potency and efficacy (i.e., **61**). Substitution of the ester with an amide (**60**) also reduced potency and efficacy. This is in agreement with the results obtained with tropic acid esters, where the amide group proved unable to maintain analgesic activity.[2] In this respect, it is interesting that compound **78**, the (4-chlorophenoxy)butyric acid analogue of tropicamide, was completely devoid of analgesic activity, in contrast to the high analgesic potency of tropicamide itself.[1]



Tropicamide



78

7. Substituting the phenyl with a naphthalene ring gave poor results, as only β-naphthoxy derivatives showed some degree of activity.

8. The compounds studied showed enantioselectivity in their antinociceptive action, confirming the findings in the tropic acid and 2-phenylpropionic acid series.[1]

As regards (+)-**42**, because of the low optical purity of the compound, absolute configuration was not firmly established but is very likely the same as the other corresponding compounds in Table 5 (*R*). In fact, (+)-**42** was the more potent of the two enantiomers.

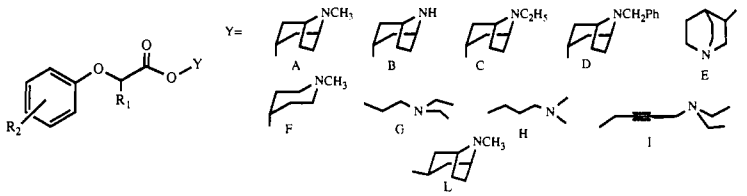
While there are only minor differences among the ED_{50}'s of the enantiomers, a clear-cut difference was unexpectedly observed in their efficacies, the *R*-(+)-isomers being much more efficacious then their *S*-(−)-counterparts. The analgesic potency and efficacy of the compounds defined as described in the Experimental Section are reported in Tables 1–5.

Discussion

Like the tropic and 2-phenylalkanoic acid esters described in the previous paper,[1] this new class of compounds seems to act through a facilitation of central ACh release by modulating presynaptic receptors.

The analgesic activity was indeed reversed by suitable doses of atropine, as shown in Figure 1 for the most

Table 1. 2-Phenoxyalkanoic Acid Esters



no.	R_1	R_2	Y	salt[a] (rec solv)	formula[b]	mp (°C)	analgesic activity[c] maximum level of analgesia[d]	analgesic activity[c] ED_{50} (SE) (mg/kg sc)
1	H	H	A	maleate (A)	$C_{20}H_{25}NO_7$	53–55	inact	
2	CH_3	H	A	maleate (A)	$C_{21}H_{27}NO_7$	89–90	inact	
(±)-3	CH_3	4-F	A	maleate (B)	$C_{21}H_{26}FNO_7$	82–83	91	33 (3.7)
(±)-4[e]	CH_3	4-Cl	A	maleate (A)	$C_{21}H_{26}ClNO_7$	112–114	90	32 (2.3)
5	CH_3	4-Cl	B	maleate (A)	$C_{20}H_{24}ClNO_7$	132–134	inact	
6	CH_3	4-Cl	C	maleate (A)	$C_{22}H_{28}ClNO_7$	92–94	42	42 (3.0)
7	CH_3	4-Cl	D	oxalate (A)	$C_{25}H_{28}ClNO_7$	117–119	inact	
8	CH_3	4-Cl	E	hydrochloride (B)	$C_{16}H_{21}Cl_2NO_3$	88–90	inact	
9	CH_3	4-Cl	F	oxalate (A)	$C_{17}H_{22}ClNO_7$	142–143	intact	
10	CH_3	4-Cl	G		$C_{15}H_{22}ClNO_3$	*f*	inact	
11	CH_3	4-Cl	I	oxalate (A)	$C_{19}H_{24}ClNO_7$	90–92	inact	
12	CH_3	4-Cl	L	maleate (A)	$C_{21}H_{26}ClNO_7$	90–92	26	21 (1.6)
13	CH_3	2-Cl	A	oxalate (C)	$C_{19}H_{24}ClNO_7$	100–102	inact	
14	CH_3	3-Cl	A	oxalate (B)	$C_{19}H_{24}ClNO_7$	137–140	62	26 (1.9)
15	CH_3	4-Br	A	oxalate (E)	$C_{19}H_{24}BrNO_7$	100–104	86	31 (2.4)
16	CH_3	4-CF_3	A	oxalate (C)	$C_{20}H_{24}F_3NO_7$	110–113	44	26 (2.0)
17	CH_3	4-CN	A	maleate (B)	$C_{22}H_{26}N_2O_7$	125–126	53	31 (2.5)
18	CH_3	4-$C(CH_3)_3$	A	maleate (B)	$C_{25}H_{35}NO_7$	171–173	inact	
19	CH_3	4-$C(CH_3)_3$	E	maleate (D)	$C_{24}H_{33}NO_7$	104–105	inact	
20	CH_3	4-SCH_3	A	maleate (A)	$C_{22}H_{29}NO_7S$	82–84	21	43 (3.1)
21	CH_3	4-$SOCH_3$	A		$C_{18}H_{25}NO_4S$	*f*	inact	
22	CH_3	3,4-Cl	A	maleate (A)	$C_{21}H_{25}Cl_2NO_7$	123–126	50	22 (1.5)
23	CH_3	3,4-Cl	E	maleate (C)	$C_{20}H_{23}Cl_2NO_7$	102–104	inact	
24	CH_3	3-CH_3,4-Cl	A	oxalate (D)	$C_{20}H_{26}ClNO_7$	87–90	inact	
25	CH_3	4-C_6H_5	A	maleate (B)	$C_{27}H_{31}NO_7$	160–162	inact	
26	C_2H_5	H	A	oxalate (C)	$C_{20}H_{27}NO_7$	97–99	35	31 (1.9)
27	C_2H_5	4-F	A	citrate (B)	$C_{24}H_{32}FNO_{10}$	174–176	94	37 (2.1)
(±)-28	C_2H_5	4-Cl	A	maleate (A)	$C_{22}H_{28}ClNO_7$	102–103	99	20 (1.8)
29	C_2H_5	4-Cl	C	oxalate (A)	$C_{21}H_{28}ClNO_7$	150–152	45	32 (2.9)
30	C_2H_5	4-Cl	F	oxalate (A)	$C_{18}H_{24}ClNO_7$	136–138	inact	
31	C_2H_5	4-Cl	H	oxalate (A)	$C_{17}H_{24}ClNO_7$	128–129	inact	
32	C_2H_5	4-Cl	I	oxalate (A)	$C_{20}H_{26}ClNO_7$	78–80	inact	
33	$CH(CH_3)_2$	4-Cl	A	maleate (D)	$C_{23}H_{30}ClNO_7$	124–126	inact	
34	C_6H_5	4-Cl	A	oxalate (A)	$C_{24}H_{26}ClNO_7$	142–145	inact	
35	CH_2-C_6H_5	4-Cl	A	maleate (D)	$C_{27}H_{30}ClNO_7$	127–129	inact	
36	CH_2OCOCH_3	4-Cl	A		$C_{19}H_{24}ClNO_5$	*f*	25	23 (1.5)

