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Synthesis of pyrrolinylnaphthalenes and evaluation of their antinociceptive activity

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Abstract

In this paper the regioselective preparation of (*R*/*S*)-1,2-dimethyl-3-[2-(6-substituted naphthyl)]-2*H*,5*H*-pyrrolines **2a**–**d** is reported. These compounds were prepared by thermal dehydration of the corresponding alcohols (2*R*,3*S*/2*S*,3*R*)-1,2-dimethyl-3-[2-(6-substituted naphthyl)]-3-hydroxy-pyrrolidines (2*R*,3*S*/2*S*,3*R*)-**1a**–**d** with anhydrous $FeCl_3$–SiO_2, under vacuum. Pharmacological properties of (*R*/*S*)-**2a**–**d** are also described. Analgesic activity was investigated by the hot plate test, also in the presence of selective antagonists of μ, δ and κ opioid receptors. Preliminary analysis of the side-effects was also accomplished using the rota-rod test. Interesting antinociceptive activity was shown by all compounds and in particular by (*R*/*S*)-**2a** ($AD_{50} = 0.31$ mg/kg); δ opioid receptors were found to be mainly involved in the pharmacological process and, in general, it was found that the compounds influenced locomotory activity to a much lesser extent than did morphine.

Keywords: Pyrrolinylnaphthalenes; Regioselective synthesis; Antinociceptive activity; Opioid receptor antagonists

1. Introduction

In recent years our research has focused on the design, synthesis and pharmacological evaluation of new molecules with analgesic antinociceptive activity as potential safe and effective drugs. Our aim has also been to establish their mode of action in order to acquire knowledge concerning their structure–activity relationships.

In fact, we have already examined the non-peptide antinociceptive agents pyrrolidinylnaphthalenes (2*R*,3*S*/2*S*,3*R*)-**1a**–**d** [1,2] (Fig. 1), which possess strong analgesic properties with potency relative to morphine ranging from 1.0 to 8.3 (according to the results of the hot plate test (HPT) performed on mice, and expressed as AD_{50} mg/kg). These compounds were selected to obtain information about the influence of the substituent in the naphthalene nucleus on the pharmacological properties of structural analogues of the heterosteroid 17-methyl-17-azaequilenine, that was previously investigated in opioid analgesia studies [3,4]. Experimental results indicated that the analgesic activity has to be related to the structural features of this moiety since substantial differences were recorded in the response to the painful stimulus, according to the following sequence: $OH < F < OCH_3 < H$ [2].

Furthermore, the antinociceptive properties were been evaluated by HPT, also in the presence of selective opioid antagonists. The activity of all compounds was proved to be influenced by naloxone, naltrindole and nor-binaltorphimine, which are specific antagonists for μ, δ and κ receptors respectively [5–8]. In particular, the activation of mainly δ receptors seems to be involved in the analgesic properties, even if the micromolar order of K_i values of the in vitro binding assays show low affinity of the compounds for all these opioid receptors.

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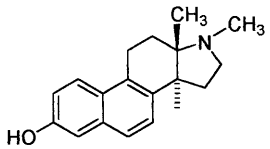
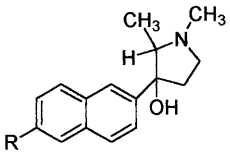


17-methyl-17-azaequilenine



		AD_{50} (mg/kg)
1a	R= F	4.12
1b	OH	5.41
1c	OCH_3	2.11
1d	H	0.65

Fig. 1. Antinociceptive agents pyrrolidinylnaphthalenes (2*R*,3*S*/2*S*,3*R*)-**1a**–**d**.

