Enantioselective Synthesis and Preliminary Pharmacological Evaluation of the Enantiomers of Unifiram (DM232), a Potent Cognition-Enhancing Agent

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Abstract: The enantiomers of the potent cognition-enhancer DM232 ((**1**), unifiram) and of its isopropylsulfonyl analog (**2**), which is endowed with amnesic properties, have been synthesized using (*S*)- and (*R*)-5-(hydroxymethyl)-2-pyrrolidinone as chiral precursors. The enantiomeric excess was determined by means of capillary electrophoresis, and found higher than 99.9 %. DM232 enantiomers were tested as cognition-enhancers in the passive-avoidance and social learning tests, and their ability to induce ACh release from rat cerebral cortex was also determined; in all the performed essays, (*R*)-(+)-(**1**) displayed higher potency than its (*S*)-(-) enantiomer, being able to elicit comparable effects at 3-fold to 10-fold lower doses. On the contrary, (*R*)-(+) and (S)-(-)-(**2**) showed the same amnesic potency when tested in the passive-avoidance test. These findings may be useful to clarify the mechanism of action of these substances.

Key Words: Cognition-enhancer, nootropic, chiral synthesis.

INTRODUCTION

Cognition-enhancers are compounds able to facilitate learning and memory and to overcome natural or induced cognitive impairment. They belong to heterogeneous chemical classes, and may exert their pharmacological effects through interaction with different biological targets (see, for instance [1] and references therein). Drugs with cognition-enhancing properties may be useful in the management of mild cognitive disorders and of the cognitive aspects of neurodegenerative diseases such as AD [2, 3]. So far, only a few compounds of this kind have reached the market and they belong practically to two families: the acethylcholinesterase inhibitors and the piracetam-like nootropics. Nootropics are not widely used and their efficacy has been questioned [4]. Acetylcholinesterase inhibitors seem to be more reliable but their reduced effects and the high number of non-responders limit their use [5].

We have recently reported the cognition-enhancing properties of DM232 (unifiram, (**1**)), a 1,4-diazabicyclo [4.3.0]nonan-9-one, and of DM235 (sunifiram), its product from molecular simplification (Chart **1**) [6, 7]. At doses as low as 0.001 mg/kg, both compounds are able to revert amnesia induced by several drugs in the mouse passive avoidance test and by scopolamine in the rat Morris Watermaze test; such a potency is rare among cognition-enhancing drugs. Moreover, unifiram and sunifiram were found to improve memory in the rat social learning paradigm, a test where no cognitive impairment has been induced in animals [7-9].

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Unifiram and sunifiram did not show affinity for the most important receptors and channels in the CNS [8], however they were found to increase, in a concentration-dependent way, excitatory synaptic transmission in rat hippocampus *in vitro* [10]: from this and from other experimental evidence it was hypothesized that AMPA-mediated neurotransmission was involved in their cognition-enhancing effect. As a matter of fact, unifiram and sunifiram, being arylsulfonyl or aroylamides, share structural similarity with some allosteric modulators of the AMPA receptor [11, 12]. Moreover unifiram and sunifiram, at the dose of 0.01 mg/kg, were able to increase the central cholinergic tone [7], a feature shared by ortosteric and allosteric AMPA receptor activators [13, 14]. Nevertheless, the doses necessary to increase excitatory synaptic transmission in rat hippocampus were not consistent with those effective in behavioural tests, suggesting that AMPA activation is not the only mechanism of action of these compounds.

Unifiram possesses a stereogenic center, and we thought it interesting to study its enantioselectivity. Therefore, a stereoselective synthesis was carried out, starting from a chiral precursor, in order to obtain both enantiomers with known absolute configuration. Moreover, we extended the study of enantioselectivity also to compound (**2**) (Chart (**1**)) [11]: this molecule, the isopropylsulfonyl analogue of DM232, synthesized during the study of the structure-activity relationships in this class of compounds, is endowed with the opposite action with respect to DM232, being able to induce amnesia in mice; it was reasoned that the study of enantioselectivity could help to clarify whether its amnesing activity is due to interaction with the same biological target as the parent compound.

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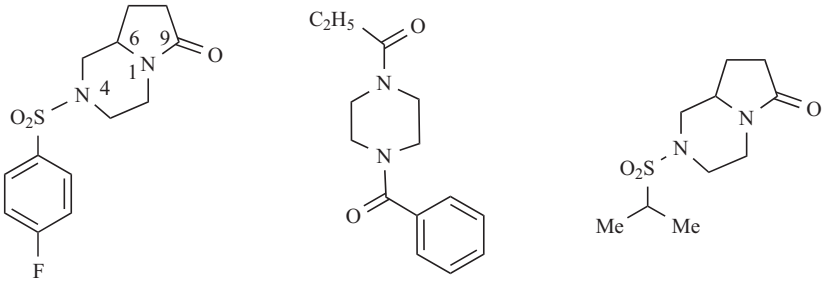


1 DM232
unifiram

DM235
sunifiram

2

Chart. (1).

CHEMISTRY

To obtain the enantiomers of 1,4-diazabicyclo[4.3.0] nonan-9-one we chose a synthetic pathway different from those previously employed to obtain the racemate (±)-(**3**). In the first approach [6] (**3**) was obtained in 6 steps *via* compound (**4**) (Figure (**1**)) in 30% overall yields. More recently, we obtained (±)-(**3**) starting from 2-methylpyrazine [11]; this synthetic method was faster (3 steps) and less expensive, but not suitable for a chiral synthesis. Compound (**5**), a possible intermediate in the first pathway, was later synthesized by other researchers in its *R*-form starting from *S*-serine, but the entire synthetic pathway was reported to give enantiopure diazabicyclo[4.3.0]nonan-9-ones with overall yields not exceeding 12% [15].

