Reduced Flexibility Analogs of Analgesic and Cognition Enhancing α-Tropanyl Esters

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***Key Words**: analgesics; cognition enhancers; reduced flexibility; ACh release*

Summary

A series of semirigid analogs of compounds 1 and 2, two potent analgesics and cognition enhancers, have been synthesized and tested for antinociceptive activity (hot plate test) and for muscarinic affinity (binding in rat cerebral cortex). They were found to be in general less potent than the reference compounds; only one of them (22) shows a good affinity for the muscarinic receptor and an antinociceptive efficacy comparable with those of the reference compounds. At a dose of 30 mg/kg 22 is also able to reverse the amnesic effect of dicyclomine. Since the analgesic effect of these compounds is affected by the 5-HT$_4$ antagonist SDZ 205557, the possible role of this receptor is discussed.

Introduction

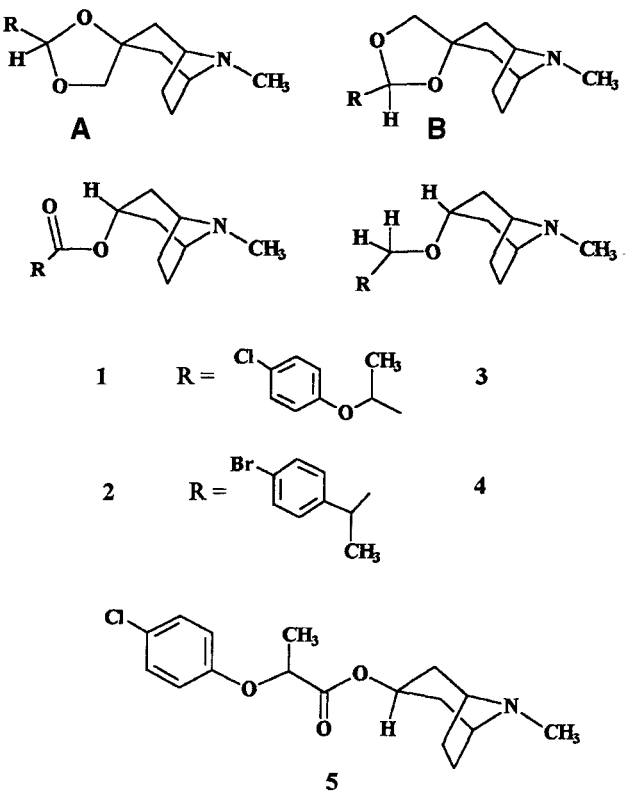
We recently reported the synthesis of a new series of α-tropanyl esters that are endowed with analgesic and cognition enhancing activity due to an increased release of central acetylcholine [1,2].

These compounds act at a presynaptic level possibly by a dual mechanism of action that very likely involves selected inhibition of a presynaptic autoreceptor[3] and activation of a 5-HT$_4$ heteroreceptor[4], both controlling the release of ACh.

In order to gain further insight into the structure-activity relationships of this new class of compounds we have designed a few spiro-analogs of compounds **1** and **2**[1,2] (general structures A and B) which are potent members of the series (see Chart 1). Moreover, since it is well known that reducing the conformational freedom of flexible ligands can induce receptor selectivity[5], we were interested in evaluating the consequences of conformational restraint on the selectivity toward the receptors involved in the activity of compounds like **1** and **2**.

In this class of compounds analgesic and cognition enhancing activity are due to the same mechanism of action, namely central presynaptic release of ACh. Since the available models for testing nootropic activity are time-consuming and fairly expensive and antinociception can be evaluated much more simply, we have followed the results of molecular modifications through the hot-plate test, checking that analgesia was always reversed by suitable doses of atropine and of the ACh depletor hemicholinium-3 (HC-3). Under these conditions we have already verified[1,2] that compounds acting as analgesic also show cognition enhancing activity. For comparative purposes we have also taken into consideration compounds **3** and **4**, which are the ethers corresponding to the esters **1** and **2**, and compound **5**, which is the β-tropanyl ester corresponding to **1**[1,2]. Compounds **14–16** and **19–20**, which are not strictly related to **1** and **2**, were also synthesized to extend further structure-activity relationships. In particular compounds **15**, **16**, and **20** that carry bulky groups in position 2 of the 1,3-dioxolane ring and that for this reason were expected to behave as antagonists, were synthesized to check wether they were also able to specifically block the muscarinic presynaptic receptor and therefore show analgesic properties.

Chart 1



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On the other hand, compounds **14** and **19**, that lack such bulky groups in position 2, were expected to behave as agonists and activate the presynaptic muscarinic receptor thus impairing, more than facilitating, ACh release.

