Pergamon

Tetrahedron: *Asymmetry* 13 (2002) 1073–1081

TETRAHEDRON: *ASYMMETRY*

Chemical and biological profile of racemic and optically active dialkylaminoalkylnaphthalenes with analgesic activity

Ornella Azzolina,[a,*] Simona Collina,[a] Gloria Brusotti,[a] Daniela Rossi,[a] Athos Callegari,[b] Laura Linati,[c] Annalisa Barbieri[d] and Victor Ghislandi[a]

[a]*Dipartimento di Chimica Farmaceutica, Università di Pavia, Viale Taramelli 12, 27100, Pavia, Italy*
[b]*Dipartimento di Scienze della Terra, Università di Pavia, Via Ferrata 1, 27100 Pavia, Italy*
[c]*Centro Grandi Strumenti, Università di Pavia, Via Bassi 21, 27100 Pavia, Italy*
[d]*Dipartimento di Farmacologia Sperimentale ed Applicata, Università di Pavia, Viale Taramelli 14, 27100 Pavia, Italy*

Received 24 April 2002; accepted 14 May 2002

Abstract—The racemic mixtures and the enantiomers of dialkylaminoalkylnaphthalenes are described here as novel analgesic agents with potencies similar, or superior to that of morphine. The synthesis and isolation of the pure enantiomers and a study of the absolute configuration are reported. The resolution of racemates was accomplished by preparative liquid chromatography using a Chiralpak AD column. The configurational assignment was performed on the basis of the X-ray crystallographic analysis of (+)-benzyl-(3-hydroxy-3-naphthalen-2-yl-butyl)dimethylammonium bromide and by comparative study of CD and NOESY ^{1}H NMR spectra of the resolved enantiomers. Pharmacological evaluation of the analgesic activity by means of the hot plate test is described.

1. Introduction

Our studies during recent years have focused on pain control and have been aimed at finding new ligands with high affinity for receptors and sub-receptors involved in the regulation of nociceptive stimuli. The availability of novel pharmacological compounds with analgesic effects for the treatment of post-operative, neurogenic and osteoarthritic pain would be very useful in order to avoid the many side effects of morphine and its derivatives.[1–3] We therefore directed our research toward the design, synthesis and pharmacological evaluation of possible analgesic compounds as potential effective and safe drugs. Dialkylaminoalkylnaphthalenes (Fig. 1, series I) and cycloaminoalkylnaphthalenes (Fig. 1, series II) were synthesized by us and their pharmacological activities were investigated by hot plate test (HPT) in mice.[4–8] Several compounds were found to possess antinociceptive activity comparable or superior to that of morphine as shown by their AD_{50} values. In particular, in series II it was shown that the naphthalene nucleus influences the in vivo activity, because substantial differences in the response to the pain stimulus were recorded according to structural modifications at the 6-position of the aromatic ring.

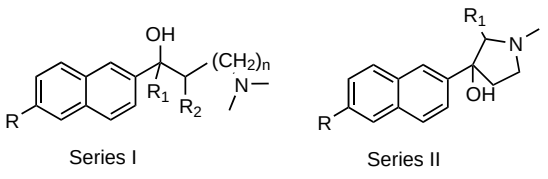
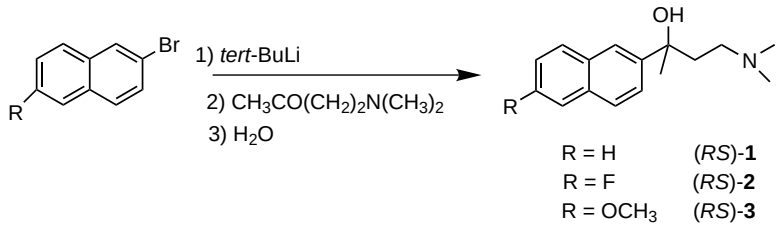


Figure 1. Dialkylaminoalkyl- and cycloaminoalkylnaphthalenes.

Herein, we report a study of the novel tertiary amino alcohols **1**–**3** (Scheme 1) to investigate the role of the substituent in the aromatic portion of the molecule, also in the open chain series I. Due to a hypothetical stereoselectivity of the drug–receptor interaction, we also describe the preparation of optically active **1**–**3**. To the best of our knowledge neither enantioselective synthesis or the resolution of open chain tertiary amino alcohols, with only one stereogenic center, has been reported. Therefore, on the basis of our previous experiences, all of the enantiomerically pure compounds **1**–**3** were obtained by chiral resolution of racemates **1**–**3**, using a direct chromatographic method with a chiral stationary phase (CSP).[9–11] The absolute configurations of the two enantiomers of all compounds were determined by X-ray crystallographic analysis, CD and ^{1}H NMR spectroscopy. Finally, a preliminary profile of

* Corresponding author. Tel.: 00390382507356; fax: 00390382422975; e-mail: ornella.azzolina@unipv.it

0957-4166/02/$ - see front matter
PII: S0957-4166(02)00241-0



Scheme 1.

the biological properties was established by measuring the analgesic activity of the compounds with HPT performed on mice.

2. Results and discussion

The synthesis of racemic **1**–**3** was accomplished following the procedure that we had used for previous compounds.[4] The results of elemental analyses, chromatographic tests, IR and ^{1}H NMR spectra agreed with the assigned structures for all compounds. Differential scanning calorimetry (DSC) showed the absence of crystallization solvent in these compounds.

In order to develop a rapid, direct method for the resolution of racemic **1**–**3** we attempted several liquid chromatography procedures using a number of chiral stationary phases. Baseline separations of all compounds were successfully achieved on analytical scale with Chiralcel OB-H, Chiralpak AD and Chiralpak AS CSPs: good separation factor values and very short analysis times were obtained, as shown by the results reported in Table 1.