Therefore, to obtain the enantiomers of (**1**) and (**2**) we chose the synthetic pathway, reported in Scheme **1**, which started from the commercially-available (*S*)-5-(hydroxymethyl)-2-pyrrolidinone, which was transformed into the methanesulfonate (*S*)-(+)-(**6**) and then reacted with benzylamine to give (*S*)-(+)-(**7**). Alkylation of the basic nitrogen with 2-bromoethanol afforded the aminoalcohol (*S*)-(+)-(**8**)**,** which was transformed, *via* the methanesulfonate, into (*S*)-(-)-(**9**) under basic conditions. Catalityc hydrogenation then gave (*S*)-(-)-(**3**) which was converted into the desired sulfonamides (*S*)-(-)-(**1**) and (*S*)-(-)-(**2**) as previously reported [6, 11]. The same synthetic pathway was used for the *R*-enantiomers starting from commercially available (*R*)-5-(hydroxymethyl)-2-pyrrolidinone.

ENANTIOMERIC EXCESS

The enantiomeric excess (e.e.) of (*S*)-(-) and (*R*)-(+)-(**3**) was checked by means of capillary electrophoresis.

Sodium heparin was used as chiral selector. This glycosaminoglycan is characterized by high aqueous solubility and relatively good UV transparency. Sodium heparin was supplemented at different levels to a pH 4.5 Tris-citrate buffer, which was particularly useful as a background electrolyte (BGE) as observed in previous studies [16, 17]. At the concentration of 4 % (w/v), sodium heparin provided the baseline enantioresolution of (±)-(**3**) (Figure (**2a**)). In Figure (**2b**) the same electropherograms of Figure (**2a**) concerning the non-racemic mixtures were reported; the response axis was magnified in order to verify the presence of the optical impurity. The e.e. of (*R*)- (+)- and (*S*)-(-)-(**3**) resulted higher than 99.9%.

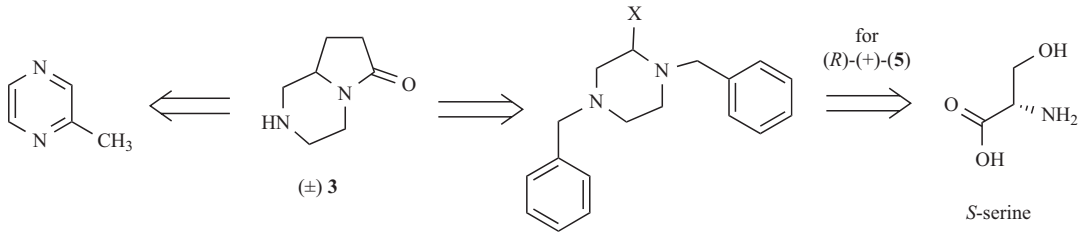
PHARMACOLOGY

Compounds (*S*)-(-)-(**1**) and (*R*)-(+)-(**1**) were tested as cognition enhancers in the mouse passive avoidance test on mice [18], using scopolamine as amnesizing agent, and in the social learning test on adult rats with unimpaired memory [19], according to an already published protocol [6, 7, 11]. Compounds (*S*)-(-)-(**1**) and (*R*)-(+)-(**1**) were also tested by microdialysis studies on rat cerebral cortex to evaluate their ability to facilitate acetylcholine release, in accordance with the method of Giovannini [20].

The passive avoidance test was used to evaluate the amnesic properties of (*S*)-(-)-(**2**) and (*R*)-(+)-(**2**).

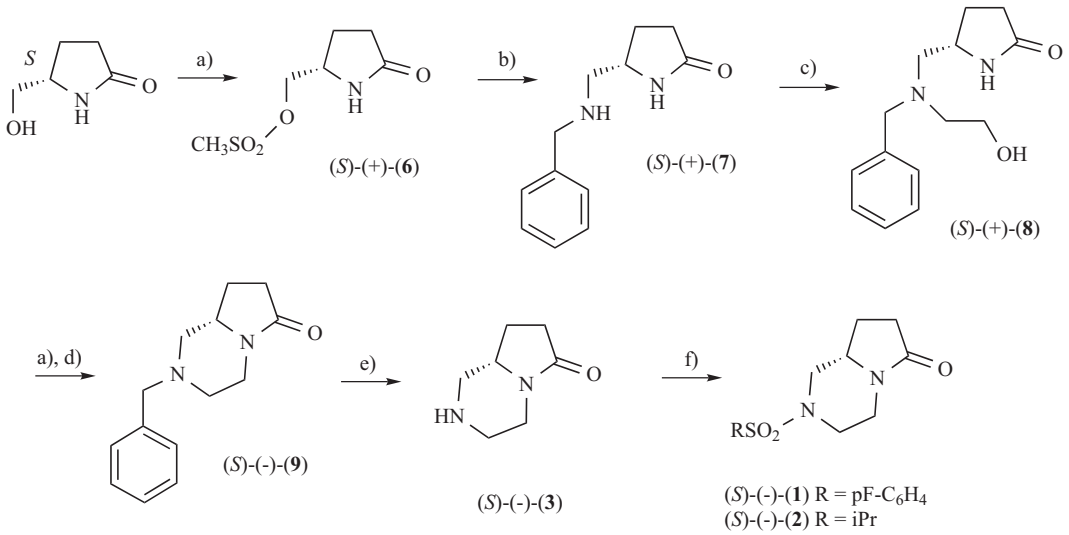
RESULTS AND DISCUSSION

The results of the passive-avoidance test in mice are shown in Table (**1**); the racemate DM232 [(±)-(**1**)] is reported as reference drug. Similarly to DM232, (*R*)-(+)-(**1**) was able to revert the amnesic effect of scopolamine at a