[a] A = absolute EtOH/Et_2O; B = absolute EtOH; C = ethyl acetate; D = ethyl acetate/Et_2O; E = dioxane. [b] All compounds were analyzed for C, H, and N. The results are within ±0.4% of the theoretical value. IR and NMR spectra are in agreement with the proposed structures. [c] Evaluated on male albino Swiss–Webster mice with hot-plate test; plate temperature 52.5 °C; cutoff time 45 s. [d] Compared to morphine as reference; see text for calculations and statistical evaluation. In this reference system, the value of atropine is 100. [e] See ref 32. [f] Oil.

interesting compounds, (±)-28 and (±)-42. Moreover, pretreatment with hemicholinium prevented the analgesic action, showing that acetylcholine release is necessary for activity (Figure 1).

As reported in the accompanying paper,[1] the involvement of a cholinergic mechanism is also substantiated by the effect of the compounds on guinea pig ileum electrically and nicotine-evoked responses, as shown for compounds (±)-4, (±)-28, (±)-39, and (±)-42 in Figure 2. Accordingly, the involvement of opioidergic, serotoninergic, and GABAergic systems has been ruled out.[1]

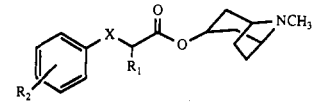
Since the analgesic profile of the compounds of the present series is practically identical in terms of potency and efficacy to that of the 2-phenylpropionic acid derivatives described in the previous paper,[1] it is conceivable that in this case, too, receptor systems besides the muscarinic one could be involved. To date, we have no sound data for solving this problem, although some indications can be derived from the analgesic activity of the enantiomers.

The different efficacies of the enantiomers in producing analgesia can be assumed to be a consequence of their different abilities to facilitate ACh release. If this is true, the most efficacious enantiomer might act on two (or more) receptors controlling ACh release, while the less efficacious isomers might act on only one.

Bearing in mind that (*R*)-(+)-hyoscyamine is active, that its *S*-(−)-enantiomer is inactive on analgesia, and that (*R*)-(+)-hyoscyamine very probably acts on the muscarinic autoreceptor[2] (see, also, the previous paper in this issue), we could speculate that the *R*-(+)-enantiomers of the present series act both on the muscarinic autoreceptors and on another receptor (5-HT?) controlling ACh release. The *S*-(−)-compounds, on the other hand, being unable to block the presynaptic muscarinic receptor, probably only act on the second receptor system.

