Presynaptic Cholinergic Modulators as Potent Cognition Enhancers and Analgesic Drugs. 1. Tropic and 2-Phenylpropionic Acid Esters

Fulvio Gualtieri,*,† Gabriele Conti,†,§ Silvia Dei,† Maria Paola Giovannoni,†,§ Francesca Nannucci,†,§ Maria Novella Romanelli,† Serena Scapecchi,†,§ Elisabetta Teodori,† Luisa Fanfani,‡,§ Carla Ghelardini,‡ Alberto Giotti,‡ and Alessandro Bartolini‡

Dipartimento di Scienze Farmaceutiche, Università di Firenze, Via G. Capponi 9, 50121 Firenze, Italy, and Dipartimento di Farmacologia Preclinica e Clinica, Università di Firenze, Viale G. B. Morgagni 65, 50134 Firenze, Italy

Received October 27, 1993®

Previous studies have shown that (*R*)-(+)-hyoscyamine has analgesic activity as a consequence of increased ACh release following antagonism of central muscarinic autoreceptors. Since the enhancement of central cholinergic transmission could be beneficial for cognitive disorders, we manipulated (*R*)-(+)-hyoscyamine, synthesizing several derivatives of tropic and 2-phenylpropionic acids, with the aim of obtaining drugs which are able to increase ACh release and consequently to show analgesic and nootropic activities. The results showed that several new compounds are indeed potent analgesics (with an analgesic efficacy comparable to that of morphine) and that the most potent one ((±)-**19**, PG_9) also has remarkable cognition-enhancing properties. Our study confirmed that the mechanism of action involves ACh release even if it is still unclear whether only muscarinic autoreceptors or, also, heteroreceptors are involved.

Introduction

The central cholinergic system has various actions, one of which is its prominent role in cognitive processes (learning and memory)[1] and in pain perception.[2,3]

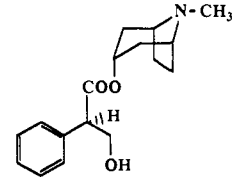
Much of the pertinent data concerning cognitive functions has emerged from studies on Alzheimer's and Alzheimer-like diseases (AD, ALD) where the cholinergic hypothesis[1,4] has stimulated an immense amount of research. This in turn has led to the study of several groups of drugs that potentiate the cholinergic system: ACh precursors,[5] cholinesterase inhibitors,[5] ACh releasers,[6] and directly acting AChR agonists.[7] However, the therapeutic usefulness of most of the compounds is compromised by the meager benefits achieved with them in clinical trials in proportion to the central and peripheral undesiderable side effects observed at potential therapeutic doses.[8]

Direct and indirect cholinomimetics such as oxotremorine, arecoline, pilocarpine, and physostigmine, etc., have been reported to possess analgesic activity.[9] In this case, too, their use as antinociceptives has been prevented by their side effects.

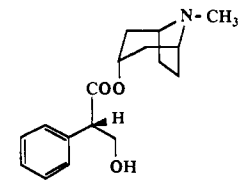
On principle, most of the problems connected with the unwanted side effects of cholinergic modulators which are potentially useful in analgesia and pathological states characterized by a cholinergic deficit could be prevented by selective inhibition of the central presynaptic receptors controlling ACh release. In practice, as has already been pointed out by Earl et al.,[10] muscarinic presynaptic inhibition gives high ACh levels only when its release is triggered by excitation of the cholinergic neurons. This action also results in an improvement of the signal-to-noise ACh ratio during cholinergic impulse transmission, without the potential of ACh overload which is typical of cholinergic inhibitors and without the distortion of the temporal pattern in cholinergic transmission caused by direct cholinergic agents. The final result should be a physiological amplification of central cholinergic transmission, a condition which would be beneficial for many symptoms caused by reduced cholinergic activity, with elimination of the side effects seen with other cholinergic agents.

We have recently reported that atropine[11] (like some local anaesthetics[12] and antiarrhythmic drugs[13]) is able to induce a selective blockade of central muscarinic autoreceptors which results in increased ACh release. Blockade of the muscarinic autoreceptor is obtained at very low concentrations of atropine (10^{-14}–10^{-12} M), while at high doses, the postsynaptic receptors are also antagonized. In rodents, the postsynaptic inhibition by atropine in fact begins at 1 mg/kg ip while the antinociceptive effect begins at 1 μg/kg ip.[11,14]

We have also shown that antinociception is peculiar to only one of the enantiomers present in atropine, namely the (*R*)-(+)-hyoscyamine, the other enantiomer ((*S*)-(−)-hyoscyamine) being devoid of any activity of this kind.[15] In this case, since (*R*)-(+)-hyoscyamine maintains a high level of activity on presynaptic receptors while being some hundred times less potent than (*S*)-(−)-hyoscyamine on postsynaptic receptors,[16] the selectivity of action is obviously increased.



R-(+)-hyoscyamine (R-(+)-1)



S-(-)-hyoscyamine (S-(-)-1)

The higher sensitivity of the autoreceptors to the block by antagonistic drugs has already been reported for other neurotransmitters. It has been shown[17-19] that very low doses of naloxone can induce analgesia and that such analgesia is due to increased enkephalin release produced by blocking autoinhibition.[20] Moreover, Starke[21] has reported that yohimbine enhances noradrenaline neural

† Dipartimento di Scienze Farmaceutiche.
‡ Dipartimento di Farmacologia Preclinica e Clinica.
§ Recipient of a FIDIA SpA fellowship.
® Abstract published in *Advance ACS Abstracts*, April 15, 1994.

0022-2623/94/1837-1704$04.50/0

release at lower concentrations than those reported to block postsynaptic receptors.

On this basis, taking atropine and its *R*-(+)-enantiomer as the leads, we started a research project aimed at developing drugs which are able to increase ACh release by specific inhibition of central muscarinic presynaptic receptors and which would therefore be potentially useful as analgesics and/or in pathological conditions characterized by cholinergic deficit.

Since the available pharmacological models for testing nootropic activity are complex, time-consuming, and fairly expensive, whereas antinociceptive activity can be evaluated much more simply, we decided to follow the results of molecular manipulation of the lead through the hot-plate test of analgesia. At the same time, we checked whether this effect was reversed by suitable doses of atropine and the ACh depletor hemicholinium-3 (HC-3), in order to be sure that analgesia was actually due to a cholinergic mechanism. When these conditions were fulfilled, we could logically expect that selected molecules would also show nootropic activity.

