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Synthesis and pharmacological evaluation of new *N*-methyl-arylpyrrolidinols with analgesic activity

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Abstract

In the present paper, we report on the synthesis and antinociceptive activity of a new series of *N*-methyl-arylpyrrolidinols that we designed for a rational structure–activity relationship (SAR) study. The antinociceptive properties were investigated in vivo by the hot plate and formalin tests in mice and control on the locomotory activity was also monitored by the rota rod test. With this aim, the evaluation of the lipophilicity of all compounds was performed by the Daylight computational method in order to better understand the SAR. Interesting properties were proven for the compounds of the entire series.

Keywords: Pyrrolidinols; Diastereoselective synthesis; Antinociceptive activity; Antiinflammatory activity

1. Introduction

The design and study of new molecules potentially useful in the control of pain and particularly in the management of oncological pain is a very important target today. It is well known that the mechanism of pain transmission is very complex and involves numerous neuromodulators of pain response [1]. The therapeutical approach consists in the use of non steroidal antiinflammatory drugs (NSADs) and opiates, both characterized by many severe side effects.

In our search for new molecules with analgesic activity, numerous dialkylaminoalkyl-naphthalenes and cycloaminoalkyl-naphthalenes (series 1 and 2 in Fig. 1) have been prepared in the last few years [2–5] and we have focused our interest on the design, synthesis and biological evaluation of pyrrolidinols for a rational study of the structure–activity relationships (SAR). Among the already investigated pyrrolidinyl-naphthalenes, we discovered the hit compound (2*R*,3*S*/2*S*,3*R*)-1,2-dimethyl-3-(2-naphthyl)-3-hydroxypyrrolidine [(2*R*,3*S*/2*S*,3*R*)-**1**] (Fig. 1) which shows interesting analgesic properties in the hot plate test (HPT) ($AD_{50} = 0.18$ mg/kg) and no influence on the locomotory activity. Further in vivo investigation has evidenced that the antinociceptive activity is antagonized by naloxone [5]. Thus, we decided to prepare a series of simple analogues of this hit. In the new series of pyrrolidinols [compounds (2*R*,3*S*/2*S*,3*R*)-**2**–**13**], (Fig. 2) the aromatic moiety was modified by introducing, respectively 2-naphthyl, 9-phenantrenyl, phenyl and 4-substituted-phenyl group to verify the importance of this moiety on the pharmacological activity.

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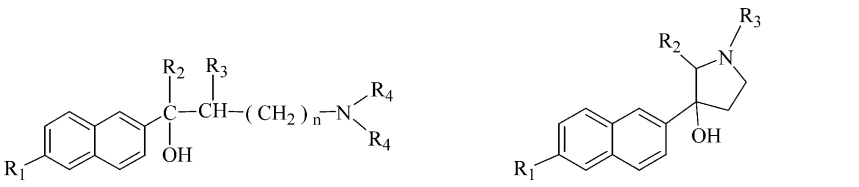


series I series II

(2R,3S/2S,3R)-**1** R_1=H
R_2=R_3=CH_3

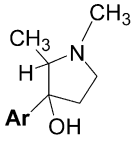
Fig. 1.

2. Chemical experimental section

Commercially available reagents and solvents were used as received from the supplier. Diethylether was dried and distilled according to standard procedures.

Starting materials **14**–**24** (Fig. 3) and solvents were purchased from commercial suppliers and employed without further purification; we prepared compound **25** (Fig. 3) according to the procedure already published [6].

Melting points were measured on SMP3 Stuart Scientific apparatus and are uncorrected. Elemental analyses were performed on a Carlo Erba 1106 C, H, N analyzer and the results were within ±0.4% of the calculated values. TLC analyses were carried out on silica gel Kieselgel 60 F_{254} (Merck), using n-hexane 87/IPA 13/DEA 2/MeOH 3 (v/v/v) as mobile phase. TLC analysis of compound (2*R*,3*S*/2*S*,3*R*)-**11** was accomplished by elution with EtOAc 90/MeOH 10/NH_4OH 0.2 v/v/v/v). IR spectra were recorded on a Perkin–Elmer FT-IR 1605 spectrophotometer; only noteworthy absorbtions are given. NMR spectra were performed at 9.4 T (TMS as internal standard $\delta = 0$) with an ADVANCE 400 spectrometer (Bruker, Germany) and a BBI 5 mm probe; chemical shifts are given in ppm. Anhydrous sodium sulphate was used to dry organic solutions. Evaporation of solvents was performed in vacuo with a rotatory evaporator.



compound	Ar	compound	Ar
(2R,3S/2S,3R)-**2**		(2R,3S/2S,3R)-**8**	
(2R,3S/2S,3R)-**3**		(2R,3S/2S,3R)-**9**	
(2R,3S/2S,3R)-**4**		(2R,3S/2S,3R)-**10**	
(2R,3S/2S,3R)-**5**		(2R,3S/2S,3R)-**11**	
(2R,3S/2S,3R)-**6**		(2R,3S/2S,3R)-**12**	
(2R,3S/2S,3R)-**7**		(2R,3S/2S,3R)-**13**	

Fig. 2. Pyrrolidinols (2*R*,3*S*/2*S*,3*R*)-**2**–**13**.

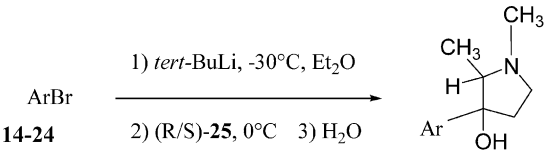
2.1. General procedure for the preparation of (2R,3S/2S,3R)-2–8 and (2R,3S/2S,3R)-10–13

The syntheses of (2*R*,3*S*/2*S*,3*R*)-**2**–**8** and (2*R*,3*S*/2*S*,3*R*)-**10**–**13** were essentially performed as reported in Scheme 1, according to the procedure that we have already described [4].

Tert-BuLi (1.7 M in pentane) was added, under N_2, to a stirring solution of the appropriate bromoarylderivative (Fig. 3) in dry Et_2O, cooled at −30 °C. After 1 h the mixture was allowed to reach 0 °C, then a solution of (*R*/*S*)-**25** in dry Et_2O was added to obtain 1:2 molar ratio. Stirring was continued for 3 h at 0 °C and then the

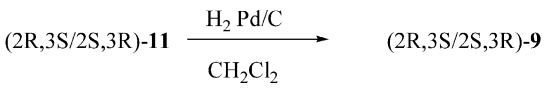
Starting materials			
14		**20**	
15		**21**	
16		**22**	
17		**23**	
18		**24**	
19		**25**	

Fig. 3.