Careful binding studies of the compounds and of their enantiomers on receptors known to control ACh release and the evaluation of M_2 (presynaptic)/M_1 (postsynaptic) selectivity should help to answer the question. These

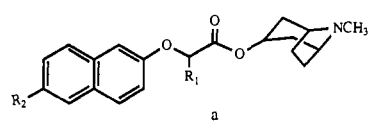
Table 2. 2-(Phenylthio)- and 2-(Phenylamino)alkanoic Acid α-Tropanyl Esters



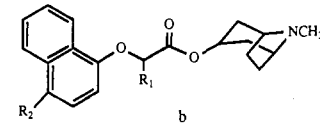
no.	R_1	R_2	X	salt[a] (rec solv)	formula[b]	mp (°C)	analgesic activity[c] maximum level of analgesia[d]	analgesic activity[c] ED_{50} (SE) (mg/kg sc)
37	CH_3	H	S	maleate (B)	$C_{21}H_{27}NO_6S$	62–64	62	30 (2.6)
38	CH_3	4-F	S	oxalate (B)	$C_{19}H_{24}FNO_6S$	112–115	41	15 (1.3)
(±)-39	CH_3	4-Cl	S	maleate (B)	$C_{21}H_{26}ClNO_6S$	120–122	72	19 (1.4)
40	CH_3	4-Br	S	oxalate (B)	$C_{19}H_{24}BrNO_6S$	102–104	33	35 (2.6)
41	CH_3	4-CH_3	S	maleate (B)	$C_{22}H_{29}NO_6S$	105–106	33	7 (0.7)
(±)-42	C_2H_5	H	S	oxalate (A)	$C_{20}H_{27}NO_6S$	143–145	103	18 (1.6)
(±)-43	C_2H_5	4-Cl	S	oxalate (A)	$C_{20}H_{26}ClNO_6S$	130–133	79	20 (2.1)
44	$CH(CH_3)_2$	4-Cl	S	maleate (D)	$C_{23}H_{30}ClNO_6S$	102–104	41	41 (2.8)
45	CH_3	H	SO	maleate (B)	$C_{21}H_{27}NO_7S$	145–147	inact	
46	CH_3	H	NH	hydrochloride (B)	$C_{17}H_{25}ClN_2O_2$	218–220	74	32 (2.5)
47	CH_3	4-Cl	NH	oxalate (A)	$C_{19}H_{25}ClN_2O_6$	138–140	33	31 (2.1)
48	CH_3	4-Cl	NCH_3	maleate (B)	$C_{22}H_{29}ClN_2O_6$	118–120	86	29 (3.0)
49	C_2H_5	4-Cl	NCH_3	maleate (B)	$C_{23}H_{31}ClN_2O_6$	138–140	85	38 (2.7)

[a–d] See corresponding footnotes of Table 1.

Table 3. 2-(Naphthyloxy)alkanoic Acid α-Tropanyl Esters



a

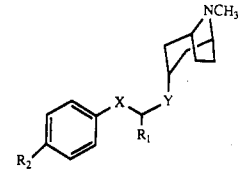


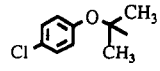
b

no.	R_1	R_2	structure	salt[a] (rec solv)	formula[b]	mp (°C)	analgesic activity[c] maximum level of analgesia[d]	analgesic activity[c] ED_{50} (SE) (mg/kg sc)
50	CH_3	H	a	maleate (A)	$C_{25}H_{29}NO_7$	115–117	86	34 (1.6)
51	C_2H_5	H	a	maleate (D)	$C_{26}H_{31}NO_7$	99–102	inact	
52	CH_3	6-Br	a	maleate (A)	$C_{25}H_{28}BrNO_7$	117–120	inact	
53	C_2H_5	6-Br	a	maleate (D)	$C_{26}H_{30}BrNO_7$	117–119	32	42 (2.4)
54	CH_3	H	b	maleate (D)	$C_{25}H_{29}NO_7$	141–143	inact	
55	C_2H_5	H	b	maleate (D)	$C_{26}H_{31}NO_7$	122–124	inact	
56	CH_3	4-Cl	b	maleate (A)	$C_{25}H_{28}ClNO_7$	152–153	inact	
57	C_2H_5	4-Cl	b	maleate (B)	$C_{26}H_{30}ClNO_7$	148–150	inact	

[a–d] See corresponding footnotes of Table 1.