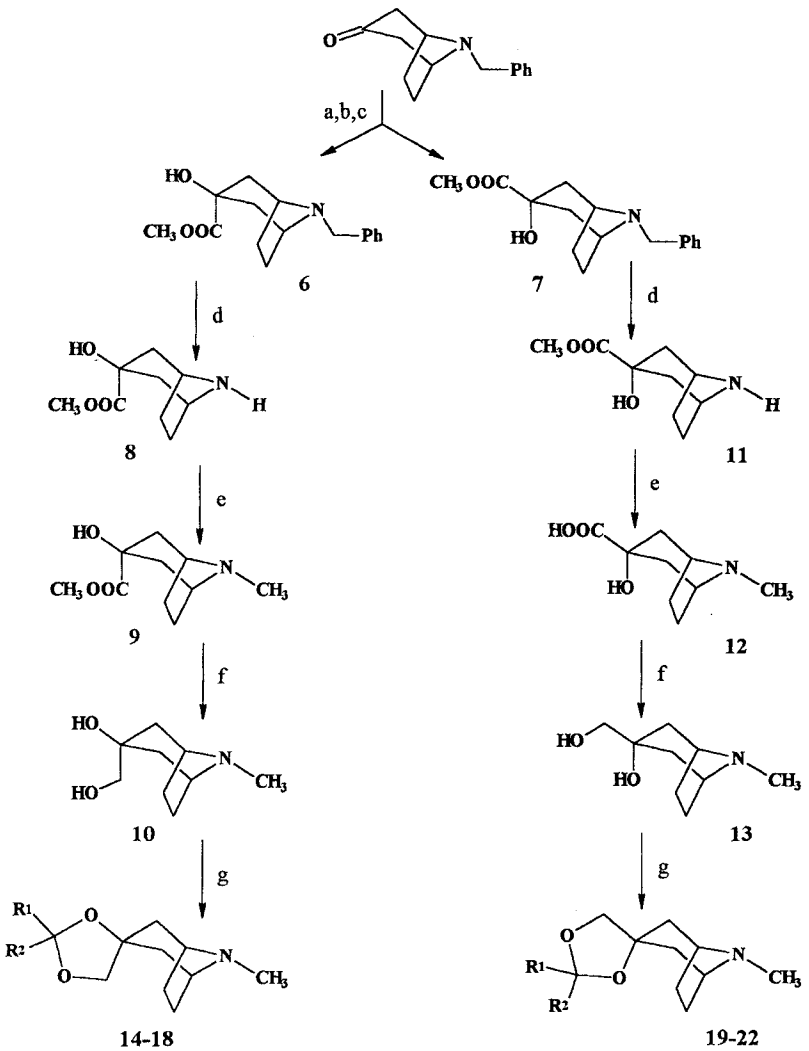
Chemistry

The diol **10** (Scheme 1) can be easily obtained according to Heusner.[6] Several attempts to synthesize its isomer **13** through nucleophilic addition to the carbonyl group of tropinone of a suitable hydroxymethyl synthon (i.e. methoxymethoxymethyltributylstannane,[7] chloromethyldimethylisopropoxysilane,[8] dimethyloxosulphonium methylide or dimethylsulphonium methylide,[9] dithiane[10]) were unsuccessful. Diol **13** was then obtained from *N*-benzyltropinone[11] according to the procedure reported in Scheme 1. The addition of HCN to the carbonyl group, followed by hydrolysis and esterification, gave the two isomeric esters **6** and **7**[12] that were transformed into the diols **10** and **13** through standard procedures. It must be noted that the *N*-methylation with formaldehyde and formic acid on **8** gave the ester **9**, while the same procedure on **11** resulted also in the hydrolysis of the ester group to give the acid **12**.

2-(4-Chlorophenoxy)propionaldehyde **23** was prepared through Swern oxidation[13] of 2-(4-chlorophenoxy)-propanol,[14] since neither the direct reduction of ethyl 2-(4-chlorophenoxy)propionate[15] with DiBAL nor the oxidation of the corresponding alcohol with pyridinium chlorochromate gave satisfactory results. Accordingly, the same procedures used to prepare 4-bromo-α-methyl-benzeneacetaldehyde (**24**), starting from ethyl 2-(4-bromophenyl)propionate, always resulted in mixtures in different proportions of **24** and 4-bromo-acetophenone, which could not be completely separated by either column chromatography or by fractional distillation. Swern oxidation gave the fewer side products, and was eventually chosen as the best method; the aldehyde was used without further purification.

Dioxolanes **14–22** were then obtained from the diols and the appropriate aldehyde or ketone with $BF_3{\cdot}Et_2O$. Several attempts at the chromatographic separation of the diastereoisomers of compounds **17,18**, **21**, and **22** were unsuccessful; therefore these compounds have been tested as isomeric mixtures.

SCHEME 1



a) NaCN, HCl; b) conc. HCl; c) MeOH, HCl; d) H_2,Pd/C; e) HCOOH, HCHO; f) $LiAlH_4$; g) R_2COR_1, $BF_3.Et_2O$.

Pharmacology

Analgesic activity was evaluated with the hot-plate test[16] and nootropic activity with a modified Jarvick and Kopp method[17] on mice.