(2R,3S/2S,3R)-**2-8**

(2R,3S/2S,3R)-**10-13**



Scheme 1.

reaction mixture was treated with water. With regard to compounds (2*R*,3*S*/2*S*,3*R*)-**2**, (2*R*,3*S*/2*S*,3*R*)-**3** and (2*R*,3*S*/2*S*,3*R*)-**12**, the organic phase was separated from the aqueous one, that contained a white solid in the form of a very fine suspension which was filtered off.

Regarding compounds (2*R*,3*S*/2*S*,3*R*)-**4**–**8**,**10**,**11**,**13** the aqueous phase was separated and extracted with Et_2O. The combined organic phases were extracted with 5% DL-tartaric acid aqueous solution and the acidic layer was made alkaline (pH 8) with $NaHCO_3$ [3]. After extraction with CH_2Cl_2 and evaporation of the solvent, the expected compounds were obtained as white solids.

After crystallization from proper solvents, all compounds were transformed into the corresponding hydrochlorides and crystallized.

*2.1.1. (2R,3S/2S,3R)-1,2-Dimethyl-3-hydroxy-3-(1-naphthyl)-pyrrolidine **2** [(2R,3S/2S,3R)-**2**]*

Yield 54.9%. White crystals, m.p. 133–135 °C [$(CH_3)_2CHOH$]. (2*R*,3*S*/2*S*,3*R*)-**2**·HCl. White crystals, m.p. 239–240 °C [$(CH_3)_2CHOH$]; $R_f = 0.56$; IR (Nujol): cm^{-1} 3267, 2922, 2483, 1378, 1187, 1022, 932, 777; ^1H NMR (CD_3OD): δ 1.55 (d, 3H, C*H*$_3$–CH, $J = 6.4$); 2.51 (ddd, 1H, CH*H*–CH_2–N, $J_{gem} = 12.1$ $J_{vic} = 2.8$–7.2), 2.88 (ddd, 1H, C*H*H–CH_2–N, $J_{gem} = 13.4$ $J_{vic} = 4.9$–6.4), 3.04 (s, 3H, CH_3–N), 3.40 (dt, 1H, CH*H*–N, $J_{gem} = 10.9$ $J_{vic} = 8.2$–8.2), 3.95 (dt, 1H, CH*H*–N, $J =$ 8.12), 4.25 (q, 1H, C*H*CH_3, $J = 6$), 7.45–7.51 (m, 4H, aromatic, $J = 9.1$), 7.87 (m, 2H, aromatic, $J = 7.42$), 8.76 (d, 1H, aromatic, $J = 7.48$). *Anal.* $C_{16}H_{20}ClNO$.

*2.1.2. (2R,3S/2S,3R)-1,2-Dimethyl-3-hydroxy-3-(9-phenantrenyl)-pyrrolidine **3** [(2R,3S/2S,3R)-**3**]*

Yield 56.8%. White crystals, m.p. 168–169 °C (CH_3CH_2OH). (2*R*,3*S*/2*S*,3*R*)-**3**·HCl. White crystals, m.p. 173–181 °C, [$(CH_3)_2CHOH$]; $R_f = 0.38$; IR (Nujol): cm^{-1} 3209, 2924, 1735, 1599, 1285, 950, 816, 767; ^1H NMR (CD_3OD): δ 1.55 (d, 3H, C*H*$_3$–CH, $J = 6.36$), 2.50 (ddd, 1H, CH*H*–CH_2–N, $J_{gem} = 12.1$ $J_{vic} = 3.9$–7.6), 2.92 (ddd, 1H, C*H*H–CH_2–N, $J_{gem} = 13.42$ $J_{vic} =$ 4.6–8.8), 3.05 (s, 3H, CH_3–N), 3.37 (dt, 1H, CH*H*–N, $J_{gem} = 10.95$ $J_{vic} = 4.24$), 3.92 (dt, 1H, CH*H*–N, $J_{gem} =$ 12 $J_{vic} = 6$), 4.37 (q, 1H, C*H*CH_3, $J = 6.4$), 7.63 (m, 4H, aromatic, $J = 7.78$, 7.72 (s, 1H, aromatic), 7.96 (d, 1H, aromatic, $J = 7.77$), 8.69 (d, 1H, aromatic, $J = 7.78$), 8.82 (m, 2H, aromatic, $J = 7.06$). *Anal.* $C_{20}H_{22}ClNO$.

*2.1.3. (2R,3S/2S,3R)-1,2-Dimethyl-3-hydroxy-3-phenyl-pyrrolidine **4** [(2R,3S/2S,3R)-**4**]*

Yield 34.6%. Yellow crystals, m.p. 78–79 °C (CH_3CH_2OH 1/H_2O 1 v/v). (2*R*,3*S*/2*S*,3*R*)-**4**·HCl. Yellow crystals, m.p. 201–202 °C (CH_3COCH_3), $R_f =$ 0.19; IR (Nujol): cm^{-1} 3223, 2923, 1895, 1599, 1317, 1230, 973, 754; ^1H NMR (CD_3OD): δ 1.16 (d, 3H, C*H*$_3$–CH, $J = 6.71$), 2.27 (ddd, 1H, CH*H*–CH_2–N, $J_{gem} = 12.72$ $J_{vic} = 3.5$–8.5), 2.69 (ddd, 1H, C*H*H–CH_2–N, $J_{gem} = 10.3$ $J_{vic} = 2.12$–8.5), 2.99 (s, 3H, N–CH_3), 3,46 (dt, 1H, CH*H*–N, $J_{gem} = 11.3$ $J_{vic} = 3.5$), 3.68 (q, 1H, C*H*–CH_3, $J = 6.4$), 3.92 (dt, 1H, C*H*H–N, $J_{gem} = 12.01$ $J_{vic} = 2.83$–8.12), 7.30 (t, 1H, aromatic, $J = 7.42$),7.40 (m, 2H, aromatic, $J = 7.77$), 7,57 (m, 2H, aromatic, $J = 7.77$). *Anal.* $C_{12}H_{18}ClNO$.

