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Further structure–activity relationships in the series of tropanyl esters endowed with potent antinociceptive activity

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Abstract

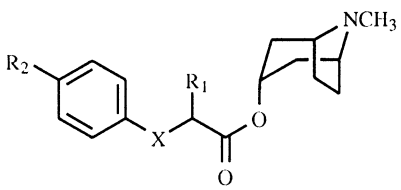
Several analogs of the α-tropanyl esters of 2-(4-chlorophenoxy)butyric acid (SM21) and 2-phenylthiobutyric acid (SM32), endowed with potent antinociceptive and cognition enhancing activity, were synthesized, aimed at obtaining more potent and safe drug candidates. Variation of the acyl moiety (**4**–**11**), as well as the conformational restriction of atropine to give the α-tropanyl ester of 2,3-dihydrobenzofurane-3-carboxylic acid (**18**), practically abolished activity. In the case of **18**, the antimuscarinic activity was also severely affected by the conformation restrain. On the contrary, conformational restriction of phenoxybutyric and phenylthiobutyric acid derivatives to give the α-tropanyl ester of 2,3-dihydro-benzofurane-2-carboxylic acid and 2,3-dihydro-benzothiophene-2-carboxylic acid (**12**–**17**), afforded potent analgesic drugs that unfortunately were too toxic to be reliable drug candidates. A series of related esters of benzofurane-3-carboxylic acid (**20**–**27**) and benzothiophene-3-carboxylic acid (**28**) were also studied and found to be potent but toxic analgesics.

Keywords: Tropanyl esters; Antinociceptive activity; Structure–activity relationships

1. Introduction

In the past few years we have developed a new series of compounds derived from *R*-(+)-hyoscyamine that are endowed with strong antinociceptive activity and which also show cognition enhancing properties, possibly due to their cholinergic mechanism of action [1–5]. As a matter of fact, these compounds, as it has been shown by microdialysis studies [3,4], enhance the central release of acetylcholine which in turn is responsible of antinociceptive (hot plate) and cognition enhancing (passive avoidance) activities. The mechanism by which acetylcholine is released is still unclear even if presynaptic muscarinic and 5-HT_4 receptors seem to be involved [3,4].

Three compounds, labeled PG9 (**1**), SM21 (**2**), and SM32 (**3**), respectively, have emerged from these researches and are now undergoing preclinical studies. In the meantime we have continued to study structure–activity relationships [6], particularly in the series of 2-phenoxy- (**2**) and 2-phenylthio-butyric acid esters (**3**); in this paper we also comment on the results obtained.



$R_1 = CH_2OH$; $R_2 = H$; X = none; Atropine
$R_1 = CH_3$; $R_2 = Br$; X = none; **1**
$R_1 = C_2H_5$; $R_2 = Cl$; X = O; **2**
$R_1 = C_2H_5$; $R_2 = H$; X = S; **3**

The first variation in the structure concerned the nature of the acyl group. It is known that the hypolipidaemic compounds related to clofibrate have a definite hepatic toxicity in the rat [7]. Since SM21, on prolonged treatment, also presents this problem, it is possible that part of the toxicity of the compound could be connected with the 4-chloro-phenoxyalkanoic moiety. We have therefore designed and synthesized a series of

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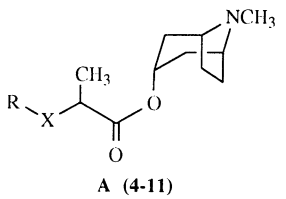
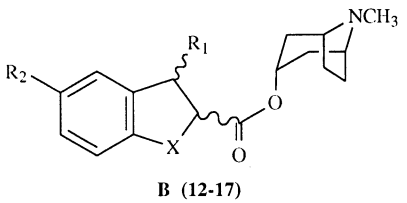


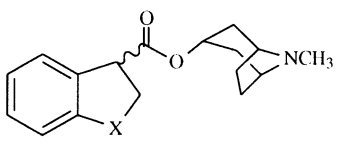
A (4-11)

R = Alkyl, cycloalkyl, alkene, alkyne, pyridine, pyrimidine
X = O, S



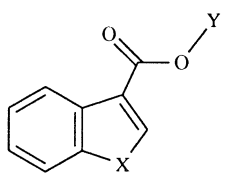
B (12-17)

R_1 = H, CH_3
R_2 = H, Cl
X = O, S



C (18,19)

X = O, S



D (20-28)

X = O, S
Y = see Tab. 7

analogs lacking this group and presenting isosteric or bioisosteric alkyl, cycloalkyl, alkenyl, alkynyl and heterocyclic moieties (general structure **A**; compounds **4**–**11**).