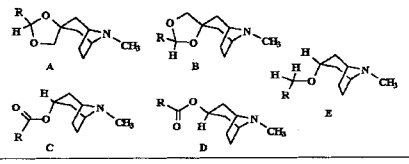
Muscarinic binding was performed on rat brain according to the method described by Franchini[18] using [^{3}H] QNB as labeled ligand.

Reversion of analgesia with the 5-HT_4 antagonist SDZ 205557[19] was evaluated on mice following the procedure reported in the experimental part.

Results

The results of the mouse hot-plate tests for analgesia[16] expressed as ED_{50} (mg/kg i.p.) and efficacy relative to morphine (see experimental part for definition), as well as binding affinities in rat cortex expressed as pK_i are reported in Table 1. The results obtained with the 5-HT_4 antagonist SDZ 205557[19] are shown in the same table, expressed as percentage of reversal of analgesia given by each compound at the dose of maximum effect. For comparative purpose the results obtained with compounds **1–5** are also reported.

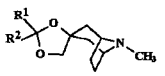
Table 1. Pharmacological activities and muscarinic binding of selected compounds.



N	Structure	R	Muscarinic binding (pK_i [a])	Analgesic activity: ED_{50} [b] mg/kg i.p.	max. level of analgesia [c]	% of reversion with SDZ 205 557 [d]
1	C		6.57	32(2.3)	90	47
2	C		6.96	21(2.2)	101	55
3	E		n.t.	44(2.7)	64	n.t.
4	E		n.t.	32(2.5)	45	n.t.
5	D		6.45	21(1.6)	26	0
17	A		6.03	37(4.3)	38	100
18	A		6.80	32(4.4)	29	100
21	B		6.07	27(2.9)	37	100
22	B		7.36	36(3.1)	97	73

[a] Values are means of at least three experiments with standard errors less than 10 % of the means. [b] Standard errors in brackets. [c] Referred to Morphine (8 mg/kg). See experimental for details [d] Reversion of analgesia obtained by an i.p. injection of 10 mg/kg of the 5-HT_4 antagonist SDZ 205557. n.t. not tested

Table 2. Functional muscarinic and analgesic activity of selected compounds [25].



N	R^1	R^2	pK_b [a]: M_2 g. p. heart (F)	M_3 g. p. ileum	M_3 g. p. bladder	Analgesic activity: ED_{50} [b] mg/kg i.p.	maximum level of analgesia [c]
14	H	CH_3	4.80 (0.04)	4.75 (0.08)	4.59 (0.06)	33 (3.2)	59
15	H	C_6H_5	5.31 (0.07)	5.18 (0.03)	5.07 (0.16)	36 (4.0)	51
16	C_6H_5	C_6H_{11}	6.14 (0.03)	6.40 (0.07)	<6	38 (3.7)	23

[a] Obtained from the Van Rossum equation lg(DR-1)=log[Ant]-logKb [34] using carbachol as agonist. The protocols used are reported in ref 33. [b] and [c] See the corresponding footnotes of Table 1.

Shapiro et al.[20] have reported that compounds **14** and **19** are devoid of any muscarinic receptor activity in functional tests on ileum and ganglion models. In fact, compounds **14–16** tested on guinea-pig heart (force), ileum and bladder behave as very weak antagonists[21]. Their analgesic activity is very poor and not reversed by atropine (Table 2).

Compound **22**, which maintains a high level of analgesic activity was tested on the passive-avoidance test[17] and found to be, as expected, fairly active as nootropic (see Fig. 1).

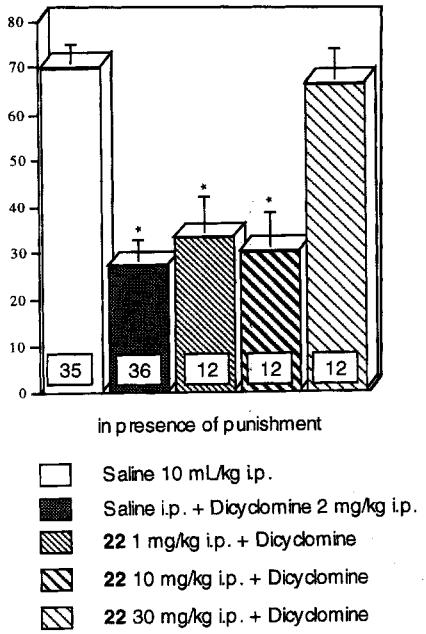


Figure 1. Dose-response curve of **22** on dicyclomine-induced amnesia in mouse passive-avoidance test. Punishment: cold water bath. Vertical lines give s.e. mean. * $P < 0.01$ in comparison with saline-treated mice. **22** was administered 20 min before training; dicyclomine was administered immediately after training.

Discussion

Pharmacological tests showed that, among the compounds synthesized and studied, only those maintaining chlorophenoxyethyl or bromophenylethyl groups in position 2 on the 1,3-dioxolane ring show analgesic properties.

The data reported in Table 1 show that restriction of flexibility on phenoxy- and phenylpropionic acid derivatives has different consequences on analgesic activity.