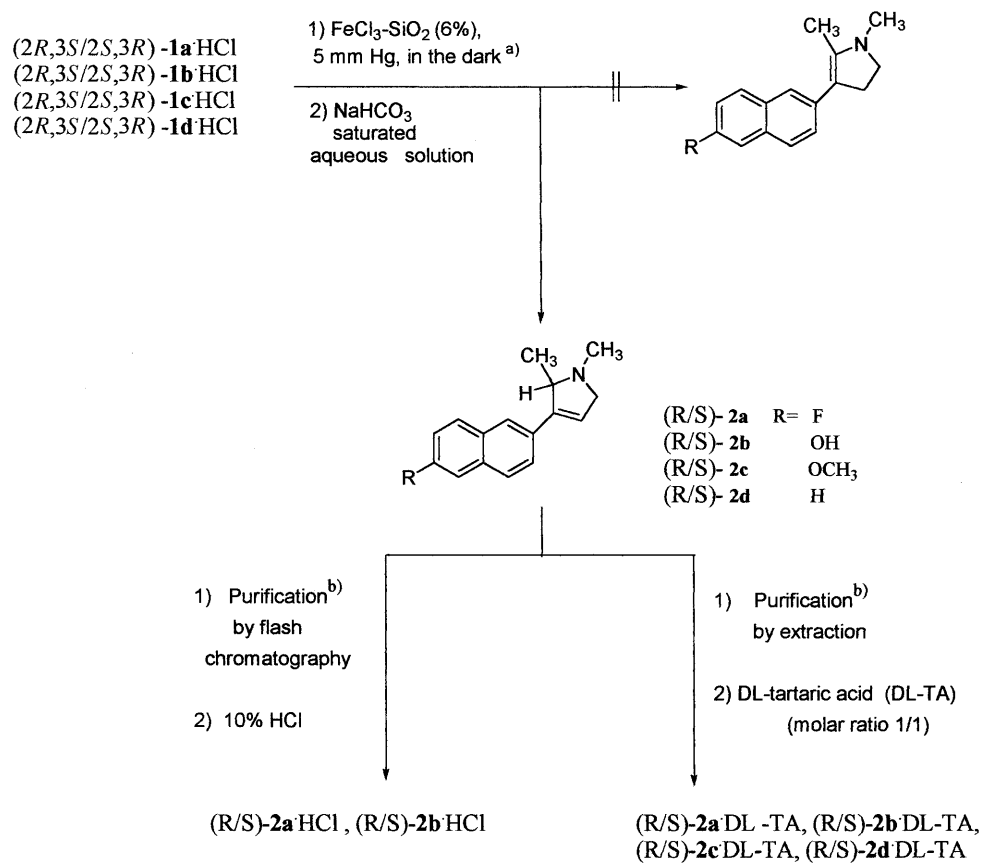
At present we can hypothesize that compounds (2*R*,3*S*/2*S*,3*R*)-**1a**–**d** produce antinociceptive effects by an indirect activation of the opioid system. This hypothesis could justify the results of HPT in the presence of selective opioid antagonists if an inhibition of specific enzymes, responsible for degradation of endogenous opioid peptides, is invoked.

Additional studies to assess the mechanism of action of these compounds are still in progress.

Possible effects on motor coordination were also investigated by rota-rod test (RRT) and it was shown that the influence of the compounds (2*R*,3*S*/2*S*,3*R*)-**1a**–**d** on the locomotory activity was less than that of morphine. Like the analgesic activity also the effects on motor coordination seem to be related to the chemical properties of the naphthalene moiety.

Here we report our study on the structure–activity relationships of (*R*/*S*)-1,2-dimethyl-3-[2-(6-substituted naphthyl)]-2*H*,5*H*-pyrrolines (*R*/*S*)-**2a**–**d** (Scheme 1), which were conceived to investigate the influence of the structural features of the heterocyclic moiety on their pharmacological properties. Thus (*R*/*S*)-**2a**–**d** were synthesized by dehydration of the alcohols (2*R*,3*S*/2*S*,3*R*)-**1a**–**d** to verify the analgesic properties of the compounds without the alcohol group and with a double bond in C3–C4. To obtain exclusively the 3-pyrrolinyl-isomers, which keep the stereogenic center in position 2 of the corresponding precursors, a regioselective dehydration method was used [9,10].

The synthesis and chemical characterization by NMR and MS spectroscopy of (*R*/*S*)-**2a**–**d** are described. Pharmacological properties in vivo were performed by HPT and RRT and compared to those of (2*R*,3*S*/2*S*,3*R*)-**1a**–**d**. The influence of the opioid receptor antagonists on the analgesic activity of the compounds was also investigated.



Scheme 1. (a) Experimental conditions are reported in Table 1. (b) See Section 3.

2. Chemistry

The synthetic route for obtaining the 3-pyrrolinylnaphthalenes is summarized in Scheme 1. Structures and experimental conditions are reported in Table 1.

Compounds (*R*/*S*)-**2a**–**d** were obtained by dehydration of the heterocyclic moiety of the corresponding 3-pyrrolidinols (2*R*,3*S*/2*S*,3*R*)-**1a**–**d**·HCl, using anhydrous ferric chloride adsorbed on silica gel ($FeCl_3$–SiO_2), an effective reagent for the dehydration of tertiary alcohols [9,10]. The reaction was performed by dissolving the substrate in methanol, mixing it either 10 or 30 times its weight (Table 1) of reagent and evaporating to dryness under vacuum (5 mmHg) in the dark. The compounds were recovered by extraction with methanol and treatment with a saturated aqueous solution of sodium bicarbonate.