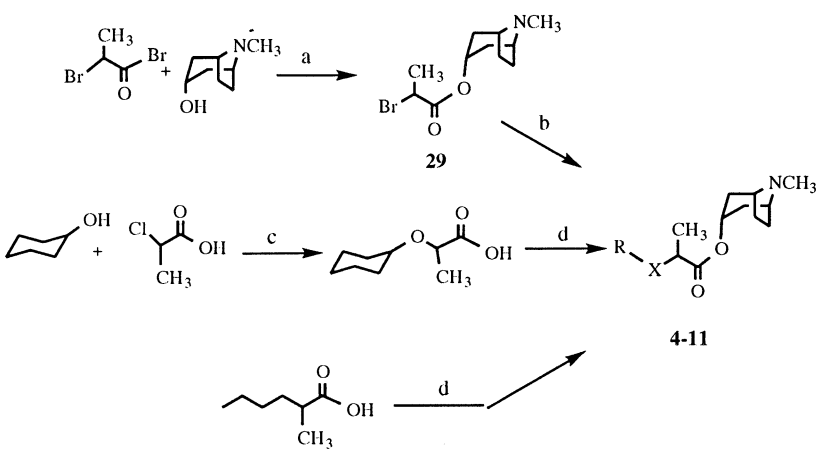
The second variation regarded the reduction of flexibility of the parent compounds to give the unsubstituted dihydrobenzofurane and dihydrobenzothiophene derivatives **15** and **17**. The other compounds with general structure **B** (**12**,**13**,**14**,**16**) correspond to frozen analogs of compounds related to **2** and **3**, but with lower analgesic activity [2]. It is known that restriction of conformational freedom can induce receptor selectivity in flexible drugs acting with multiple mechanisms of action [8]; since the release of acetylcholine of **1**–**3** apparently involves both muscarinic and serotonergic receptors [3,4], we reasoned that this modification could induce selectivity for only one of the receptors involved, thus reducing unwanted side-effects. This was also the rationale for the synthesis of compounds **18** and **19**, which are positional isomers of compounds **12** and **16** and can be regarded as a frozen analog (**18**) and an isoster thereof (**19**) of atropine (the racemic form of *R*-(+)-hyoscyamine).

Finally, compounds **20**–**28** were synthesized as aromatic analogs of **18** and **19** on the basis of the potent antinociceptive activity of the previously studied compound **20**, which seems also to act by the same acetylcholine-releasing mechanism [9].

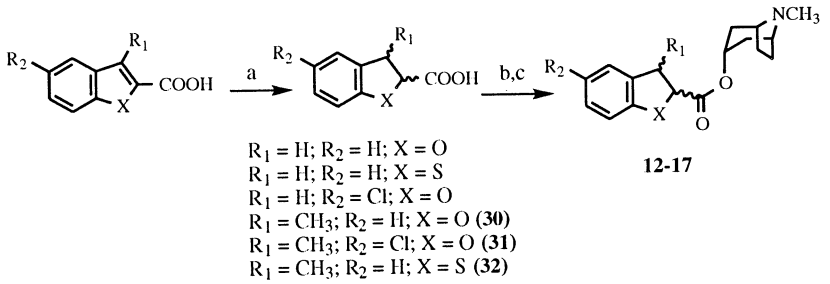
2. Chemistry

The synthesis of compounds **4**–**11** was performed by standard methods according to Scheme 1; most of the compounds were obtained through the key intermediate **29**, which had been already used by us [2] but not characterized.

The synthesis of compounds **12**–**17** is reported in Scheme 2. Benzofurane-2-carboxylic acid is commercially available; the other aromatic acids used as starting materials were obtained as described in the literature: 5-chlorobenzofurane-2-carboxylic acid [10]; 3-methylbenzofurane-2-carboxylic acid [11]; 3-methyl-5-chlorobenzofurane-2-carboxylic acid [12]; benzothiophene-2-carboxylic acid [13]; 3-methylbenzothiophene-2-carboxylic acid [14]. The aromatic acids were reduced to the corresponding 2,3-dihydro derivatives with Na/Hg. 2,3-Dihydrobenzofurane-2-carboxylic acid [15], 2,3-dihydro-5-chlorobenzofurane-2-carboxylic acid [16]



Scheme 1. (a) Anhydrous C_6H_6, reflux; (b) NaOH, C_2H_5OH, R–XH; (c) NaOH, C_2H_5OH; (d) CDI, α-tropanol.