*2.1.4. (2R,3S/2S,3R)-1,2-Dimethyl-3-hydroxy-3-(4-methylphenyl)-pyrrolidine **5** [(2R,3S/2S,3R)-**5**]*

Yield 54.33%. White crystals, m.p. 113–114 °C (CH_3OH 1/H_2O 1.5 v/v). (2*R*,3*S*/2*S*,3*R*)-**5**·HCl. White crystals, m.p. 223–224 °C (CH_3COCH_3); $R_f = 0.39$; IR (Nujol): cm^{-1} 3374, 3235, 2655, 1159, 1118, 971, 940, 724; ^1H NMR (CD_3OD): δ 1.18 (d, 3H, C*H*$_3$–CH, $J =$ 6.85), 2.26 (ddd, 1H, CH*H*–CH_2–N, $J_{gem} = 12.72$ $J_{vic} = 3.42$–8.8), 2.35 (s, 3H, CH_3), 2.68 (ddd, 1H, C*H*H–CH_2–N, $J_{gem} = 11.25$ $J_{vic} = 2.45$–8.31), 2.99 (s, 3H, CH_3–N), 3.43 (dt, 1H, CH*H*–N, $J_{gem} = 11.25$ $J_{vic} = 3.42$), 3.61 (q, 1H, C*H*CH_3, $J = 6.36$), 3.92 (dt, 1H, CH*H*–N, $J_{gem} = 11.74$ $J_{vic} = 3.42$–8.31), 7.24 (d, 2H, aromatic, $J = 7.85$), 7.44 (d, 2H, aromatic, $J =$ 8.31). *Anal.* $C_{13}H_{20}ClNO$.

*2.1.5. (2R,3S/2S,3R)-1,2-Dimethyl-3-hydroxy-3-(4-trifluoromethylphenyl)-pyrrolidine **6** [(2R,3S/2S,3R)-**6**]*

Yield 68.52%. White crystals, m.p. 85–88 °C (CH_3CH_2OH 1/H_2O 1 v/v). (2*R*,3*S*/2*S*,3*R*)-**6**·HCl. White crystals, m.p. 255–256 °C (CH_3COCH_3); $R_f =$ 0.55; IR (Nujol): cm^{-1} 3243, 2673, 1375, 1326, 1164, 1069, 947, 725; ^1H NMR (CD_3OD): δ 1.20 (d, 3H, C*H*$_3$–CH, $J = 6.36$), 2.34 (ddd, 1H, CH*H*–CH_2–N, $J_{gem} = 12.72$ $J_{vic} = 3.42$–8.8), 2.76 (ddd, 1H, C*H*H–CH_2–N, $J_{gem} = 11.25$ $J_{vic} = 2.45$–8.8), 3.02 (s, 3H, CH_3–N), 3.49 (dt, 1H, CH*H*–N, $J_{gem} = 11.25$ $J_{vic} =$ 2.45), 3.71 (q, 1H, C*H*CH_3, $J = 6.36$), 3.96 (dt, 1H, CH*H*–N, $J_{gem} = 9.29$ $J_{vic} = 4.4$), 7.74 (m, 2H, aromatic, $J = 8.31$), 7.80 (m, 2H, aromatic, $J = 8.31$). *Anal.* $C_{13}H_{17}ClF_3NO$.

2.1.6. (2R,3S/2S,3R)-1,2-Dimethyl-3-hydroxy-3-(4-fluorophenyl)-pyrrolidine 7 [(2R,3S/2S,3R)-7]

Yield 67.86%. White crystals, m.p. 83–84 °C (CH_3CH_2OH 1/H_2O 1 v/v). (2*R*,3*S*/2*S*,3*R*)-**7**·HCl. White crystals, m.p. 215–216 °C (CH_3COCH_3); R_f = 0.46; IR (Nujol): cm^{-1} 3361, 3248, 2361, 1160, 1116, 972, 946, 722; ^1H NMR (CD_3OD): δ 1.19 (d, 3H, C*H*$_3$–CH, $J = 6.85$), 2.29 (ddd, 1H, CH*H*–CH_2–N, J_{gem} = 13.2 $J_{vic} = 3.9–9.3$), 2.70 (ddd, 1H, C*H*H–CH_2–N, $J_{gem} = 10.8$ $J_{vic} = 2.45–9.3$), 3.00 (s, 3H, CH_3–N), 3.45 (dt, 1H, CH*H*–N, $J_{gem} = 9.78$ $J_{vic} = 3.42$), 3.64 (q, 1H, C*H*CH_3, $J = 6.36$), 3.92 (dt, 1H, CH*H*–N, $J_{gem} = 9.78$ $J_{vic} = 2.93$), 7.16 (m, 2H, aromatic, $J = 8.8$), 7.60 (m, 2H, aromatic, $J = 5.38$). *Anal.* $C_{12}H_{17}ClFNO$.

2.1.7. (2R,3S/2S,3R)-1,2-Dimethyl-3-hydroxy-3-(4-chlorophenyl)-pyrrolidine 8 [(2R,3S/2S,3R)-8]

Yield 73.57%. White crystals, m.p. 106–107 °C (CH_3CH_2OH 1/H_2O 1.5 v/v). (2*R*,3*S*/2*S*,3*R*)-**8**·HCl. White crystals, m.p. 219–220 °C (CH_3COCH_3), R_f = 0.30; IR (Nujol): cm^{-1} 3317, 2679, 2360, 1162, 1082, 976, 946, 721; ^1H NMR (CD_3OD): δ 1.19 (d, 3H, C*H*$_3$–CH, $J = 6.85$), 2.29 (ddd, 1H, CH*H*–CH_2–N, J_{gem} = 12.72 $J_{vic} = 3.42–8.8$), 2.70 (ddd, 1H, C*H*H–CH_2–N, $J_{gem} = 10.76$ $J_{vic} = 2.45–8.8$), 2.99 (s, 3H, CH_3–N), 3.45 (dt, 1H, CH*H*–N, $J_{gem} = 10.76$ $J_{vic} = 2.45$), 3.63 (q, 1H, C*H*CH_3, $J = 6.4$), 3.93 (dt, 1H, CH*H*–N, $J_{gem} = 8.8$ $J_{vic} = 2.93$), 7.44 (m, 2H, aromatic, $J = 4.4$), 7.57 (m, 2H, aromatic, $J = 4.4$). *Anal.* $C_{12}H_{17}Cl_2NO$.