So as to obtain sufficient quantities of both enantiomers of **1**–**3** for configurational study and pharmacological investigation, the resolution of the racemates **1**–**3** was performed by semi-preparative HPLC with a Chiralpak AD column (25×2 cm) (Table 2). Chromatographic resolutions were scaled up to the maximum tolerated level by increasing the amounts of samples injected into the column until the maximum amount compatible with the best yield of both enantiomers was reached. Mixtures of *n*-hexane/2-propanol (IPA), containing a small amount of diethylamine (DEA), were used as eluents, at a flow rate of 8 mL/min. The injection of the racemic samples was repeated every 45 min to optimize the efficiency of the chromatographic resolution. In this way about 12 mg of enantiopure **1**–**3** was obtained from each chromatographic run. Enantiomeric excess (e.e.) measurements were completed on analytical scale and all enantiomers were obtained with e.e. of $\geq$99.3% with a recovery from the preparative resolution of about 90% (Fig. 2, Table 3). The first eluting enantiomers are (*R*)-(+)-**1**, (*S*)-(−)-**2** and (*S*)-(−)-**3**, highlighting the influence of the naphthalene substituent in the stability of diastereoisomeric complexes deriving from the solute–CSP interaction.

The absolute configurations of the resolved enantiomers were established on the basis of crystallographic studies as well as CD and ^{1}H NMR studies. The absolute configuration of the dextrorotatory enan-

Table 1. Analytical resolution of racemic mixtures **1**–**3**

Compd	Column (250×4.6 mm)	Mobile phase	Flow rate (mL/min)	rt_1 (min) (config.)	rt_2 (min) (config.)
1	AD	95/5/0.01[a]	0.7	10.6 (*R*)	12.5 (*S*)
2	AS	98.5/1.5/0.1[a]	1.0	7.3 (*R*)	9.4 (*S*)
	AD	97/3/0.01[a]	1.0	12.0 (*S*)	14.4 (*R*)
3	OB-H	98/2/0.1[b]	1.0	11.1 (*S*)	13.1 (*R*)
	AD	95/5/0.01[a]	1.0	8.7 (*S*)	10.9 (*R*)

[a] Solvent mixture: *n*-hexane/IPA/DEA (v/v/v).
[b] Solvent mixture: *n*-hexane/EtOH/DEA (v/v/v). Detector: λ=273 nm.

Table 2. Semi-preparative resolution of racemic mixtures **1**–**3** on a Chiralpak AD column (25×2 cm)

Compd	Mobile phase[a]	Injection volume (mL)	Conc.[b] (mg/mL)	rt_1 (min) (config.)	rt_2 (min) (config.)
1	95/5/0.01	3	11.2	17.6 (*R*)	21.0 (*S*)
2	97/3/0.01	3	12.5	20.0 (*S*)	24.0 (*R*)
3	95/5/0.01	3	13.0	20.5 (*S*)	26.0 (*R*)

[a] *n*-hexane/IPA/DEA (v/v/v); flow rate=8 mL/min.
[b] In the mobile phase. Detector: λ=273 nm.

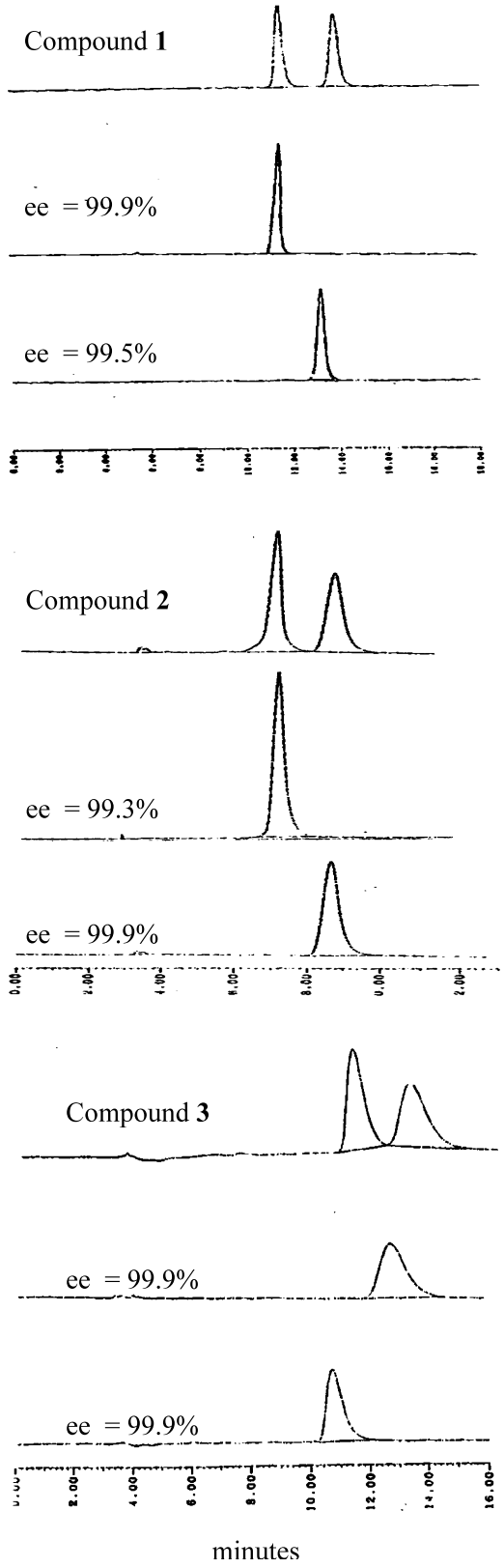


Figure 2. Analytical resolutions of racemic **1**–**3** and control of the fractions collected in the semi-preparative separations. Experimental conditions are reported in Table 1.

tiomer of **1** was determined by crystallographic analysis of its *N*-benzylammonium bromide derivative (+)-**5** (Fig. 3), suitable for structural study by X-ray diffraction spectroscopy because of the presence of the high density bromine atom.[12–14] Crystalline sample of (+)-**5** was obtained by slow crystallization from EtOH. A stereoscopic view, with the atomic numbering scheme of the compound is shown in Fig. 4 and the molecular structure packing, obtained using the ORTEP 3 program, is shown in Fig. 5.[12] In this way compound (+)-**5** was found to have (*R*)-configuration. Therefore, the (*R*)-configuration was also assigned to (+)-**1** because its transformation into (+)-**5** did not involve reaction at the stereogenic center.