In the phenoxypropionic acid derivatives **17** and **21**, while potency shows little changes, analgesic efficacy is strongly reduced. This result is quite reasonable for compound **17** since the corresponding β-tropanyl ester **5** also shows a low level of analgesia compared to the α-tropanyl counterpart **1**. The reduction in analgesic efficacy of **21** might be due, as well, to the substitution of an sp^2 with an sp^3 hybridized carbon atom. The corresponding ether **3** also shows reduced potency and efficacy with respect to the ester analog **1**.

However, this is not likely to be the reason for the drop in analgesic efficacy since the phenylpropionic acid analog **22** shows an analgesic efficacy comparable to that of **2**, even if there is a similar reduction in the level of analgesia going from the ester **2** to the corresponding ether **4**. Even in the phenylpropionic acid series compound **18**, which corresponds to a β-tropanyl derivative, has a reduced analgesic activity.

As observed previously[2] there is no apparent correlation between the binding to central muscarinic receptors and analgesic activity. Since one of the mechanisms of action claimed for these compounds is interaction with presynaptic muscarinic receptors, this could appear an inconsistent result. However the binding affinities reported in Table 1 refer to all the muscarinic receptors present in the cortex (mainly M_1 and M_3), while our compounds might interact selectively with receptor subtypes (M_2, M_4) not yet established.[22]

The reversal of analgesia in mouse by the 5-HT_4 antagonist SDZ 205557 gave interesting results. In principle this experiment, by canceling the contributions that activation of the putative 5-HT_4 receptors gives to ACh release[4], would allow an estimate of the respective role of muscarinic and 5-HT_4 receptors in ACh release and as a consequence on analgesic activity. Actually, it is apparent from the data reported in Table 1 that while the contribution of muscarinic and 5-HT_4 receptors is nearly equivalent in the parent compounds **1** and **2**, the contribution of 5-HT_4 is higher for **22** and total for derivatives **17–21**.

This implies that, in particular for the phenoxypropionic acid series, the reduction of flexibility shifts the activity of the compounds from muscarinic to 5-HT_4 receptors. As a consequence, the amount of the ACh released is lower and the analgesic efficacy reduced with respect to **1** which acts on both systems. In this regard compound **22**, which maintains some ability to activate muscarinic autoreceptors, is able to reach a substantially higher analgesic activity.

Of course the reliability of these results is totally dependent on the selectivity of SDZ 205557 for 5-HT_4 receptor, and it is fair to say that this selectivity has recently been challenged as similar affinities for 5-HT_3 and 5-HT_4 receptors have been reported.[23] It is known that 5-HT_3 antagonists like ICS 205930 and bemesetron are able to increase the pain threshold through facilitation of ACh release by an indirect mechanism.[24, 25] However in our case there is evidence that seems to exclude the involvement of 5-HT_3 receptors in the analgesic activity measured. We have already reported[26] that SDZ 205557 is not able to reverse the analgesia induced by ICS 205930 and bemesetron while it blocks the analgesia induced by known 5-HT_4 agonists like cisapride and metoclopramide. Thus, at least in these *in vivo* tests, SDZ 205557 seems a reliable tool to selectively block 5-HT_4 receptors. However more experiments (e.g. binding studies) are needed to confirm the involvement of 5-HT_4 receptors in the analgesic action of our compounds.

In conclusion, the results of this research can be summarized as follows:

1) molecular flexibility restriction of phenoxypropionic acid α-tropyl ester **1** reduces its analgesic activity.

2) The reduction seems most likely due to the lost ability to interact with muscarinic receptor since the new compounds seem to behave exclusively as putative 5-HT_4 agonists.

3) Molecular flexibility reduction of the corresponding phenylpropionic acid derivative **2** maintains some activity at the muscarinic autoreceptor and as a consequence **2** maintains high analgesic efficacy and cognition enhancing activity.

Experimental

Chemistry. All melting points were measured 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.

Methyl 8-benzyl-3β-hydroxy-8-azabicyclo[3.2.1]octane-3α-carboxylate (**6**) *and methyl 8-benzyl-3α-hydroxy-8-azabicyclo[3.2.1]octane-3β-carboxylate* (**7**)[12]

