Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 15 (2004) 3601–3608

Microwave assisted synthesis of chiral pyrrolines with biological activity

Simona Collina,[a,*] Guya Loddo,[a] Annalisa Barbieri,[b] Laura Linati,[c] Stefano Alcaro,[d] Paola Chimenti[e] and Ornella Azzolina[a]

[a]*Dipartimento di Chimica Farmaceutica, Università di Pavia, Via Taramelli, 12, 27100 Pavia, Italy*
[b]*Dipartimento di Farmacologia Sperimentale ed Applicata, Università di Pavia, Via Taramelli, 14, 27100 Pavia, Italy*
[c]*Centro Grandi Strumenti, Università di Pavia, Via Bassi 21, 27100 Pavia, Italy*
[d]*Dipartimento di Scienze Farmacobiologiche 'Complesso Ninì Barbieri' Università di Catanzaro 'Magna Graecia', 88021 Roccelletta di Borgia (CZ), Italy*
[e]*Dipartimento di Studi di Chimica e Tecnologia delle Sostanze Biologicamente Attive, Università degli Studi di Roma 'La Sapienza', P.le A.Moro 5, 00185 Rome, Italy*

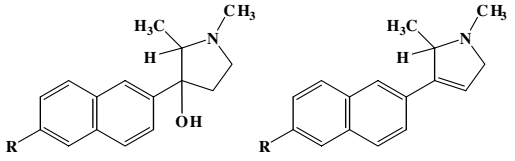
Received 2 September 2004; accepted 1 October 2004

Abstract—A new, high yield regioselective reaction affording racemic and enantiomeric 1,2-dimethyl-3-[2-(6-substituted naphthyl)]-2*H*,5*H*-pyrrolines is reported. Compounds were provided by dehydration of the corresponding 1,2-dimethyl-3-[2-(6-substituted naphthyl)]-3-hydroxy-pyrrolidines under microwave irradiation and solvent-free conditions. Pharmacological properties of enantiomeric compounds are also described; the analgesic activity was investigated by the hot plate test. A conformational analysis of the compounds was carried out by molecular modeling techniques with the aim to get information about the fitting to known analgesic pharmacophore models.

1. Introduction

Nowadays, the discovery of new drugs for reducing pain levels in the treatment of oncological pain is a very important target. Over the last few years research has been aimed at the design and synthesis of new analgesic analogues of nonpeptide antinociceptive agents (2*RS*,3*SR*)-**1a**–**c** (Fig. 1), as they exert significant biological activity.[1] To simplify the SAR study, racemic 1,2-dimethyl-3-[2-(6-substituted naphthyl)]-2*H*,5*H*-pyrrolines (*RS*)-**2a**–**c** (Fig. 1), characterized by a stereogenic center only, were prepared.[2] The analgesic activity (determined by the hot plate test, HPT) of racemic **2a**–**c** is noticeable when compared to morphine. It is noteworthy that compound **2a**, shows an AD_{50} of 0.31 mg/kg.

Herein we have focused our attention on the synthesis of enantiomers **2a**–**c** in order to investigate the influence of stereochemistry on the biological activity. To the best of our knowledge, the preparation of enantiomeric 1,2-dimethyl-2*H*,5*H*-pyrrolines has never been published.



1a-c	2a-c
Compound	R
a	H
b	F
c	OCH_3

Figure 1.

Compounds (*RS*)-**2a**–**c** were synthesized by dehydration under vacuum of the corresponding pyrrolidinols (2*RS*,3*SR*)-**1a**–**c**, using anhydrous $FeCl_3$ adsorbed on silica gel. However, all compounds were afforded in very long reaction times and with low yields.[2] Herein, we

* Corresponding author. Tel.: +39 0382507356; fax: +39 0382422975; e-mail: simona.collina@unipv.it

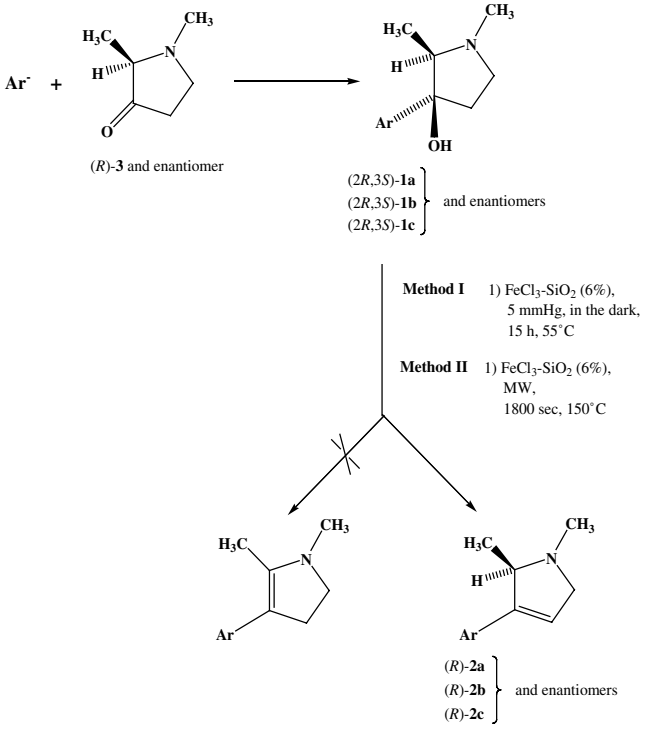
0957-4166/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2004.10.001

report a new synthetic procedure suitable for affording both racemic and enantiopure **2a–c** compounds. Thus, we studied the potential of microwave irradiation as a nonconventional energy source for the promotion of organic reactions associated with slow reaction rates.[3] Microwave dielectric heating promotes organic reactions, mainly in solvent free systems, leading to significant increases in reaction rates and yields, when compared to the conventional thermal processes, with significant advantages for the outcome of the reaction.