Table 3. Chiroptical and physico-chemical data for the resolved enantiomers of compounds **1**–**3**

Compound	$[\alpha]_{405}^{22}$	E.e. %[a]	Column	Mp (°C)
(*R*)-**1**	+14.8[b]	99.9	AD	85–87
(*S*)-**1**	−14.1[b]	99.5	AD	85–87
(*R*)-**2**	+6.4[c]	99.3	AS	89–91
(*S*)-**2**	−6.5[c]	99.9	AS	90–92
(*R*)-**3**	+42.4[b]	99.9	OB-H	108–109
(*S*)-**3**	−42.5[b]	99.9	OB-H	108–109

[a] Table 1 and Fig. 2.
[b] $c=1$, MeOH.
[c] $c=0.5$, MeOH.

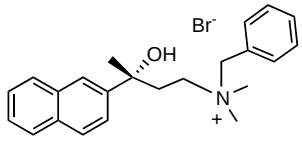


Figure 3. (*R*)-(+)-**5**.

The configurational assignment of **2** and **3** was effected by comparative CD curve analysis of the levorotatory isomers **1**–**3**. The spectra show analogous profiles, with the same Cotton effects in the spectral region between 200 and 250 nm (Fig. 6). Therefore, the (*S*)-absolute configuration of (−)-**1** may also be assigned to (−)-**2** and (−)-**3**. Further confirmation of the configurational assignment was obtained by performing ^{1}H,^{1}H-COSY and ^{1}H-NOESY NMR experiments on the L-tartrate salts of (*S*)-(−)-**2** and (*S*)-(−)-**3**. The NOESY spectra clearly show a significant NOE effect corresponding to the proton interactions between the methyl group linked to the asymmetric carbon atom and the H1 of the aromatic nucleus. This interaction is possible only for the (*S*)-enantiomer, as confirmed by a molecular modeling study, according to Ewig et al.[15] Fig. 7 shows the NOESY spectrum (A) and a molecular view (B) of the L-tartrate salt of (*S*)-(−)-**3**.

To establish a preliminary pharmacological profile, the analgesic activity of the L-tartrate salts of **1**–**3** was investigated by HPT in mice.[16] AD_{50} values were determined using a computer program.[17]

The experimental data (Table 4) clearly show that all new compounds possess very interesting antinociceptive properties with AD_{50} values ranging from 4.97 (4.54–

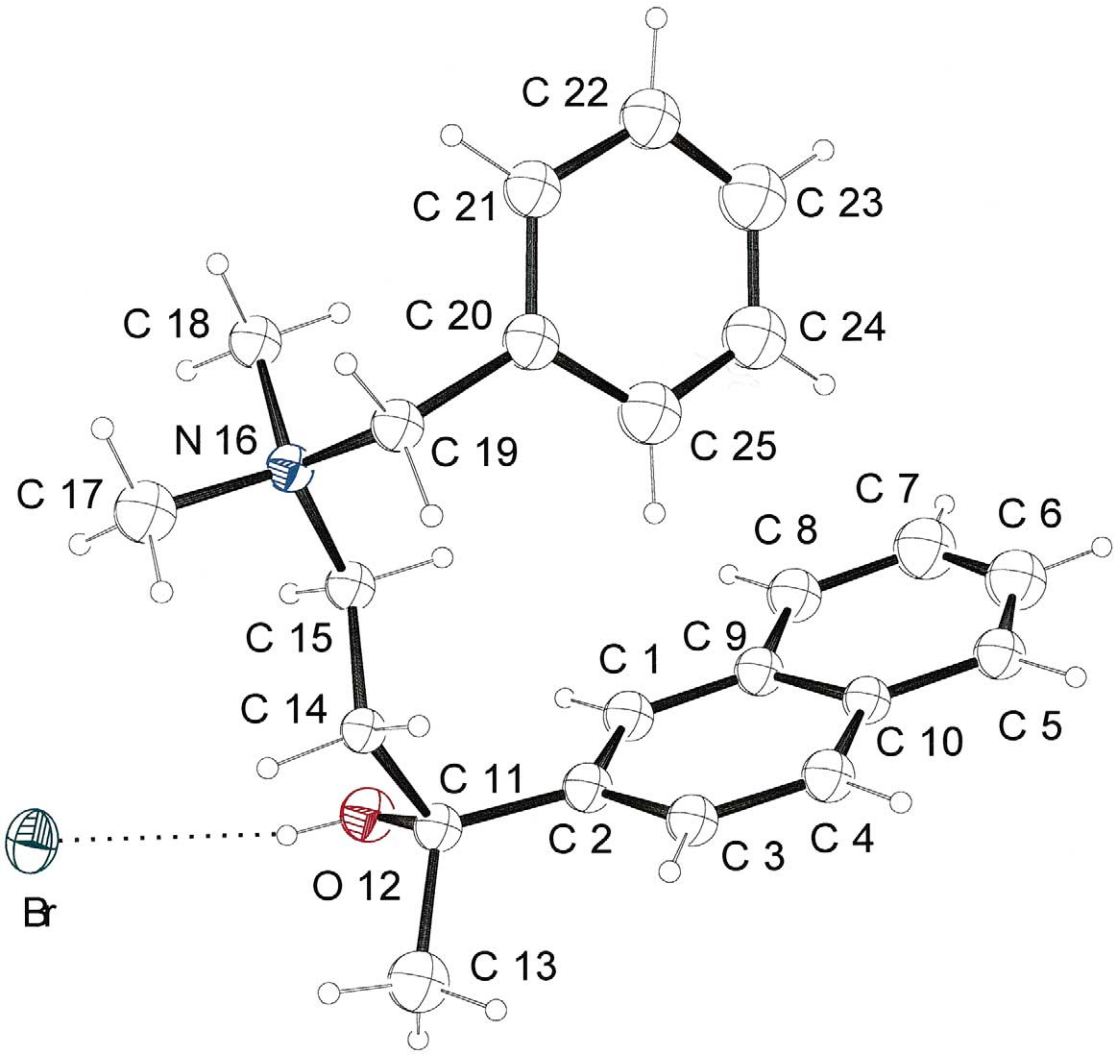


Figure 4. ORTEP III view of molecular structure of (*R*)-(+)-**5**.