Our results show that this reaction is regioselective. In fact only the 3-pyrrolinyl derivatives (*R*,*S*)-**2a**–**d**, were obtained, as evidenced by ^{1}H NMR spectroscopy: an accurate research of the signals due to the 2-pyrrolinyl derivatives was unsuccessful.

To date there is no evidence in the literature concerning the preparation of 2*H*,5*H*-pyrrolines by dehydration of 3-pyrrolidinols. The regioselectivity of this reaction has only been shown in the case of some cyclopentanols where a $-CH_2$, instead of a $-CH$ group, near to the OH function, was involved in the dehydration process [9]. Crude products (*R*,*S*)-**2a**–**b** were purified by flash chromatography (Table 1) and converted into their respective hydrochlorides. This drastic procedure was a critical step: the high amount of diethylamine in the mobile phase (necessary to prevent strong interactions of the aminic group of compounds with silica gel) as well as the use of 37% HCl were probably responsible for the low yields of (*R*,*S*)-**2a**–**b** (18.1% and 4.0%, respectively). To achieve mild conditions, the products were purified with an extractive route and DL-tartaric acid (DL-TA) was employed to obtain the corresponding salts. Crude products were taken up in methylene chloride and extracted three times with 5% DL-TA, sodium bicarbonate saturated aqueous solution and ethyl ether, successively. Salification with DL-TA (molar ratio 1/1) and crystallization (Table 1) gave pure (*R*/*S*)-**2a**–**b**·DL-TA. With this procedure an increase of 7% of the yield was achieved. On the basis of this result also compounds (*R*/*S*)-**2c**–**d** were purified by extraction and isolated as tartrates.

The chemical structure of the compounds was elucidated by elemental analysis, IR, MS, ^{1}H NMR spectroscopy and by differential scanning calorimetry.

The MS spectrum of (*R*/*S*)-**2b**·DL-TA presents a signal at $m/z = 477.3$, which indicates the presence of the corresponding binaphthol as by product, owing to the oxidative coupling of phenols [11]. That was also shown by ^{1}H NMR spectroscopy (Fig. 2A). Signals of the aromatic protons are well identified and confirmed by bidimensional H–H COSY experiment, which also permits the absolute identification of the signal of H4′ protons of the dimeric compound (Fig. 2B). On the basis of the ratio of the intensity of the signals relative to H4 in monomeric (7.56 ppm) and H4′ in dimeric (8.03 ppm) compounds the amount of the latter compound is less than 5%.

3. Experimental

3.1. Chemistry

Melting points were determined with a SMP3 apparatus (Stuard Scientific) and are uncorrected. Elemental analyses were carried out with a Perkin–Elmer 240 C, H, N analyser and were within $\pm 0.4\%$ of the theoretical values. IR spectra were obtained on a Perkin–Elmer 682 spectrophotometer and ^{1}H NMR and ^{13}C NMR spectra [TMS as internal standard ($\delta = 0.00$)] were obtained using a Bruker AMX 400 (1H 400

Table 1
Reaction and purification conditions of the prepared compounds

Comp.	Reaction conditions			Purification [a]	Crystallization solvent	Yield (%)
	Time (h)	Temperature (°C)	Ratio substrate/reactant (w/w)			
(*R*/*S*)-**2a**·HCl	8	65	1/30	flash chromatography	IPA [b] 1/petroleum ether 1	18.1
(*R*/*S*)-**2a**·DL-tartrate				extraction	IPA [b] 10/H_2O 1	25.2
(*R*/*S*)-**2b**·HCl	24	45	1/30	flash chromatography	IPA [b] 1/petroleum ether 1	4.0
(*R*/*S*)-**2b**·DL-tartrate				extraction	methanol 1/acetone 1	11.5 [c]
(*R*/*S*)-**2c**·DL-tartrate	15	55	1/10	extraction	IPA [b] 1/H_2O 1	17.7
(*R*/*S*)-**2d**·DL-tartrate	15	55	1/30	extraction	methanol	35.0

[a] See Section 3.
[b] Isopropyl alcohol.
[c] Impure of dimeric compound (see Section 3).