The successful preparation of (*R*)- and (*S*)-**2a–c** via regioselective synthesis under microwave irradiation is described without racemization. The resolution of racemic **2a** via fractional crystallization of the diastereoisomeric salts (−)-L-tartrate of (*R*)-**2a** and (+)-D-tartrate of (*S*)-**2a** is also reported. Chemical characterization of compounds by NMR, MS spectroscopy, polarimetry, and chiral HPLC analysis was effected. Pharmacological profiles of all enantiomers **2a–c** were drawn by HPT and compared to the corresponding racemates. Opioid receptor binding assays, as well as a molecular modeling studies were accomplished on compounds (2*R*,3*S*)-**1a** and (*R*)-**2a** to elucidate their mechanism of action.

2. Results and discussion

The synthetic procedure of 1,2-dimethyl-3-[2-(6-substituted naphthyl)]-2*H*,5*H*-pyrrolines **2a–c** is reported in Scheme 1.



Scheme 1.

Racemic **2a–c** were already afforded (Method I) by heating under vacuum a mixture of the corresponding 3-pyrrolidinols (2*RS*,3*SR*)-**1a–c** and anhydrous ferric chloride adsorbed on silica gel, a solid support in dry media, as an effective reagent for the dehydration of tertiary alcohols.[4,5] This regioselective procedure allowed us to gain the 3-pyrrolinyl-isomers, but with very low yields.[2]

Microwave heating (Method II) was investigated to improve yields, varying the reaction time and the power. All reactions were performed by dissolving the substrate in methanol, mixing it either with 10 or 30 times its weight of reagent and evaporating to dryness under vacuum in the dark. In a closed borosil vessel, the reaction mixture was then irradiated inside a microwave oven at 150 °C until the reaction was found to have gone to completion (1800 s). The inorganic solid supported reagent was separated after eluting the product with diethyl ether and aqueous ammonia. The evaporation of the solvent was performed at 30 °C, in the dark and, after acid–base work-up, the crude product was purified with the ion-exchange sulfonic resin Amberlyst 15, affording all compounds with high yields (from 50% up to 90%). LC–MS analyses of (*RS*)-**2a–c** detected only one peak at the UV detector, corresponding to the molecular ion of the expected compounds. Moreover, an accurate research of the signals of the 2-pyrrolinyl derivatives by ^{1}H NMR spectroscopy was unsuccessful, confirming the regioselectivity of the procedure. LC–MS and ^{1}H NMR analyses of crude **2a** are reported in Figure 2. It is worth noting that microwave irradiation affords products with higher yields than those obtained with conventional heating (Table 1). Therefore (*R*)-**2a–c** or (*S*)-**2a–c** were prepared via enantioselective synthesis by nucleophilic addition of the naphthalenic anion, obtained via metal–halogen exchange with *tert*-BuLi, to (*R*)-**3** or (*S*)-**3**, respectively,[6] followed by dehydration with microwave heating.

As already shown for the intermediate pyrrolidinols,[6] the C2 absolute configuration is the same as precursor **3**. Since the C2 stereogenic center is not involved in the dehydration process, enantiopure **2a–c** actually show the same configuration as the corresponding ketone **3**.

Resolution of (*RS*)-**2a** via fractional crystallization from 100% methanol using (+)-L-tartaric and (−)-D-tartaric acids as resolving agents, affording diastereoisomeric salts (−)-(*R*)-L-tartrate and (+)-(*S*)-D-tartrate, was also performed. Owing to the low yields (30–33%) of the resolution, the stereospecific synthesis was found to be more convenient, giving rise to pyrrolines with high enantiomeric excess (ee).

In order to develop a suitable method for the evaluation of the enantiomeric excess of **2a–c**, several HPLC procedures were attempted. Baseline separations were successfully achieved on an analytical Chiralpak AD column. Chromatographic parameters, enantiomeric excesses and specific rotations are reported in Table 2.

In vivo pharmacological profile of enantiomeric **2a–c** was investigated by HPT in mice. Data in Table 3 clearly show that all compounds exhibit noticeable analgesic