2.1.8. (2R,3S/2S,3R)-1,2-Dimethyl-3-hydroxy-3-(4-methoxyphenyl)-pyrrolidine 10 [(2R,3S/2S,3R)-10]

Yield 73.80%. White crystals, m.p. 91–92 °C (CH_3CH_2OH 1/H_2O 2 v/v). (2*R*,3*S*/2*S*,3*R*)-**10**·HCl. White crystals, m.p. 190–191 °C (CH_3COCH_3), R_f = 0.43; IR (Nujol): cm^{-1} 3223, 2654, 2360, 1174, 1106, 973, 948, 724; ^1H NMR (CD_3OD): δ 1.20 (d, 3H, C*H*$_3$–CH, $J = 6.85$), 2.28 (ddd, 1H, CH*H*–CH_2–N, J_{gem} = 12.72 $J_{vic} = 3.42–8.8$), 2.67 (ddd, 1H, C*H*H–CH_2–N, $J_{gem} = 10.76$ $J_{vic} = 2.45–8.8$), 2.91 (s, 3H, CH_3–N), 3.42 (dt, 1H, CH*H*–N, $J_{gem} = 11.25$ $J_{vic} = 3.42$), 3.60 (q, 1H, C*H*CH_3, $J = 6.85$), 3.81 (s, 3H, CH_3–O), 3.90 (dt, 1H, CH*H*–N, $J_{gem} = 8.31$ $J_{vic} = 3.91$), 7.05 (d, 2H, aromatic, $J = 8.8$), 7.33 (d, 1H, aromatic, $J = 6.85$), 7.38 (t, 2H, aromatic, $J = 7.83$), 7.45 (m, 4H, aromatic, $J = 8.8$). *Anal.* $C_{13}H_{20}ClNO_2$.

2.1.9. (2R,3S/2S,3R)-1,2-Dimethyl-3-hydroxy-3-(4-benzyloxyphenyl)-pyrrolidine 11 [(2R,3S/2S,3R)-11]

Yield 54.26%. White crystals, m.p. 162–164 °C (CH_3CH_2OH 10/H_2O 1 v/v). (2*R*,3*S*/2*S*,3*R*)-**11**·HCl. White crystals, m.p. 223–224 °C (CH_3COCH_3), R_f = 0.24; IR (Nujol): cm^{-1} 3353, 1511, 1459, 1179, 1074, 1001, 944, 724; ^1H NMR (CD_3OD): δ 1.19 (d, 3H, C*H*$_3$–CH, $J = 6.36$), 2.29(ddd, 1H, CH*H*–CH_2–N, $J_{gem} = 12.72$ $J_{vic} = 3.18–8.12$), 2.66 (ddd, 1H, C*H*H–CH_2–N, $J_{gem} = 11.30$ $J_{vic} = 2.83–9.18$), 2.97 (s, 3H, CH_3–N), 3.40 (dt, 1H, CH*H*–N, $J_{gem} = 10.60$ J_{vic} = 3.89), 3.55 (q, 1H, C*H*CH_3, $J = 6.36$), 3.89 (dt, 1H, CH*H*–N, $J_{gem} = 10.24$ $J_{vic} = 3.53$), 5.12 (s, 2H, CH_2–O), 7.05 (m, 2H, aromatic $J = 7.42$), 7.33 (m, 1H, aromatic, $J = 7.77$), 7.38 (m, 2H, aromatic, $J = 7.6$), 7.45 (m, 4H, aromatic, $J = 7.8$). *Anal.* $C_{19}H_{24}ClNO_2$.

2.1.10. (2R,3S/2S,3R)-1,2-Dimethyl-3-hydroxy-3-(4-biphenyl)-pyrrolidine 12 [(2R,3S/2S,3R)-12]

Yield 69.5%. White crystals, m.p. 135–140 °C, dec. (2*R*,3*S*/2*S*,3*R*)-**12**·HCl. White crystals, m.p. 247–248 °C [$(CH_3)_2CHOH$ 20/H_2O 1 v/v], $R_f = 0.40$; IR (Nujol): cm^{-1} 3197, 2652, 1230, 1166, 1068, 949, 839, 729; ^1H NMR (CD_3OD): δ 1.19 (d, 3H, C*H*$_3$–CH, J = 6.36), 2.29 (ddd, 1H, CH*H*–CH_2–N, $J_{gem} = 12.72$ $J_{vic} = 3.18–8.12$), 2.73 (ddd, 1H, C*H*H–CH_2–N, $J_{gem} = 11.3$ $J_{vic} = 2.93–9.18$), 2.98 (s, 3H, CH_3–N), 3.45 (dt, 1H, CH*H*–N, $J_{gem} = 10.6$ $J_{vic} = 3.89$), 3.69 (q, 1H, C*H*CH_3, $J = 6.36$), 3.92 (dt, 1H, CH*H*–N, $J_{gem} = 10.24$ $J_{vic} = 3.53$), 7.32 (m, 1H, aromatic, J = 7.42), 7.42 (m, 2H, aromatic, $J = 7.77$), 7.60 (m, 2H, aromatic, $J = 7.6$), 7.65 (m, 4H, aromatic, $J = 7.3$). *Anal.* $C_{18}H_{22}ClNO$.

2.1.11. (2R,3S/2S,3R)-1,2-Dimethyl-3-hydroxy-3-(4-dimethylaminophenyl)-pyrrolidine 13 [(2R,3S/2S,3R)-13]

Yield 45.63%. White crystals, m.p. 109–110 °C [$(CH_3)_2CHOH$ 1/H_2O 1.5 v/v]. (2*R*,3*S*/2*S*,3*R*)-**13**·2HCl. White crystals, m.p. 176–177 °C (CH_3COCH_3), $R_f = 0.37$; IR (Nujol): cm^{-1} 3449, 3309, 2638, 2361, 1118, 978, 941, 725; ^1H NMR (CD_3OD): δ 1.08 (d, 3H, C*H*$_3$–CH, $J = 6.36$), 2.22 (ddd, 1H, CH*H*–CH_2–N, $J_{gem} = 12.72$ $J_{vic} = 3.42–8.8$), 2.65 (ddd, 1H, C*H*H–CH_2–N, $J_{gem} = 11.25$ $J_{vic} = 2.45–8.8$), 2.92 (s, 3H, CH_3–N), 3.23 (s, 6H, $(CH_3)_2$–N), 3.39 (dt, 1H, CH*H*–N, $J_{gem} = 10.76$ $J_{vic} = 2.45$), 3.63 (q, 1H, C*H*CH_3, $J = 6.85$), 3.85 (dt, 1H, CH*H*–N, $J_{gem} = 8.8$ $J_{vic} = 2.45$), 7.67 (m, 2H, aromatic, $J = 8.8$), 7.74 (m, 2H, aromatic, $J = 8.8$). *Anal.* $C_{14}H_{24}Cl_2N_2O$.