Scheme 2. (a) Na/Hg; (b) $SOCl_2$; (c) α-tropanol.

and 2,3-dihydrobenzothiophene-2-carboxylic acid [17] have already been described. Their 3-methyl homologs (**30**–**32**) are described here for the first time.

The final esters **12**–**17** were obtained by standard esterification with α-tropanol; compounds **15** and **17** (Scheme 2, $R_1 = CH_3$) are mixtures of *cis*/*trans* isomers that have not been separated in consideration of the unsatisfactory biological results obtained.

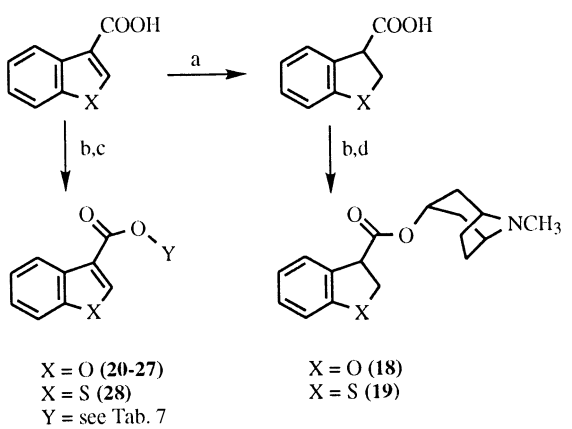
Compounds **18** and **19** and **20**–**28** were obtained according to Scheme 3, starting from benzofurane-3-carboxylic acid [18], benzothiophene-3-carboxylic acid [19], 2,3-dihydrobenzofurane-3-carboxylic acid [20] and 2,3-dihydrobenzothiophene-3-carboxylic acid [17], respectively. Even in this case, due to the poor pharmacological profile of the compounds, no attempts were made to separate the enantiomers of **18** and **19**.

3. Pharmacology

The analgesic activity of the compounds was tested on mice, subcutaneously (s.c.) or intraperitoneally (i.p.), with the hot-plate test [21], using the previously reported methods [1]. The results are reported in Table 1 and are expressed as the dose (mg/kg) giving the maximal analgesic effect; as a rule, because of the high toxicity of the compounds, ED_{50} values were not determined, except for the most efficacious ones and for comparative purposes. Analgesic efficacy was evaluated with respect to the effect of 8 mg/kg of morphine. Whenever available the minimal dose giving evident toxic effects (convulsions and death) was reported.

Muscarinic antagonism of **18** was evaluated on rabbit vas deferens (M_1), guinea pig atrium (M_2), guinea pig ileum (M_3) and guinea pig immature uterus (putative M_4) tissues, using McN-A-343, charbacol and ACh as agonists, as reported in Section 5. The results are reported in Table 2 and are expressed as Schild's pA_2 [22] for the reference compound atropine and as pK_b ($-\log K_b$), calculated according to Van Rossum [23], for **18**. Each concentration was tested between four and seven times. No reduction in the maximum effect was observed at the doses used.

Although the active compounds would very likely show the non-atropic action of their parent compounds, considering their toxicity, no experiments to evaluate cognitive effects were made.



Scheme 3. (a) Na/Hg; (b) $SOCl_2$; (c) aminoalcohol of Table 7; (d) α-tropanol.

4. Results and discussion

The data regarding analgesic activity, reported in Table 1, show that the modifications introduced into the parent molecules have different consequences on their activity.

The first modification, concerning the acyl moiety, was unsuccessful as, among the compounds synthesized and studied (**4**–**11**), only **10** and **11** possess a weak analgesic activity which, however, is not reversed by atropine and therefore is of non-cholinergic nature.

Conformationally restricted analogs gave mixed results. The frozen analog of atropine (**18**) is somehow more efficacious, but definitely less potent than atropine (Tables 1 and 2). It is interesting to observe that conformational restriction has major consequences also on the antimuscarinic activity of atropine, as **18** is some 100 times less potent on the tissues studied (Table 2). Its isoster **19** is fairly efficacious, much less potent, and highly toxic, showing a trend that unfortunately is characteristic of most of the compounds studied.