(±)-(**4**)**:** X = CHO [ref. 5]
(*R*)-(+)-(**5**) X = CH_2OH [ref. 15]

Fig. (1).



a) CH_3SO_2Cl, Et_3N; b) $PhCH_2NH_2$; c) $BrCH_2CH_2OH$, Et_3N; d) tBuOK; e) H_2/Pd/C; f) RSO_2Cl, Et_3N.
The *R* forms were synthesized in the same way, starting from (*R*)-(-)-5-(hydroxymethyl)-2-pyrrolidinone.

Scheme (1).

dose as low as 0.001 mg/kg i.p., while the minimal effective dose (MED) of its (*S*)-(-) enantiomer was higher (0.003 mg/kg). As for (±)-(**1**), the antiamnesic effect is elicited without changing the animal's behavior; rota-rod and Animex tests did not show any impairment of motor coordination, spontaneous motility and curiosity of the treated animals (data not shown).

In the social learning test on adult rats (Figure (**3**)) (*R*)-(+)-(**1**) was more active than its (*S*)-(-) enantiomer, being able to reduce, at lower doses, the active exploration of the familiar partner at the second pairing after 24 h.

Both DM232 enantiomers were shown to facilitate the release of acetylcholine from rat cerebral cortex (Figure (**4**)) at a dose comparable to those of the racemate; again, (*R*)-(+)-(**1**) was more active, being able to increase ACh release at doses as low as 0.001 mg/kg ip while (*S*)-(-)-**1** was active only at higher doses (0.01 mg/kg ip).

Compounds (*S*)-(-)-(**2**) and (*R*)-(+)-(**2**) were tested in the passive avoidance test in order to measure their amnesic activity (Table (**1**)). Both enantiomers as well as the racemate were effective at the dose of 1 mg/kg i.p., with a potency similar to that of scopolamine. However, differently from **1**, (*S*)-(-)-(**2**) and (*R*)-(+)-(**2**) did not show enantioselectivity in their action.

The data reported above show that both enantiomers of (**1**) are active at very low doses, confirming the outstanding potency of the corresponding racemate. In all the assays performed, (*R*)-(+)-(**1**) displayed higher potency than its (*S*)-(-)-enantiomer, being able to elicit comparable effects at 3-fold to 10-fold lower doses. Enantioselectivity is indeed low;

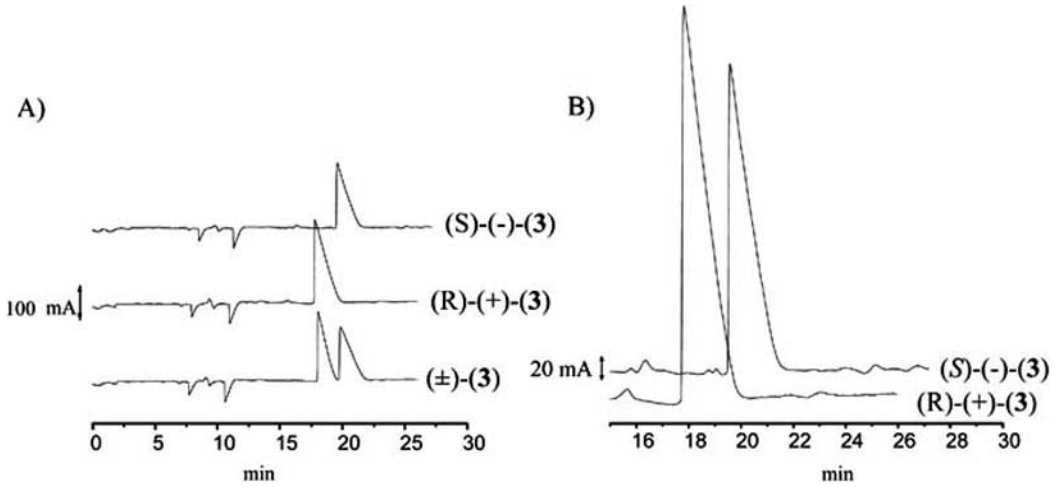


Fig. (2). Electropherograms of racemate and single enantiomers (*S*)-(-)-(**3**) and (*R*)-(+)-(**3**) under chiral CE conditions: fused-silica capillary (38.5 cm, ID 50 µm); Tris-citrate buffer (25 mM) pH 4.5 containing sodium heparin 4 %.
Voltage 15 kV; detection 200 nm; hydrodynamic injection 50 mbar for 5 s; T 15 °C.