2.2. (2R,3S/2S,3R)-1,2–Dimethyl-3-hydroxy-3-(4-hydroxy-phenyl)-pyrrolidine 9 [(2R,3S/2S,3R)-9]

(2*R*,3*S*/2*S*,3*R*)-**11** (15 g, 50.9 mmol) was dissolved in CH_2Cl_2 (250 ml) and Pd–C (10%, 200 mg) was added. The mixture was stirred overnight under H_2 balloon, then filtered through Celite. Evaporation of the solvent in vacuo gave pure compound. Yield 98%. White crystals, m.p. 190–193 °C. (2*R*,3*S*/2*S*,3*R*)-**9**·HCl. Pure (2*R*,3*S*/2*S*,3*R*)-**9**·HCl (white crystals, m.p. 213–215 °C) was obtained from (2*R*,3*S*/2*S*,3*R*)-**9** after treatment with 10% HCl and crystallization from CH_3COCH_3. $R_f = 0.18$; IR (Nujol): cm^{-1} 3292, 3151, 2695, 1614, 1379, 1172, 972, 693; ^1H NMR (CD_3OD): δ

1.10 (d, 3H, CH_3–CH, $J = 6.36$), 2.14 (ddd, 1H, CH*H*–CH_2–N, $J_{gem} = 12.72$ $J_{vic} = 2.93$–8.31), 2.56 (ddd, 1H, C*H*H–CH_2–N, $J_{gem} = 10.76$ $J_{vic} = 2.45$–8.31), 2.88 (s, 3H, CH_3–N), 3.31 (dt, 1H, CH*H*–N, $J_{gem} = 10.76$ $J_{vic} = 2.45$), 3.47 (q, 1H, C*H*CH_3, $J = 6.35$), 3.81 (dt, 1H, CH*H*–N, $J_{gem} = 8.8$ $J_{vic} = 2.93$), 6.74 (d, 2H, aromatic, $J = 8.8$), 7.28 (d, 2H, aromatic, $J = 8.31$). *Anal.* $C_{12}H_{18}ClNO_2$.

3. Pharmacological experimental section

3.1. In vitro assays

3.1.1. Materials

^{3}H-DAMGO (S.A = 50 Ci/mmol), ^{3}H-Nociceptin (S.A = 87.7 Ci/mmol), ^{3}H-Naltrindole (S.A = 50 Ci/mmol), ^{3}H-U69593 (S.A = 40 Ci/mmol), were purchased from NEN Perkin–Elmer Life Sciences. All other substances and reagents were purchased from Sigma–Aldrich.

3.1.2. Methods

Brain cellular membranes of male Hartley guinea pig (Charles River, Calco, Italy) were prepared according to Wang et al [7].

The crude membrane preparation was suspended in cold Tris–Cl 50 mM, $MgCl_2$ 5 mM, pH 7.4 and further diluted in suitable buffer solutions. Buffer binding solutions were composed as follows: Tris–Cl 50 mM, $MgCl_2$ 5 mM, pH 7.4 (for μ-receptors binding assay), Tris–Cl 50 mM, $MgCl_2$ 5 mM, PMSF 100 μM (for δ-receptors binding assay), Tris–Cl 50 mM, $MgCl_2$ 5 mM, bestatin 30 μM, bovine serum albumine (BSA) 0.1 mg/ml, Bacitracin 0.1 mg/ml (for k-receptors binding assay) and Tris–Cl 50 mM, $MgCl_2$ 5 mM, EDTA 2 mM, PMSF 100 μM, BSA 1 mg/ml (for ORL1-receptors binding assay).

Different concentrations of the competing agent under investigation were incubated with an aliquot of 1 ml of tissue suspension (0.5 mg/ml protein) and selective radioligands for μ-receptors (0.5 nM ^{3}H-DAMGO), δ-receptors (0.3 nM ^{3}H-Naltrindole), k-receptors (1.5 nM ^{3}H-U69593) and ORL1-receptors (0.1 nM ^{3}H-Nociceptin). The protein concentration was determined by the method of Bradford [8], using BSA as a standard.

Incubation (2 ml final volume) was performed in polystirene microplates (6 × 4) at 25 °C for 60 min (120 min for ^{3}H-Nociceptin binding assay). The bound radioligand was separated by rapid filtration on Unifilter GFB/24 plates (Packard Biosciences), pre-coated in Tris–Cl 50 mM pH 7.4 buffer or PEI 0.1%/Tris–Cl 50 mM pH 7.4 (for ^{3}H-Naltrindole and ^{3}H-Nociceptin binding assays). Filtrates were washed three times with 2.5 ml of ice cold buffer. The filter disks were dried for 30 min at 30 °C and then 0.2 ml of MICROSCINT-20 (Packard Biosciences) were added. Plates were counted after at least 1 h of stabilization in a solid scintillation spectrometer (TopCount, Packard Biosciences; 40% efficiency).

The specific binding of radioligands (except for ^{3}H-Nociceptin) was determined as the difference between binding in the absence or presence of 10 μM Naloxone. It represents about 95 (μ-receptor), 55 (δ-receptor) and 60% (k-receptor) of total binding. The specific binding of ^{3}H-Nociceptin (about 98%) was calculated subtracting the residual binding, in the presence of 1 μM Nociceptin, from the total binding.

3.2. In vivo assays

3.2.1. Animals

The experiments were carried out on male adult Swiss mice (30 ± 5 g). Control and experimental groups consisted of 8–10 animals each. The investigated compounds as hydrochlorides were dissolved in saline solution and administered subcutaneously within 1 h of dissolution. Morphine·HCl was used as a standard antinociceptive agent in order to make a suitable comparison with the tested compounds.

3.2.2. Hot plate test (HPT)

The HPT was employed to assess the antinociceptive effects. The response to a thermal stimulus was evaluated using a copper plate heated to 55 °C and proceeding according to a previously reported method [3]. Mouse responded by sitting on its hind legs and licking. The experiment was conducted on mice treated with increasing doses of the compound. The reaction time to the pain stimulus was measured 20 min after the injection. The reaction time of the control animals was 20.8 ± 0.3 s. AD_{50} mg/kg were calculated using a computerized program [9]. Experimental data are reported in Table 1.