Chemistry

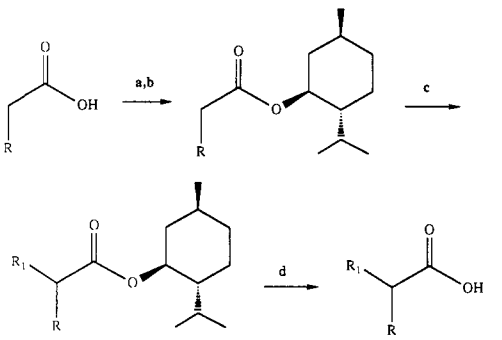
The structure and chemical–physical characteristics of the compounds synthesized and studied are reported in Tables 1–3 together with their analgesic activity.

The synthesis of optically pure (>98%)[15] enantiomers of atropine, (*R*)-(+)-hyoscyamine ((+)-1) and (*S*)-(−)-hyoscyamine ((−)-1), and racemic acid esters **2–10** was performed using the methods already described in the literature.[22] 4-Chloro- and 4-methoxytropic acids were obtained according to Caldwell.[23] We attempted to obtain 4-nitroatropine or 4-nitrotropic acid which would have provided a useful entry to several 4-substituted derivatives. However, although 4-nitroatropine has been described,[24,25] we obtained only α-tropanyl 2-(4-nitrophenyl)acrylate.[26] Accordingly, all attempts to synthesize 4-nitrotropic acid with a variety of methods (e.g., direct nitration of tropic acid, treatment of *p*-nitrophenylacetic acid with isopropylmagnesium bromide and formaldehyde, hydrolysis of α-chloromethyl-4-nitrophenylacetic acid) failed, as 2-(4-nitrophenyl)acrylic acid was invariably obtained.

2-(4-Nitrophenyl)propionic acid and 2-(4-isobutylphenyl)propionic acid are commercially available. Apart from 2-(2-bromophenyl)propionic acid (**42**) and 2-(4-trifluorophenyl)propionic acid (**43**), which have not been described before, all other 2-phenylpropionic acids and analogues that were used as intermediates are known and have been obtained with a variety of methods.[27] We chose to synthesize them with a common synthetic pathway that uses the hindered (1*R*,2*S*,5*R*)-menthyl esters of the corresponding phenylacetic acids as a substrate for alkylation (Scheme 1). In this way, double alkylation was minimized, and the yields were reasonably high. 2-(4-Bromophenyl)butyric acid was also obtained by this method.[37] Finally, the ether **40** was obtained from the corresponding ester (±)-**19** by reduction with sodium borohydride in boron trifluoride etherate[38] (Scheme 2).

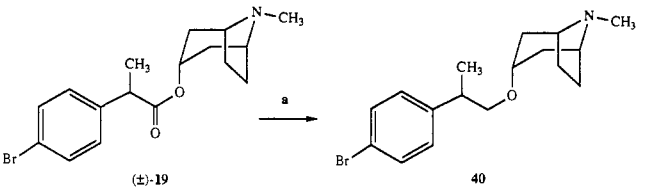
(*S*)-(+)- and (*R*)-(−)-**11** were obtained from the corresponding enantiomeric acids, which are commercially available at an optical purity of 97%. The synthetic pathway shown in Scheme 1 is potentially useful for a chiral synthesis of the enantiomers of 2-(4-bromophenyl)-propionic acid (**41**), the starting material of one of the most interesting compounds studied, (±)-**19**. However,

Scheme 1[a]



[a] (a) $SOCl_2$; (b) (1*R*,2*S*,5*R*)-menthol; (c) R_1I, $NaNH_2$; (d) KOH, C_2H_5OH. R and R_1 are as reported in Tables 1–3.

Scheme 2[a]



[a] (a) $NaBH_4$, $BF_3{\cdot}Et_2O$.

probably because of the smallness of the entering methyl group, the enantiomeric excess obtained was quite low (about 38% as evaluated by ^1H NMR; see the Experimental Section). Moreover, hydrolysis with KOH/C_2H_5OH brought about nearly complete racemization. Other chemical resolution methods did not afford complete separation, and for this reason, resolution of (±)-**41** is currently incomplete as the two enantiomers obtained ((−)- and (+)-**41**) have ees of 30 and 44, respectively (evaluated by ^1H NMR with chiral shift reagents and HPLC chiral chromatography; see the Experimental Section). Consequently, (−)- and (+)-**19** are scalemic mixtures with similar optical purities.

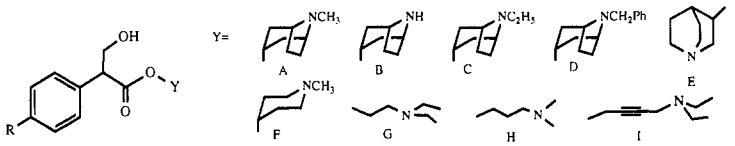
Results

Tropic Acid Derivatives 1-10. The first attempt to modulate the lead (atropine, **1**) was made by substituting the phenyl ring of tropic acid. 4-Chloro substitution (**7**) left potency and efficacy nearly unchanged, while 4-OCH_3 substitution (**6**) completely abolished analgesic activity.

Substitution of the α-tropanyl moiety with other amino alcohols gave variable results. We have already reported[15] that α-tropanol can be substituted by *N*-methylpiperidin-4-ol and 2-(diethylamino)ethanol. Here, α-nortropanol (**2**), and *N*-ethyl-α-nortropanol (**3**) substitution still gave active compounds, while 3-(dimethylamino)propanol (**4**) and 3-quinuclidinol (**8**) substitution abolished analgesic activity. It is remarkable that in the case of tropic acid, 2-(diethylamino)ethanol substitution gave an active compound while the corresponding compound with 4-chlorotropic acid (**10**) was inactive. Although the tropic acid tertiary amide tropicamide is active as an analgesic,[39] no attempts to substitute the ester with the isosteric amide function were made, as we had found and already reported that other tropic acid amides were inactive.[15]

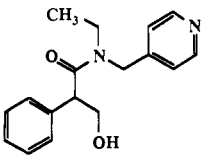
Substituted 2-Phenylpropionic Acid Derivatives and Related Compounds 11–40. Since the synthesis of substituted tropic acids is troublesome due to their poor stability and since none of the active compounds in this series exceeded 50% of the analgesic effect of morphine,