Table 1
Analgesic activity of compounds **2**,**3** and **10**–**28** on the hot-plate test (mouse)

Compound	Analgesic activity			
	Maximal effect dose (MED)[a] (mg/kg, i.p.)	ED_{50} ($\pm$SE) (mg/kg, i.p.)	Efficacy[b] (%)	Maximal non-toxic dose[c] (mg/kg, i.p.)
2 (SM21)	40	21.2 (1.8)	114.4	400
3 (SM32)	40	16.6 (1.6)	107.5	380
10	50[d]	–	–	–
11	50[d]	–	–	–
12	50	–	–	–
13	50	–	–	–
14	50	–	48.4	80
15	50[e]	17.6 (1.3)	78.7	100
16	25	11.1 (2.5)	79.7	40
17	30[f]	14.3 (1.9)	100.9	40[f]
18	25	–	73	40
19	50	34.2 (3.8)	111.9	80
20	50	26.3 (2.7)	129.5	100
21	40	–	75.3	50
22	50	–	48.4	n.d.
23	50	–	83.6	70
24	50	–	59.4	<75
25	50	–	48.4	n.d.
26	50	–	47.9	n.d.
27	50	–	21.0	>200
28	50	32.7 (2.6)	132.6	100

[a] The maximal dose reported was limited to 50 mg/kg, even if some compounds (**12**,**13**) show some activity at higher doses.
[b] Efficacy is expressed as % of the effect of MED respect to that of a dose of 8 mg/kg of morphine (i.p.).
[c] The toxic dose is that at which the animals present convulsions and death. Even in the cases where the maximal non-toxic dose has not been evaluated (n.d.) the compounds show toxicity at doses very close to those tested.
[d] The analgesic effect is not atropine sensitive and for this reason the compound has not been further studied.
[e] The analgesic effect produced by this dose is almost completely reversed by SDZ-205557.
[f] The compound was tested subcutaneously (s.c.).

The frozen analogs of SM21 (**15**) and SM32 (**17**), which have been studied in more detail, are practically equiactive with their parent compounds, but their acute toxicity is much higher (Table 1). The same happens for the analog **16**, while **12**, **13**, **14** are practically inactive at the dose tested.

Among the analogs of **20**, which have already been studied by us [9], only the sulfur isoster **28** is interesting, since it shows a remarkable efficacy as analgesic. We have previously shown that the antinociceptive activity of **20**, despite its structural similarity with 5-HT_3 antagonists, is not due to interaction with this kind of receptors but, more likely, to activation of central 5-HT_4 receptors. It is possible that its isoster **28** behaves in the same way. However, due to its high toxicity, that would prevent any useful use as analgesic, we did not investigate any further its mechanism of action.

In conclusion, it appears that reduction of molecular flexibility of the lead compounds maintains or slightly reduces analgesic activity but, at the same time, increases their toxicity. During our researches in this field we have noticed [9] that toxicity somehow parallels 5-HT_4 affinity. As a matter of fact, the analgesic activity of **15** at the maximal effect dose (MED = 50 mg/kg) is almost completely reversed (66%) by SDZ-205557, a 5-HT_4 antagonist. The reversal of the analgesic activity of SM21 (**2**) was, under the same conditions, quite lower (44%) [3]. We may conclude that conformational restriction of atropine, SM21 and SM32, apparently and contrary to our expectations, favors interaction with the 5-HT_4, respect to presynaptic muscarinic receptors.

5. Experimental

5.1. 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. 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

Table 2
Antimuscarinic and analgesic activity of atropine and **18**

Comp.	pK_b ($\pm$SE)				Analgesic activity	
	M_1[a]	M_2[b]	M_3[c]	Mu[d]	MED[e] (mg/kg)	Efficacy[f] (%)
Atropine	9.46[g]	8.92[g]	9.00[g]	9.50[g]	0.005	42
18	7.43[h] (0.03)	6.55[h] (0.01)	6.57[h] (0.09)	7.25[i] (0.02)	25	73

[a] Rabbit vas deferens.
[b] Guinea pig atrium (force).
[c] Guinea pig ileum.
[d] Guinea pig uterus.
[e] Maximal effect dose (i.p.).
[f] Effect of the maximal effect dose compared to that of 8 mg/kg of morphine.
[g] pA_2 calculated according to Arunlakshana and Schild [22].
[h] 10^{-6} M.
[i] 3×10^{-7} M.

analyses are indicated by symbols, the analytical results are within $\pm$0.4% of the theoretical values. Table 3 shows the elemental analyses for compounds **4–32**.