Table 4. Other α-Tropanyl Esters and Ethers



no.	R_1	R_2	X	Y	salt[a] (rec solv)	formula[b]	mp (°C)	analgesic activity[c] maximum level of analgesia[d]	analgesic activity[c] ED_{50} (SE) (mg/kg sc)
58[e]	CH_3	Cl	CO	COO	oxalate (A)	$C_{20}H_{24}ClNO_7$	175–178	30	34 (2.4)
59	H	H	O-CH_2	COO	maleate (A)	$C_{21}H_{27}NO_7$	88–89	28	22 (1.2)
60	CH_3	Cl	O	CONH	hydrochloride (A)	$C_{17}H_{24}Cl_2O_2N_2$	70–71	33	36 (1.9)
61	CH_3	Cl	O	CH_2O	oxalate (A)	$C_{19}H_{26}ClNO_6$	134–136	64	44 (2.7)
62	C_2H_5	Cl	O	CH_2O	tartrate (A)	$C_{22}H_{32}ClNO_8$	65–68	inact	
63	CH_3	Cl	S	CH_2O	tartrate (A)	$C_{21}H_{30}ClNO_7S$	83–85	inact	
64	C_2H_5	Cl	S	CH_2O	tartrate (A)	$C_{22}H_{32}ClNO_7S$	70–73	53	39 (3.0)
65				COO	oxalate (A)	$C_{20}H_{26}ClNO_7$	153–155	52	23 (1.8)

[a–d] See corresponding footnotes of Table 1. [e] The acid used as starting material was obtained according to ref 33.

studies are already underway. The first results indicate that the members of this series bind to the central muscarinic receptor with affinities in the micromolar range (1.74×10^{-7} M for (±)-28 and 2.27×10^{-8} M for (±)-42 against [^{3}H]QNB on rat brain), but there is no apparent correlation between analgesic activities and binding.[27] This can be partly explained pharmacokinetically, as discussed below, but the involvement of other receptor systems cannot be excluded.

As expected, compounds showing analgesic activity also possess nootropic properties. Figure 3 shows the results of a passive avoidance test on mice. In this experiment,

Table 5. Chemical–Physical Properties and Analgesic Activity of the Enantiomers of **3**, **4**, **28**, **39**, **42**, and **43**

compd	$[\alpha]^{20}_D$ (absolute EtOH) (deg)	salt[a] (rec solv)	mp (°C)	analgesic activity[c] maximum level of analgesia[d]	analgesic activity[c] ED_{50} (SE) (mg/kg sc)
(*R*)-(+)-**3**	+29.6	citrate (B)	155–157	106	21 (2.0)
(*S*)-(−)-**3**	−28.7	citrate (B)	155–157	65	23 (1.9)
(*R*)-(+)-**4**	+42.2	maleate (A)	111–114	68	16 (0.7)
(*S*)-(−)-**4**	−40.8	maleate (A)	112–115	37	17 (1.3)
(*R*)-(+)-**28**	+48.1	maleate (B)	83–85	89	14 (0.8)
(*S*)-(−)-**28**	−49.4	maleate (B)	83–85	56	18 (1.7)
(*R*)-(+)-**39**	+70.6	maleate (B)	112–114	61	14 (1.7)
(*S*)-(−)-**39**	−71.0	maleate (B)	110–113	34	16 (0.9)
(+)-**42**[b]	+35.4	oxalate (A)	147–148	86	16 (1.5)
(−)-**42**[b]	−33.8	oxalate (A)	144–146	53	13 (2.1)
(*R*)-(+)-**43**	+85.2	maleate (A)	74–77	64	11 (0.8)
(*S*)-(−)-**43**	−87.6	maleate (A)	74–77	38	14 (0.7)

[a,c,d] See the corresponding footnotes of Table 1. [b] The compounds are only partially resolved (scalemic) with an ee = 40% (see the Experimental Section). Accordingly, their absolute stereochemistry is not yet firmly established but is very probably identical to that of the other members of the series (*R*-(+) and *S*-(−), respectively).

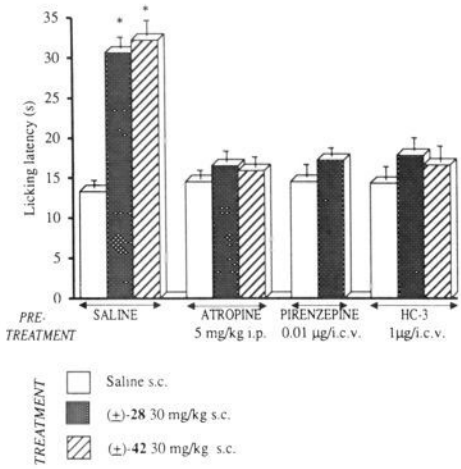


Figure 1. Effect of atropine, pirenzepine, and hemicholinium-3 (HC-3) on (±)-**28** and (±)-**42** antinociception in mouse hot-plate test (52.5 °C). Vertical lines give se of the mean. $*P < 0.01$ in comparison with saline controls. Atropine, pirenzepine, and HC-3 were administered respectively 30 min, 20 min, and 5 h before the test. (±)-**28** and (±)-**42** were injected 15 min before the test. Each column represents the mean of at least 10 mice.