Table 1. Tropic Acid Esters



no.	R	Y	salt[a] (rec solv)	formula[b]	mp (°C)	analgesic activity[c]	
						% analgesic efficacy[d]	ED_{50} (SE) (mg/kg sc)
(±)-1[e]	H	A			114–116	42	$0.7\ (0.1) \times 10^{-3}$
2[f]	H	B	oxalate (B)	$C_{18}H_{23}NO_7$	255–258	35	$5.0\ (0.4) \times 10^{-3}$
3[f]	H	C	tartrate (A)	$C_{22}H_{31}NO_9$	122–124	33	$7.3\ (0.5) \times 10^{-3}$
4	H	H	dibenzoyltartrate (A)	$C_{32}H_{35}NO_{11}$	72–75	inact	
5	H	I	dibenzoyltartrate (A)	$C_{35}H_{37}NO_{11}$	83–85	inact	
6[g]	4-OCH_3	A			89–92	inact	
7[g]	4-Cl	A			188–190	37	$0.6\ (0.1) \times 10^{-3}$
8	4-Cl	E		$C_{16}H_{20}ClNO_3$	*h*	inact	
9	4-Cl	F	oxalate (B)	$C_{17}H_{22}ClNO_7$	67–70	26	5 (0.8)
10	4-Cl	G		$C_{15}H_{22}ClNO_3$	*h*	inact	

[a] A = absolute EtOH/Et_2O; B = absolute EtOH; C = cyclohexane; D = ethyl acetate. [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 the hot-plate test; plate temperature 52.5 °C; cutoff time 45 s. [d] Compared to morphine used as reference; see text for calculations and statistical evaluation. In this reference system, the value of atropine is 100. [e] Atropine; see ref 15. [f] See ref 49. [g] See ref 23. [h] Oil.



Tropicamide

we decided to start with molecular manipulation of the 2-phenylpropionic acid ester of α-tropanol, (±)-11,[15] which is less potent than atropine but shows the same efficacy. The decision was to overlook the potency (which seems to be related to the presence of the CH_2OH group) in order to concentrate on looking for more efficacious compounds which give a comparable analgesic effect to that of morphine.

Compounds (±)-14, (±)-19, (±)-28, and (±)-30 were readily obtained. These are indeed less potent than atropine and (±)-11 itself but have a comparable analgesic efficacy to that of morphine.

Optimization of both potency and efficacy was attempted, and the many variations performed on the basic structure are indicated in Tables 2 and 3. It should be noted that, in this series, only esterification with α-tropanol gave active compounds. The shift in the ortho and meta positions of bromine and chlorine affected the potency and, to a lesser extent, the efficacy. On the other hand, double substitution with chlorine gave a compound, 18, which has a comparable potency to that of atropine. However, its efficacy is definitely lower than that of other less potent compounds.

All other modifications usually reduced both potency and efficacy. Homologation of 14 to the corresponding 3-(4-chlorophenyl)isobutyric acid α-tropanyl ester (34) greatly affected potency and efficacy, resulting in an inactive compound. On the other hand, similar modification of (±)-19 to the 2-(4-bromophenyl)butyric acid α-tropanyl ester (33) maintained high activity.

As previously reported,[15] chirality appears to be critical for activity, and only (*R*)-(+)-1 and (*S*)-(+)-11 (which despite the nomenclature show identical absolute configurations) are active. The insufficient resolution of (±)-19 did not allow us to verify if this also holds true for the most efficacious compounds. In this case, too, the most active enantiomer was, however, (+)-19 (Table 4), for which an *S*-absolute configuration is very likely.[40] The analgesic potency and efficacy of the compounds defined as described in the Experimental Section are reported in Tables 1–4.

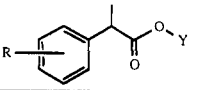
Discussion

It must be borne in mind that the choice of an *in vivo* test, while obviously speeding up the selection of the compounds with the most promising clinical properties, does not allow sound structure–activity relationships to be established. In fact, the resulting activity is the consequence of both pharmacokinetic and pharmacodynamic properties that are differently affected by structural modifications. This may explain some unexpected results, such as the lack of activity of compounds like 4 or 21, and, more generally, the wide variation of activity observed following small structural changes.

The action of the active compounds does arise from a cholinergic mechanism, since the analgesic effect is prevented by suitable doses of antimuscarinic drugs such as atropine, dicyclomine, and pirenzepine[11,12] (Figure 1). Moreover, the fact that the depletion of CNS ACh content by pretreatment of mice with hemicholinium-3 (Figure 1) prevents the analgesic effect of the active compounds indicates that the activity of such compounds depends on endogenous ACh, and a presynaptic mechanism can therefore be hypothesized for their mechanism of action. The potentiating effect of the compounds on electrically and nicotine-evoked guinea pig ileum responses, which was absent for ACh-induced contraction, confirms the involvement of a presynaptic cholinergic mechanism (Figure 2). In fact, the effect of exogenous ACh, which acts predominantly on muscarinic postsynaptic receptors, was not potentiated, while endogenous ACh release, evoked presynaptically with both electrical and nicotinic stimulation, was.

On the other hand, the fact that the antinociception induced by compounds (*R*)-(+)-1 and (±)-19 was not prevented by naloxone (1 mg/kg ip), reserpine (2 mg/kg ip administered twice 48 and 24 h before the analgesic test), and CGP-35348, a new GABA-B antagonist[41] (100 mg/kg ip; data not shown), rules out the involvement of

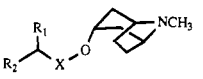
Table 2. 2-Phenylpropionic Acid Esters

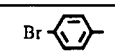
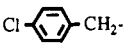
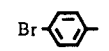
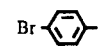