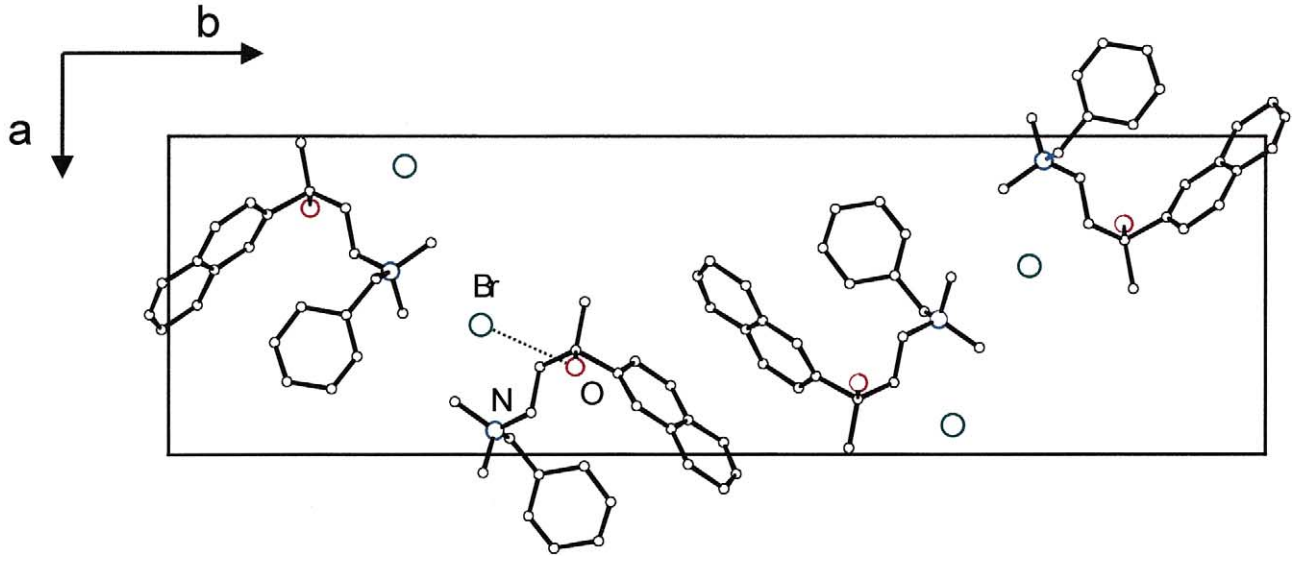
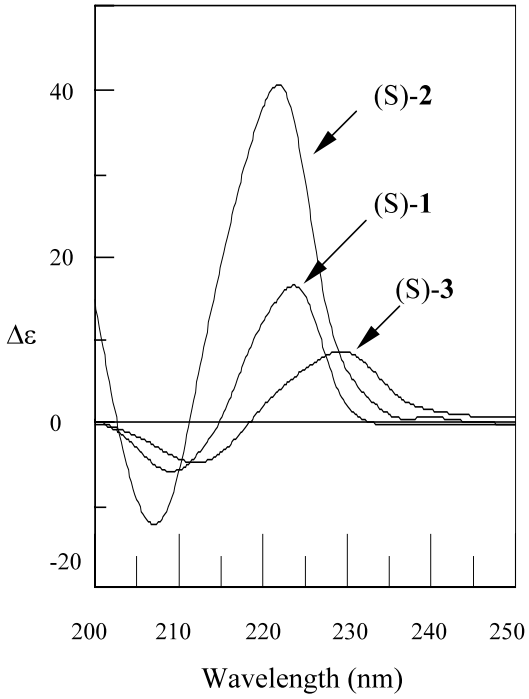


Figure 5. ORTEP view along [*c*] axis of (*R*)-(+)-**5**.



Compound	λ (nm)	Δε
(S)- **1**	209.13	-5.97
	223.56	+17.00
(S)- **2**	206.79	-12.18
	221.76	+40.25
(S)- **3**	212.09	-3.56
	229.48	+9.89

Figure 6. CD spectra and Δε data of compounds (*S*)-(−)-**1**, (*S*)-(−)-**2** and (*S*)-(−)-**3**.

5.44) to 0.22 (0.08–0.58). Their potency is similar or superior to that of morphine [AD_{50} = 4.18 (3.11–5.80)]. The naphthalene nucleus influences the in vivo activity: the pain stimulus was substantially decreased by the presence of a substituent on this hydrophobic portion. Furthermore, the activity of the two enantiomers was very different for compounds **2** and **3**. Thus, the enantioselectivity also seems to be strictly correlated with the presence of a substituent on the aromatic ring. The most active compounds are (*R*)-**2** and (*R*)-**3**. On the contrary, no significant difference was observed in the activity of the two enantiomers of **1**.

Due to their interesting analgesic activity, all compounds investigated in this work and, in particular, the most active compounds (*R*)-**2** and (*R*)-**3** will be further investigated to acquire more information about their pharmacological properties in vivo. Moreover, in vitro tests will also be executed to investigate their possible influence on receptor systems involved in pain control.