5.2. 3-α-Tropanyl 2-bromopropionate (29)

A 1.5 ml sample of 2-bromopropionyl bromide was added to a solution of α-tropanol (2 g) in 20 ml of anhydrous benzene, and the solution refluxed for 13 h. The resulting mixture was cooled and evaporated to dryness. The residue was dissolved in CH_2Cl_2, and the organic layer shaken with a saturated solution of Na_2CO_3, washed with water and dried. Evaporation of the solvent gave a white solid (3.6 g), m.p. 178–180°C. ^{1}H NMR ($CDCl_3$) δ 1.82 (d, $J=6.1$ Hz, 3H, CHCH_3); 1.85–1.95 (m, 2H, tropanol); 2.08–2.22 (m, 4H, tropanol); 2.49 (s, 3H, N–CH_3); 3.34–3.41 (m, 2H, CH bridge); 4.36 (q, $J=6.1$ Hz, 1H, CHCH$_3$); 5.10 (t, 1H, CH–O) ppm.

5.3. General method for the synthesis of the esters 6–11

A solution of 2.5 mmol of the appropriate commercial alcohol or thioalcohol in 2 ml of absolute (abs.) ethanol were added to a solution of 2.5 mmol of NaOH in 2 ml of abs. ethanol. The mixture was refluxed for 0.5 h and then evaporated to dryness. To the white residue dissolved in anhydrous DMF a solution of **29** (0.5 g, 1.8 mmol) dissolved in anhydrous DMF was added. The mixture was refluxed for 18–30 h, depending from the reagent used. In every case, the solvent was removed at the end of the reaction and the residue dissolved in $CHCl_3$ and washed with a saturated solution of Na_2CO_3. Evaporation of the solvent gave an oil that was purified by flash chromatography with $CHCl_3$:petrol ether:abs. EtOH:conc. NH_4OH = 340:60:65:8 (**6–9**) or $CHCl_3$:MeOH = 75:25 (**10–11**) as the eluent, yielding the desired product, that was transformed into the salt reported in Table 4.

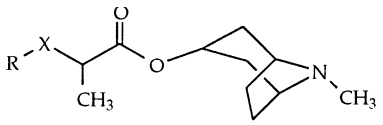
5.4. Synthesis of the esters 5 and 4

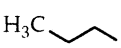
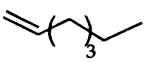
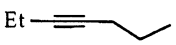
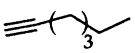
A total of 2 g (20 mmol) of cyclohexanol was added to a solution of 20 mmol of NaOH in 15 ml of abs. ethanol; 3.2 g (30 mmol) of 2-chloropropionic acid were then added to this solution and the mixture refluxed for 4 h and evaporated to dryness. The residue was dis-

Table 3
Elemental analyses of compounds **4–19** and **21–32**

Comp.	Calculated (%)			Found (%)		
	C	H	N	C	H	N
4	59.44	8.53	4.08	59.25	8.86	3.89
5	59.19	8.12	3.63	58.80	8.27	3.85
6	59.19	8.12	3.63	59.41	7.80	3.91
7	59.50	7.64	3.65	59.26	7.91	3.87
8	59.50	7.64	3.65	59.87	7.31	3.95
9	56.82	6.37	7.37	57.10	6.05	7.59
10	54.52	6.11	7.07	54.36	6.25	7.33
11	51.36	5.84	10.57	51.05	6.03	10.23
13	55.40	5.39	3.40	55.18	5.31	3.72
14	61.36	6.45	3.58	61.22	5.91	3.28
15	56.40	5.69	3.29	56.74	5.37	3.51
16	57.99	5.90	3.56	58.23	5.61	3.72
17	58.94	6.20	3.44	59.18	5.92	3.71
18	63.04	6.86	4.33	62.85	7.05	4.02
19	60.07	6.54	4.12	59.81	6.81	3.95
21	64.37	6.62	4.17	64.51	6.85	3.88
22	69.42	6.09	3.52	69.11	5.81	3.77
23	62.43	5.91	4.55	62.71	6.07	4.27
24	60.90	6.15	4.74	61.21	5.89	4.98
25	62.02	6.52	4.52	61.78	6.79	4.71
26	60.90	6.15	4.74	61.18	5.88	4.51
27	60.49	6.78	4.70	60.80	6.55	4.91
28	60.42	5.98	4.15	60.71	6.21	3.83
30	67.40	5.67	–	67.21	5.81	–
31	56.48	4.27	–	56.91	4.01	–
32	61.82	5.20	–	62.18	4.85	–

Table 4
Chemical and physical characteristics of compounds **4–11**



No.	R	X	M.p. (°C)[a]	Formula[b] (salt)
5		O	[c]	$C_{17}H_{29}NO_3 \cdot C_2H_2O_4$
4		CH_2	158–160	$C_{15}H_{27}NO_2 \cdot C_2H_2O_4$
6		O	68–71	$C_{17}H_{29}NO_3 \cdot C_2H_2O_4$
7		O	90–91	$C_{17}H_{27}NO_3 \cdot C_2H_2O_4$
8		O	78–81	$C_{17}H_{27}NO_3 \cdot C_2H_2O_4$
9		O	140–143	$C_{16}H_{22}N_2O_3 \cdot C_2H_2O_4$
10		S	142–144	$C_{16}H_{22}N_2O_2S \cdot C_2H_2O_4$
11		S	138–140	$C_{15}H_{21}N_3O_2S \cdot C_2H_2O_4$

[a] All compounds are oxalic acid salts and were crystallized from ethylacetate.