no.	R	Y*	salt[a] (rec solv)	formula[b]	mp (°C)	analgesic activity[c] maximum level of analgesia[d]	analgesic activity[c] ED_{50} (SE) (mg/kg sc)
(±)-11[e]	H	A	hydrochloride (B)		178–181	35	$5.0\ (0.7) \times 10^{-2}$
12	4-NO_2	A	hydrochloride (B)	$C_{17}H_{23}ClN_2O_4$	190–192	38	18 (1.9)
13	4-NH_2	A	hydrochloride (C)	$C_{17}H_{25}ClN_2O_2$	170–172	41	6 (1.7)
14	4-Cl	A	maleate (B)	$C_{21}H_{26}ClNO_6$	137–139	83	36 (2.1)
15	4-Cl	E	hydrochloride (A)	$C_{16}H_{21}Cl_2NO_2$	121–125	inact	
16	2-Cl	A	maleate (A)	$C_{21}H_{26}ClNO_6$	138–140	83	36 (2.6)
17	3-Cl	A	maleate (A)	$C_{21}H_{26}ClNO_6$	130–132	71	37 (2.1)
18	3,4-Cl	A	maleate (A)	$C_{21}H_{25}Cl_2NO_6$	146–148	42	$1.0\ (0.2) \times 10^{-2}$
(±)-19	4-Br	A	maleate (B)	$C_{21}H_{26}BrNO_6$	148–150	101	21 (2.2)
20	4-Br	B	maleate (A)	$C_{20}H_{24}BrNO_6$	89–91	inact	
21	4-Br	C	oxalate (A)	$C_{20}H_{26}BrNO_6$	172–174	inact	
22	4-Br	D	oxalate (A)	$C_{25}H_{28}BrNO_6$	118–120	34	28 (3.2)
23	4-Br	F	maleate (B)	$C_{19}H_{24}BrNO_6$	116–119	inact	
24	4-Br	G	oxalate (B)	$C_{17}H_{24}BrNO_6$	118–119	inact	
25	4-Br	H	maleate (A)	$C_{18}H_{24}BrNO_6$	87–88	inact	
26	4-Br	I	oxalate (B)	$C_{19}H_{24}BrNO_6$	88–90	inact	
27	2-Br	A	hydrochloride (D)	$C_{17}H_{23}BrClNO_2$	192–193	62	30 (3.7)
28	4-F	A	maleate (A)	$C_{21}H_{26}FNO_6$	132–135	79	31 (4.1)
29	4-$CH_2CH(CH_3)_2$	A	maleate (B)	$C_{25}H_{35}NO_6$	103–106	40	27 (2.9)
30	4-OCH_3	A	maleate (A)	$C_{22}H_{29}NO_7$	105–108	97	32 (2.8)
31	4-CF_3	A	maleate (A)	$C_{22}H_{26}F_3NO_6$	156–157	26	31 (3.1)
32	4-$N(CH_3)_2$	A	oxalate (A)	$C_{21}H_{30}N_2O_6$	113–116	inact	

* See Table 1. [a–d] See corresponding footnotes of Table 1. [e] See refs 15 and 50.

Table 3. Structural Variations on molecule (±)-19



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)
33	C_2H_5		CO	maleate (B)	$C_{22}H_{28}BrNO_6$	116–118	81	33 (2.0)
34[e]	CH_3		CO	oxalate (A)	$C_{20}H_{26}ClNO_6$	159–162	inact	
35	H		CO	hydrochloride (B)	$C_{16}H_{22}ClNO_2$	140–145	inact	
36[f]	Cl		CO	maleate (D)	$C_{20}H_{24}ClNO_6$	152–155	inact	
37	CH_3		CO	oxalate (A)	$C_{17}H_{23}NO_6S$	144–145	21	5.4 (0.7)
38	CH_3		CO	oxalate (B)	$C_{17}H_{23}NO_6S$	141–143	44	22 (1.3)
39	H		CO	maleate (A)	$C_{20}Y_{24}BrNO_6$	136–138	inact	
40	CH_3		CH_2	oxalate (A)	$C_{19}H_{26}BrNO_5$	156–158	45	32 (2.5)

[a–d] See the corresponding footnotes of Table 1. [e] The acid starting material was obtained according to ref 51. [f] The acid starting material was obtained according to ref 52.

the opioidergic, serotoninergic, adrenergic, and GABAergic systems; thus, the argument in favor of the cholinergic involvement is reinforced.

As expected, the compounds showing high antinociceptive activity were also potent nootropic drugs, as shown for compounds (*R*)-(+)-1 and (±)-19 in Figure 3.

We tested the lead compound (*R*)-(+)-hyoscyamine and also (±)-19, the most active analgesic compound of the series, on dicyclomine-induced amnesia. As reported in Figure 3, both compounds were able to prevent this amnesia, the potency of (*R*)-(+)-hyoscyamine being, however, 1000 times higher than that of compound (±)-19. Again as expected, the two reference drugs physostigmine and piracetam were also able to prevent dicyclomine amnesia, but the protective effect of the anticholinesterase agent physostigmine was accompanied by typical cholinergic symptoms such as tremors, scialorrhea, diarrhea, and lacrimation, etc., which do not appear with the other three drugs.

It is still unclear whether the active compounds act through presynaptic muscarinic receptors (autoreceptors) or heteroreceptors. It is known, in fact, that many kinds of receptors and ion channels control ACh release at the nerve ending[42,43] (including 5-HT_3, 5-HT_4, D_2, adrenergic receptors, and K^+ and Ca^{2+} channels) and that their modulation can modify the pain threshold.[9]

Table 4. Enantiomers of 1, 11, and 19

compd	$[\alpha]^{20}_D$ (absolute EtOH)	salt[a] (rec solv)	mp (°C)	analgesic activity[c] maximum level of analgesia[d]	analgesic activity[c] ED_{50} (SE) (mg/kg sc)
(*R*)-(+)-**1**	+21.0		108–109	41	$0.7\ (0.1) \times 10^{-3}$
(*S*)-(−)-**1**	−21.0		108–109	inact	
(*S*)-(+)-**11**	+37.6[b]	hydrochloride (B)	152–154[b]	29	$3.1\ (0.4) \times 10^{-2}$
(*R*)-(−)-**11**	−37.6[b]	hydrochloride (B)	152–154[b]	inact	
(+)-**19**[e]	+14.2	maleate (D)	137–139	92	17 (2.0)
(−)-**19**[e]	−11.6	maleate (D)	141–143	83	16 (1.1)

[a,c,d] See the corresponding footnotes of Table 1. [b] In ref 15, these data have been erroneously reported as −3.0° and +3.0° and 178–180 °C, respectively. [e] The compounds are incompletely resolved and have an ee of about 40% (see text).