3. Conclusions

Chromatographic resolution of the naphthylamino alcohols **1**–**3**, at the 400–500 mg scale, has been performed using a Chiralpak AD column (25×2 cm). In this way both enantiomers of all compounds examined could be obtained with very high enantiomeric excess in relatively short times. The derivatization of (+)-**1** allowed us to obtain a compound suitable for X-ray analysis and, consequently, to assign the (*R*)-configuration to (+)-**1**. CD and NOESY NMR studies permitted us to assign (*R*)-configuration to (+)-**2** and (+)-**3**. Pharmacological investigation highlighted the activity of (*R*)-enantiomers of compounds **2** and **3**.

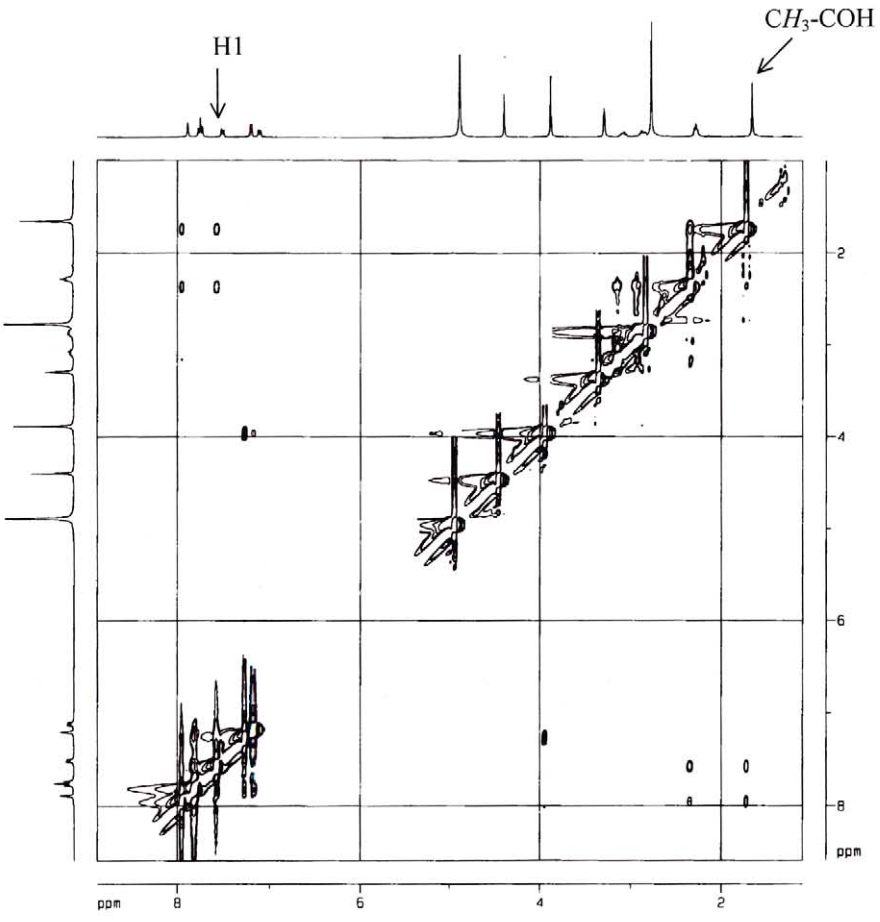
4. Experimental

4.1. General

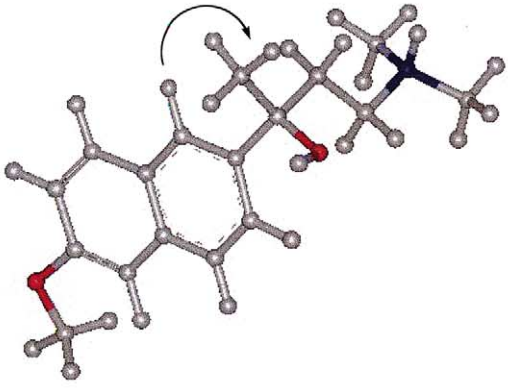
Commercially available reagents and solvents were used as received from the supplier. Diethyl ether was dried and distilled according to standard procedures.[18] Melting points were measured on SMP3 Stuart Scientific apparatus and are uncorrected. Elemental analyses were executed by Carlo Erba 1106 C, H, N analyzer. TLC analyses were carried out on silica gel Kieselgel 60 F_{254} (Merck) and the chromatograms were detected with UV light. UV spectra were measured on Beckman

Table 4. Analgesic activity in the HPT

Compound	AD_{50} (mg/kg)	Conf. limits	Relative potency (versus morphine)
Morphine	4.18	3.11–5.80	1.0
(*RS*)-**1**	2.39	1.05–5.44	1.7
(*R*)-(+)-**1**	2.58	1.91–3.48	1.6
(*S*)-(−)-**1**	1.12	0.42–2.95	3.7
(*RS*)-**2**	0.68	0.22–2.03	6.1
(*R*)-(+)-**2**	0.22	0.08–0.58	19.0
(*S*)-(−)-**2**	4.97	4.54–5.44	0.8
(*RS*)-**3**	0.71	0.15–3.33	5.9
(*R*)-(+)-**3**	0.63	0.31–1.29	6.6
(*S*)-(−)-**3**	3.59	2.29–5.62	1.2



A)



B)

Figure 7. ^{1}H NMR and NOESY spectra (A) and conformational view of (*S*)-(−)-**3**·L-tartrate.