Table 1. Effect of (*S*)-(-)-(1), (*R*)-(+)-(1) (DM-232 Enantiomers)[a], (*S*)-(-)-(2) and (*R*)-(+)-(2)[b] in the Mouse Passive-Avoidance Test

PRE-TREATMENT	TREATMENT	Training session	Retention session	Δ
SALINE	SALINE	14.3 ± 3.3	95.2 ± 7.9	80.9
SALINE	SCOPOLAMINE	16.0 ± 4.0	38.2 ± 8.1	22.2
(S)-(-)-(**1**) 0.0003 mg/kg	SCOPOLAMINE	17.3 ± 3.9	40.6 ± 9.2	23.3
(S)-(-)-(**1**) 0.001 mg/kg	SCOPOLAMINE	14.3 ± 3.5	51.4 ± 8.2	37.1
(S)-(-)-(**1**) 0.003 mg/kg	SCOPOLAMINE	15.2 ± 3.6	78.7 ± 9.3*	63.5
(S)-(-)-(**1**) 0.01 mg/kg	SCOPOLAMINE	17.1 ± 4.1	97.2 ± 7.7*	80.1
(R)-(+)-(**1**) 0.0003 mg/kg	SCOPOLAMINE	16.5 ± 3.8	51.1 ± 9.4	34.6
(R)-(+)-(**1**) 0.001 mg/kg	SCOPOLAMINE	14.4 ± 3.5	75.4 ± 8.8*	61.0
(R)-(+)-(**1**) 0.003 mg/kg	SCOPOLAMINE	15.4 ± 3.8	84.9 ± 7.3*	69.5
(R)-(+)-(**1**) 0.01 mg/kg	SCOPOLAMINE	18.2 ± 4.2	96.5 ± 9.0*	78.3
(R)-(+)-(**1**) 0.03 mg/kg	SCOPOLAMINE	15.8 ± 3.7	99.2 ± 8.3*	83.4
(±)-**1** [c] 0.001 mg/kg	SCOPOLAMINE	19.7 ± 5.8	104.4 ± 10.6	93.8
(S)-(-)-(**2**) 0.1 mg/kg	SALINE	16.6 ± 4.1	93.2 ± 9.6*	76.6
(S)-(-)-(**2**) 1.0 mg/kg	SALINE	15.5 ± 3.9	56.9 ± 8.3	41.4
(S)-(-)-(**2**) 10 mg/kg	SALINE	13.8 ± 3.8	26.6 ± 7.6	12.8
(R)-(+)-(**2**) 0.1 mg/kg	SALINE	14.3 ± 4.0	95.1 ± 10.2*	80.8
(R)-(+)-(**2**) 1.0 mg/kg	SALINE	16.2 ± 4.1	52.0 ± 9.3	35.8
(R)-(+)-(**2**) 10 mg/kg	SALINE	13.7 ± 3.8	31.4 ± 8.5	17.7
(±) **2** (1.0 mg/kg)	SALINE	13.6 ± 3.9	61.5 ± 10.4	47.9
(±) **2** (10 mg/kg)	SALINE	13.7 ± 3.2	20.5 ± 9.4	6.8

[a] (*S*)-(-)-(**1**) and (*R*)-(+)-(**1**) were injected s.c. 20 min before training session while scopolamine (3.0 mg/kg i.p.) was administered immediately after punishment; each value represents the mean of 25-31 mice. [b] (*S*)-(-)-(**2**) and (*R*)-(+)-(**2**) were administered s.c. 30 min before training session; each value represents the mean of at least twelve mice. [c] From ref. [5].

* $P < 0.01$ in comparison with scopolamine-treated mice.

this might be due to the characteristics of the interaction with the biological target but could be a consequence of pharmacokinetic factors, as well. Unfortunately, since at the moment we do not know the mechanism of action of these compounds, we have no means to decide which is the case. In fact, we cannot perform any *in vitro* experiment where pharmacokinetic phenomena are absent or under control, to compare with the *in vivo* experiments performed, where the enantiomers may undergo enzymatic racemization, different metabolism, different active transport and so on.

More studies are necessary to clarify this aspect; they are under way and the results will be reported in due time. At the moment we can only exclude chemical racemisation since, as also suggested by the structure of the compounds and by the characteristics of the hydrogen atom involved, the two enantiomers appear to be optically stable under physiological conditions. The same reasoning can be done to explain the lack of enantioselectivity of the amnesing analogue (**2**) that should reasonably interact with the same target.

In conclusion, while the present data confirm that the central cholinergic system is eventually involved in the cognition enhancing activity of this class of compounds, the molecular target responsible for acetylcholine release remains unidentified. It is hoped that the availability of the enantiomers of the potent cognition enhancer (**1**) and of the closely related amnesing drug (**2**) will be useful to identify this target and to clarify the mechanism of action.

EXPERIMENTAL PART

Chemistry

General Considerations

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 (200 MHz for ^{1}H, 50.3 MHz for ^{13}C). Optical rotation was measured at a concentration of 1 g/100 mL (c = 1), unless

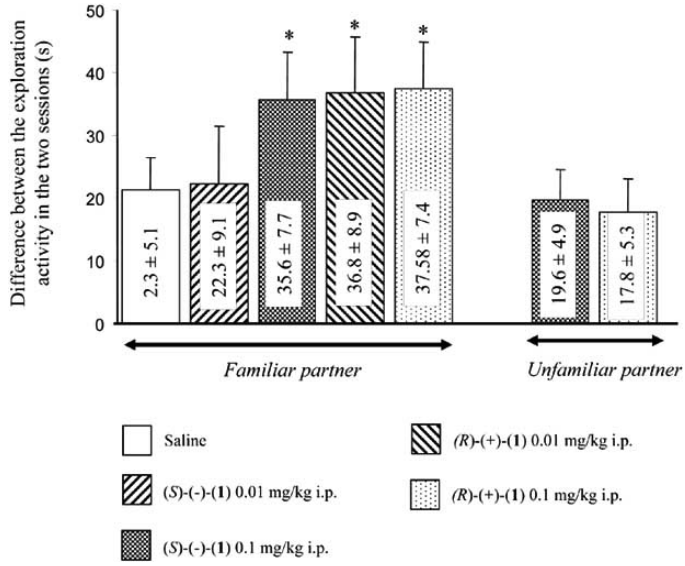


Fig. (3). Effect of (*S*)-(-)-(**1**) and (*R*)-(+)-(**1**) in the social learning test. Each column represents the means of five rats. * P<0.01.

otherwise stated, with a Perkin-Elmer 241 polarimeter (accuracy ± 0.002°). 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.