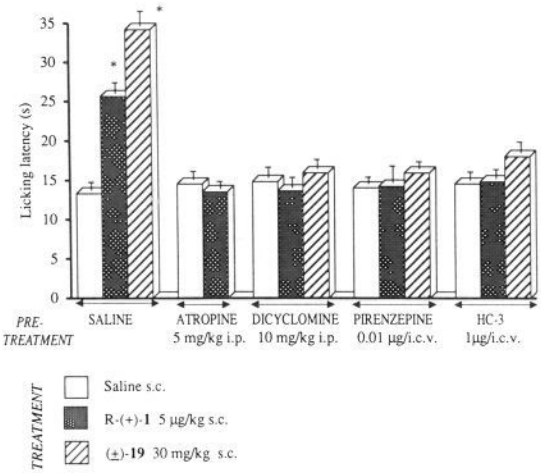


Figure 1. Effect of atropine, dicyclomine, pirenzepine, and hemicholinium-3 (HC-3) on (*R*)-(+)-**1** and (±)-**19** antinociception in mouse hot-plate test (52.5 °C). Vertical lines give SE of the mean. $*P < 0.01$ in comparison with saline controls. Atropine, dicyclomine, pirenzepine, and HC-3 were administered respectively 30 min, 30 min, 20 min, and 5 h before the test. (*R*)-(+)-**1** and (±)-**19** were injected 15 min before the test. Each column represents the mean of at least 10 mice.

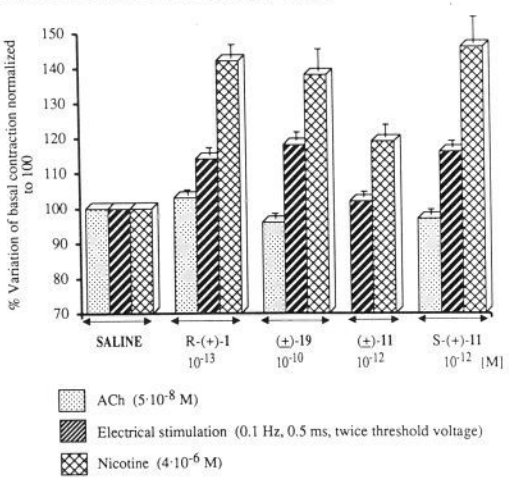


Figure 2. Effect of (*R*)-(+)-**1**, (±)-**19**, (±)-**11**, and (*S*)-(+)-**11** on chemically and electrically evoked contractions of guinea pig ileum myenteric plexus longitudinal muscle strip. Each column represents the mean of at least four experiments, and vertical lines give SE of the mean.

As already discussed in the Introduction and suggested by one of the referees, it seems reasonable that (*R*)-(+)-**1** antinociceptive activity depends on selectivity toward presynaptic receptors rather than postsynaptic muscarinic receptors. However, the results concerning 2-phenylpropionic acid derivatives **14** and **19** suggest that other heteroreceptors might be involved. In fact, while (*R*)-

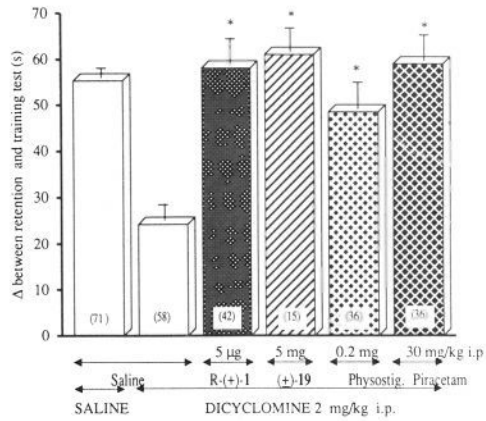


Figure 3. Effect of (*R*)-(+)-**1** and (±)-**19** on dicyclomine-induced amnesia in mouse passive avoidance test. (*R*)-(+)-**1**, (±)-**19**, and physostigmine were administered 20 min before training. Piracetam was administered 30 min before training. Dicyclomine was injected immediately after the 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.

Table 5. Effect of Selected Drugs on Specific [^{3}H]QNB Binding to Membrane Preparations from Rat Brain Tissue[53]

compd	pK_i[a]
(*R*)-(+)-**1**	7.30
14	7.02
(±)-**19**	6.96

[a] Affinity estimates are given as pK_i values calculated using the computer program LIGAND.[54] Values are means of at least three experiments with standard errors less than 10% of the means.

(+)-**1** bound quite well to the muscarinic receptor, as expected (Table 5), **14** and (±)-**19** showed a similar affinity toward this receptor, although their analgesic efficacy is clearly higher. Moreover, it is not yet clear which kind of muscarinic autoreceptor is involved in the analgesic activity of atropine and related compounds. M_2 subtypes seem probable candidates, but there are indications that M_4 receptors might also be responsible for the presynaptic modulation of ACh release.[9]

As regards the possible clinical usefulness of our compounds, it must be emphasized that the animals treated with (*R*)-(+)-**1** and (±)-**19** did not show the symptoms of cholinergic excitation that prevent use of other cholinomimetic drugs. In fact, mice treated with active doses behaved undistinguishably from controls, did not present salivation, tremors, or diarrhea, and maintained their normal motility and coordination in the Animex and rota-rod tests, respectively.

In conclusion, we have developed a new class of compounds which, by virtue of their ability to increase central ACh release, have promising nootropic and analgesic properties. The α-tropanyl ester of 2-(4-bromo-

phenyl)propionic acid (±)-19 (PG_9) has been selected from these compounds for further study.

Experimental Section

Chemistry. All melting points were taken on a Büchi apparatus and are uncorrected. Infrared spectra were recorded with a Perkin-Elmer 681 spectrophotometer in a Nujol mull for solids and neat for liquids. Unless otherwise stated, NMR spectra were recorded 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 (accuracy ±0.5°).

General Method for the Synthesis of Tropic Acid Esters 1–10. The amino alcohols used as starting materials are commercially available or were obtained according to the literature.[44-46] Their esters can be obtained with the following general procedure: 0.01 mol of the appropriate tropic acid was dissolved in 20 mL of acetyl chloride and the mixture refluxed for 20 min. The excess of the reagent was eliminated and the oily residue stirred at 60 °C for 2.5 h with 25 mL of $SOCl_2$. The excess of $SOCl_2$ was removed under reduced pressure, the residue dissolved in cyclohexane, and the solvent eliminated under vacuum; this operation was repeated three times.

The oily acid chloride obtained was treated with 0.01 mol of the hydrochloride of the appropriate amino alcohol and the mixture stirred under nitrogen for 2 h at 80 °C. When cool, the mixture was dissolved in 3 mL of water, treated with 1.5 mL of 6 N HCl, and stirred at room temperature for 24 h. After alkalinization with 10% Na_2CO_3, the solution was extracted with $CHCl_3$ and evaporated to give an oil from which minor impurities were removed by silica gel column chromatography using $CHCl_3$/petroleum ether/absolute ethanol/NH_4OH 340/60/65/8 as eluent.