DU-7000. Differential scanning calorimetry (DSC) was carried out using a Mettler TA 4000 apparatus equipped with DSC 20 cell and TC 10 processor. IR spectra were recorded on a Perkin–Elmer FT-IR 1605 spectrophotometer; only noteworthy absorbtions are given in cm^{-1}. X-Ray crystallographic analysis was performed on Philips Pw 1100 computer-controlled four circle diffractometer, graphite-monochromated Mo Kα radiation, ω scan technique (±*h*, ±*k*, ±*l*), scan width 1.5°, scan speed 0.05°/min, range 2–20° theta. NMR spectra were performed at 9.4 T (TMS as internal standard $\delta=0$) with an AVANCE 400 spectrometer (Bruker, Germany) and a BBI 5 mm probe, chemical shifts are given in ppm. Optical rotations were measured on a Jasco DIP-1000 photoelectric polarimeter (0.5 dm cell). Circular dichroism spectra were recorded on a Jasco J-710 dichrograph. Chiral analyses were performed by liquid chromatography on Chiralcel OB-H, Chiralpak AS and Chiralpak AD columns (250×4.6 mm) of Daicel Chemical Industries, Tokyo, Japan. The chiral stationary phases of these columns were cellulose tris-4-methylbenzoate, amylose 1-phenylethylcarbamate and amylose tris-3,5-dimethylphenylcarbamate, respectively, coated on a 5 μm silica-gel substrate. Semi-preparative resolutions of racemic **1**–**3** were obtained by Chiralpak AD column (25×2 cm, 10 μm). The HPLC system consisted of two Gilson pumps, mod. 306, a Reodyne 7125 injector (20 μL or 3 mL sample loop, respectively, for analytical or semi-preparative resolutions) and a Gilson, mod.119, double wavelength UV detector. Experimental data were analyzed with the Gilson 715 HPLC software.

4.2. 4-Dimethylaminobutan-2-one 4

The synthesis of 4-dimethylaminobutan-2-one **4** was performed essentially according to the method reported by Swaminathan for the preparation of 4-diethylaminobutan-2-one.[19] Methylvinylketone (91 mL, 1.23 mol) was added dropwise, over 50 min, to a stirred solution of dimethylamine (1.23 mol, 220 mL) and glacial acetic acid (3 mL) in absolute EtOH (190 mL). Stirring was continued for 2 h and, after this time, the solvent was removed under reduced pressure affording a brown oil. By fractional distillation pure compound was obtained as a colorless oil (90.0 g; yield 64%; $bp_{20\ mmHg}$ 60–62°C). IR (film): 2944, 2861, 2817, 1712, 1460, 1229, 1158, 1041, 867, 812. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): $\delta_H=2.57$ (m, 4H, CH_2CH_2); 2.20 (s, 6H, $N(CH_3)_2$); 2.16 (s, 3H, CH_3CO). Elemental analysis: $C_6H_{13}NO$ requires C, 62.57; H, 11.38; N, 12.16. Found C, 62.61; H, 11.29; N, 12.20%.

4.3. General procedure for the preparation of compounds 1–3 in racemic form

The syntheses of (*RS*)-**1**–**3** were essentially performed as reported in the Scheme 1, according to the procedure that we have already described.[4]

To a solution of the appropriate 2-bromonaphthalene in dry Et_2O, cooled to –40°C, *tert*-BuLi was added with stirring under N_2. After 1 h the mixture was allowed to warm to –10°C then a solution of ketone **4** in dry Et_2O was added. Stirring was continued for 3 h at 0°C and then the reaction mixture was treated with water. The aqueous phase was extracted with Et_2O and the combined organic phases were extracted with 5% DL-tartaric acid aqueous solution.[8] The acid aqueous layer was made alkaline with $NaHCO_3$ to pH 8 and, after extraction with CH_2Cl_2 and evaporation of the solvent, an oily product was obtained.

The crude products were crystallized from the appropriate solvents and transformed into the salts (*RS*)-**1**–**3**·DL-tartrate (molar ratio 1/1). DSC analysis evidenced only an endothermic process corresponding to the melting points of the tartrates; no thermal phenomena attributable to the evaporation of crystallization solvents were observed.

4.3.1. (*RS*)-4-Dimethylamino-2-(naphthalen-2-yl)-butan-2-ol, (*RS*)-1. White solid, yield 5.37 g (62%); mp 64–66°C (acetone 1/H_2O 1), $R_f=0.29$ (*n*-Hex 87/IPA 13/DEA 2); IR (Nujol): 3160 (broad), 3040, 1605, 1280, 1255, 1215, 1125, 1010, 865, 820. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): $\delta_H=7.87$ (s, 1H, aromatic); 7.76 (m, 3H, aromatic, $J=4.8$, 3.2, 5.3); 7.46 (d, 1H, aromatic, $J=8.6$); 7.37 (m, 2H, aromatic, $J=2.8$, 1.7, 4.6); 2.27 (dt, 1H, HC*H*C-OH, $J=8.6$, 4.9, 5.1); 2.10 (s, m, 7H, -N(CH_3)$_2$ and *H*CHC-OH); 2.02 (m, 2H, CH_2-N); 1.53 (s, 3H, -CH_3). Elemental analysis: $C_{16}H_{21}NO$ requires C, 78.97; H, 8.70; N, 5.76. Found C, 78.64; H, 9.03; N, 5.59%.

4.3.2. (*RS*)-1·DL-Tartrate. White solid, mp 130–133°C. Elemental analysis: $C_{16}H_{21}NO\cdot C_4H_6O_6$ requires C, 61.10; H, 6.92; N, 3.56. Found C, 61.24; H, 7.08; N, 3.35%.

4.3.3. (*RS*)-4-Dimethylamino-2-(6-fluoronaphthalen-2-yl)butan-2-ol, (*RS*)-2. White solid, yield 1.4 g (25%), mp 98–100°C (acetone 1/H_2O 1), $R_f=0.34$ (*n*-Hex 87/IPA 13/DEA 2); IR (Nujol): 3125 (broad), 3058, 1610, 1280, 1250, 1195, 1175, 1085, 898, 870. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): $\delta_H=7.95$ (s, 1H, aromatic); 7.87 (q, 1H, aromatic, $J=5.5$, 3.5); 7.78 (d, 1H, aromatic, $J=9.2$); 7.56 (d, 1H, aromatic H, $J=8.5$); 7.46 (dd, 1H, aromatic $J=9.2$); 7.26 (dt, 1H, aromatic $J=9.2$, 2.8); 2.32 (m, 2H, CH_2-N); 2.15 (s, 6H, -N(CH_3)$_2$); 2.05 (m, 2H, CH_2C-OH); 1.57 (s, 3H, -CH_3). Elemental analysis: $C_{16}H_{20}FNO$ requires C, 73.53; H, 7.71; N, 5.36. Found C, 73.64; H, 7.75; N, 5.35%.