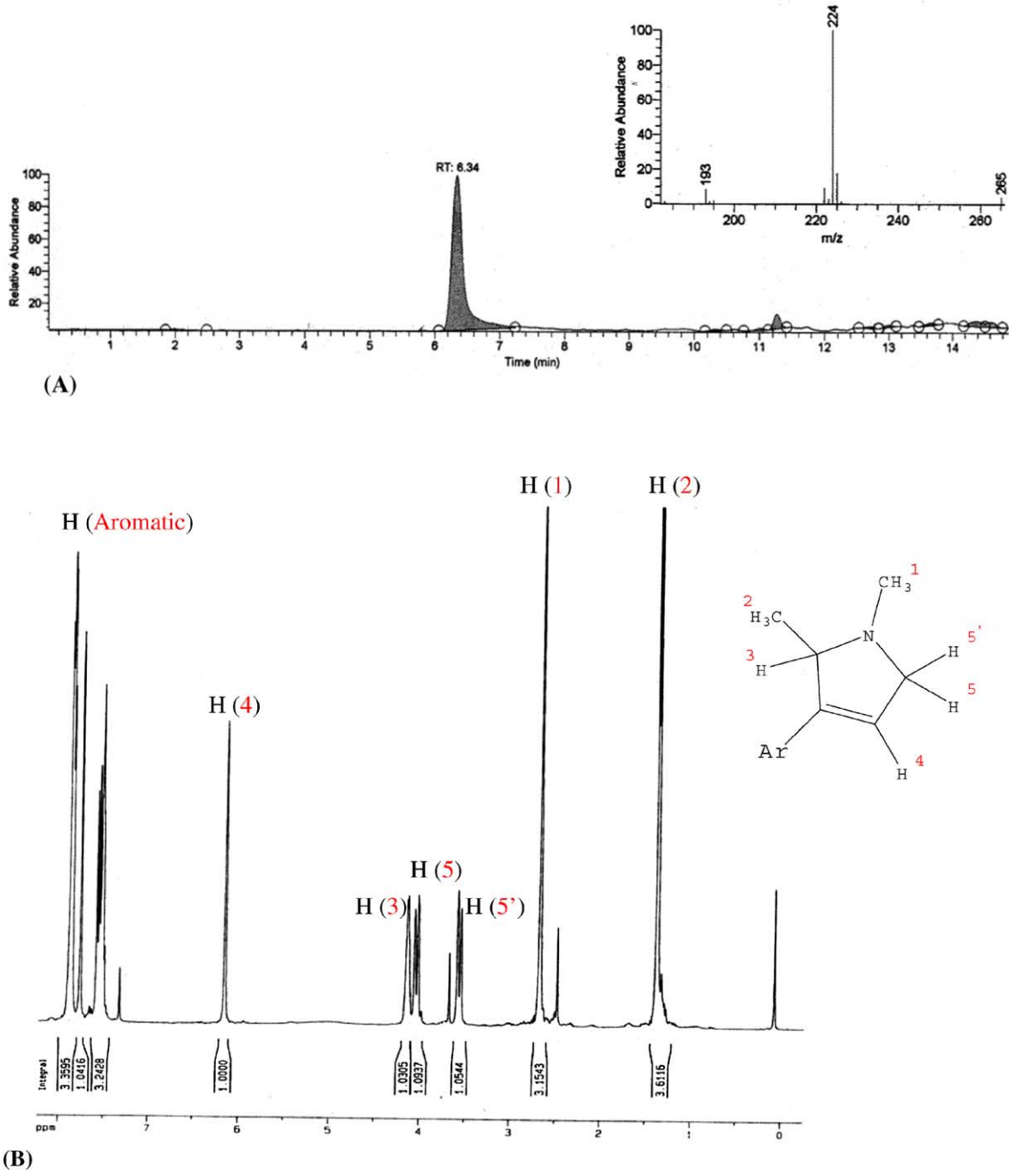


Figure 2. LC–MS (A) and ^{1}H NMR (B) spectra of crude (*RS*)-**2a** afforded according to Method II (Scheme 1).

Table 1. Experimental conditions of the dehydration process comparing the conventional heating with MW irradiation

Compound	Heating	Time	*T* (°C)	Yield (%)
2a	Oil bath	15 h	55	35
	MW	30 min	150	90
2b	Oil bath	8 h	65	18
	MW	30 min	150	70
2c	Oil bath	15 h	55	18
	MW	30 min	150	50

properties, with similar or even better DA_{50} values than morphine. Moreover, the activity is related to the structural features of the aromatic moiety, since substantial differences were revealed in response to the painful stimulus. Furthermore the analgesic activity seems to be strictly related to the configuration, having the eutomer with an (*R*)-configuration. It is noteworthy that the influence of the fluorine atom on the activity, in particular, (*R*)-**2b** was found to be the most enantioselective (eudismic ratio: 15.5) and the most potent (relative potency vs morphine: 15.0, E_{max} comparable with morphine).

In vitro binding assays of racemic **1a** and **2a**, were also performed. Notwithstanding the in vivo activity, there

Table 2. Analytical resolution on a Chiralpak AD column (10 μm, 250×4.6 mm) and optical activity of **2a–c**

Compound	Mobile phase[a]	α	Rs	t_R (min) (config.)	Ee%	[α][b]
2a	98.5/1.5/0.1	1.28	2.97	9.6 (*S*)	99.9	+23.3
				11.5 (*R*)	99.9	−23.4
2b	99.0/1.0/0.1	1.34	2.11	10.5 (*S*)	92.1	+23.2
				13.5 (*R*)	99.9	−26.6
2c	97.0/3.0/0.2	1.72	2.80	10.3 (*S*)	92.8	+24.5
				16.5 (*R*)	99.9	−26.2

[a] Solvent mixture: *n*-hexane/IPA/DEA (v/v/v); flow rate: 0.5 mL/min; UV detector: λ 273 nm.
[b] Performed on **2a–c** tartrates, *c* 0.5, MeOH; λ 589 nm.

Table 3. Analgesic activity in the HPT

Compound	AD_{50} (μmol/kg)	Conf. limits	$E_{max\,sec}$ (mg)	Eudismic ratio
Morphine	11.12	8.27–14.90	59.8 (12)	
(*RS*)-**2a**	5.78	4.98–6.70	44.5 (12)	
(*R*)-**2a**	4.66	3.24–6.75	37.1 (12)	2.0
(*S*)-**2a**	9.43	8.49–10.47	41.3 (12)	
(*RS*)-**2b**	0.79	0.33–1.89	41.7 (8)	
(*R*)-**2b**	0.74	0.05–1.33	50.6 (8)	15.5
(*S*)-**2b**	11.45	7.82–16.76	42.3 (16)	
(*RS*)-**2c**	4.02	2.21–7.26	51.1 (12)	
(*R*)-**2c**	2.55	1.81–3.62	54.6 (12)	2.3
(*S*)-**2c**	5.92	2.68–13.14	35.5 (12)	

is evidence that compounds show very poor affinity for μ, δ, and κ opioid receptors (K_i values in the order of micromoles).

Taking into account that opioid analgesia is influenced by many factors, including the sigma-1 receptor system,[7] and in order to elucidate the mechanism of action of the examined compounds, a molecular modeling study was performed. The features of the most stable conformers were compared to common features of two different pharmacophore models developed for nonspecific opioid ligands[8] and for sigma-1 ligands.[9]

The conformational search was carried out by molecular mechanics methodologies (see Section 4.5) on the simplest derivatives (2*R*,3*S*)-**1a** and (*R*)-**2a**, since both pharmacophore models were not built to take into account the functionalization of the aromatic ring. As expected, the results indicated a consistent conformational distribution difference between the two compounds. Within 12.5 kcal/mol above the energy global minima, (2*R*,3*S*)-**1a** and (*R*)-**2a** were, respectively, characterized by 16 and 2 conformers. The estimation of the Boltzmann population allowed us to reduce the number of **1a** most probable conformers to 4, that at 300 K collect more than 97% of probability (Table 4). In Figure 3 and in Tables 4 and 5, the most populated conformations of both compounds are reported.