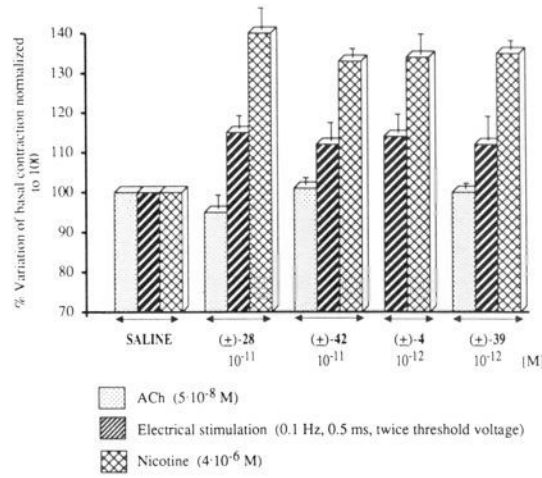


Figure 2. Effect of (±)-**28**, (±)-**42**, (±)-**4**, and (±)-**39** on chemically and electrically evoked contractions of guinea pig ileum myenteric plexus longitudinal muscle strip. Each column represents the mean of at least three experiments, and vertical lines give se of the mean.

compounds (±)-**28** and (±)-**42** show reversion of dicyclomine-induced amnesia at doses that are about 10 times lower than that required for analgesia with compound (±)-**28** (Figure 3).

Structure–activity relationships based on analgesic activity measured on the hot-plate model obviously reflect not only the interaction with the receptor(s) but also the pharmacokinetics of each single compound, so that they must be regarded with some caution. In fact, only the so far unexplored balance of pharmacodynamic and pharmacokinetic properties can explain the numerous inconsistencies in structure–activity relationships which are found in the data reported in Tables 1–4.

Consider, for instance, the inactivity of **2** as compared to the high analgesic effects of (±)-**3** and (±)-**4** and the fact that *p*-chloro substitution, which is critical for phenoxy derivatives ((±)-**28**), does not seem to be a requirement for phenylthio derivatives ((±)-**42**).

Despite these differences in structure–activity relationships, the compounds studied show an identical enantioselectivity, the most active enantiomer invariably being the *R*-(+)-one (see Table 5). Their absolute configuration is identical to that of the most potent enantiomers of the α-tropanyl esters of tropic acid ((*R*)-(+)-hyoscyamine) and 2-phenylpropionic acid (*S*-(+)).[1]

We tried to overlap the minimized conformations of the eutomers ((*R*)-(+)-hyoscyamine, (*S*)-(+)-α-tropanyl 2-phenylpropionate, (*R*)-(+)-**28**, and (+)-**42**) and found that an acceptable degree of superimposition is indeed possible,[28] suggesting that the compounds probably interact with common receptor site(s). However, the real significance of such overlapping is highly questionable since, as discussed above, different receptor types seem to be involved in the production of the pharmacological activity of some of the compounds studied.

Like the compounds studied before,[1] this new series of compounds does not provoke any symptom of cholinergic excitation (salivation, lacrimation, tremors, and hypothermia) when administered to the animals. This makes the compounds quite promising clinical candidates for analgesia and treatment of pathological states characterized by cholinergic deficit. Compounds (±)-**28** (SM_{21}) and (±)-**42** (SM_{32}) have been selected for further study on the basis of their activity and acute toxicity.

Experimental Section

Chemistry. All melting points were taken on a Büchi apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 681 spectrophotometer in a Nujol mull for

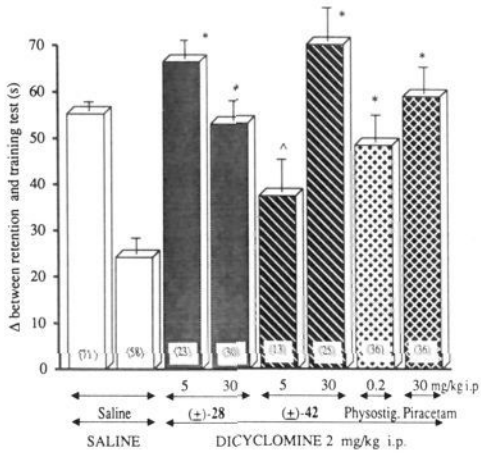


Figure 3. Effect of (±)-**28** and (±)-**42** in comparison with physostigmine and piracetam on dicyclomine-induced amnesia in mouse passive avoidance test. (±)-**28**, (±)-**42**, and physostigmine were administered 20 min before training. Piracetam was administered 30 min before training. Dicyclomine was injected immediately after the training test. Vertical lines give se of the mean. In parentheses is the number of mice. $*P < 0.01$ in comparison with dicyclomine-treated mice.