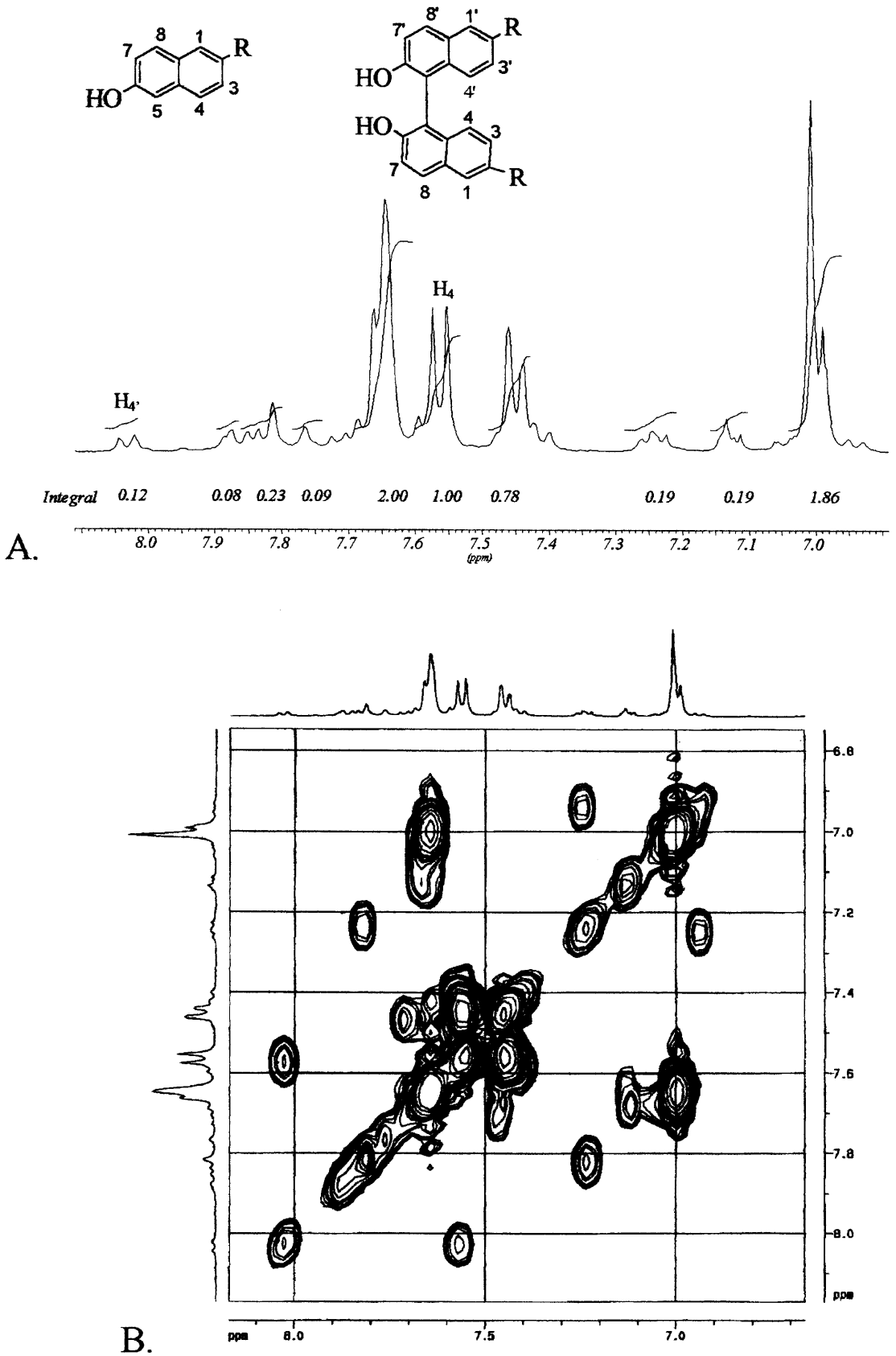


Fig. 2. (A) ^{1}H NMR spectrum of the aromatic region. (B) H–H Cosy spectrum of the aromatic region.

MHz, 13C 100.617 MHz) apparatus. Mass spectra were obtained on a Finningan LCQ apparatus. Differential scanning calorimetry (DSC) was carried out using a Mettler TA 4000 apparatus equipped with DSC 25 cell. Compounds in TLC were detected using UV light. ICN silica gel 60 (70–230 mesh) was used for flash chromatography. Anhydrous sodium sulfate was always used to dry organic solutions. Evaporation of solvents was performed in vacuum with a rotatory evaporator. All reagents and solvents were purchased from commercial suppliers and employed without further purification.