Methanesulfonic acid (S)-(+)-[5-(oxo)-2-pyrrolidinyl]methyl Ester [(S)-(+)-(6)]

To a solution of commercially-available (*S*)-(+)-5-(hydroxymethyl)-2-pyrrolidinone (0.29 g, 2.5 mmol) in ethanol-free $CHCl_3$ (10 mL) were added at 0°C Et_3N (0.38 g, 3.8 mmol) and methanesulfonyl chloride (0.35 g, 3.0 mmol). The mixture was left stirring at room temperature for 3 h, then it was diluted with CH_2Cl_2 and washed several times with a saturated solution of $NaHCO_3$. After drying (Na_2SO_4), the solvent was removed under reduced pressure obtaining the title compound in 86% yield. The product was used as such in the next step. [^{1}H]-NMR ($CDCl_3$) δ 1.76-2.04 (m, 1H); 2.20-2.45 (m, 3H); 3.08 (s, 3H, CH_3); 3.95-4.13 (m, 2H, CHN and CH*H*O); 4.25 (dd, 1H, J = 9.5 Hz, 3.3 Hz, C*H*HO); 7.11 (bs, 1H, NH) ppm. [^{13}C]-NMR ($CDCl_3$) δ 22.99 (t), 30.04 (t), 37.76 (q), 53.27 (d), 71.90 (t), 179.01 (s) ppm. A small sample was purified by column chromatography ($CHCl_3$/MeOH 9:1): white solid, m.p. 40°C; $[\alpha]_{20}^{D}$ = +20° (EtOH 96%, c = 1) Anal. $C_6H_{11}NO_4S$ (C, H, N).

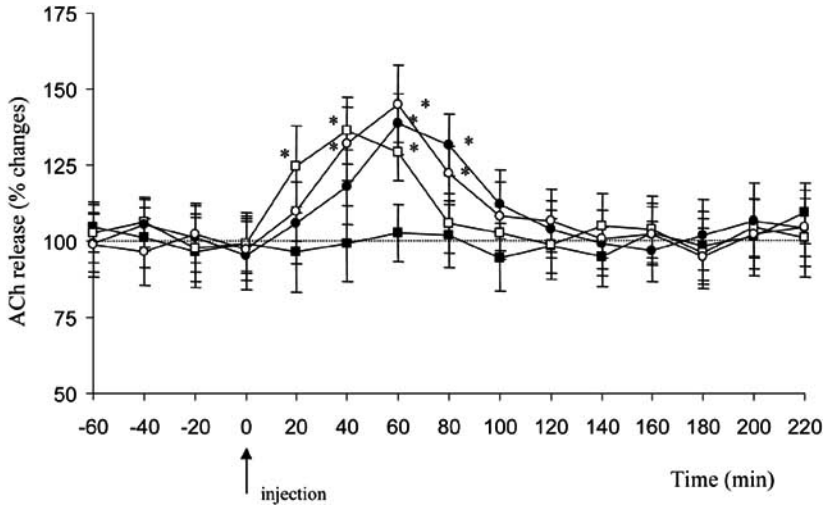


Fig. (4). Dose response curve for compound (*S*)-(-)-(**1**) (squares) and (*R*)-(+)-(**1**) (circles) on ACh release from rat cerebral cortex. Compounds were injected at time 0, as shown by the arrow. All values are expressed as changes over basal output. Vertical lines give SEM. Each point represents the mean of six rats. Closed squares: (*S*)-(-)-(**1**) 0.001 mg/kg i.p.; open squares: (*S*)-(-)-(**1**) 0.01 mg/kg i.p.; closed circles: (*R*)-(+)-(**1**) 0.001 mg/kg i.p.; open circles: (*R*)-(+)-(**1**) 0.01 mg/kg i.p. * P<0.01 in comparison to controls.

Methanesulfonic acid (R)-(-)-[5-(oxo)-2-pyrrolidinyl]methyl Ester [(R)-(-)-(6)]

Following the same procedure as for (*S*)-(+)-(**6**), starting from commercially available (*R*)-(+)-5-(hydroxymethyl)-2-pyrrolidinone, the title compound was obtained in 62% yield. $[\alpha]_{20}^{D}$ = -20° (EtOH 96%, c = 1) The [^{1}H]- and [^{13}C]-NMR spectra are identical to those of (*S*)-(+)-(**6**). Anal. $C_6H_{11}NO_4S$ (C, H, N).

(S)-(+)-5-(Benzylaminomethyl)-2-pyrrolidinone [(S)-(+)-(7)]

A mixture of (*S*)-(+)-(**6**) (2.0 g, 10 mmol) and benzylamine (2.2 g, 20 mmol) in CH_2Cl_2 (20 mL) were heated at 40°C for 20 h, then the mixture was diluted with CH_2Cl_2 and washed with water. The organic layer was dried and the solvent was removed to yield a residue, which was purified by column chromatography (CH_2Cl_2/MeOH 9:1) obtaining the title compound as an oil in 84% yield.