The comparison between the selected conformers and opioid and sigma-1 pharmacophores was carried out

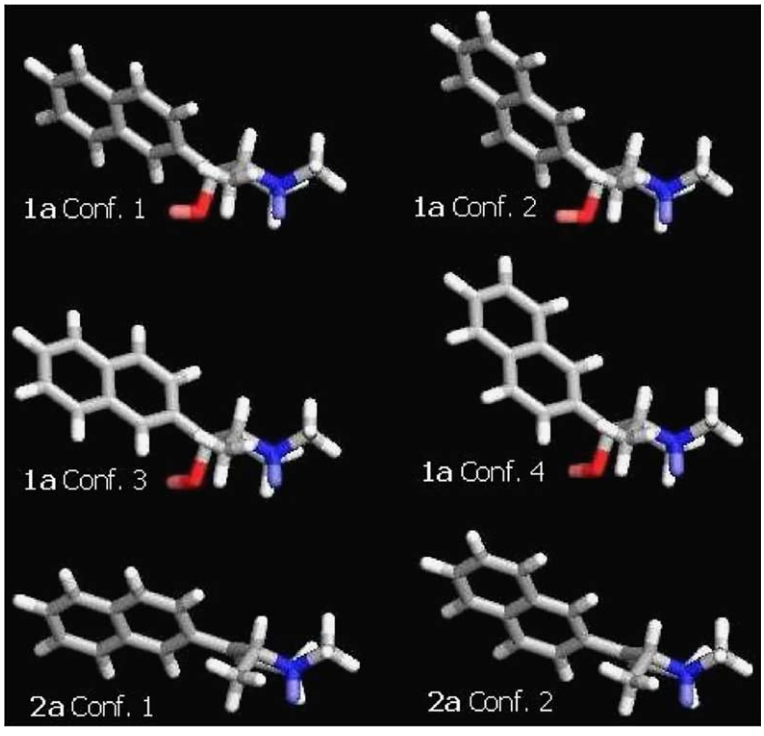


Figure 3. Polytube models of the most populated (2*R*,3*S*)-**1a** and (*R*)-**2a** conformers.

Table 4. Properties of the most populated (2*R*,3*S*)-**1a** conformers

Conformer	Relative internal energy (kcal/mol)	Boltzmann population at 300 K (%)	C3′–C2′–C3–C2 (deg)	H–O–C3–C2 (deg)	C4–C3–C2–C1 (deg)
1	0.00	40.29	−0.02	99.06	−38.65
2	0.19	29.59	179.63	100.07	−38.65
3	0.57	15.52	96.20	168.37	−37.41
4	0.72	12.20	−82.87	167.54	−37.38

Table 5. Properties of the most populated (*R*)-**2a** conformers

Conformer	Relative internal energy (kcal/mol)	Boltzmann population at 300 K (%)	C3′–C2′–C3–C2 (deg)
1	0.00	53.10	29.49
2	0.11	49.90	−149.62

while considering that both models have in common one aromatic ring, one hydrophobic moiety, and one protonated nitrogen. The three distances between the aromatic centroid, the hydrophobic moiety corresponding to the methyl substituent at the 2 position and the protonated nitrogen were evaluated. The distances of the two models (Table 6) clearly indicate two quite different receptors geometries with absolutely no relationship to each other. Measurements of the considered conformers are shown in Table 6.

As expected, the comparison of the most populated conformer distances with the opioid pharmacophore model clearly indicated no matching of any descriptor. Also considering the variability of this model due to the presence of two hydrophobic moieties it is impossible to fit any distance within an error range lower than 10%. Conversely, the match of descriptors of both compounds with respect to the sigma-1 model is relatively better at least in two of three features, highlighting the possible involvement of the sigma-1 receptors.

3. Conclusions

Racemic and enantiomeric 1,2-dimethyl-3-[2-(6-substituted naphthyl)]-2*H*,5*H*-pyrrolines have been afforded by microwave promoted high speed (minutes instead of hours) regioselective dehydration under solvent free conditions without racemization. There is striking evidence that the new synthetic strategy is much more convenient than the conventional heating procedure, as suggested by the higher yields as well as the reduced reaction time (Fig. 4). Enantiomeric compounds have been afforded with high ees, as shown by chiral HPLC analysis.

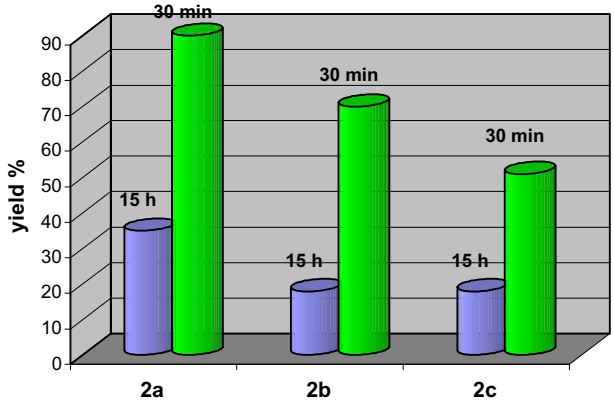


Figure 4. Comparison between conventional (blue) and microwave heating (green) with regards to yields and reaction times.

Pharmacological data evidenced that the analgesic activity, expressed as AD_{50} (μmol/kg), is noticeable if compared to morphine and strictly related to the configuration. For all compounds, the eutomer is the (*R*)-enantiomer. More importantly, compound (*R*)-**2b**, being 15 times more potent than morphine and with a eudismic ratio 15, has to be highly considered (Fig. 5). At present, no clear correlation can be made between the structural features of these compounds and their biological activity. Both binding assays and molecular modeling studies exclude a direct involvement of the opioid receptors, while the computational work highlights the possible involvement of sigma-1 receptors. Therefore, chiral pyrrolines will be considered for further biological investigation.