*3.1.1. Procedure for the synthesis of (R/S)-1,2-dimethyl-3-[2-(6-substituted-naphthyl)]-2H,5H-pyrroline hydrochlorides [(R/S)-**2a**–**b**·HCl]*

The synthesis of (*R*/*S*)-**2a**–**b** was essentially effected (Scheme 1) according to the procedure proposed by Keinan and Mazur [9] for the dehydration of some tertiary alcohols. Anhydrous $FeCl_3$–SiO_2 (30 g, 6%) was added to a solution of (2*R*,3*S*/2*S*,3*R*)-**1a**·HCl or -**1b**·HCl (3.1 mmol) in 100 ml of MeOH. After evaporation of the solvent on a rotatory evaporator, the reaction mixture was stirred under vacuum (5 mmHg), in the dark, while the colour turned from yellow to yellowish-brown. The progress of the reaction was monitored by TLC. The reaction conditions and purification are reported in Table 1. The powder was then added with 120 ml MeOH and the silica gel filtered off; the filtrate was made alkaline with a saturated solution of $NaHCO_3$ to pH 8 and the precipitated ferric hydroxide was filtered off. The evaporation of the organic solvent gave a brown oil which was purified by flash chromatography. Three fractions were collected. The first and the third fraction were discarded, the second one was acidified with 37% HCl to pH 2 and evaporated to furnish a brown oil which was directly crystallized from a proper solvent.

DSC evidenced only an endothermic process corresponding to the melting point of the substances; no thermal phenomena attributable to the evaporation of the crystallization solvent were present.

*3.1.1.1. (R/S)-**2a**·HCl.* 18.1% yield; m.p. 223–224°C (IPA 1/petroleum ether 1). Flash chromatography: column $h = 180$ mm, diameter 35 mm; mobile phase *n*-hexane 75/acetone 25/diethylamine 2. TLC analysis [stationary phase Merck silica gel 60 F_{254}; mobile phase *n*-hexane 87/IPA 13/methanol 3/diethylamine 2]: R_f 0.49. IR (Nujol) main absorptions (cm^{-1}): 3320, 3260, 2680, 1615, 1510, 1485, 1255, 1080, 960, 805. ^{1}H NMR (in CD_3OD) δ: 1.50 (d, 3H, C*H*$_3$CH, $J = 6.5$); 3.00 (s, 3H, C*H*$_3$N); 4.10 (d, 1H, *H*CHN, $J_{gem} = 15.20$); 4.42 (d, 1H, HC*H*N, $J_{gem} = 15.20$); 4.93 (q, 1H, *H*CCH$_3$); 6.3 (s, 1H, HC=C); 7.27 (dd, 1H, aromatic); 7.46 (dd, 1H, aromatic); 7.62 (d, 1H, aromatic); 7.80 (m, 3 H, aromatic). MS: *m*/*z* 242.3 [*M* + 1]. *Anal.* Calc. $C_{16}H_{16}FN$·HCl (C, H, N).

*3.1.1.2. (R/S)-**2b**·HCl.* 4.0% yield; m.p. 240–242°C (ethanol 1/petroleum ether 1). Flash chromatography: column $h = 170$ mm, diameter 25 mm; mobile phase *n*-hexane 55/acetone 35/methanol 10/diethylamine 4. TLC analysis [stationary phase Merck silica gel 60 F_{254}; mobile phase *n*-hexane 87/IPA 13/methanol 3/diethylamine 2]: R_f 0.28. IR (Nujol) main absorptions (cm^{-1}): 3395, 3080, 2605, 1630, 1570, 1510, 1290, 1150, 875, 805, 725. ^{1}H NMR (in CD_3OD) δ: 1.48 (d, 3H, C*H*$_3$CH, $J = 6.60$); 3.00 (s, 3H, CH$_3$N); 4.10 (d, 1H, *H*CHN, $J_{gem} = 14.00$); 4.40 (d, 1H, HC*H*N, $J_{gem} = 14.00$); 4.90 (q, 1H, *H*CCH$_3$); 6.16 (s, 1H, HC=C); 7.00 (m, 2H, aromatic); 7.46 (dd, 1H, aromatic); 7.58 (d, 1H, aromatic); 7.66 (m, 2 H, aromatic). MS *m*/*z* 240.2 [*M* + 1]. *Anal.* Calc. $C_{16}H_{17}NO$·HCl (C, H, N).

*3.1.2. Procedure for the synthesis of (R/S)-1,2-dimethyl-3-[2-(6-substituted-naphthyl)]-2H,5H-pyrroline DL-tartrates [(R/S)-**2a**–**d**·DL-tartrates]*

The reaction proceeds under the conditions already described for the corresponding hydrochlorides until the filtration of the ferric hydroxide and the evaporation of the solvent. A mixture of CH_2Cl_2/water 1/1 was added to the recovered brown oil. The aqueous phase was extracted with methylene chloride and the organic layer was extracted with 5% DL-tartaric acid aqueous solution. The acid aqueous layer was made alkaline with $NaHCO_3$ to pH 8 and, after extraction with ethyl ether and evaporation of the solvent, an oily product was obtained. By reaction with DL-tartaric acid (molar ratio 1/1) the crude (*R*/*S*)-**2a**–**d**·DL-tartrates were obtained and directly crystallized from proper solvents (Table 1).