When necessary, the product was transformed into the salt reported in Table 1.

General Method for the Synthesis of Ring-Substituted 2-Phenylpropionic Acids and Like Compounds. A 0.1-mol portion of the appropriate phenylacetic acid was dissolved in 20 mL of $SOCl_2$ and stirred at 60 °C for 1 h; the reaction was then worked up as already described.

The oily product was dissolved in 50 mL of CH_2Cl_2; 0.1 mol of (+)-(1*R*,2*S*,5*R*)-menthol was added and the mixture kept at 40 °C for 1 h. After removal of the solvent, the residue was dissolved in 30 mL of anhydrous toluene and 0.2 mol of $NaNH_2$ in 30 mL of toluene was added. The mixture was kept at room temperature for 10 min, and after it had cooled at 0 °C, 0.2 mol of alkyl iodide was added. The reaction mixture was then stirred at room temperature for 40 min. Dilution with H_2O and extraction with $CHCl_3$ gave an oil, which was hydrolized as follows: 0.1 mol of menthyl ester was dissolved in 30 mL of ethanol, 30 mL of a saturated ethanolic solution of KOH was added, and the mixture was refluxed for 24 h. Acidification with 6 N HCl and extraction with $CHCl_3$ gave the acids, which were purified by crystallization or distillation. Yields ranged from 60% to 80%.

Methylation of *p*-bromophenylacetic acid menthyl ester (44) in order to obtain 41 was studied in detail to establish the stereoselectivity of the reaction. Under the conditions reported above, 44 gives the menthyl ester of *p*-bromophenylpropionic acid (45) with $[\alpha]^{20}_D = -57.1°$ ($c = 0.5$, absolute EtOH). The ^1H NMR spectrum shows two overlapping quartets at 3.62 and 3.64 ppm (CH_3-*CH*-COO). These do not however allow a confident evaluation of the enantiomeric excess. ^1H NMR spectrum in the presence of tris(3-trifluoroacetyl-*d*-camphorate)europium(III) (5 mg + 15 mg of 45 in 2 mL of $CDCl_3$) shows two doublets in a 69.5:31.5 ratio (CH_3-CH-COO), indicating an enantiomeric excess of about 38. Hydrolysis of the ester, with either acid or alkali, however, afforded only racemic 41.

2-(2-Bromophenyl)propionic Acid (42). Mp 104–106 °C from ethanol/H_2O. Yield 85%. Anal. ($C_9H_9BrO_2$) C, H.

2-[4-(Trifluoromethyl)phenyl]propionic Acid (43). Mp 128–130 °C from ethanol/H_2O. Yield 80%. Anal. ($C_{10}H_9F_3O_2$) C, H.

General Method for the Synthesis of 2-Phenylpropionic Acid Esters 11–32 and Like Compounds 33–40. A 0.01-mol portion of the appropriate acid was heated at 60 °C for 3 h in 25 mL of $SOCl_2$, and the reaction was worked up as already described. The acyl chloride was dissolved in 25 mL of CH_2Cl_2, 0.03 mol of the proper amino alcohol was added, and the solution was left at room temperature for 12 h. Evaporation of the solvent, alkalinization with 6 N NaOH, and ether extraction gave oils that could be purified by silica gel column chromatography using $CHCl_3$/petroleum ether/absolute ethanol/NH_4OH 340/60/65/8 as eluting system.

The oils were usually transformed into the salts reported in Tables 1 and 2. Yields ranged from 65% to 85%.

Compound 13 was obtained by reduction of the corresponding nitro ester 12 with H_2/Pd/C. Compound 32 was obtained from compound 13 by methylation with CH_2O/HCOOH.

General Method for the Synthesis of Chiral α-Tropanyl Esters (*R*)-(−)-11, (*S*)-(+)-11, (−)-19, and (+)-19. A 0.01-mol portion of the proper acid enantiomer was transformed into the acyl chloride by the method already described.

The oil obtained was 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 then removed, the residue alkalinized with a 10% solution of Na_2CO_3, and the solution extracted with ether. The organic layer was carefully washed with water and dried and the solvent evaporated under vacuum to leave an oil that was transformed into the salt reported in Table 3.

α-Tropanyl 2-(4-Bromophenyl)propyl Ether (40). A 0.002-mol portion of (±)-19 was dissolved in 5 mL of boron trifluoride etherate and a solution of $NaBH_4$ (0.01 mol) in 15 mL of anhydrous THF added while cooling to 0 °C. The mixture was left at room temperature for 10 h; then, the excess of hydride was destroyed with acetone and the mixture evaporated under vacuum. Alkalinization of the residue with a 10% solution of NaOH and extraction with $CHCl_3$ gave an oil which was transformed into the oxalate (Table 2).

Resolution of Racemic (4-Bromophenyl)propionic Acid (41). A 6.55-mmol portion of racemic (*p*-bromophenyl)propionic acid (41) was dissolved in the minimum amount of acetone and 6.55 mmol of (*R*)-(+)-1-phenylethylamine added. The crystals formed at room temperature were collected and recrystallized several times from acetone to constant rotation, $[\alpha]^{20}_D = +6.2°$ ($c = 0.5$, absolute EtOH). Mp 139–142 °C. The salt was dissolved in the minimum amount of water and the solution acidified with 2 N H_2SO_4 and extracted with chloroform to give 1.22 mmol of the acid, $[\alpha]^{20}_D = -30.0°$ ($c = 0.5$, absolute EtOH). Mp 87–90 °C.

The mother liquors were evaporated to dryness, and the residue was recrystallized from water several times, $[\alpha]^{20}_D = +7.8°$ ($c = 0.5$, absolute EtOH). Mp 136–139 °C. The acid recovered as described above (0.87 mmol) has $[\alpha]^{20}_D = +33.0°$ ($c = 0.5$, absolute EtOH). Mp 88–92 °C.

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) showed that the separation was incomplete and the two samples had ees of 30 and 44, respectively. The same results were obtained with ^1H NMR (Varian VXR 300) using quinine as chiral ligand (acid/quinine 1/3; solvent $CDCl_3$).

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 original 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 chiral HPLC and ^{1}H NMR determination of the optical purity of (+)- and (−)-41 and to Dr. Cristina Bellucci for her excellent technical assistance.