Compounds **6** and **7** were synthesized starting from benzyltropinone[6] according to Clarke,[12] and separated by column chromatography using $CHCl_3$:CH_3OH 9:1 as eluent. The first fraction (R_f 0.8) was **6** (oil, 24 % overall yield). ^{1}H-NMR ($CDCl_3$) δ=1.58–1.68 (m, 2H), 1.80–1.91 (m, 2H), 2.12–2.43 (m, 4H), 3.28–3.42 (m, 2H, CHN), 3.47 (s, 2H, CH_2Ph), 3.75 (s, 3H, OCH_3), 7.22–7.38 (m, 5H, aromatic H) ppm. ^{13}C-NMR ($CDCl_3$) δ = 29.19 (t), 43.66 (t), 53.00 (q), 56.59 (d), 56.75 (t), 72.56 (s), 127.84 (d), 128.89 (d), 129.06 (d), 139.13 (s), 175.11 (s) ppm. The second fraction (R_f 0.5) was **7** (oil, 28% overall yield). ^{1}H-NMR ($CDCl_3$) δ = 1.58–1.70 (m, 2H), 1.92–2.28 (m, 4H), 2.36–2.48 (m, 2H), 3.11–3.32 (m, 3H, CHN and OH), 3.63 (s, 2H, CH_2Ph), 3.78 (s, 3H, OCH_3), 7.23–7.46 (m, 5H, aromatic H) ppm. ^{13}C-NMR ($CDCl_3$) δ = 26.30 (t), 41.16 (t), 53.50 (q), 56.50 (t), 58.32 (d), 73.63 (s), 127.38 (d), 128.71 (d), 129.07 (d), 139.92 (s), 178.40 (s) ppm.

Methyl 3β-hydroxy-8-azabicyclo[3.2.1]octane-3α-carboxylate (**8**)

Compound **6** (1 g, 0.004 mol) was hydrogenated over Pd/C 10% (1 g) in absolute ethanol at 80 psi for 24 h. After filtration, the solvent was removed under vacuum to give **8** (98 % yield). IR (Nujol) ν = 3600–3000 (OH), 3260 (NH), 1740 (CO) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ = 1.61–1.90 (m, 4H), 1.95–2.25 (m, 4H), 2.79 (bs, 2H, NH and OH), 3.59–3.71 (m, 2H, CHN), 3.76 (s, 3H, OCH_3) ppm. Anal ($C_9H_{15}NO_3$) C, H, N.

Methyl 3β-hydroxy-8-methyl-8-azabicyclo[3.2.1]octane-3α-carboxylate (**9**)[27]

2 mL of HCOOH and 1 mL of a 40% aqueous solution of HCHO were added to a solution of **8** (0.69 g, 0.00373 mol) in H_2O (4 mL). After 9 h heating under reflux, the excess acid was neutralized with $NaHCO_3$ and the solvent evaporated under vacuum. The semisolid residue was extracted with $CHCl_3$; after rendering anhydrous (Na_2SO_4) the solvent was removed to give 0.35 g of **9**; m.p. 105 °C (47% yield). ^{1}H-NMR ($CDCl_3$) δ = 1.48–1.60 (m, 2H), 1.82–1.94 (m, 2H), 2.08–2.14 (m, 2H), 2.22–2.38 (m, 2H), 3.18–3.30 (m, 2H, CHN), 3.74 (s, 3H, OCH_3) ppm. ^{13}C-NMR ($CDCl_3$) δ = 26.90 (t), 39.30 (q), 41.08 (t), 52.75 (q), 59.26 (d), 71.63 (s), 175.07 (s) ppm.

3α-Hydroxymethyl-8-methyl-8-azabicyclo[3.2.1]octan-3β-ol (**10**)[6]

To a solution of **9** (2.4 g, 0.012 mol) in anhydrous ether (60 mL) $LiAlH_4$ (0.92 g, 0.024 mol) was added at 0 °C. After one night at room temperature, the excess of hydride was destroyed with ice, the solvent was removed under vacuum and the residue extracted several times with CH_2Cl_2. After rendering anhydrous and removal of solvent, 1.77 g of **10** were obtained; m. p. 110 °C. (86% yield). ^{1}H-NMR ($CDCl_3$) δ = 1.18–1.53 (m, 4H), 2.10–2.35 (m, 4H), 2.22 (s, 3H, CH_3N), 3.18–3.28 (m, 2H, CHN), 3.36 (s, 2H, CH_2O) ppm. This compound is identical to that obtained by Heusner[6].

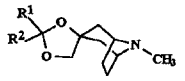
Methyl 3α-hydroxy-8-azabicyclo[3.2.1]octane-3β-carboxylate (**11**)

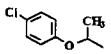
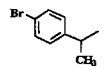
With the same procedure as for **8**, starting from **7**, compound **11** was obtained with 95% yield. IR (Nujol) ν = 3500–3000 (OH), 3300 (NH), 1740 (CO) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ = 1.71–1.98 (m, 4H), 2.25–2.52 (m, 4H), 3.75–3.88 (m, 2H, CHN), 3.75 (s, 3H, CH_3O), 4.68 (bs, 2H, NH and OH) ppm. Anal ($C_9H_{15}NO_3$) C, H, N.

3α-Hydroxy-8-methyl-8-azabicyclo[3.2.1]octane-3β-carboxylic acid (**12**)

The same procedure as for **9**, starting from **11**, resulted in a simultaneous hydrolysis of the ester function to give the acid **12** in 34% yield. ^{1}H-NMR ($CDCl_3$) δ = 1.65–1.75 (m, 2H), 1.98–2.10 (m, 2H), 2.43–2.55 (m, 2H), 2.68–2.79 (m, 2H), 2.70 (s, 3H, CH_3N), 3.56–3.68 (m, 2H, CHN) ppm. Anal ($C_9H_{15}NO_3$) C, H, N.