DSC evidenced only an endothermic process corresponding to the melting point of the substances; no thermal phenomena attributable to the evaporation of the crystallization solvent were present.

*3.1.2.1. (R/S)-**2a**·DL-tartrate.* 25.2% yield; m.p. 162–165°C (IPA 10/H_2O 1). TLC analysis [stationary phase Merck silica gel 60 F_{254}; mobile phase *n*-hexane 87/IPA 13/methanol 3/diethylamine 2]: R_f 0.49. ^{1}H NMR (in CD_3OD) δ: 1.44 (d, 3H, C*H*$_3$CH, $J = 6.50$); 2.90 (s, 3H, CH$_3$N); 3.95 (d, 1H, *H*CHN, $J_{gem} = 15.0$); 4.32 (d, 1H, HC*H*N, $J_{gem} = 15.0$); 4.95 (q, 1H, C*H*CH$_3$); 6.23 (s, 1H, HC=C); 7.26 (m, 1H, aromatic); 7.45 (dd, 1H, aromatic); 7.60 (d, 1 H, aromatic); 7.82 (m, 3 H, aromatic). MS *m*/*z* 242.3 [*M* + 1]. *Anal.* Calc. $C_{16}H_{16}NF$·$C_4H_6O_6$ (C, H, N).

*3.1.2.2. (R/S)-**2b**·DL-tartrate.* 11.5% yield; m.p. 204–206°C (IPA 95/H_2O 5); TLC analysis [stationary phase Merck silica gel 60 F_{254}; mobile phase *n*-hexane 87/IPA

Table 2
Analgesic activity in the hot plate test

Comp.	AD_{50} (mg/kg)	Confidence limits
Morphine·HCl·$3H_2O$	4.18	3.11–5.60
(2*R*,3*S*/2*S*,3*R*)-**1a**·HCl	3.14	1.26–7.78
(2*R*,3*S*/2*S*,3*R*)-**1b**·HCl	4.63	4.36–4.93
(2*R*,3*S*/2*S*,3*R*)-**1c**·HCl	2.66	1.39–5.09
(2*R*,3*S*/2*S*,3*R*)-**1d**·HCl	0.19	0.05–0.69
(*R*/*S*)-**2a**·DL-TA	0.31	0.13–0.74
(*R*/*S*)-**2b**·HCl	2.00	0.76–5.30
(*R*/*S*)-**2c**·DL-TA	1.62	0.89–2.93
(*R*/*S*)-**2d**·DL-TA	0.71	0.22–2.27

13/methanol 3/diethylamine 2]: R_f 0.17. ^{1}H NMR (in CD_3OD) δ: 1.60 (d, 3H, C*H*$_3$CH, $J = 6.80$); 3.10 (s, 3H, CH_3N); 4.20 (d, 1H, *H*CHN, $J = 13.8$); 4.50 (d, 1H, HC*H*N, $J = 13.80$); 5.00 (q, 1H, CH_3CH); 6.28 (s, 1H, HC=C); 7.05 (d + s, 2H, aromatic, H7 and H5); 7.44 (d, 1H, aromatic, H3); 7.56 (d, 1 H, aromatic, H4); 7.65 (d + s, 2 H, aromatic, H8 and H1, $J = 4.50$); 8.03 (d, 2H, aromatic, H4′ of the dimeric compound of Fig. 2A). MS *m*/*z* 240.2 [*M* + 1]; *m*/*z* 477.3 [*M* + 1] (dimeric compound). *Anal.* Calc. $C_{16}H_{17}NO{\cdot}C_4H_6O_6$ (C, H, N).

*3.1.2.3. (R/S)-**2c**·DL-tartrate*. 17.7% yield; m.p. 172–174°C (IPA 1/H_2O 1). TLC analysis [stationary phase Merck silica gel 60 F_{254}; mobile phase *n*-hexane 87/IPA 13/methanol 3/diethylamine 2]: R_f 0.47. ^{1}H NMR (in CD_3OD) δ: 1.50 (d, 3H, C*H*$_3$CH, $J = 6.50$); 2.98 (s, 3H, CH_3N); 3.83 (s, 3H, CH_3O); 4.05 (d, 1H, *H*CHN, $J_{gem} = 14.30$); 4.43 (d, 1H, HC*H*N, $J_{gem} = 14.30$); 4.90 (q, 1H, CH_3CH); 6.19 (s, 1H, HC=C); 7.08 (dd, 1H, aromatic); 7.20 (d, 1H, aromatic); 7.53 (dd, 1H, aromatic); 7.75 (m, 3H, aromatic). MS *m*/*z* 254.2 [*M* + 1]. *Anal.* Calc. $C_{17}H_{19}NO{\cdot}C_4H_6O_6$ (C, H, N).