$[\alpha]_{20}^{D}$ = +28° (EtOH 96%, c = 1). [^{1}H]-NMR ($CDCl_3$) δ 1.64-1.80 (m, 1H, pyrrolidine); 1.96 (bs, 1H, BzNH); 2.09-2.34 (m, 3H, pyrrolidine protons); 2.56 (dd, 1H, J = 11.7 Hz, 8.4 Hz) and 2.76 (dd, 1H, J = 11.7 Hz, 3.3 Hz) (CH_2N); 3.63-3.84 (m, 3H, CH_2-Ph + CHN); 6.62 (bs, 1H, NHCO); 7.30 (s, 5H, aromatic protons) ppm. [^{13}C]-NMR ($CDCl_3$) δ 25.25 (t), 30.59 (t), 54.19 (t), 54.79 (d), 54.86 (t), 127.48 (d), 128.48 (d), 128.81 (d), 140.06 (s), 178.74 (s) ppm. Anal. $C_{12}H_{16}N_2O$ (C, H, N).

(R)-(-)-5-(Benzylaminomethyl)-2-pyrrolidinone [(R)-(-)-(7)]

Following the same procedure as for (S)-(+)-(**7**), starting from (*R*)-(-)-(**6**), the title compound was obtained in 39% yield. $[\alpha]_{20}^{D}$ = -30° (EtOH 96%, c = 1). The [^{1}H]- and [^{13}C]-NMR spectra are identical to those of (*S*)-(+)-(**7**). Anal. $C_{12}H_{16}N_2O$ (C, H, N).

(S) - (+)-5 -[[Benzyl-(2-hydroxyethyl)amino]methyl]pyrrolidin-2-one [(S)-(+)-(8)]

A mixture of (*S*)-(+)-(**7**) (1.81 g, 8.9 mmol), Et_3N (2.63 g, 26 mmol) and 2-bromoethanol (2.89 g, 23 mmol) in anhydrous CH_3CN (10 mL) were heated under reflux for 6 h. The mixture was then partitioned between CH_2Cl_2 and HCl 2N; the water layer was basified and extracted with CH_2Cl_2. After drying (Na_2SO_4) and removal of the solvent, the residue was purified by flash chromatography (CH_2Cl_2/ MeOH 9:1) to obtain the title compound as an oil in 66% yield.

$[\alpha]_{20}^{D}$ = +35° (EtOH 96%, c = 1). [^{1}H]-NMR ($CDCl_3$) δ 1.42-1.63 (m, 1H); 1.99-2.57 (m, 6H); 2.69-2.88 (m, 1H); 3.42-3.91 (m, 5H, CH_2Ph + CH_2O + CHN); 4.08 (bs, 1H, OH); 7.19-7.40 (m, 5H, aromatic protons); 8.42 (bs, 1H, NHCO) ppm. [^{13}C]-NMR ($CDCl_3$) δ 25.16 (t), 30.99 (t), 53.40 (d), 57.72 (t), 59.92 (t), 60.07 (t), 61.19 (t), 127.52 (d), 128.70 (d), 129.32 (d), 138.99 (s), 179.12 (s) ppm. Anal. $C_{14}H_{20}N_2O_2$ (C, H, N).

(R)-(-)-5-[[Benzyl-(2-hydroxyethyl)amino]methyl]pyrrolidin-2-one [(R)-(-)-(8)]

Following the same procedure as for (*S*)-(+)-(**8**), starting from (*R*)-(-)-(**7**), the title compound was obtained in 50% yield. $[\alpha]_{20}^{D}$ = -35° (EtOH 96%, c = 1). The [^{1}H]- and [^{13}C]-NMR spectra are identical to those of (*S*)-(+)-(**8**). Anal. $C_{14}H_{20}N_2O_2$ (C, H, N).

(S)-(-)-4-Benzyl-1,4-diazabicyclo[4.3.0]nonan-9-one [(S)-(-)-(9)]

To a solution of (*S*)-(+)-(**8**) (0.12 g, 0.48 mmol) in ethanol-free $CHCl_3$ (5 mL), Et_3N (0.08 g, 0.72 mmol) and methanesulfonyl chloride (0.07 g, 0.6 mmol) were added at 0°C. The mixture was allowed to warm to room temperature and left stirring for 1h, then it was diluted with CH_2Cl_2 and washed with a saturated solution of $NaHCO_3$. After drying (Na_2SO_4) and removal of the solvent, a residue was obtained (methanesulfonic acid (*S*)-2-[N-benzyl-N-(5-oxo-pyrrolidin-2-yl)methyl]aminoethyl ester) which was used as such for the following step: it was dissolved in anhydrous THF and tBuOK (0.058 g, 0.48 mmol) was added at 0°C. After 2 h at this temperature, the mixture was allowed to warm to room temperature and left stirring overnight. The solvent was then removed under reduced pressure, the residue was treated with water and extracted with CH_2Cl_2. After drying (Na_2SO_4) and removal of the solvent, the residue was purified by column chromatography (CH_2Cl_2/abs. EtOH/ petroleum ether/ NH_4OH/diethyl ether 300:180:900:9.9:360) obtaining the title compound as an oil (30% yield). $[\alpha]_{20}^{D}$ = -52° (EtOH 96%, c = 1). [^{1}H]-NMR ($CDCl_3$) δ 1.40-1.78 (m, 2H); 1.85-2.19 (m, 2H); 2.26-2.32 (m, 2H); 2.72-2.99 (m, 3H); 3.42-3.70 (m, 3H); 3.96-4.01 (m, 1H); 7.25-7.38 (m, 5H, aromatic protons) ppm. [^{13}C]-NMR ($CDCl_3$) δ 22.56 (t), 30.70 (t), 39.96 (t), 52.27 (t), 56.01 (d), 60.16 (t), 63.07 (t), 127.61 (d), 128.66 (d), 129.33 (d), 137.95 (s), 173.46 (s) ppm. Anal. $C_{14}H_{18}N_2O$ (C, H, N).