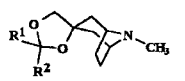
Table 3. Chemical and physical characteristics of compounds **14–18**.

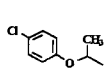
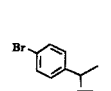


N	R^1	R^2	mp (°C)	yield (%)	^{1}H-NMR ($CDCl_3$) (δ)
14[a]	CH_3	H	52[b]	76	1.28-1.78 (m, 4H), 1.31 9d, 3H, CH_3C), 1.92-2.20 (m, 4H), 2.36 (s, 3H, CH_3N), 3.14-3.26 (m, 2H, CHN), 3.73 (d, 1H) and 3.91 (d, 1H) (CH_2O), 5.05 (q, 1H, CHMe) ppm.
15	C_6H_5	H	160[c]	25	1.52-1.62 (m,2H), 1.70-1.93 (m,2H), 1.98-2.12 (m, 2H), 2.21-2.33 (m, 2H), 2.41 (s, 3H, NCH_3), 3.18-3.30 (m, 2H, CHN), 3.94 (d, 1H) and 4.06 (d, 1H) (CH_2O), 5.82 (s, 1H, CHPh), 7.31-7.49 (m, 5H, aromatics) ppm.
16	C_6H_{11}	C_6H_5	205[c]	20	1.19-1.35 (m, 4H), 1.52-1.79 (m, 10H), 1.85-2.27 (m, 5H), 2.35 (s, 3H, NCH_3), 3.00-3.09 (m, 1H) and 3,12-3.21 (m, 1H) (CHN), 3.52 (d, 1H) and 3.98 (d, 1H) (CH_2O), 7.20-7.45 (m, 5H, aromatics) ppm.
17		H	65-8[c]	35	1.27 (dd, 3H, CH_3C), 1.42-1.83 (m, 4H), 2.02-2.30 (m, 4H), 2.35 (s, 3H, CH_3N), 3.12-3.28 (m, 2H, CHN), 3.78-3.92 (m, 2H, CH_2O), 4.19-4.35 (m, 1H, CHMe), 4.99 (dd, 1H, CHO_2), 6.85 (d, 2H) and 7.19 (d, 2H) (aromatics) ppm.
18		H	110[c]	29	1.28 (dd, 3H, CH_3C), 1.35-1.61 (m, 4H),1.95-2.21 (m, 4H), 2.33 (s, 3H, NCH_3), 2.80-2.95 (m, 1H, CHMe), 3.08-3.22 (m, 2H, CHN), 3.61-3.75 (m, 2H, CH_2O), 4.93 (dd, 1H, CHO), 7.12 (d, 2H) and 7.39 (d, 2H) (aromatics) ppm.

[a] Ref. 20. [b] Purified by column chromatography on Al_2O_3, with $CHCl_3$/MeOH 99:1 as eluent. The oxalate melts at 173 °C. [c] As oxalate salt.

Table 4. Chemical and physical characteristics of compounds **19–22**.



N	R^1	R^2	mp (°C)	yield (%)	^{1}H-NMR ($CDCl_3$) (δ)
19[a]	CH_3	H	-[b]	32	1.30 (d, 3H, CH_3C), 1.58-2.23 (m, 8H), 2.35 (s, 3H, CH_3N), 3.22-3.32 (m, 2H, CHN), 3.44 (d, 1H) and 3.59 (d, 1H) (CH_2O), 5.08 (q, 1H, CHMe) ppm.
20	C_6H_{11}	C_6H_5	-[c]	15	1.05-2.19 (m, 17H),2.28-2.48 (m, 2H), 2.45 (s, 3H, NCH_3), 3.06-3.18 (m, 1H) and 3,19-3.28 (m, 1H) (CHN), 3.35 (d, 1H) and 3.72 (d, 1H) (CH_2O), 7.24-7.43 (m, 5H, aromatics) ppm.
21		H	65-8[d]	35	1.32 (dd, 3H, CH_3C), 1.60-2.08 (m, 8H), 2.28 (s, 3H, CH_3N), 3.08-3.23 (m, 2H, CHN), 3.50-3.60 (m, 2H, CH_2O), 4.25-4.45 (m, 1H, CHOAr), 5.04 (dd, 1H, CHO_2), 6.92 (d, 2H) and 7.20 (d, 2H) (aromatics) ppm.
22		H	115-9[d]	19	1.31 (d, 3H, CH_3C), 1.52-2.08 (m, 8H), 2.28 (s, 3H, NCH_3), 2.89-3.01 (m, 1H, CHPh), 3.08-3.21 (m, 2H, CHN), 3.32 (d, 1H) and 3.68 (d, 1H) (CH_2O), 4.97 (d, 1H, CHO_2), 7.18 (d, 2H) and 7.41 (d, 2H) (aromatics) ppm.