[b] The compounds were analyzed after vacuum drying for 5 h at a temperature below the melting point. The analytical results were within ±0.4 of the theoretical values. Their IR and proton NMR are consistent with the proposed structure.

[c] The compound melts at r.t.

solved in $CHCl_3$ and washed with 2 N HCl. Evaporation of the solvent gave 1.8 g of 2-cyclohexyloxypropionic acid that was used as such in the following reaction.

A 0.48 g (3 mmol) sample of 1,1′-carbonyldiimidazole (CDI) was dissolved in anhydrous CH_2Cl_2, 0.5 g (3 mmol) of 2-cyclohexyloxypropionic acid was added, and the mixture was kept at room temperature (r.t.) for 2 h. Evaporation of the solvent gave a residue that was dissolved in 5 ml of anhydrous acetone and added to a solution of 0.45 g (3.2 mmol) of α-tropanol in anhydrous acetone. The mixture was refluxed for 22 h and evaporated to dryness. The residue was dissolved in CH_2Cl_2 and the organic layer washed with water and Na_2CO_3 sat. solution. Evaporation of the solvent gave an oil that was purified by flash chromatography using $CHCl_3$:petrol ether:abs. EtOH:conc. NH_4OH = 340:60:65:8 as the eluent, yielding 0.45 g of **5**.

In the same way, starting from commercial 2-methylhexanoic acid, compound **4** was prepared. The chemical and physical characteristics of these compounds are reported in Table 4.

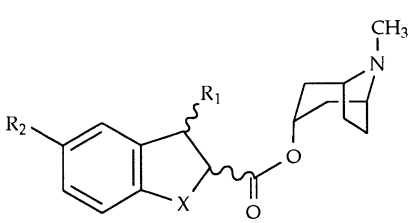
5.5. General method for the synthesis of esters *13–28*

A 0.5 mmol portion of the appropriate carboxylic acid, synthesized as reported below or in the literature, was transformed into the chloride by reaction with $SOCl_2$ (1 ml) at 60°C for 5 h, and purified by fractional distillation. Acyl chlorides were then added to a solution of α-tropanol (0.16 g, 1.1 mmol) in 5 ml anhydrous benzene and the solution refluxed for 12 h. The solvent was removed at the end of the reaction and the residue dissolved in $CHCl_3$, washed with $NaHCO_3$ sat. solution and dried over Na_2SO_4. Evaporation of the solvent gave a residue that was transformed into the salts reported in Tables 5–7.

5.6. 2,3-Dihydro-3-methylbenzofurane-2-carboxylic acid (*30*)

A 0.2 g (0.9 mmol) sample of 3-methylbenzofurane-2-carboxylic acid [12] was suspended in a solution of 15 ml of water containing 0.3 g of KOH. 1 g of Na/Hg 5% amalgam was then added, the mixture kept at r.t. for 4 h and then filtered. The yellow solution obtained was acidified giving **30** as a white solid (150 mg of a 1:2 diastereomeric mixture; m.p. 145–147°C from water). ^{1}H NMR (DMSO) δ 1.32 (d, $J = 6.5$ Hz, 3H, CH_3) and 1.51 (d, $J = 6.5$ Hz, 3H, CH_3) in a 1:2 ratio; 3.60–3.88 (m, 1H, C*H*–CH_3); 4.55 (d, $J = 6.3$ Hz, 1H, CH–O)

Table 5
Chemical and physical characteristics of compounds **12–17**



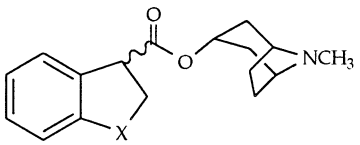
No.	R_1	R_2	X	M.p. (°C)[a]	Formula[b] (salt)
12[c]	H	H	O	–	–
13	H	Cl	O	156–159	$C_{17}H_{20}ClNO_3 \cdot C_2H_2O_4$
14	CH_3	H	O	170–171	$C_{18}H_{23}NO_3 \cdot C_2H_2O_4$
15	CH_3	Cl	O	154–157	$C_{18}H_{22}ClNO_3 \cdot C_2H_2O_4$
16	H	H	S	195–198	$C_{17}H_{21}NO_2S \cdot C_2H_2O_4$
17	CH_3	H	S	190–191	$C_{18}H_{23}NO_2S \cdot C_2H_2O_4$

[a] All compounds are oxalic acid salts and were crystallized from ethylacetate except **17** that was crystallized from abs. ethanol.