4.3.4. (*RS*)-2·DL-Tartrate. White solid, mp 149–151°C. Elemental analysis: $C_{16}H_{20}FNO\cdot C_4H_6O_6$ requires C, 58.39; H, 6.37; N, 3.40. Found C, 58.52; H, 6.59; N, 3.24%.

4.3.5. (*RS*)-4-Dimethylamino-2-(6-methoxynaphthalen-2-yl)butan-2-ol, (*RS*)-3. White solid, yield 4.10 g (69%), mp 98–100°C (acetone 1/H_2O 1), $R_f=0.38$ (*n*-Hex 87/IPA 13/DEA 2); IR (Nujol): 3095 (broad), 1632, 1604, 1260, 1218, 1192, 1125, 1010, 915, 865. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): $\delta_H=7.89$ (s, 1H, aromatic); 7.75 (t, 2H, aromatic, $J=9.2$); 7.50 (d, 1H, aromatic, $J=$

8.5); 7.20 (sb, 1H, aromatic); 7.11 (dd, 1H, aromatic, $J=8.5$); 3.89 (s, 3H, CH_3O); 3.10 (q, 1H, HC*H*C-$N(CH_3)_2$, $J=5.7$); 2.86 (q, 1H, *H*CHC-$N(CH_3)_2$, $J=7.1$); 2.78 (s, 6H, -$N(CH_3)_2$); 2.29 (m, 2H, CH_2-N); 1.66 (s, 3H, -CH_3). Elemental analysis: $C_{17}H_{23}NO_2$ requires C, 74.69; H, 8.48; N, 5.12. Found C, 74.82; H, 8.65; N, 5.05%.

4.3.6. (*RS*)-3·DL-Tartrate. White solid, mp 131–133°C. Elemental analysis: $C_{17}H_{23}NO_2{\cdot}C_4H_6O_6$ requires C, 59.56; H, 6.9; N, 3.31. Found C, 59.72; H, 7.05; N, 3.28%.

4.4. (*R*)-(+)-Benzyl-(3-hydroxy-3-naphthalen-2-ylbutyl)-dimethylammonium bromide, (*R*)-(+)-5

Benzyl bromide (29.2 mg, 0.171 mmol) was added to a solution of (+)-**1** (41.6 mg, 0.171 mmol, $[\alpha]_D^{22}=+14.8$ ($c=1$ MeOH), e.e. 99.9% HPLC) in absolute EtOH (1 mL) and the solution was stirred for 24 h at room temperature. After solvent evaporation and addition of diethyl ether (1 mL), a white solid precipitated, corresponding to (+)-**5** (62 mg, 88%), mp 209–211°C, $[\alpha]_{405}^{22}=+29.9$ ($c=0.6$ MeOH). ^{1}H NMR (400 MHz, $CDCl_3$, TMS): $\delta_H=7.98$ (s, 1H, aromatic); 7.85 (m, 3H, aromatic, $J=5.7$); 7.60 (dd, 1H, aromatic, $J=8.5$); 7.49 (m, 2H, aromatic, $J=4.2$); 7.40 (t, 1H, aromatic, $J=7.1$); 7.27 (m, 4H, aromatic, $J=8.5$); 4.4 (s, 2H, -CH_2-Ph); 3.43 (dt, 2H, -CH_2-$N(CH_3)_2$, $J_{gem}=12$, $J_{vic}=4.9$); 2.92 (d, 6H, -$N(CH_3)_2$, $J=9.9$); 2.45 (dt, 2H, CH_2-C-OH, $J_{gem}=13.4$, $J_{gem}=4.8$); 1.74 (s, 3H, -CH_3). Elemental analysis: $C_{23}H_{28}BrNO$ requires C, 66.67; H, 6.81; N, 3.38. Found C, 66.74; H, 6.73; N, 3.34%.

4.5. Chiral chromatographic separation of racemic compounds 1–3

The chromatographic resolution of (*RS*)-**1**–**3** was accomplished by semi-preparative high-pressure chromatography with a Chiralpak AD column (250×20 mm, 10 μm) at room temperature, with the experimental conditions reported in Table 2. The enantiomers were recovered by evaporating the eluate, which was partitioned according to the profile of the chromatogram. The isolated enantiopure products were characterized by their enantiomeric excesses (e.e.) and specific rotation data (Tables 1 and 3).

4.6. X-Ray crystallographic analysis

Single crystals of (+)-**5** were grown by slow crystallization from EtOH and used for data collection. A colourless needle-shaped crystal, (0.17×0.17×0.43 mm), was used. The crystal structure was solved by direct methods using MULTAN 80 and refined isotropically (except Br atom) on F^2.[13,14] Crystal data: $C_{23}H_{28}NOBr$, orthorhombic, Mr = 414.36, orthorhombic, space group $P2_12_12_1$, $a=10.23$ (3), $b=35.19$ (20), $c=6.02$ (2) Å, $V=2167.16$ Å^3, $Z=4$, $D_x=1.27$ g cm^{-3}, $\mu=1.908$ mm^{-1}, $F(000)=864.00$, $T=293°$ K, reflections collected 8067, unique reflections 2023 ($R_{int}=0.278$), data/parameters = 2023/113, goodness of fit on $F^2=0.925$, $R_1=0.0809$ [($I>2s(I)$] and $wR_2=0.1094$. The absolute structure parameter is 0.02(4). Cell parameters were determined from a least-squares refinement of the setting angles of 60 reflections. The H atoms were calculated and inserted with an isotropic displacement factor proportional to those of their neighboring atoms and not refined. Crystallographic data for (+)-**5** reported in this paper have been deposited with Cambridge Crystallographic Centre (CCDC 182723).