[a] Ref. 20. [b] Purified by transformation into the oxalate salt which was obtained as a gummy solid. [c] As free base. [d] As oxalate salt.

3β-Hydroxymethyl-8-methyl-8-azabicyclo[3.2.1]octan-3α-ol (**13**)

Following the same procedure as for **10**, starting from **12**, compound **13** was obtained in 43% yield. ^{1}H-NMR ($CDCl_3$) δ = 1.55–1.68 (m, 2H), 1.78–2.75 (m, 6H), 2.27 (s, 3H, CH_3N), 3.12–3.20 (m, 2H, CHN), 3.28 (s, 2H, CH_2O) ppm. ^{13}C-NMR ($CDCl_3$) δ = 25.82 (t), 26.39 (t), 40.86 (q), 41.07 (t), 41.21 (t), 58.42 (t), 60.62 (d), 73.31 (s) ppm. Anal ($C_9H_{17}NO_2$) C, H, N.

General Procedure for the Synthesis of the dioxolanes **14–22**

To a CH_2Cl_2 solution of the diol (**10** or **13**) (1 eq) and the appropriate aldehyde or ketone (2 eq) a 48% solution of $BF_3.Et_2O$ (2 eq) is added at 0 °C. After some time at 0 °C, the mixture is treated with a 10% solution of NaOH, and extracted with CH_2Cl_2. After rendering anhydrous (Na_2SO_4) and removal of the solvent the residue is purified by column chromatography (Al_2O_3) or by transformation into the oxalate salt with an equimolar amount of oxalic acid in ethyl acetate. The chemical and physical characteristics of the compounds obtained are reported in Tables 3 and 4.

2-(4-Chlorophenoxy)propionaldehyde (**23**)

$LiAlH_4$ (0.5 g, 0.0175 mol) was suspended in 40 mL of anhydrous THF and 4 g (0.0175 mol) of ethyl 2-(4-chlorophenoxy)propionate[15] (prepared from the commercially available acid with standard procedure) were added at 0 °C. After 2 h at 0 °C the mixture was treated with ice and extracted with ether. After rendering anhydrous (Na_2SO_4) and removal of solvent, 2-(4-chlorophenoxy)propanol[14] was obtained as an oil (95% yield). ^{1}H-NMR ($CDCl_3$) δ = 1.25 (d, 3H, CH_3C), 2.05 (br. s, 1H, OH), 3.69–3.75 (m, 2H, CH_2O), 4.35–4.54 (m, 1H, CHCO), 6.86 (d, 2H) and 7.23 (d, 2H) (aromatic H) ppm. The product was used in the next step without purification.

Anhydrous DMSO (0.4 mL) in anhydrous CH_2Cl_2 was cooled to –50 °C and trifluoroacetaldehyde (0.56 mL, 0.004 mol) was added dropwise. After 10 min 2-(4-chlorophenoxy)propanol (0.5 g, 0.0027 mol) dissolved in 3 mL of CH_2Cl_2 was added. The mixture was allowed to warm to room temperature; the solvent was evaporated and the residue treated with ether and water; the organic layer was collected and dried (Na_2SO_4). After removal of solvent, the residue was purified by column chromatography (cyclohexane/ethyl acetate 70:30) to give the title compound as an oil (51% yield). ^{1}H-NMR ($CDCl_3$) δ = 1.48 (d, 3H, J 7.0 Hz, CH_3C), 4.61 (m, 1H, J 1.6 and 7.0 Hz, CH), 6.83 (d, 2H) and 7.25 (d, 2H) (aromatic H), 9.70 (t, 1H, J 1.6 Hz, CHO) ppm. Anal ($C_9H_9ClO_2$) C, H.

4-Bromo-α-methyl-benzeneacetaldehyde (**24**)

1.7 g of ethyl 4-bromo-α-methyl-benzeneacetate **25** (prepared from 4-bromo-α-methyl-benzeneacetic acid[1] with standard procedures), dissolved in 5 mL of anhydrous THF, was added dropwise to a suspension of $LiAlH_4$ (0.2 g, 0.0053 mol) in anhydrous THF (5 mL) at 0 °C. After 2 h at 0 °C the excess of $LiAlH_4$ was destroyed with ice and the solution extracted with ether. After rendering anhydrous and removal of solvent, 1.25 g of 4-bromo-α-methyl-benzeneethanol **26** was obtained as an oil (88 % yield). ^{1}H-NMR ($CDCl_3$) δ = 1.28 (d, 3H, CH_3C), 2.93 (m, 1H, CH), 3.67 (d, 2H, CH_2O), 7.11 (d, 2H) and 7.46 (d, 2H) (aromatic H) ppm. The product was used in the next step without purification.

Following the same procedure as for **23**, **24** was obtained in 10% yield. From the NMR spectrum and GC-MS a mixture of **24** (88%) and 4-bromoacetophenone (12%) resulted (comparison with an authentic sample). Attempts to purify the product by column chromatography or fractional distillation were unsuccessful. The product was used without further purification for the following step.