3.2.3. Rota-rod test (RRT)

The integrity of motor coordination was controlled with RRT on mice after treatment with the investigated substances [5]. Randomly selected mice, were examined 15 min. after treatment with doses corresponding to AD_{50} of HPT. Results (Table 1) are expressed as the percentage of mice remaining on the rod during a 30 s period.

3.2.4. Formalin test (FT)

Nociception was induced by injecting of 20 μl dilute formalin (1% in saline solution) under the skin of the dorsal surface of the hind paw of the mouse [10]. The licking of the treated hind paw was used as a measure of nociception. Each mouse was injected with formalin 10 min after being pretreated with analgesic compounds

Table 1
Analgesic activity in the hot plate test and locomotory activity in the rota-rod test

Comp.	HPT (55 °C)	RRT
	AD_{50} mg/kg (confidence limits)	% of mice which remain on the rod after 30 s
Morfina·HCl·$3H_2O$	4.18 (3.11–5.60)	63 *
(2*R*,3*S*/2*S*,3*R*)-**1**·HCl	0.18 (0.05–0.69)	100
(2*R*,3*S*/2*S*,3*R*)-**2**·HCl	1.63 (0.64–4.17)	87 *
(2*R*,3*S*/2*S*,3*R*)-**3**·HCl	10.04 (6.30–15.99)	50 *
(2*R*,3*S*/2*S*,3*R*)-**4**·HCl	0.82 (0.16–4.09)	100
(2*R*,3*S*/2*S*,3*R*)-**5**·HCl	0.55 (0.15–1.93)	63 *
(2*R*,3*S*/2*S*,3*R*)-**6**·HCl	4.82 (2.80–8.30)	44 *
(2*R*,3*S*/2*S*,3*R*)-**7**·HCl	1.87 (0.80–4.38)	100
(2*R*,3*S*/2*S*,3*R*)-**8**·HCl	0.10 (0.06–0.71)	88 *
(2*R*,3*S*/2*S*,3*R*)-**9**·HCl	0.98 (0.80–1.20)	75 *
(2*R*,3*S*/2*S*,3*R*)-**10**·HCl	0.74 (0.26–2.09)	100
(2*R*,3*S*/2*S*,3*R*)-**11**·HCl	0.90 (0.78–1.03)	89 *
(2*R*,3*S*/2*S*,3*R*)-**12**·HCl	0.58 (0.32–1.05)	100
(2*R*,3*S*/2*S*,3*R*)-**13**·2HCl	1.36 (0.51–3.61)	89 *

* $P < 0.05$.

and then placed into a transparent plastic cage and observed. The licking response was monitored until 30 min starting immediately after the injection of formalin. Experiments were performed on two separate groups of animals: the control group, receiving only formalin, and the treated one receiving formalin and compounds (2*R*,3*S*/2*S*,3*R*)-**4**, (2*R*,3*S*/2*S*,3*R*)-**8**, (2*R*,3*S*/2*S*,3*R*)-**11** administered at maximal doses tested with HPT (6, 2, 4 mg/kg, respectively). Antinociception was defined as a statistically significant reduction in the time spent licking, in comparison with the vehicle control group during the early (0–5 min.) and late phase (20–25 min.). Results obtained are expressed as licking time in seconds.

3.2.5. Statistical analysis

The one-way ANOVA test was used to calculate the significance of the difference among the AD_{50} values (HPT) of the examined compounds. The results of RRT and FT were expressed as means ± SEM and the means were compared using Student's *t*-test, * (*P* values < 0.05) or ** ($P < 0.01$) being considered as statistically significant or highly significant, respectively.

All the statistical analyses were performed using the statistical software package SYSTAT.

4. Results and discussion

The syntheses of racemic compounds (2*R*,3*S*/2*S*,3*R*)-**2**–**13** were accomplished (Scheme 1) following the same procedure previously described for analogous compounds [4]. The pyrrolidinols (2*R*,3*S*/2*S*,3*R*)-**2**–**8** and (2*R*,3*S*/2*S*,3*R*)-**10**–**13** were prepared by reacting compounds **14**–**24** with *tert*-BuLi and followed by nucleophylic condensation with racemic 1,2-dimethyl-3-pyrrolidone (*R*,*S*)-**25** [6]. According to previous findings [4], the reaction mechanism led only to the racemic couples (2*R*,3*S*/2*S*,3*R*), as evidenced by ^{1}H NMR analysis of the crude products. All compounds were purified by crystallization from proper solvents and transformed into the biologically compatible and water soluble hydrochloride salts. The catalytic reduction of pure pyrrolidinol (2*R*,3*S*/2*S*,3*R*)-**11**·HCl gave compound (2*R*,3*S*/2*S*,3*R*)-**9**·HCl. The results of elemental analyses (Table 2), IR and ^{1}H NMR spectra confirmed the expected structures for all compounds.

In order to understand the SAR and predict the bioavailability, pharmacokinetic properties and in vivo distribution, the hydrophobicity of all compounds was estimated by calculating log *P*. In fact, lipophilicity is known to have a relevant influence on the pharmacokinetic profile of drugs and, as a consequence, on their biological activity [11,12]. The log *P* values of the examined compounds were calculated using the Daylight computational method (version 4.01) that combines fragment lipophilicity contributions carefully parameterised with experimental data and calculated 'from scratch' values [13]. We had already validated this method for analogue compounds by comparison with chromatographic hydrophobicity values [14].