solid and neat for liquids. NMR spectra were measured on a Gemini 200 spectrometer. Chromatographic separations were performed on a silica gel column by gravity chromatography (Kieselgel 40, 0.063–0.200 mm, Merck) or flash chromatography (Kieselgel 40, 0.040–0.063 mm, Merck). Yields are given after purification, unless otherwise stated. Where analyses are indicated by symbols, the analytical results are within ±0.4% of the theoretical values. Optical activity was measured at a concentration of 1 g/100 mL (c = 1) with a Perkin-Elmer 241 polarimeter with an accuracy of ±0.5°. GC-MS spectra were obtained with a Perkin-Elmer ITD connected to a Perkin-Elmer 8420 capillary gas chromatograph.

General Method for the Synthesis of 2-Phenoxy- and 2-(Phenylthio)alkanoic Acids and Like Compounds. A 0.2-mol portion of NaOH was dissolved in 300 mL of absolute EtOH; to this solution were added 0.1 mol of the appropriate phenol or thiophenol and 0.1 mol of the appropriate α-bromoalkanoic acid, and the mixture was refluxed for 24 h. This procedure works nicely for thiophenols and most phenols; for less reactive phenols, it may be useful to remove the solvent and dissolve the residue in 100 mL of DMF, keeping the solution at 80 °C for 48 h.

In every case, the solvent was removed at the end of the reaction and the residue dissolved in 100 mL of H_2O, acidified with 6 N HCl, and extracted with ether. The organic layer was extracted several times with a saturated solution of Na_2CO_3, and the extracts were collected and acidified to give the final product which was filtered or extracted with ether if oily. Yields ranged from 60% to 95%. Table 6 shows the acids not previously described.

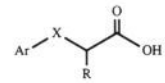
2-(4-Chlorophenoxy)-3-hydroxypropionic acid (**70**) was obtained from the corresponding 2-bromo-3-hydroxypropionic acid[29] with a similar procedure.

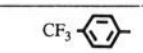
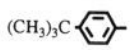
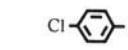
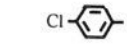
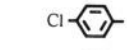
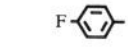
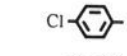
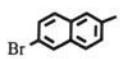
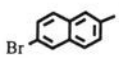
General Method for Ester Synthesis. A 0.01-mol portion of the appropriate acid was refluxed with 25 mL of $SOCl_2$ for 1 h. The excess of thionyl chloride was removed at reduced pressure and the oily residue dissolved in cyclohexane and evaporated again to dryness. This procedure, repeated twice, gave a sufficiently pure acid chloride that was used as such in the following reaction.

A 0.01-mol portion of the appropriate amino alcohol was added to 0.01 mol of acyl chloride in 25 mL of CH_2Cl_2 and stirred for 12 h in the presence of an excess of solid anhydrous Na_2CO_3. At the end of the reaction, the organic layer was washed with a saturated solution of Na_2CO_3 and with H_2O and then dried over Na_2SO_4. Evaporation of the solvent gave a residue that was transformed into the salts reported in Tables 1–4.

The amide **60** was obtained in the same way, starting from α-tropamine. The ester **36** was obtained in the same way after protection of the hydroxy group with acetyl chloride. The ester **45** was obtained by oxidation of **37** with H_2O_2 in glacial acetic acid.

Table 6. (Aryloxy)- and (Arylthio)alkanoic Acids



no.	Ar	X	R	mp (°C) (rec solv)[a]	formula[b]
66		O	CH_3	73–75	$C_{10}H_9F_3O_3$
67		O	CH_3	87–89 (A)	$C_{13}H_{18}O_3$
68		O	C_6H_5	160–162 (B)	$C_{14}H_{11}ClO_3$
69		O	CH_2-C_6H_5	*c*	$C_{15}H_{13}ClO_3$
70		O	CH_2OH	136–138 (A)	$C_9H_9ClO_4$
71		S	CH_3	50–51 (A)	$C_9H_9FO_2S$
72		S	C_2H_5	*d*	$C_{10}H_{12}O_2S$
73		S	$CH(CH_3)_2$	*d*	$C_{11}H_{13}ClO_2S$
74		O	CH_3	182–183 (A)	$C_{13}H_{11}BrO_3$
75		O	C_2H_5	135–136 (C)	$C_{14}H_{13}BrO_3$
76		O	CH_3	158–160 (B)	$C_{13}H_{11}ClO_3$
77		O	C_2H_5	133–134 (B)	$C_{14}H_{13}ClO_3$

[a] Solvent: A = EtOH/H_2O, B = ethyl acetate/cyclohexane, and C = cyclohexane. [b] All compounds were analyzed for C, H, and N. The results are within ±0.4% of the theoretical value. IR and NMR spectra are in agreement with the proposed structures. [c] The sample could not be purified from traces of *p*-chlorocinnamic acid and was used as such. [d] Oil.