4. Experimental

4.1. General

All reagents and solvents were purchased from commercial suppliers and employed without further purification. Technical grade Amberlyst resin was used after washing with suitable solvents (H_2O, CH_3OH, CH_2Cl_2). Unless otherwise specified, reactions involving polymers were

Table 6. Pharmacophore distance comparison of the most populated (2*R*,3*S*)-**1a** and (*R*)-**2a** conformers (all data are expressed in Å)

Pharmacophore descriptor	Opioid[8]	σ-1[9]	**1a**-1	**1a**-2	**1a**-3	**1a**-4	**2a**-1	**2a**-2
Protonated nitrogen—aromatic centroid	4.31	7.14	6.43	6.25	6.31	6.26	6.40	6.28
Hydrophobic moiety—aromatic centroid	2.51–3.53[a]	8.66	5.78	5.33	5.13	5.25	5.41	5.10
Hydrophobic moiety—protonated nitrogen	3.75–3.98[a]	2.51	2.49	2.49	2.49	2.49	2.47	2.47

[a] Average values for both hydrophobic moieties.[8]

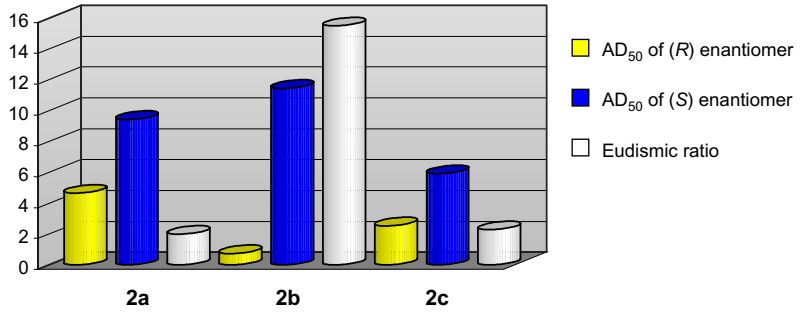


Figure 5. AD_{50} values (μmol/kg) and eudismic ratio of **2a–c**.

carried out on an IKA KS 130 basic laboratory shaker at 250 rpm.

Microwave dielectric heating was performed in a Smith Creator single mode microwave cavity Personal Chemistry from Biotage GmbH, Germany, producing continuous irradiation at 2.45 GHz. The reaction vessel was a round-bottomed 100 mm Duran™ glass tube with a Schott GL 18 screw cap, provided with a Teflon septa as a pressure relief device.

NMR spectra were performed at 9.4 T (TMS as internal standard $\delta = 0$) with an ADVANCE spectrometer at 400 MHz, mod. Bruker Germany and a BB1 5 mm probe; chemical shifts are given in ppm. Elemental analyses were performed on a Carlo Erba 1106 C, H, N analyzer. Analytical TLC were performed using precoated glass-backed plates (Fluka Kieselgel 60 F_{254}) and visualized by ultra-violet radiation, acidic ammonium molybdate(IV) or potassium permanganate. Melting points were measured on SMP3 Stuart Scientific apparatus. LC–MS analyses were performed on a HP1100 instrument with UV detector wavelength of 220 nm and Waters Symmetry C8 column (150 × 4.6 mm) and a Thermofinnigan Navigator single quadrupole LC/MS/MS mass spectrometer with APCI ion source.

HPLC analyses were performed on a Jasco system consisting of a PU-1580 pump, a Reodyne 7125 injector (20 μL sample loop) and a MD-1510 Diode Array detector (wavelength 273 nm). Experimental data were acquired and interpreted with Borwin PDA and Borwin chromatograph software. Chiral HPLC analyses were performed on Chiralpak AD column (Daicel) (250 × 4.6 mm, 5 μm); elution was carried out using *n*-hexane, isopropyl alcohol (IPA) and diethylamine (DEA). All the solvents were purchased by Carlo Erba and are HPLC grade. Optical rotations were recorded at room temperature on a Jasco DIP 1000 polarimeter.

4.2. General procedure for the arylation of ketone 3

The synthesis of (2*R*,3*S*)- or (2*S*,3*R*)-**1a–c** was performed essentially according to the procedure for the preparation of an analogous compound already published.[1]

To a solution of the appropriate aromatic bromide precursor (31 mmol) in dry diethyl ether (80 mL), *t*-BuLi was added (62 mmol, 36.5 mL 1.7 M in pentane) and the reaction mixture kept at −78 °C for 1 h. A solution of (*RS*)-**3**, (*R*)-**3** $\{[\alpha]_D^{23} = +58.5$ (*c* 1.0, MeOH)$\}$ or (*S*)-**3** $\{[\alpha]_D^{23} = -56.1$ (*c* 1.0, MeOH)$\}$ (25.9 mmol in 20 mL of dry diethyl ether) was then added. After 3 h the mixture was allowed to warm at room temperature, then quenched with HCl 10% (70 mL) and stirred for 1 h. The precipitate obtained was filtered to afford the corresponding crude product, crystallized from a proper solvent.

4.2.1. (+)-(2*R*,3*S*)-1a·HCl. White solid (IPA 9/H_2O 1), mp 168–170 °C. $[\alpha]_{365} = +23.5$ (*c* 1.0, MeOH). ^{1}H NMR (400 MHz, CD_3OD, TMS): $\delta_H = 7.83$–8.01 (s and m, 4H, aromatic); 7.61 (dd, 1H, aromatic, C*H* 4, $J = 8.5$ Hz); 7.45 (m, 2H, aromatic); 3.91 (dt, 1H, *H*CH–N, $J_{gem} = 11.0$ Hz, $J_{vic} = 8.0$ Hz); 3.75 (q, 1H, CH_3–C*H*, $J = 6.3$ Hz); 3.45 (dt, 1H, HC*H*–N, $J_{gem} = 11.0$ Hz, $J_{vic} = 3.4$ Hz); 2.97 (s, 3H, CH_3–N); 2.78 (ddd, 1H, *H*CH–CH_2N, $J_{gem} = 14.0$ Hz, $J_{vic} = 8.3$–10.5 Hz); 2.28 (ddd, 1H, HC*H*–CH_2N, $J_{gem} = 14.0$ Hz, $J_{vic} = 3.5$–8.7 Hz); 1.15 (d, 3H, CH_3–CH, $J = 6.3$ Hz). Elemental analysis: $C_{16}H_{20}NOCl$ requires C, 69.18; H, 7.26; N, 5.04. Found: C, 69.29; H, 7.56; N, 4.88.