4.7. Circular dichroism analysis

Solutions of the *levo*-isomers **1**–**3** in *n*-hexane ($c=2.5\times10^{-5}$ M; optical path 1 cm) were analyzed in the spectral region between 200 and 250 nm. CD spectra are reported in Fig. 6.

4.8. ^{1}H NMR spectroscopy

^{1}H,^{1}H-COSY and ^{1}H-NOESY NMR spectra of (*S*)-(−)-**2** and **3**·L-tartrate salts were performed at 9.4 T, in CD_3OD at room temperature (TMS as internal standard $\delta=0$).

4.9. Molecular modelling study

A systematic conformational search of compound (*S*)-(−)-**3**·L-tartrate was performed using the MMFF94 force field as implemented in the MOE 2001.01 software.[15] Only those conformations with energies within 10 kcal/mol of the lowest energy structure were considered. One of the low-energy conformations obtained is represented in Fig. 7B.

4.10. Pharmacology

4.10.1. Antinociception activity. Male adult Swiss mice weighing 30±5 g were used. Antinociception was estimated by means of the hot plate test (HPT).[16] Compounds were dissolved in saline solution and administered within 1 h of dissolution. The response to the thermal stimulus was evaluated using a copper plate heated to 55°C. The time at which the mouse displayed a nociceptive response, by sitting on its hind legs and licking, was measured in seconds. Once the basal animal reaction time was determined, to establish the dose–response curve, groups of 10 mice were treated (via s.c. injection) with increasing doses of the compounds. Control animals received the same volume of saline solution. The reaction time to the pain stimulus was measured 20 min following injection. The reaction time of the control animals (cut off time) was 23±2 s.

AD_{50} values and their 95% confidence intervals were determined using a computer program.[17]

Experimental data are reported in Table 4.

Acknowledgements

This work was supported by Grant from MIUR (Ministero dell'Istruzione, dell'Università e della Ricerca, Italy).

References

1. Williams, M.; Kowaluk, E. A.; Arneric, P. *J. Med. Chem.* **1999**, *42*, 1481.
2. Millan, M. J. *Trends Pharmacol. Sci.* **1990**, *11*, 70.
3. Rees, D. C. In *Progress in Medicinal Chemistry*; Ellis, G. P.; Luscombe, D. K., Eds.; Elsevier Science: Amsterdam, 1992; Vol. 29, pp. 109–139.
4. Ghislandi, V.; Azzolina, O.; Collina, S.; Paroli, E.; Antonilli, L.; Giuseppetti, G.; Tadini, C. *Chirality* **1994**, *6*, 389.
5. Ghislandi, V.; Collina, S.; Azzolina, O.; Barbieri, A.; Lanza, E.; Tadini, C. *Chirality* **1999**, *11*, 21.
6. Collina, S.; Azzolina, O.; Vercesi, D.; Benevelli, F.; Callegari, A.; Tadini, C.; Lanza, E.; Barbieri, A.; Ghislandi, V. *Bioorg. Med. Chem.* **2000**, *8*, 769.
7. Collina, S.; Azzolina, O.; Vercesi, D.; Sbacchi, M.; Scheideler, M. A.; Barbieri, A.; Lanza, E.; Ghislandi, V. *Bioorg. Med. Chem.* **2000**, *8*, 1925.
8. Collina, S.; Azzolina, O.; Vercesi, D.; Barbieri, A.; Lanza, E.; Linati, L.; Ghislandi, V. *Farmaco* **2000**, *55*, 611.
9. Azzolina, O.; Collina, S. *J. Liq. Chromatogr.* **1995**, *18*, 81.
10. Azzolina, O.; Dal Piaz, V.; Collina, S.; Giovannoni, M. P.; Tadini, C. *Chirality* **1997**, *9*, 681.
11. Azzolina, O.; Collina, S.; Linati, L.; Anzini, M.; Cappelli, A.; Scheideler, M. A.; Sbacchi, M. *Chirality* **2001**, *13*, 606.
12. Farrugia, L. J. J. ORTEP3 for Windows, *Appl. Crystallogr.* **1997**, *30*, 565.
13. Main, P.; Fiske, S. J.; Hull, S. E.; Lessinger, L.; Germain, G.; Declercq, J. P.; Woolfson, M. M. *MULTAN 80*. University of York, York, UK, and University of Louvain, Louvain, Belgium, 1980.
14. Sheldrick, G. M. SHELX-97. *Programs for Crystal Structure Analysis (Release 97-2)*. Institüt für Anorganische Chemie der Universität, Tammanstrasse 4, D-3400 Göttingen, Germany, 1998.
15. Ewig, C. S.; Berry, R.; Dinur, U.; Hill, J. R.; Hwang, M. J.; Li, H.; Maple, J.; Peng, Z.; Stockfish, T. P.; Thacher, T. S.; Yan, L.; Ni, X.; Hagler, A. T. *J. Comput. Chem.* **2001**, *22*, 1782.
16. Woolfe, G.; MacDonald, A. D. *J. Pharmacol. Exp. Ther.* **1944**, *80*, 300.
17. Tallarida, R. J.; Murray, R. B. *Manual of Pharmacological Calculation with Computer Programs*; Springer Verlag: Berlin, 1987; pp. 26–31.
18. Perrin, D. D.; Armarego, W. L. F.; Perrin, D. R. *Purification of Laboratory Chemicals*; Pergamon Press: Oxford, 1980.
19. Swaminathan, S.; Newman, M. *Tetrahedron* **1958**, *2*, 88.