The pyrrolidinols (2*R*,3*S*/2*S*,3*R*)-**1**–**13** have clog *P* values ranging from 1.17 to 3.68 (Table 3), except for compound (2*R*,3*S*/2*S*,3*R*)-**3** with a clog *P* of 4.14. It is known that in general the optimal value of log *P* for CNS agents is 2 ± 0.5 [12]. However, compounds such as morphine (clog *P* 0.48), methadone (clog *P* 2.97) and phentanyl (clog *P* 3.81) have a high analgesic activity even though their clog *P* are out of the above-mentioned

Table 2
Analytical data for the synthesized compounds

Comp.	Molecular formula	Found (required) (%)		
		C	H	N
(2*R*,3*S*/2*S*,3*R*)-**2**·HCl	$C_{16}H_{20}ClNO$	68.96 (69.18)	7.18 (7.26)	4.99 (5.04)
(2*R*,3*S*/2*S*,3*R*)-**3**cHCl	$C_{20}H_{22}ClNO$	73.45 (73.27)	6.67 (6.76)	4.51 (4.27)
(2*R*,3*S*/2*S*,3*R*)-**4**·HCl	$C_{12}H_{18}ClNO$	63.01 (63.29)	8.03 (7.97)	6.22 (6.15)
(2*R*,3*S*/2*S*,3*R*)-**5**·HCl	$C_{13}H_{20}ClNO$	64.21 (64.59)	8.18 (8.34)	5.65 (5.79)
(2*R*,3*S*/2*S*,3*R*)-**6**·HCl	$C_{13}H_{17}ClF_3NO$	52.95 (52.80)	5.58 (5.79)	4.83 (4.74)
(2*R*,3*S*/2*S*,3*R*)-**7**·HCl	$C_{12}H_{17}ClFNO$	58.89 (58.66)	7.02 (6.97)	5.75 (5.70)
(2*R*,3*S*/2*S*,3*R*)-**8**·HCl	$C_{12}H_{17}Cl_2NO$	54.93 (54.97)	6.63 (6.54)	5.18 (5.34)
(2*R*,3*S*/2*S*,3*R*)-**9**·HCl	$C_{12}H_{18}ClNO_2$	58.98 (59.13)	7.23 (7.44)	5.43 (5.73)
(2*R*,3*S*/2*S*,3*R*)-**10**·HCl	$C_{13}H_{20}ClNO_2$	60.72 (60.58)	7.66 (7.82)	5.19 (5.43)
(2*R*,3*S*/2*S*,3*R*)-**11**·HCl	$C_{19}H_{24}ClNO_2$	68.12 (68.35)	6.99 (7.25)	4.37 (4.20)
(2*R*,3*S*/2*S*,3*R*)-**12**·HCl	$C_{18}H_{22}ClNO$	71.12 (71.16)	7.44 (7.30)	4.58 (4.61)
(2*R*,3*S*/2*S*,3*R*)-**13**·2HCl	$C_{14}H_{24}Cl_2N_2O$	54.55 (54.73)	7.93 (7.87)	9.15 (9.12)

range. Therefore, our compounds possess lipophilic properties suitable for in vivo investigation.

Analgesic activity was investigated in mice using HPT (AD_{50} values were determined using a computerized program) in order to define the pharmacological profile of (2*R*,3*S*/2*S*,3*R*)-**2**–**13**·HCl. The influence on the locomotory activity of the animals was determined with the RRT to exclude motor changes which were associated with the analgesic effect.

According to the experimental data (Table 1, Fig. 4), all new pyrrolidinols [except compound (2*R*,3*S*/2*S*,3*R*)-**3** with $AD_{50} = 10.04$ (6.30–15.99) mg/kg] show promising antinociceptive properties [AD_{50} values: from 4.82 (2.80–8.30) of compound **6** to 0.10 (0.06–0.71) mg/kg of compound **8**] with a potency similar or superior to that of morphine [$AD_{50} = 4.18$ (3.11–5.80) mg/kg]. Their maximal effect (E_{max}) ranges from 41.8 + 1.0 to 55.5 + 0.5 s.

The aromatic moiety influences in vivo activity: the response to the pain stimulus was substantially affected by its structural features. The most hindered and lipophilic compound (2*R*,3*S*/2*S*,3*R*)-**3** shows the least analgesic activity. The substituent on the aromatic moiety also influences the in vivo activity. Compound (2*R*,3*S*/2*S*,3*R*)-**8** was proved to be the most potent of the series with an AD_{50} value of 0.10 mg/kg. As regards the maximal effect (E_{max}), the most interesting compounds are (2*R*,3*S*/2*S*,3*R*)-**6** and (2*R*,3*S*/2*S*,3*R*)-**11**, with an E_{max} of 55.5 ± 0.5 and 54.4 ± 0.4 s. respectively, comparable with that of morphine (59.8 ± 0.2).

Moreover, RRT data highlights that the animal's locomotory control is not significantly affected by the compounds with the exception of the trifluoromethyl derivative **6** (Table 1).

Therefore, we selected the compounds (2*R*,3*S*/2*S*,3*R*)-**8** and (2*R*,3*S*/2*S*,3*R*)-**11**, bearing a chlorine atom and a benzyloxy group on the aromatic moiety, respectively, for further in vitro and in vivo investigation of their biological activity. The study was also extended to unsubstituted (2*R*,3*S*/2*S*,3*R*)-**4**, analogous to the hit compound **1**.

A preliminary in vitro screening with μ, κ, δ and ORL1 opioid receptors was performed owing to the great relevance of the opioid system's activation in the analgesic process. However, the experimental data suggests a low involvement of opioid receptors. In

Table 3
log *P* calculated (clog *P*) by Daylight method

Comp.	clog *P*
(2*R*,3*S*/2*S*,3*R*)-**1**	2.97
(2*R*,3*S*/2*S*,3*R*)-**2**	2.96
(2*R*,3*S*/2*S*,3*R*)-**3**	4.14
(2*R*,3*S*/2*S*,3*R*)-**4**	1.79
(2*R*,3*S*/2*S*,3*R*)-**5**	2.29
(2*R*,3*S*/2*S*,3*R*)-**6**	2.68
(2*R*,3*S*/2*S*,3*R*)-**7**	1.94
(2*R*,3*S*/2*S*,3*R*)-**8**	2.51
(2*R*,3*S*/2*S*,3*R*)-**9**	1.13
(2*R*,3*S*/2*S*,3*R*)-**10**	1.71
(2*R*,3*S*/2*S*,3*R*)-**11**	3.48
(2*R*,3*S*/2*S*,3*R*)-**12**	3.68
(2*R*,3*S*/2*S*,3*R*)-**13**	1.96

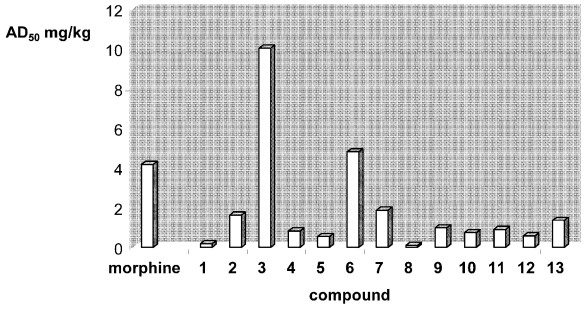


Fig. 4. AD_{50} (mg/kg) data for compounds (2*R*,3*S*/2*S*,3*R*)-**1**–**13** and morphine in the HPT.