4.2.2. (−)-(2*S*,3*R*)-1a·HCl. White solid (IPA 9/H_2O 1), mp 170–171 °C. $[\alpha]_{365} = -25.6$ (*c* 1.0, MeOH). ^{1}H NMR spectrum is identical to that of the corresponding enantiomer. Elemental analysis: $C_{16}H_{20}NOCl$ requires C, 69.18; H, 7.26; N, 5.04. Found: C, 69.32; H, 7.31; N, 4.96.

4.2.3. (+)-(2*R*,3*S*)-1b·HCl. White solid (IPA 8/H_2O 2), mp 210–212 °C. $[\alpha]_{365} = +22.9$ (*c* 1.0, MeOH). ^{1}H NMR (400 MHz, CD_3OD, TMS): $\delta_H = 8.03$ (s, 1H, aromatic); 7.90 (dd, 1H, aromatic, C*H* 8, $J = 5.6$ Hz); 7.85 (d, 1H, aromatic, C*H* 4, $J = 8.7$ Hz); 7.62 (d, 1H, aromatic, C*H* 3, $J = 8.7$ Hz); 7.49 (d, 1H, aromatic, C*H* 5, $J = 9.8$ Hz); 7.28 (dt, 1H, aromatic, C*H* 7, $J = 2.5$–9.0–9.0 Hz); 3.90 (dt, 1H, *H*CH–N, $J_{gem} = 11.3$ Hz, $J_{vic} = 8.4$–8.4 Hz); 3.70 (q, 1H, CH_3–C*H*, $J = 6.4$ Hz); 3.42 (t, 1H, HC*H*–N, $J_{gem} = 11.3$ Hz, $J_{vic} = 4$ Hz); 2.75 (ddd, 1H, *H*CH–CH_2N, $J_{gem} = 13.7$ Hz, $J_{vic} = 8.6$–11.6 Hz); 2.28 (ddd, 1H, HC*H*–CH_2N, $J_{gem} = 13.7$ Hz, $J_{vic} = 3.4$–8.6 Hz); 1.15 (d, 3H, CH_3–CH, $J = 6.4$ Hz). Elemental analysis: $C_{16}H_{19}NOFCl$ requires C, 64.97; H, 6.47; N, 4.74. Found: C, 64.95; H, 6.59; N, 4.99.

4.2.4. (−)-(2*S*,3*R*)-1b·HCl. White solid (IPA 8/H_2O 2), 210–211 °C. $[\alpha]_{365} = -23.9$ (*c* 1.0, MeOH). ^{1}H NMR spectrum is identical to that of the corresponding enantiomer. Elemental analysis: $C_{16}H_{19}NOFCl$ requires C, 64.97; H, 6.47; N, 4.74. Found: C, 65.08; H, 6.34; N, 4.80.

4.2.5. (+)-(2*R*,3*S*)-1c·HCl. White solid (IPA 9/H_2O 1), mp 215–217 °C. $[\alpha]_{405} = +34.6$ (*c* 0.5, MeOH). ^{1}H NMR (400 MHz, CD_3OD, TMS): $\delta_H = 7.91$ (s, 1H, aromatic, CH 1); 7.77 (d, 1H, aromatic, C*H* 8, $J = 8.7$ Hz); 7.73 (d, 1H, aromatic, C*H* 4, $J = 9.0$ Hz); 7.53 (dd, 1H, aromatic, C*H* 3, $J = 2.0$–9.0 Hz); 7.19 (dt, 1H, aromatic, C*H* 7, $J = 2.5$ Hz); 7.10 (dd, 1H, aromatic, C*H* 5, $J = 3.9$ Hz); 3.895 (dt, 1H, *H*CH–N, $J_{gem} = 11.3$ Hz, $J_{vic} = 8.5$, 8.5 Hz); 3.85 (s, 3H, OCH_3); 3.68 (q, 1H, CH_3–C*H*, $J = 6.5$ Hz); 3.42 (dt, 1H, HC*H*–N, $J_{gem} = 11.3$ Hz, $J_{vic} = 3.5$ Hz); 2.95 (s, 3H, CH_3–N); 2.74 (ddd, 1H, *H* CH–CH_2N, $J_{gem} = 13.5$ Hz, $J_{vic} = 8.6$–11.5 Hz); 2.28 (ddd, 1H, HC*H*–CH_2N, $J_{gem} = 13.5$ Hz, $J_{vic} = 3.5$–8.5 Hz); 1.15 (d, 3H, CH_3–CH, $J = 6.5$ Hz). Elemental analysis: $C_{17}H_{22}NO_2Cl$ requires C, 66.33; H, 7.20; N, 4.55. Found: C, 66.36; H, 7.41; N, 4.68.

4.2.6. (−)-(2*S*,3*R*)-1c·HCl. White solid (IPA 9/H_2O 1), mp 214–217 °C. $[\alpha]_{405} = -33.8$ (*c* 0.5, MeOH). ^{1}H NMR spectrum is identical to that of the corresponding enantiomer. Elemental analysis: $C_{17}H_{22}NO_2Cl$ requires C, 66.33; H, 7.20; N, 4.55. Found: C, 66.10; H, 7.32; N, 4.89.