Pharmacology

Analgesic Activity

Analgesic activity was evaluated using the hot-plate test on male Swiss-Webster mice. The method described by O'Callaghan was adopted[16], using a stainless steel container (36 × 28 × 30 cm), thermostatically set at 52.5 ± 0.1 °C, in a precision water-bath. Mice with a licking latency below 12 and over 18 s in the test before drug administration (30%) were rejected. An arbitrary cut-off time of 45 s was adopted. The number of mice treated in each test varied from 8 to 20.

The analgesic potency of the compounds is reported as the ED_{50} (Table 1). This potency does not however indicate the level of analgesia reached. To evaluate this parameter, the analgesic effect of the new products injected at their maximal non-toxic dose was compared to that of morphine, taken as the reference compound and injected at 8 mg/kg s.c., a dose that does not alter the 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 time of X)/(maximum reaction time of morphine – pretest reaction time of morphine) × 100.

The maximal non-toxic dose is the highest dose of X at which treated mice exhibit, on the rota-rod test, the same motor coordination and resistance to fatigue than saline-control mice (data not shown). Rota-rod test was performed according to the method described by Vaught;[28] the apparatus consists of a base platform and a rotating rod of 3 cm in diameter. The integrity of motor coordination was assessed on the basis of the number of falls from the rotating rod during 30 s of observation, before and 15, 30, and 45 min after drug administration. Saline injected mice progressively reduced the number of their falls as a function of experimental sessions on the rotating rod.

Standard errors on the value expressed as percentage were not evaluated. Original data, however, have been statistically analyzed. The statistical significance of the differences between the means obtained by administering various doses of drugs in treated group and the mean obtained in saline control group were evaluated with Dunnett's test. Differences were considered statistically significant when $P \leq 0.05$. Percent values were calculated only for those differences that resulted statistically significant; in the other cases, drugs were considered inactive. Since the reaction times were measured with an accuracy of ± 15%, the errors in the percent values calculated through the formula reported above should be in the same range.

Nootropic Activity

The test was performed according to the step-through method described by Jarvik and Kopp[17] modified by us for testing drugs endowed with analgesic properties. The apparatus is constituted by a two compartment acrylic box, with a lighted conpartment connected by a guillotine door to a darkened one. In the original method mice would receive a punishing electrical shock as soon as they enter in the dark compartment, while in our modified method mice, after entry into the dark compartment, received a non-painful punishment consisting of a fall into cold water (10 °C). For this purpose the dark chamber was constructed with a pitfall floor. The latency times for entering the dark compartment were measured in the training test and after 24 h in the ritention test. Compound **22** was injected intraperitoneally 20 min before the training session, while the amnesic drug dicyclomine (2 mg/kg) was injected i.p. immediately after termination of the training session. The maximum entry latency allowed in the retention session was 120 s. The memory degree of received punishment (fall into cold water) was expressed as the difference (Δs) between retention and training latencies.

Binding

Rat membranes for [^{3}H]-QNB binding assay were prepared as described by Franchini[18] and were used fresh for the binding experiments. Briefly, aliquots of membranes (30–50 μg protein/tube) were incubated in a final volume of 2 mL with 0.2 nmol/L [^{3}H]-QNB and increasing concentrations of unlabelled drugs (0.1–10000 nmol/L) at 25 °C in 50 mmol/L Na^+/K^+ phosphate buffer, pH 7.4 . After 90 min incubation, membranes were filtered through Whatman GF/B filter strips, presoaked in 0.01% aqueous polyethyleneimine solution[29], using the Brandel M-48R 48-well cell harvester (Gaithersburg, MD, USA). Filters were washed three times with 5 mL of ice cold buffer and the radioactivity trapped on the filters was extracted in 10 mL scintillation cocktail (Instagel, Packard Instrument, B. V., Groningen, The Netherlands) and counted in a B counter (TRI-CARB 1900TR, Packard) at an efficiency of about 48%.

Protein concentration was determined according to the method of Bradford,[30] using the bovine serum albumin as the standard.

The binding data were evaluated quantitatively with non linear least squares curve fitting using the computer program LIGAND.[31]

The computer program ALLFIT[32] was used for analysis of sigmoidal dose-response curves obtained in binding; this program uses the constrained 4-parameter logistic model to obtain estimates of IC_{50} values and the logit-log slope (pseudo Hill coefficient).

SDZ 205557 Reversion of Analgesia

SDZ 205557 was administered at the dose of 10 mg/kg i.p. 30 min before the hot-plate test. The reversion of analgesia was calculated using the following formula:

Reversion of analgesia (%) = 100 – [(licking latency for X after administration of SDZ 205557 – pretest time)/(licking latency for X without SDZ 205557 – pretest time)] × 100.

The licking latency times in absence and in presence of SDZ 205557 were taken at the same time after administration of the drug X, time at which the maximum level of analgesia was reached. When the residual analgesia after administration of SDZ 205557 was not significantly different from the pretreatment value, the reversion of analgesia was considered as 100%.

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