References

(1) Bartus, R. T.; Dean, R. L., III; Beer, B.; Lippa, A. S. The Cholinergic Hypothesis of Geriatric Memory Dysfunction. *Science* **1982**, *217*, 408–417.
(2) Harris, L. S.; Dewey, W. L.; Howes, J. F.; Kennedy, J. S.; Pars, H. Narcotic antagonist analgesics: interactions with cholinergic systems. *J. Pharmacol. Exp. Ther.* **1969**, *169*, 17–22.
(3) Herz, A. Action of arecoline on the central nervous system. *Arch. Exptl. Pathol. Pharmakol.* **1962**, *242*, 414–429.
(4) Drachman, D. A.; Leavitt, J. L. Human memory and the cholinergic system. Relation to aging. *Arch. Neurol.* **1974**, *30*, 113–121.
(5) Becker, R. E.; Giacobini, E. Mechanism of cholinesterase inhibition in senile dementia of the Alzheimer type: clinical, pharmacological and therapeutical aspects. *Drug Dev. Res.* **1988**, *12*, 163–195.
(6) Wesseling, H.; Agoston, S. Effects of 4-aminopyridine in elderly patients with Alzheimer's disease. *N. Engl. J. Med.* **1984**, *310*, 988–989.
(7) Baker, R.; Saunders, J. Central Muscarinic Ligands and Receptors. *Annu. Rep. Med. Chem.* **1989**, *24*, 31–40.
(8) Porsolt, R. D. The silent epidemic: the search for treatment of age-related memory impairment. *TIPS* **1988**, *10*, 3–6.
(9) Ghelardini, C.; Giotti, A.; Gualtieri, F.; Matucci, R.; Romanelli, M. N.; Scapecchi, S.; Teodori, E.; Bartolini, A. Presynaptic auto- and heteroreceptors in the cholinergic regulation of pain. In *Trends in Receptor Research*; Angeli, P., Gulini, U., Quaglia, W., Eds., Elsevier Science Publishers S. V.: Amsterdam, 1992; pp 95–114.
(10) Earl, R. A.; Myers, M. J.; Johnson, A. L.; Scribner, R. M.; Wuonola, M. A.; Boswell, G. A.; Wilkerson, W. W.; Nickolson, V. J.; Tam, S. W.; Brittelli, D. R.; Chorvat, R. J.; Zaczek, R.; Cook, L.; Wang, C.; Zhang, X.; Lan, R.; Mi, B.; Wenting, H. Acetylcholine-releasing agents as cognition enhancers. Structure-activity relationships of pyridinyl pendant groups on selected core structures. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 851–854.
(11) Ghelardini, C.; Malmberg-Aiello, P.; Giotti, A.; Malcangio, M.; Bartolini, A. Investigation into atropine induced antinociception. *Br. J. Pharmacol.* **1990**, *101*, 49–54.
(12) Bartolini, A.; Galli, A.; Ghelardini, C.; Giotti, A.; Malcangio, M.; Malmberg-Aiello, P.; Zucchi, P. L. Antinociception induced by systemic administration of local anaesthetics depends on a central cholinergic mechanism. *Br. J. Pharmacol.* **1987**, *92*, 711–721.
(13) Bartolini, A.; Ghelardini, C.; Meini, S.; Malmberg-Aiello, P.; Malcangio, M.; Giotti, A. The stimulating action of very low doses of antagonist drugs, or the otherwise generally higher sensitivity of presynaptic autoreceptors. *Pharmacol. Res. Commun.* **1988**, *20* (Suppl 1), 59–60.
(14) Bartolini, A.; Ghelardini, C.; Fantetti, L.; Malcangio, M.; Malmberg-Aiello, P.; Giotti, A. Role of muscarinic receptors subtypes in central antinociception. *Br. J. Pharmacol.* **1992**, *105*, 77–82.
(15) 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.
(16) Barlow, R. B.; Franks, F. M.; Pearson, J. D. M. Studies on the Stereospecificity of Closely Related Compounds Which Block Postganglionic Acetylcholine Receptors in Guinea-Pig Ileum. *J. Med. Chem.* **1973**, *16*, 439–446.
(17) Levine, J. D.; Gordon, N. C.; Fields, H. L. Naloxone dose-dependently produces analgesia and hyperalgesia in postoperative pain. *Nature* **1979**, *278*, 740–741.
(18) Woolf, C. J. Analgesia and hyperalgesia produced in the rat by intrathecal naloxone. *Brain. Res.* **1980**, *189*, 593–597.
(19) Kaiser, V.; Guilbaud, G. Dose-dependent analgesic and hyperalgesic effects of systemic naloxone in arthritic rats. *Brain Res.* **1981**, *226*, 344–348.
(20) Ueda, H.; Fukushima, N.; Kitao, T.; Ge, M.; Takagi, H. Low doses of naloxone produce analgesia in the mouse brain by blocking presynaptic autoinhibition of enkephaline release. *Neurosci. Lett.* **1986**, *65*, 247–252.
(21) Starke, K. Regulation of noradrenaline release by presynaptic receptor systems. *Rev. Physiol. Biochem. Pharmacol.* **1977**, *77*, 1–124.
(22) Fodor, G.; Rakoczi, J.; Csepreghy, Gy. Resolution of tropic acid and a direct synthesis of (−)hyoscyamine [S(−)atropine]. *Acta Chim. Hung.* **1961**, *28*, 409–411.
(23) Caldwell, H. C.; Finkelstein, J. A.; Arbakov, D.; Pelikan, C.; Groves, W. G. Spasmolytics. I. 3-Tropanyl 2-Arylacrylates and 3-Tropanyl 2-Arylhydracrilates. *J. Med. Chem.* **1969**, *12*, 477–480.
(24) Einhorn, A.; Fischer, L. Ueber nitroatropin. (On nitroatropine.) *Ber. Dtsch. Chem. Ges.* **1892**, *25*, 1390–1391.
(25) Repke, H.; Matthies, H.; Dittrich, G.; Werchan, H. G. Synthesis in affinity gels for the isolation of muscarinergic acetylcholine receptors. *Z. Chem.* **1980**, *20*, 60.
(26) Schwenker, G. Zur Kenntnis der Vitalischen Farbreaktion. (On the knowledge of vital colon reactions.) *Arch. Pharm.* **1965**, *298*, 826–838.
(27) 2-(4-Chlorophenyl)-,[28] 2-(2-chlorophenyl)-,[29] 2-(3-chlorophenyl),[30] 2-(3,4-dichlorophenyl)-,[31] 2-(4-bromophenyl)-,[32] 2-(4-fluorophenyl)-,[33] and 2-(4-methoxyphenyl)propionic acid[34] and 2-thienyl-[35] and 3-thienylpropionic acid[36] have been already described.
(28) Wilds, A. L.; Meader, A .L. The use of higher diazohydrocarbons in the Arndt-Eistert synthesis. *J. Org. Chem.* **1948**, *13*, 763–779.
(29) Herbicidal compositions of ring-halogenated α-substituted phenylacetic acids and their derivatives. Neth. Appl. 6,408,190, Jan. 20, 1965; *Chem. Abstr.* **1965**, *62*, 16141d.
(30) Nerdel, F.; Haerter, H. Darstellung um Rotationdispersionen optisch aktiver m-substituierter Hydratropäsaren. (Preparation and rotary dispersion of optically active m-substituted hydrotropic acids.) *Justus Liebigs Ann. Chem.* **1959**, *621*, 22–50.
(31) Zdansky, G. Preparation of 2,4- and 3,4-dichlorohydratropic acids. *Ark. Kemi* **1955**, *7*, 577–581.
(32) Abbott, D. C.; Arcus, C. L. Addition and Substitution with the Bromine Cation in Aqueous Solution. *J. Chem. Soc.* **1952**, 2934–2935.
(33) Platinum(II) complexes as neoplasm inhibitors. PCT Int. Appl. WO 89 05,346, June 15, 1989; *Chem. Abstr.* **1990**, *112*, 151849r.
(34) Kenyon, W. G.; Kaiser, E. M.; Hauser, C. R. Alkylations of Phenylacetic, α-Alkylphenylacetic, and Diphenylacetic Esters by Means of Sodamide and Sodium Hydride. *J. Org. Chem.* **1965**, *3*, 2937–2942.
(35) Berçot-Vatteroni, M.; Moreau, R. C.; Reynaud, P. Sur Quelques acides alcoyl-(thiényl-2)-acétiques et quelques-uns de leurs dérivés. (On some alkyl-(2-thienyl)-acetic acid and some of their derivatives.) *Bull. Soc. Chim. Fr.* **1961**, 1820–1822.
(36) 2-(3-Thienyl)propionic acid. Span 487,840, Dec. 16, 1980; *Chem. Abstr.* **1981**, *95*, 42894m.
(37) Canonica, L.; Bonati, A.; Tedeschi, C. Anticolesterolemici di sintesi. (Synthetic anticholesterolemics.) *Farmaco* **1958**, *13*, 286–293.
(38) Pettit, G. R.; Ghatak, U. R.; Green, B.; Kasturi, T. R.; Piatak, D. M. Steroids and Related Natural Products. IV. Reduction of Lactones to Cyclic Ethers. *J. Org. Chem.* **1961**, *26*, 1685–1686.
(39) Tropicamide is active on the hot-plate test and shows an ED_{50} of 0.6 mg/kg sc and an analgesic efficacy of 77% compared to that of morphine.
(40) The absolute configuration has not yet been established because of the incomplete resolution of the racemate of the starting material