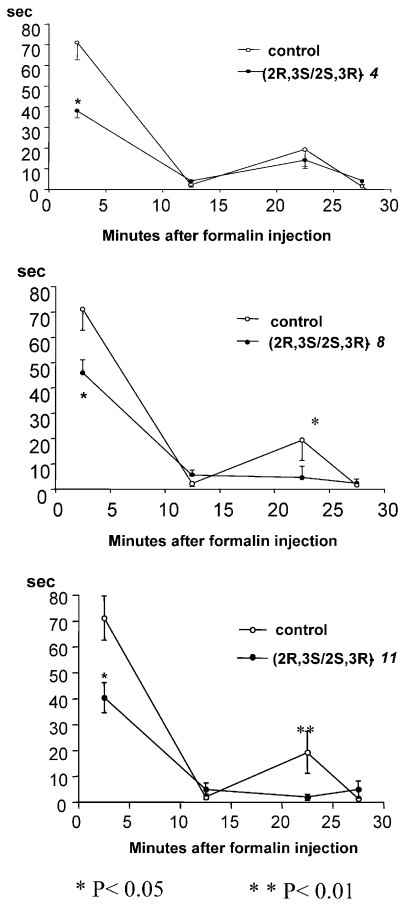


Fig. 5. Biological properties of (2*R*,3*S*/2*S*,3*R*)-**4**, **8**, **11** in the formalin test.

fact, all compounds showed a very poor affinity to opiate receptors (μM order of K_i values). These compounds will be screened by in vitro assays for the binding of targets involved in pain.

The formalin test in mice was performed in order to acquire more information about their mechanism of action. This test was chosen because it measures the response to a long-lasting nociceptive stimulus. The formalin test response of compounds (2*R*,3*S*/2*S*,3*R*)-**4**, (2*R*,3*S*/2*S*,3*R*)-**8**, (2*R*,3*S*/2*S*,3*R*)-**11** is reported in Fig. 5. All compounds show a statistically significant ($P < 0.05$) pain reduction in the early phase, demonstrating an antinociceptive activity. It is evident that compounds (2*R*,3*S*/2*S*,3*R*)-**8** and (2*R*,3*S*/2*S*,3*R*)-**11** are active also in the late phase ($P < 0.05$ and 0.01, respectively) suggesting their involvement in the inflammatory process.

In conclusion, pyrrolidinols (2*R*,3*S*/2*S*,3*R*)-**8** and (2*R*,3*S*/2*S*,3*R*)-**11** show an interesting pharmacological profile, as they have a potent antinociceptive activity and a good influence on the inflammatory process, with no influence on the locomotory control. Therefore, they will be further investigated; it would also be useful to study the corresponding enantiomers to draw a more complete profile of the biological properties in order to determine their mechanism of action.

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References

[1] M. Williams, E.A. Kowaluk, S.P. Arneric, Emerging molecular approaches to pain therapy, J. Med. Chem. 42 (1999) 1481–1500.
[2] V. Ghislandi, S. Collina, O. Azzolina, E. Paroli, L. Antonilli, G. Giuseppetti, C. Tadini, Preparation and configuration of racemic and optically active analgesic dialkylaminoalkylnaphthalenes, Chirality 6 (1994) 389–399.
[3] S. Collina, O. Azzolina, D. Vercesi, F. Benevelli, A. Callegari, C. Tadini, E. Lanza, A. Barbieri, V. Ghislandi, Dialkylaminoalkylnaphthalenes as novel opioid-like analgesics, Bioorg. Med. Chem. 8 (2000) 1–7.
[4] S. Collina, O. Azzolina, D. Vercesi, M. Sbacchi, M.A. Scheideler, A. Barbieri, E. Lanza, V. Ghislandi, Synthesis and antinociceptive activity of pyrrolidinylnaphthalenes, Bioorg. Med. Chem. 8 (2000) 1925–1930.
[5] S. Collina, O. Azzolina, D. Vercesi, A. Barbieri, E. Lanza, V. Ghislandi, Synthesis of pyrrolinylnaphthalenes and evaluation of their antinociceptive activity, Farmaco 55 (2000) 611–618.
[6] S. Collina, A. Gamba, V. Ghislandi, O. Azzolina, Preparation of (+)- and (−)-1,2-dimethyl-3-pyrrolidone and stability studies, Chirality 7 (1995) 439–445.
[7] Y.X. Wang, D.C. Geoffrey, M. Sbacchi, G. Petrone, D.P. Brooks, Contribution of alpha-2 adrenoceptors to kappa opioid agonist-induced water diuresis in the rat, J. Pharmacol. Exp. Ther. 1 (1994) 244–249.
[8] M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding, Anal. Biochem. 72 (1976) 248–254.
[9] R.J. Tallarida, R.B. Murray, Manual of Pharmacologic Calculation with Computer Programs, Springer, Berlin, 1987.
[10] C.W Murray, F. Porreca, A. Cowan, Methodological refinements to the mouse paw formalin test. An animal model of tonic pain, J. Pharm. Methods 20 (1988) 175–186.
[11] K. Valkò, C. Bevan, D. Reynolds, Chromatography hydrophobicity index by fast-gradient RP-HPLC: a high-throughput alternative to log *P*/log *D*, Anal. Chem. 69 (1997) 2022–2029.
[12] C. Hansch, J.P. Bjorkroth, A. Leo, Hydrophobicity and central nervous system agents: on the principles of minimal hydrophobicity in drug design, J. Pharm. Sci. 76 (1987) 663–697.
[13] A.J. Leo, D. Hoekman, Calculating log *P*(oct) with no missing fragments; the problem of estimating new interaction parameters, Perspectives in Drug Discovery and Design 18 (2000) 19–38.
[14] O. Azzolina, S. Collina, D. Rossi, S. Alcaro, V. Ghislandi, Experimental and computational approaches to estimate the hydrophobicity of antinociceptive agents, First Magna Grecia Medicinal Chemistry Workshop on New Perspectives in Drug Research, Copanello, Catanzaro, Italy, 10–13 June, 2001.