*3.1.2.4. (R/S)-**2d**·DL-tartrate*. 35.0% yield; m.p. 150–154°C (methanol). TLC analysis [stationary phase Merck silica gel 60 F_{254}; mobile phase *n*-hexane 87/IPA 13/methanol 3/diethylamine 2]: R_f 0.53. ^{1}H NMR (in CD_3OD) δ: 1.58 (d, 3H, C*H*$_3$CH, $J = 6.60$); 3.00 (s, 3 H, CH_3N); 4.14 (d, 1H, *H*CHN, $J_{gem} = 15.00$); 4.52 (d, 1H, HC*H*N, $J_{gem} = 15.00$); 5.01 (q, 1H, CH_3CH); 6.34 (s, 1H, HC=C); 7.46 (m, 2H, aromatic); 7.61 (dd, 1H, aromatic); 7.83 (m, 4 H, aromatic). MS *m*/*z* 224.3 [*M* + 1]. *Anal.* Calc. $C_{16}H_{17}N{\cdot}C_4H_6O_6$ (C, H, N).

3.2. Pharmacology

Pharmacological studies in vitro were performed according to the previously described procedure [12]. Opioid receptor binding was measured using cloned human μ, δ and κ receptors.

Data obtained from competition experiments were analysed using non-linear fitting analysis according to Benfenati and Guardabasso [13] and using the RS/1 software. K_i values were determined from IC_{50} using the Cheng and Prusoff equation and were > 1000 nM for all compounds.

Pharmacological studies in vivo were performed on male adult Swiss mice weighting 30 ± 5 g. To assess the antinociceptive effects the hot plate test (HPT) was utilized. The DL-TA salts were used for (*R*/*S*)-**2a**, -**2c** and -**2d** whereas the compound -**2b** was employed as hydrochloride, because the corresponding DL-TA salt was impure of the binaphthol. All compounds were dissolved in saline solution and administered subcutaneously within 1 h from dissolution.

3.2.1. Hot plate test (HPT)

The response to a thermal stimulus was evaluated using a copper plate heated to 55°C and proceded according to a previously reported method [12]. The response of the mouse included the sitting on its hind legs and licking [14].

The experiment was conducted on groups of 10 mice which were treated with increasing doses of compound. The reaction time to the pain stimulus was measured 20 min after the injection. The reaction time of the control animals was 23 ± 2 s. AD_{50} were calculated using a computerized program [15]. Experimental data are reported in Table 2.

3.2.2. Antagonist activity test

To assess any involvement of the opioid system (μ, δ and κ receptors), the antinociceptive activity of (*R*/*S*)-**2a**–**d** was determined by HPT also in the presence of the following opioid antagonists: naloxone (NLX) at high dose (10 mg/kg) as a non-selective opioid inhibitor, naloxone at low dose (0.5 mg/kg) as a μ-preferential antagonist [5,6], naltrindole (NTN) 1 mg/kg as a δ selective antagonist [8], and nor-binaltorphimine (nor-BNI) 5 mg/kg as a κ preferential antagonist [7].

NLX, NTN and nor-BNI were administered 30 min, 24 h, 5 min before the drugs, respectively. Experimental data are reported in Table 3.

3.2.3. Rota-rod test

The rota rod test was performed on mice after treatment with the investigated substances in order to verify the influence of pyrrolinylnaphthalene derivatives on the integrity of the motor coordination. The assay was carried out using the method described by Vaught et al. [16], slightly modified by us. The apparatus and the experimental conditions were already described in our previous article [12]. Mice were randomly selected and divided into groups of 10 animals for each experiment. They were examined 15 min after treatment with the substances, tested at AD_{50} of HPT. The integrity of motor coordination was evaluated by counting the number of mice of each group that remained on the rod during the periods of 30 and 120 s.

Table 3
Efficacy of NLX, NTN, nor-BNI as selective antagonists of μ, δ and κ opioid receptors

Comp.	% of inhibition [a]		
	NLX (0.5 mg/kg)	NTN (1 mg/kg)	nor-BNI (5 mg/kg)
(*R*/*S*)-**2a**·DL-tartrate	52.4 (±9.3)	97.6 (±10.0) [b]	22.6 (±3.2)
(*R*/*S*)-**2b**·HCl	40.2 (±4.6)	31.8 (±4.0)	16.2 (±1.5)
(*R*/*S*)-**2c**·DL-tartrate	40.5 (±6.2) [b]	82.9 (±12.3) [b]	15.4 (±2.4)
(*R*/*S*)-**2d**·DL-tartrate	23.7 (±3.4)	75.3 (±9.8) [b]	2.2 (±0.3)

[a] The standard error is reported in parentheses.
[b] $P<0.05$.