(R)-(+)-4-Benzyl-1,4-diazabicyclo[4.3.0]nonan-9-one [(R)-(+)-(9)]

Following the same procedure as for (*S*)-(-)-(**9**), starting from (*R*)-(-)-(**8**), the title compound was obtained in 21% yield. $[\alpha]_{20}^{D}$ = +51.6° (EtOH 96%, c = 1) The [^{1}H]- and [^{13}C]-NMR spectra are identical to those of (*S*)-(-)-(**9**). Anal. $C_{14}H_{18}N_2O$ (C, H, N).

(S)-(-)-1,4-Diazabicyclo[4.3.0]nonan-9-one [(S)-(-)-(3)]

Compound (*S*)-(-)-(**9**) was hydrogenated at 60 psi in absolute ethanol with a catalytic amount of 10% Pd/C. After filtration and evaporation of the solvent, the title compound was obtained as an oil in 76% yield. $[\alpha]_{20}^{D}$ = -35.0° (EtOH 96%, c = 1). [^{1}H]-NMR ($CDCl_3$) δ 1.58-1.61 (m, 1H, H-7); 1.89 (bs, 1H, NH); 1.96-2.18 (m, 1H, H-8); 2.20-2.36 (m,

3H, H-7 + H-8 + H-5); 2.49 (td, 1H, J = 11.4 Hz, 3.4 Hz, H-3); 2.73 (td, 1H, J = 12.4 Hz, 3.6 Hz, H-2); 2.92 (dd, 1H, J = 11.4 Hz, 3.6 Hz, H-3); 3.09 (dd, 1H, J = 12.4 Hz, 3.6 Hz, H-5); 3.39-3.44 (m, 1H, H-6); 3.88 (dd, 1H, J = 12.4 Hz, 3.0 Hz, H-2) ppm. [^{13}C]-NMR ($CDCl_3$) δ 22.56 (t, C-7), 30.31 (t, C-8), 41.02 (t, C-2), 44.99 (t, C-3), 53.18 (t, C-5), 56.64 (d, C-6), 173.46 (s, C-9) ppm. Anal. $C_7H_{12}N_2O$ (C, H, N).

(R)-(+)-1,4-Diazabicyclo[4.3.0]nonan-9-one [(R)-(+)-(3)]

Compound (*R*)-(+)-(**9**) was hydrogenated in the same conditions as for (*S*)-(-)-(**9**), obtaining the title compound in 67% yield. $[\alpha]_{20}^{D}$ = +31.0° (EtOH 96%, c = 1). The [^{1}H]- and [^{13}C]-NMR spectra are identical to those of (*S*)-(-)-(**3**). Anal. $C_7H_{12}N_2O$ (C, H, N).

(S)-(-)-4-(4-Fluorobenzenesulfonyl)-1,4-diazabicyclo[4.3.0] nonan-9-one [(S)-(-)-1)]

To a solution of (*S*)-(-)-(**3**) (0.11 g, 0.8 mmol) in anhydrous CH_3CN (3 mL) Et_3N (0.12 g, 1.2 mmol) and p-fluorobenzenesulfonyl chloride (0.154 g, 0.8 mmol) were added at 0°C. The mixture was left stirring at room temperature overnight, then it was treated with H_2O and extracted with ether. Drying (Na_2SO_4) and removal of the solvent gave a residue, which was purified by column chromatography (CH_2Cl_2/MeOH 95:5 as eluent), to yield the title compound in 68% yield. M.p. 179 °C. $[\alpha]_{20}^{D}$ = -40.2° (CH_2Cl_2, c = 1) [^{1}H]-NMR ($CDCl_3$) δ 1.42-1.68 (m, 1H); 1.83-2.05 (m, 1H,); 2.12-2.54 (m, 4H); 2.85-3.08 (m, 1H); 3.63-3.84 (m, 2H); 3.85-3.97 (m, 1H); 4.01-4.16 (m, 1H); 7.15-7.32 (m, 2H, aromatics); 7.61-7.86 (m, 2H, aromatics) ppm. Anal. $C_{13}H_{15}FN_2O_3S$ (C, H, N).

(R)-(+)-4-(4-Fluorobenzenesulfonyl)-1,4-diazabicyclo[4.3.0] nonan-9-one [(R)-(+)-(1)]

The title compound was prepared as described for (*S*)-(-)-(**1**), starting from (*R*)-(+)-(**3**). Yield: 70%.

$[\alpha]_{20}^{D}$ = +40.8° (CH_2Cl_2, c = 1) The [^{1}H]-NMR spectrum is identical to that of (*S*)-(-)-(**1**). Anal. $C_{13}H_{15}FN_2O_3S$ (C, H, N).

(S)-(-)-4-(Isopropylsulfonyl)-1,4-diazabicyclo[4.3.0]nonan-9-one [(S)-(-)-(2)]

Following the same procedure as for (*S*)-(-)-(**1**), starting from (*S*)-(-)-(**3**) and isopropylsulfonyl chloride, after purification by column chromatography (CH_2Cl_2/abs. EtOH/ petroleum ether/ NH_4OH/diethyl ether 300:180:900:9.9:360 as eluent) the title compound was obtained in 57% yield. M. p. 113 °C. $[\alpha]_{20}^{D}$ = -38.6° (CH_2Cl_2, c = 1) [^{1}H]NMR ($CDCl_3$) δ 1.33 (d, 6H, J = 6.6 Hz, CH_3); 1.52-1.68 (m, 1H); 2.13-2.32 (m, 1H); 2.34-2.48 (m, 2H); 2.60 (dd, 1H, J = 12.5 Hz, 10.7 Hz); 2.77-2.99 (m, 2H); 3.11-3.25 (m, 1H, $CHMe_2$); 3.58-3.73 (m, 1H); 3.77-3.84 (m, 1H); 3.89-3.99 (m, 1H); 4.04-4.11 (m, 1H) ppm. Anal. $C_{10}H_{18}N_2O_3S$ (C, H, N).