[b] The compounds were analyzed after vacuum drying for 5 h at a temperature below the melting point. The analytical results were within ±0.4 of the theoretical values. Their IR and proton NMR spectra are consistent with the proposed structures.

[c] See Ref. [9].

Table 6
Chemical and physical characteristics of compounds **18** and **19**



No.	X	M.p. (°C)[a]	Formula[b] (salt)
18	O	220–222	$C_{17}H_{21}NO_3 \cdot HCl$
19	S	235 (dec.)	$C_{17}H_{21}NO_2S \cdot HCl$

[a] The compounds are hydrochlorides and were crystallized from anhydrous ether/abs. ethanol.

[b] The compounds were analyzed after vacuum drying for 5 h at a temperature below the melting point. The analytical results were within ± 0.4 of the theoretical values. Their IR and proton NMR spectra are consistent with the proposed structures.

and 5.26 (d, $J = 6.3$ Hz, 1H, CH–O); 6.82–7.20 (m, 4 H, aromatics); 10.20–10.65 (bs, 1H, OH) ppm. IR (Nujol) ν 3600–2600 (OH); 1740 (CO) cm^{-1}. *Anal.* $C_{10}H_{10}O_3$ (C, H).

With the same procedure were also synthesized the other dihydro derivatives; among them the following have not been reported previously.

*5.7. 2,3-Dihydro-3-methyl-5-chlorobenzofurane-2-carboxylic acid (**31**)*

Starting from 3-methyl-5-chlorobenzofurane-2-carboxylic acid [12] (1:3 diastereomeric mixture; m.p. 145–147°C from water). ^{1}H NMR ($CDCl_3$) δ 1.35 (d, $J = 6.3$ Hz, 3H, CH_3) and 1.51 (d, $J = 6.3$ Hz, 3H, CH_3) in a 1:3 ratio; 3.60–3.85 (m, 1H, C*H*–CH_3); 4.82 (d, $J = 6.6$ Hz, 1H, CH–O) and 5.28 (d, $J = 11.9$ Hz, 1H, CH–O); 7.41–7.65 (m, 3H, aromatics) ppm. IR (Nujol) ν 3600–2500 (OH); 1740 (CO) cm^{-1}. *Anal.* $C_{10}H_9ClO_3$ (C, H).

*5.8. 2,3-Dihydro-3-methylbenzothiophene-2-carboxylic acid (**32**)*

Starting from 3-methylbenzothiophene-2-carboxylic acid [14] (1:1 diastereomeric mixture; m.p. 120–122°C from water). ^{1}H NMR ($CDCl_3$) δ 1.35–1.52 (m, 3H, CH_3); 3.80–3.93 (m, 1H, C*H*–CH_3); 4.05 (d, $J = 5.7$ Hz, 1H, CH–O) and 4.47 (d, $J = 10.8$ Hz, 1H, CH–O) in a 1:1 ratio; 7.01–7.31 (m, 4H, aromatics); 10.10 (bs, 1H, COOH) ppm. IR (Nujol) ν 3300–2500 (OH); 1720 (CO) cm^{-1}. *Anal.* $C_{10}H_{10}O_2S$ (C, H).

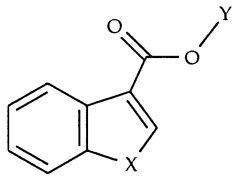
5.9. Pharmacology

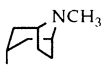
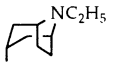
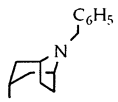
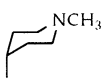
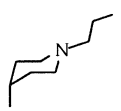
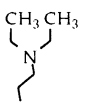
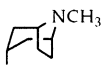
5.9.1. Analgesic activity

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 level of analgesia reached was evaluated comparing the analgesic effect of the maximal effect dose of each compound to that of morphine, taken as the reference compound, and injected at 8 mg/kg s.c., a dose that does not alter animal behavior.