Table 4
Locomotor activity in the rota-rod test: percentage of mice which remain on the rod after 30 or 120 s

Comp.	30 s	120 s
Morphine·HCl·3H_2O	65 [a]	50 [a]
(*R*/*S*)-**2a**·DL-tartrate	100	62.5 [a]
(*R*/*S*)-**2b**·HCl	90	70
(*R*/*S*)-**2c**·DL-tartrate	87.5	62.5 [a]
(*R*/*S*)-**2d**·DL-tartrate	100	62.5 [a]

[a] $P<0.05$.

Morphine·HCl was used as a standard antinociceptive agent in order to make a suitable comparison with the tested compounds.

Results are expressed as percentage of mice remaining on the rod during the fixed period of time. Statistical analysis was performed by Student's *t* test for grouped data; *P* values less than 0.05 were considered significant. Experimental results are reported in Table 4.

4. Results and discussion

Compounds (*R*/*S*)-**2a**–**d** and the precursors (2*R*,3*S*/2*S*,3*R*)-**1a**–**d** were tested in the HPT according to the conditions reported in Section 3 and their activities were compared.

The results of this study confirm that the analgesic activity, expressed as AD_{50} (mg/kg), is noticeable when compared to that of morphine. The potency of (*R*/*S*)-**2b**–**d** is similar to that of the precursors (2*R*,3*S*/2*S*,3*R*)-**1b**–**d** whereas (*R*/*S*)-**2a** is ten times more potent than (2*R*,3*S*/2*S*,3*R*)-**1a**. The AD_{50} 0.31 (mg/kg) is to be highly considered; the comparison with the value 3.14 of (2*R*,3*S*/2*S*,3*R*)-**1a** could suggest that the increased lipophilicity of the compound improves the activity, owing to better pharmacokinetic properties.

The inhibitory activity of the opioid antagonists NLX, NTN and nor-BNI was investigated to verify whether the receptor opioid system is involved in the antinociceptive activity, as already shown for the analogues (2*R*,3*S*/2*S*,3*R*)-**1a**–**d**. The experimental results also confirm that the activity of the pyrrolinylderivatives (*R*/*S*)-**2a**–**d** is lowered by the antagonists, in particular by NTN in the case of **2a**, **2c** and **2d**. Therefore δ receptors seem to be prevalently involved in the analgesic process even if the results of the in vitro binding assays exhibit, also for these compounds, very poor affinity for opioid receptors (K_i values >1000 nM). This finding supports the previously hypothesized [2] indirect activation of the opioid system, that could explain the antinociceptive properties of both pyrrolinyl- and pyrrolidinyl-naphthalenes.

The control of the muscle relaxant effects of (*R*/*S*)-**2a**–**d** by RRT was carried out to assess the possible sedative properties of the compounds and distinguish analgesia from drug-induced motor changes.

Monitoring was effected after 30 s and was repeated after 120 s. After 30 s all compounds were found to influence the locomotory activity to a lesser extent than morphine. When motor coordination was tested for a longer period of time (120 s) the sedative effects were again found to be inferior to those of morphine. As shown by the experimental data reported in Table 4, the influence of the compounds on the locomotory activity was negligible, consequently the results of HPT can be mainly attributed to their analgesic properties.

In conclusion, the experimental findings confirm, also for (*R*/*S*)-**2a**–**d**, previous evidence regarding the analgesic properties of the precursors as well as their very low side effects on the locomotory activity. This knowledge prompted us to hypothesize that the alcohol group of the heterocyclic portion of compounds (2*R*,3*S*/2*S*,3*R*)-**1a**–**d** was not relevant to their activity or, on the contrary, it could be restored in vivo.

To better understand the influence of the chemical features of this portion of the molecule on the pharmacological properties, it would be interesting to investigate the biological activities of both the isomers having unsaturation in the position 2 and their corresponding saturated compounds.

To date no clear correlation can be made between the structural features of these compounds and their intrinsic activity. Anyway, owing to their pharmacological profile, the 3-pyrrolinylnaphthalenes investigated in this work are very interesting compounds and will be considered, together with the precursors (2*R*,3*S*/2*S*,3*R*)-**1a**–**d**, for further investigation, in order to elucidate the mode of action of these novel analgesic agents.

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