3α-Tropanyl 2-(Phenylamino)propionate (46). A 6.5-g (0.042-mol) portion of 2-bromopropionic acid was transformed into the chloride by reaction with $SOCl_2$ (6.2 mL, 0.084 mol) at 50 °C. The acyl chloride was purified by fractional distillation (120–125 °C). Acyl chloride (0.68 g, 3.9 mmol) was added to a solution of α-tropanol hydrochloride (0.7 g, 3.9 mmol) in 50 mL of $CHCl_3$ and the solution kept at 50 °C for 8 h; then, 0.73 g (7.8 mmol) of aniline in 10 mL of $CHCl_3$ was added and the solution refluxed for another 6 h. The resulting mixture was then cooled and shaken with a saturated solution of Na_2CO_3 and the organic layer washed with H_2O and dried. Evaporation of the solvent gave 1.2 g of an oil that was transformed into the hydrochloride (see Table 2).

In the same way, using the appropriate aniline and alkanoic acid, compounds **47–49** were prepared (see Table 2).

3α-Tropanyl 2-(4-Chlorophenoxy)propyl Ether (61). A 0.02-mol portion of (±)-**4** (as the free base) was dissolved in 25 mL of boron trifluoride etherate, and a solution of $NaBH_4$ (0.1 M) in 75 mL of anhydrous THF was added while cooling to 0 °C. The mixture was then left at room temperature for 10 h. The excess of hydride was destroyed with acetone and the mixture evaporated at reduced pressure. Alkalinization of the residue with 10% NaOH and extraction with $CHCl_3$ gave an oil that was transformed into the oxalate. Compounds **62–64** were obtained in the same way (see Table 4).

General Method for the Synthesis of Chiral α-Tropanyl Esters. A 0.01-mol portion of the enantiomer of the acid was heated at 60 °C for 3 h in 25 mL of $SOCl_2$ and the reaction

product worked up as already described for the racemic chloride. The acyl chloride was then dissolved in 20 mL of ethanol-free $CHCl_3$, a solution of 0.012 mol of α-tropanol hydrochloride in 100 mL of ethanol-free $CHCl_3$ was added, and the mixture was refluxed for 30 h. The solvent was removed under vacuum and the residue treated with a 10% solution of $NaHCO_3$. The solution was extracted with H_2O and then dried and evaporated to give an oil that was transformed into the salt shown in Table 5.

Resolution of Racemic 2-(Phenylthio)butyric Acid (72). A 3.7-g (0.019-mol) portion of racemic **72** was dissolved in the minimum amount of CH_3CN, and an equimolar amount of (*R*)-(+)-phenylethylamine was added; the salt obtained was recrystallized several times from CH_3CN to constant rotation. $[\alpha]_D^{20}$ = +23.8° (*c* = 0.5; absolute EtOH). Mp 85–87 °C. The salt was dissolved in the minimum amount of water, the solution acidified with 6 N HCl, and the acid extracted with $CHCl_3$. Yield 0.83 g. $[\alpha]_D^{20}$ = +51.6° (*c* = 0.5; absolute EtOH). The mother liquors of previous crystallizations were collected. The acid was recovered as described above and then dissolved in CH_3CN; an equimolar amount of (*S*)-(−)-phenylethylamine was added. After several crystallizations, constant rotation was obtained. $[\alpha]_D^{20}$ = −22.0° (*c* = 0.5; absolute EtOH). Mp 86–88 °C. The acid was obtained as described above; $[\alpha]_D^{20}$ = −46.8° (*c* = 0.5; absolute EtOH).

The enantiomer with $[\alpha]_D^{20}$ = −46.8° was derivatized with (1*R*,2*S*,5*R*)-menthol and then gas chromatographed on a Perkin-Elmer 8420 capillary gas chromatograph equipped with a DB5 (J&W Scientific, CA), 0.1 μm. The results showed that the resolution was incomplete, the ee being about 40%. The same results were obtained with HPLC on chiracel OD-R (DAICEL) (eluent: $CH_3CN/NaClO_4$ (0.1 M)–$HClO_4$, pH 3, 70/30; flux 0.5 mL/min).

***N*-Ethyl-*N*-(4-pyridinylmethyl)-2-(4-chlorophenoxy)butyramide (78).** A 0.3-g (1.4-mmol) portion of 2-(4-chlorophenoxy)butyric acid was transformed into the acyl chloride by reaction with $SOCl_2$ (0.2 mL, 2.8 mmol) at 80 °C for 2 h. The excess of thionyl chloride was removed following the procedure described for the General Method for Ester Synthesis.