41; work is in progress to obtain complete resolution and to confirm the configuration of enantiomers.
(41) Bittiger, H.; Froestl, W.; Hall, R.; Karlsson, G.; Klebs, K.; Olpe, H.-R.; Pozza, M. F.; Steinmann, M. W.; Van Riezen, H. Biochemistry, electrophysiology and pharmacology of a new GABA-B antagonist: CGP 35348. In *GABA-B Receptors in Mammalian Pharmacology*, Bowery, N. G., Bittiger, H., Olpe, H.-R., Eds.; John Wiley & Sons, Chichester, U.K., 1990; pp 47–60.
(42) Baux, G.; Fossier, P. La libération d'acétylcholine et sa régulation. (Acetylcholine release and its regulation.) *Arch. Int. Physiol. Biol. Biophys.* **1992**, *100*, A3–A15.
(43) Chesselet, M. F. Presynaptic regulation of neurotransmitter release in the brain. *Neuroscience* **1984**, *12*, 347–375.
(44) α-Tropanol, 3-quinuclidinol, *N*-methylpiperidinol, (*N*,*N*-diethylamino)ethanol, and (*N*,*N*-dimethylamino)propanol are commercially available. α-Nortropanol was obtained according to Kraiss;[45] *N*-ethyl- and *N*-benzyl-α-nortropanol were obtained according to Berthold.[46]
(45) Kraiss, G.; Nádor, K. A convenient synthesis of *nor*atropine from tropine or tropinone. *Tetrahedron Lett.* **1971**, 57–58.
(46) Berthold, H.; Pfleger, R.; Schulz, W. Ueber Azoniaspiro-verbindungen. (On azoniaspiro compounds.) *Arzneim.-Forsch.* **1967**, *17*, 719–726.
(47) Woolfe, G.; Macdonald, A. A. Evaluation of the analgesic action of pethidine hydrochloride (demerol). *J. Pharmacol.* **1944**, *80*, 300–307.
(48) Jarvik, M. E.; Kopp, R. An improved one-trial passive avoidance learning situation. *Psychol. Rep.* **1967**, *21*, 221–224.
(49) Siegmann, C. M.; Wibaut, J. P. Synthesis and some pharmacological properties of esters of some N-alkyl homologues of tropinol and ψ-tropinol. *Recueil* **1954**, *73*, 203–217.
(50) Bosse, J. A.; Wasserman, O. The affinity of atropine derivatives for acetylcholine and histamine receptors of guinea pig ileum. *Naunyn-Schmiedeberg's Arch. Pharmakol. Exp. Pathol.* **1967**, *259*, 34–44.
(51) Kuchar, M.; Rejholec, V.; Roubal, Z.; Nemeck, O. Quantitative relationship between structure and fibrinolytic activity in the series of α-methyl-β-aryl propionic acid. *Collect. Czechoslov. Chem. Commun.* **1979**, *44*, 183–193.
(52) Schultz, O. E.; Krause, W. Problems of reaction centers in tropyl esters. I. Quantum chemistry studies of spasmolytics. *Pharm. Ztg.* **1972**, *117*, 1455–6.
(53) Matucci, R. Personal communication.
(54) Munson, P. J.; Rodbard, D. LIGAND: a versatile computerized approach for characterization of ligand-binding systems. *Anal. Biochem.* **1980**, *107*, 220–239.