4.3. General procedure for the preparation of 1,2-dimethyl-3-[2-(6-substituted)-naphthyl]-2*H*,5*H*-pyrroline tartrates [2a–c tartrate]

Anhydrous $FeCl_3$–SiO_2 (30 g, 6%) was added to a methanolic solution (250 mL) of (2*RS*,3*SR*)-**1a**–**c** or (+)-(2*R*,3*S*)-**1a**–**c** or (−)-(2*S*,3*R*)-**1a**–**c** (3.7 mmol) obtained by treatment of the corresponding hydrochlorides with $NaHCO_3$. After evaporation of the solvent, the reaction mixture was irradiated in a monomode microwave oven at 150 °C for 30 min. Diethyl ether (1 L) was added, and the resulting mixture stirred for 3 h before being filtered through a pad of sea sand and washed thoroughly with diethyl ether/aqueous ammonia (400 mL/5 mL).

The solvent was carefully (bath temperature 30 °C) evaporated under vacuum in the dark to afford an oil. After an acid–base work-up, the residue was re-dissolved in dichloromethane, Amberlyst A15 was added to the solution and the mixture shaken for 2 h. After filtration, the resin was suspended in 3.5 M solution of ammonia in methanol (250 mL) and shaken for 48 h. The resin was filtered off and washed thoroughly with methanol (300 mL). The evaporation of the solvent under vacuum afforded the desired products as yellow oils. After treatment with a solution of DL- or L-(+)- or D-(−)-tartaric acid (*RS*)-**2a**–**c**·DL-tartrate, (−)-(*R*)-**2a**–**c**·L-tartrate and (+)-(*S*)-**2a**–**c**·D-tartrate were obtained as white solids.

4.3.1. (−)-(*R*)-2a·L-Tartrate. Yield 93.8%. White solid, mp 161–163 °C. For [α] and ee% values, see Table 2. ^{1}H NMR (400 MHz, CD_3OD, TMS): $\delta_H = 7.83$ (m, 4H, aromatic); 7.61 (dd, 1H, aromatic); 7.46 (m, 2H, aromatic); 6.34 (s, 1H, *H*C═C); 5.01 (q, 1H, CH_3C*H*, $J = 6.2$ Hz); 4.52 (d, 1H, HC*H*N, $J_{gem} = 15.0$ Hz); 4.14 (d, 1H, *H*CHN, $J_{gem} = 15.0$ Hz); 3.00 (s, 3H, CH_3–N); 1.58 (d, 3H, CH_3–CH, $J = 6.6$ Hz). Elemental analysis: $C_{20}H_{23}NO_6$ requires C, 64.33; H, 6.21; N, 3.75. Found: C, 64.53; H, 6.44; N, 3.95.

4.3.2. (+)-(*S*)-2a·D-Tartrate. Yield 92.6%. White solid, mp 160–162 °C. For [α] and ee% values, see Table 2. ^{1}H NMR spectrum is identical to that of the corresponding enantiomer. $C_{20}H_{23}NO_6$ requires C, 64.33; H, 6.21; N, 3.75. Found: C, 64.09; H, 6.11; N, 3.81.

4.3.3. (−)-(*R*)-2b·L-Tartrate. Yield 69.6%. White solid, hygroscopic. For [α] and ee% values, see Table 2. ^{1}H NMR (400 MHz, CD_3OD, TMS): $\delta_H = 7.82$ (m, 3H, aromatic); 7.60 (d, 1H, aromatic); 7.45 (dd, 1H, aromatic); 7.26 (m, 1H, aromatic); 6.23 (s, 1H, *H*C═C); 4.95 (q, 1H, C*H*–CH_3, $J = 6.2$ Hz); 4.32 (d, 1H, HC*H*–N, $J_{gem} = 15.0$ Hz); 3.95 (d, 1H, *H* CH–N, $J_{gem} = 15.0$ Hz); 2.90 (s, 3H, CH_3–N); 1.44 (d, 3H, CH_3–CH, $J = 6.5$ Hz). Elemental analysis: $C_{20}H_{22}NO_6F$ requires C, 61.38; H, 5.67; N, 3.58. Found: C, 61.27; H, 5.73; N, 3.34.

4.3.4. (+)-(*S*)-2b·D-Tartrate. Yield 70.2%. White solid, hygroscopic. For [α] and ee% values, see Table 2. ^{1}H NMR spectrum is identical to that of the corresponding enantiomer. Elemental analysis: $C_{20}H_{22}NO_6F$ requires C, 61.38; H, 5.67; N, 3.58. Found: C, 61.48; H, 5.82; N, 3.71.

4.3.5. (−)-(*R*)-2c·L-Tartrate. Yield 51.2%. White solid, mp 182–184 °C. For [α] and ee% values, see Table 2. ^{1}H NMR (400 MHz, CD_3OD, TMS): $\delta_H = 7.75$ (m, 3H, aromatic); 7.53 (dd, 1H, aromatic); 7.20 (d, 1H, aromatic); 7.08 (dd, 1 H, aromatic); 6.19 (s, 1H, *H*C═C); 4.90 (q, 1H, CH_3–C*H*, $J = 6.2$ Hz); 4.43 (d, 1H, HC*H*–N, $J_{gem} = 14.3$ Hz); 4.05 (d, 1H, *H*CH–N, $J_{gem} = 14.3$ Hz); 3.83 (s, 3H, CH_3O); 2.98 (s, 3H, CH_3–N); 1.50 (d, 3H, CH_3–CH, $J = 6.5$ Hz). Elemental analysis: $C_{21}H_{25}NO_7$ requires C, 62.52; H, 6.25; N, 3.47. Found: C, 62.46; H, 6.28; N, 3.40.

4.3.6. (+)-(*S*)-2c·D-Tartrate. Yield 50.0%. White solid, mp 180–182 °C. For [α] and ee% values, see Table 2. ^{1}H NMR spectrum is identical to that of the corresponding enantiomer. Elemental analysis: $C_{21}H_{25}NO_7$ requires C, 62.52; H, 6.25; N, 3.47. Found: C, 62.31; H, 6.14; N, 3.37.