4-[(Ethylamino)methyl]pyridine (0.4 mg, 2.8 mmol) was added to the acyl chloride in 10 mL of ethanol-free $CHCl_3$ and the mixture heated to reflux for 14 h. At the end of the reaction, the organic layer was washed with a saturated solution of Na_2CO_3 and with H_2O and then dried over Na_2SO_4. Evaporation of the solvent gave a residue (0.4 g) that was crystallized from a mixture of ethyl acetate–cyclohexane; 0.14 g of **78** was obtained. Mp 99–100 °C. IR ν 1660 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.40–8.55 (m, 2H), 6.65–6.75 (m, 1H), 6.80–7.05 (m, 3H), 7.10–7.20 (m, 2H), 4.45–4.90 (m, 3H), 3.20–3.60 (m, 2H), 1.80–2.15 (m, 2H), 0.95–1.20 (m, 6H). MS *m/e* 332 (M^+). Anal. ($C_{18}H_{21}ClN_2O_2$) C,H,N.

Pharmacology. Analgesic Activity. Analgesic activity was evaluated using the hot-plate method according to Woolfe.[30] The plate temperature was fixed at 52.5 ± 0.1 °C. An arbitrary cutoff 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} (Tables 1–5). 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 nontoxic dose was compared to that of morphine, taken as the reference compound and injected at 8 mg/kg sc, a dose that does not alter animal behavior.

Calculations were performed using the following formula: Analgesic efficacy of X expressed as percentage of that of morphine·HCl (8 mg/kg sc) = (maximum reaction time of X − pretest reaction of X)/(maximum reaction time of morphine − pretest reaction time of morphine) × 100.

The maximal nontoxic 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 nontreated 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 the pretest reaction time. Differences were considered statistically significative when $P \leq 0.05$. Percent values were calculated only for those differences that resulted statistically significative; in the other cases, drugs were considered inactive. Since the reaction times were measured with an accuracy of ±15%, the errors on the percent values calculated through the formula reported above should be in the same range.

Nootropic Activity. Nootropic activity was evaluated in mice using the passive avoidance test according to the method described by Jarvik and Kopp.[31] The above orginal method was slightly modified by using a painless punishment (fall into cold water, 10 °C) instead of the electrical foot-shock punishment.

This modification was introduced to avoid false results arising from the analgesic properties of the tested compounds. The M_1 antagonist dicyclomine (2 mg/kg ip injected immediately after the training session) was used in order to induce amnesia for evaluating the potential protective activity of the test compounds. These were injected intraperitoneally 20 min before the training session.

Results are expressed as differences in the times of entry into the dark compartment between the first and second sessions.

Acknowledgment. This research has been supported by FIDIA SpA, Abano Terme (Pd), Italy. We are grateful to Dr. Carlo Bertucci of the University of Pisa for the determination of optical purity of (−)-**72** by chiral HPLC and to Dr. Cristina Bellucci for her excellent technical assistance.

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(2) Gualtieri, F.; Romanelli, M. N.; Scapecchi, S.; Teodori, E.; Bartolini, A.; Fantetti, L.; Ghelardini, C.; Giotti, A. Muscarinic presynaptic autoreceptors and muscarinic postsynaptic receptors have opposite stereochemical requirements. *Med. Chem. Res.* **1991**, *1*, 52–58.
(3) 2-Phenoxyacetic acid, 2-(4-chlorophenoxy)acetic acid, 2-phenoxypropionic acid, 3-phenoxypropionic acid, 2-(2-chlorophenoxy)-propionic acid, and its 3- and 4-isomers, 2-phenoxybutyric acid and chlofibric acid, were commercially available. The following acids were obtained according with the literature: 2-(4-fluorophenoxy)propionic acid;[4] 2-(4-bromophenoxy)propionic acid;[5] 2-(4-cyanophenoxy)propionic acid;[6] 2-[4-(thiomethyl)phenoxy]propionic acid;[7] 2-(3,4-dichlorophenoxy)propionic acid;[8] 2-(3-methyl-4-chlorophenoxy)propionic acid;[9] 2-(4-biphenylyloxy)propionic acid;[10] 2-(4-chlorophenoxy)butyric acid;[11] 2-(4-fluorophenoxy)butyric acid;[12] 2-(4-chlorophenoxy)isobutyric acid;[13] 2-(phenylthio)propionic acid;[14] 2-[(4-chlorophenyl)thio]propionic acid;[15] 2-[(4-bromophenyl)thio]-propionic acid;[15] 2-[(4-methylphenyl)thio]propionic acid;[16] 2-[(4-chlorophenyl)thio]butyric acid;[17] 2-β-naphthoxypropionic acid;[18] 2-β-naphthoxybutyric acid;[18] 2-α-naphthoxypropionic acid;[18] and 2-α-naphthoxybutyric acid.[18]
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