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

Table 7
Chemical and physical characteristics of compounds **20–28**



No.	X	Y	M.p. (°C)[a]	Formula[b] (salt)
20[c]	O		–	
21	O		201–204	$C_{18}H_{21}NO_3 \cdot HCl$
22	O		226–228	$C_{23}H_{23}NO_3 \cdot HCl$
23	O		209–211	$C_{16}H_{17}NO_3 \cdot HCl$
24	O		170–172	$C_{15}H_{17}NO_3 \cdot HCl$
25	O		180–183	$C_{16}H_{19}NO_3 \cdot HCl$
26	O		194–196	$C_{15}H_{17}NO_3 \cdot HCl$
27	O		134 (dec.)	$C_{15}H_{19}NO_3 \cdot HCl$
28	S		260 (dec.)	$C_{17}H_{19}NO_2S \cdot HCl$

[a] The compounds have been recrystallized from abs. ethanol/anhydrous ether.

[b] The compounds were analyzed after vacuum drying for 5 h at a temperature below the melting point. The analytical results were within ± 0.4 of the theoretical values. Their IR and proton NMR are consistent with the proposed structure.

[c] See Ref. [9].

reaction time of morphine − pretest reaction of morphine) × 100.

Standard errors (SEs) 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 significant when $P \leq 0.05$. Percent values were calculated only for those differences that were statistically significant; in all other cases, the drugs were considered inactive. Since the reaction time was measured with an accuracy of ± 15%, the errors on the percent values calculated through the formula reported above are in the same range.

5.9.2. Antimuscarinic activity

Male guinea pigs (200–300 g), female guinea pigs (150–200 g) and male New Zealand white rabbits (2.5–3 kg) were killed by cervical dislocation and the organs were set up under the appropriate tension (see below) in 13 ml organ baths containing physiological salt solution (PSS) kept at an appropriate temperature (see below) and treated with 5% CO_2–95% O_2. Dose–response curves were constructed by addition of the agonist (cumulative curves in the case of rabbit vas deferens and guinea pig atria).

The concentration of agonist in the organ bath was increased approximately 3-fold each step, each addition being made only after the response to the previous addition had attained a maximal level and remained steady. Tissues were incubated with the antagonist for 1 h and a new dose–response curve to the agonist was obtained. Contractions were recorded by means of a force transducer connected to a single channel recorder (U. Basile).

5.9.2.1. Rabbit stimulated vas deferens. Surrounding tissues were carefully removed from vasa deferentia which were then divided into four segments, two prostatic portions of 1 cm and two epididymal portions approximately 1.5 cm long. The four segments were mounted under 0.75 g tension in PSS with the following composition (mM): NaCl (118.4), KCl (4.7), $CaCl_2$ (1.8), $MgCl_2$ (0.6), KH_2PO_4 (1.18), $NaHCO_3$ (25), glucose (11.1); 10^{-6} M yohimbine was included to block α_2-adrenoceptors. The solution was maintained at 32°C and tissues were stimulated through platinum electrodes by square-wave pulses (2 ms, 0.1 Hz, 10–30 V). Contractions were measured isometrically after tissues had been equilibrated for 1 h, then a cumulative dose–response curve for the inhibitory effect of McN-A-343 was plotted [24].

5.9.2.2. Guinea pig stimulated left atria. The heart was rapidly removed and the left atria were excised and mounted under 1 g of tension in PSS with the following composition (mM): NaCl (137), KCl (2.7), $CaCl_2$ (1.8), $MgCl_2$ (1.05), NaH_2PO_4 (0.42), $NaHCO_3$ (11.9), glucose (5.6). The solution was maintained at 30°C and stimulated through platinum electrodes by square-wave pulses (1 ms, 1 Hz, 4–10 V). Inotropic activity was recorded isometrically. Tissues were equilibrated for 1 h and a cumulative dose–response curve to cabachol was plotted [25].

5.9.2.3. Guinea pig ileum. Portions of terminal ileum (2 cm) were removed at about 5 cm from the ileum–cecum junction and mounted under 1 g of tension in PSS (the same used for atria) at 37°C. Tension changes were recorded isotonically. Tissues were equilibrated for 1 h and a dose–response curve to acetylcholine was obtained [26].

5.9.2.4. Guinea pig uterus. Uterine horns were divided into four portions and mounted under 1 g of tension in PSS with the following composition (mM): NaCl (154), KCl (5.63), $CaCl_2$ (0.54), $MgCl_2$ (0.95), $NaHCO_3$ (5.95), glucose (2.78). The preparations were maintained at 30°C and after a 1 h equilibration period, isotonic contractions to carbachol were recorded. Initially the tissues were exposed to a single concentration of carbachol (3 μM) to check the responsiveness to the agonist, then a dose–response curve for carbachol was obtained [27].

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