4.4. Resolution of (*RS*)-2a

4.4.1. With (+)-L-tartaric acid to afford (−)-(*R*)-2a·L-tartrate. Compound (*RS*)-**2a** (0.9 g, 4.03 mmol) was added to a warm (60 °C) methanolic solution (10 mL) of (+)-L-tartaric acid (0.6 g, 4.03 mmol). After cooling at room temperature, 0.5 g of (−)-(*R*)-**2a**-tartrate {mp 158–160 °C, $[\alpha]_D = -13.8$ (*c* 1.0, MeOH), ee = 59.0%} precipitated. Further crystallization from methanol

(6 mL) afforded 0.3 g of (−)-(*R*)-**2a**-tartrate [mp 159–161 °C, $[\alpha]_D = -20.4$ (*c* 1.0, MeOH), ee = 87.2%].

4.4.2. With (−)-D-tartaric acid to afford (+)-(*S*)-2a·D-tartrate. The partially resolved (+)-(*S*)-**2a**·D-tartrate (0.7 g, 3.13 mmol), provided from mother liquor of the resolution described above, was crystallized from methanol (7 mL), affording 0.2 g of (+)-(*S*)-**2a**·D-tartrate {mp 158–160 °C, $[\alpha]_D = +19.8$ (*c* 1.0, MeOH), ee = 84.6%}.

4.5. Computational study

Compounds (2*R*,3*S*)-**1a** and (*R*)-**2a**, respectively, were built by the Maestro user interface of MACROMODEL ver 7.2[10] and energy minimized with MMFF forcer field[11] and GB/SA implicit model of solvation.[12] The nitrogen atom was considered as protonated, inducing another stereogenic center to the computational model. For energetic reasons, the stereochemistry of such an atom in both compounds was modeled in *trans* configuration with respect to the stereogenic center in **2**.

For compound **1a**, the automatic setup included the torsional bond corresponding to the hydroxyl–pyrroline and the naphthalene–pyrroline bonds only. In order to explore the flexibility of the five-member ring, one closure and two more torsional bonds were manually inserted in the Monte Carlo (MC) search.[13] Conformations (1000) were generated and energy minimized with the same force field and solvation conditions. For compound **2a** only one torsional bond was randomized by the MC search.

The deduplication of identical conformers after superimposition was performed according to the standard MACROMODEL criterion (max RMS deviation equal to 0.25 Å, max energy difference equal to 1 kcal/mol). The Boltzmann probability of each conformer was determined at 300 K with the thermodynamic module of the MOLINE package.[14]

The pharmacophore feature measurements were carried out with the Maestro graphical interface considering for the aromatic moiety the centroid averaging the position of the 1′–10′ naphthalene carbon atoms, for the protonated amine the nitrogen atom and for the hydrophobic moiety the carbon atom of the methyl group in **2**. All calculations were performed by an INTEL PENTIUM IV workstation equipped with LINUX REDHAT ver. 7.3.

4.6. Pharmacology

In vitro pharmacological studies were performed according to the previously described procedure.[1] Opioid receptor binding experiments were measured using cloned human κ, δ, and μ receptors. Data obtained from competition experiments were analyzed using nonlinear fitting analysis according to Benfenati and Guardabasso[15] and using the RS/1 software. K_i values were determined from IC50 using the Cheng and Prusoff equation and were >1000 nM for all compounds. In vivo pharmacological studies were performed on male adult Swiss mice weighting 30 ± 5 g. Antinociception was estimated by means of the hot plate test (HPT).[1] Compounds were dissolved in saline solution and administered within 1 h from dissolution.

4.6.1. Hot plate test (HPT). The response to the thermal stimulus was evaluated using a copper plate heated to 55 °C. The time at which mice 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 sc 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 the injection. The reaction time of the control animals (cut-off time) was 23 ± 2 s. AD50 values and their 95% confidence intervals were determined using a computerized program.[16] Experimental data are reported in Table 3.

Acknowledgements

This work was supported by Grant from MIUR (Ministero dell'Istruzione, dell'Università e della Ricerca, Italy). Special thanks for the oven support to Biotage GmbH, Germany.

References

1. Collina, S.; Azzolina, O.; Vercesi, D.; Sbacchi, M. A.; Scheideler, A.; Barbieri, A.; Lanza, E.; Ghislandi, V. *Bioorg. Med. Chem.* **2000**, *8*, 1925–1930.
2. Collina, S.; Azzolina, O.; Vercesi, D.; Barbieri, A.; Lanza, E.; Linati, L.; Ghislandi, V. *Il Farmaco* **2000**, *55*, 611–618.
3. Perreux, L.; Loupy, A. *Tetrahedron* **2001**, *57*, 9199–9223.
4. Keinan, E.; Mazur, Y. *J. Org. Chem.* **1978**, *43*, 1020–1022.
5. Fadel, A.; Salaun, J. *Tetrahedron* **1985**, *41*, 413–420.
6. Ghislandi, V.; Collina, S.; Azzolina, O.; Barbieri, A.; Lanza, E.; Tadini, C. *Chirality* **1999**, *11*, 21–28.
7. Mei, J.; Pasternak, G. W. *J. Pharmacol. Exp. Ther.* **2002**, *300*, 1070–1074.
8. Filizola, M.; Villar, H. O.; Loew, G. H. *Bioorg. Med. Chem.* **2001**, *9*, 69–76.
9. Gund, T. M.; Floyd, J.; Jung, D. *J. Mol. Graph. Modell.* **2004**, *22*, 221–230.
10. Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440–467.
11. Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 490.
12. Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. *J. Am. Chem. Soc.* **1990**, *112*, 6127.
13. Chang, G.; Guida, W. C.; Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 4379.
14. Alcaro, S.; Gasparrini, F.; Incani, O.; Mecucci, S.; Misiti, D.; Pierini, M.; Villani, C. *J. Comput. Chem.* **2000**, *21*, 515–530.
15. Benfenati, F.; Guardabasso, V. In *Principles and Methods in Receptor Binding*; Cattabeni, F., Nicosia, S., Eds.; Plenum: New York, 1984; pp 41–63.
16. Tallarida, R. J.; Murray, R. B. *Manual of Pharmacological Calculation with Computer Programs*; Springer: Berlin, 1987, pp 26–31.