(R)-(+)-4-(Isopropylsulfonyl)-1,4-diazabicyclo[4.3.0]nonan-9-one [(R)-(+)-(2)]

The title compound was prepared as described for (*S*)-(-)-(**1**), starting from (*R*)-(+)-(**3**). Yield: 64%.

$[\alpha]_{20}^{D}$ = +38.7° (CH_2Cl_2, c = 1) The [^{1}H]-NMR spectrum is identical to that of (*S*)-(-)-(**2**). Anal. $C_{10}H_{18}N_2O_3S$ (C, H, N).

PHARMACOLOGY

Passive Avoidance Test

The test was performed according to the step-through method described by Jarvik and Kopp [18]. The apparatus consists of a two-compartment acrylic box with a lighted compartment connected to a darkened one by a guillotine door. In the original method, mice received a punishing electrical shock as soon as they entered the dark compartment, while in our modified method, after entry into the dark compartment, mice receive a nonpainful punishment consisting of a fall (from 40 cm) into a cold water bath (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 (first day) and after 24 h in the retention test (second day). Mice that did not enter after 60 s latency were excluded from the experiment. For memory disruption, mice were injected i.p. with the amnesic drugs (scopolamine, diphenhydramine, baclofen and clonidine). All investigated drugs were injected 20 min before the training session, while amnesic drugs were injected immediately after termination of the training session. The maximum entry latency allowed in the retention session was 120 s. The degree of memory of received punishment (fall into cold water) was expressed as the increase in seconds between training and retention latencies.

Social Learning Test

The social learning test was performed according to Mondadori *et al.* [19]. Male Wistar rats (350-450 g) were used throughout the experiments and juvenile males (90-110 g) were used as social stimuli. All the adult animals were housed individually and placed in the testing room at least 24 h before the experiment. On the day preceding the experiment, adult rats were handled to become familiar with the operator. Juvenile rats were housed 4/cage and brought into the testing room the same day of the experiment. Each mature rat was tested in its home cage. The first day of the experiment, a juvenile rat was introduced into the adult male's cage and the time spent in social-investigatory behavior by the adult male within a 5-min fixed interval was recorded. Social investigatory behavior was defined as being proximally oriented to the juvenile or in direct contact while sniffing, following, nosing, grooming or generally inspecting any body surface of the juvenile. After 24 h, either the same juvenile or an unfamiliar one was placed again into the mature male's cage and social investigatory behavior was recorded over a 5-min interval. Compounds were injected i.p. 20 min before the first session of the experiment.

Acetylcholine Release

Microdialysis was performed in rat parietal cortex as described by Giovannini *et al.* [20]. The coordinates used for

the implantation of the horizontal microdialysis tubing (AN 69 membrane, molecular weight cut off < 15 kDa; Dasco, Italy) were AP 0.5 mm and H 2.3 mm from the bregma [21]. One day after surgery the microdialyzing tubing was perfused at a constant flow rate (2 μL min^{-1}) with Ringer's solution (NaCl 147, KCl 4.0, $CaCl_2$ 1.2 mM) containing 7 μM physostigmine sulfate. After a 1h settling period, the perfusate was collected at 15 min intervals in test tubes containing 5 μL of 0.5 mM HCl to prevent ACh hydrolysis. The samples were then assayed for ACh content either immediately or after freezing. ACh was detected and quantified by HPLC with an electrochemical detector as described by Damsma *et al.* [22]. ACh release was expressed as percent change over the mean of the first three basal samples as controls.

CAPILLARY ELECTROPHORESIS

Apparatus

The electrophoretic separations were performed using the ^{3D}HPCE system by Agilent Technologies (Waldbronn, Germany) equipped with a DAD-UV detector. A fused-silica capillary of 50 μm of internal diameter and 38.5 cm length (30 cm effective length) was purchased from BioRad (Hercules, CA, USA). The sample solutions were injected by hydrodynamic system operating at a pressure of 50 mbar for 5 s; the separations were carried out at a constant voltage of 15 kV and the temperature was thermostatted at 15°C. The detection was performed at a wavelength of 200 nm.

Materials and Solutions

Citric acid and Tris buffer were from Carlo Erba Reagenti (Milan, Italy); sodium heparin used as chiral selector was from Opocrin (Corlo di Formigine, Modena, Italy) and was characterized as follows: number average (Mn = 10180), weight average (Mw = 14630), Z- averaging (Mz = 20420), polydispersity (D = 1.438). Water was deionized by a Milli-RX system, Millipore (Milford, MA, USA). The electrophoretic running buffer consisted of a 25 mM citric acid solution adjusted to pH 4.5 with Tris base; sodium heparin was dissolved at the level of 4 % (w/v) without changing in the pH value.

Procedure

The capillary was rinsed at the beginning of each working day for 10 min with the running buffer solution; moreover a procedure of capillary rinse with water (2 min) and running buffer (3 min) was performed between each electrophoretic run.

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