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Dialkylaminoalkylnaphthalenes as Novel Opioid-Like Analgesics

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Abstract—The study of dialkylaminoalkylnaphthalenes as novel opioid-like analgesics is reported. In particular, the synthesis of (1*R*,2*R*/1*S*,2*S*)-1-ethyl-1-[2-(6-hydroxynaphthyl)]-1-hydroxy-2-methyl-2-dimethylaminoethane and its structural analogue (1*R*,2*R*/1*S*,2*S*)-1-ethyl-1-[2-(6-fluoronaphthyl)]-1-hydroxy-2-methyl-2-dimethylaminoethane and the configurational analysis by X-ray and ^{1}H NMR spectroscopy are described. Pharmacological profiles are discussed on the basis of the experimental results of analgesia tests (hot plate and writhing test) and rota-rod test, which was performed to distinguish analgesia from drug-induced motor changes. The compounds showed dose-dependent antinociception, with less potency than morphine. Motor coordination appeared to be less involved.

Introduction

It is well known that specific, but multiple, opiate receptors are present in the central nervous system (CNS) and that the activation of κ and δ specific subsets elicits analgesia with a less extent of side effects respect to the current opioid analgesics (μ agonists). Therefore the design of a safer analgesic will depend on defining the structural attributes which contribute to selective high affinity for opiate receptors subpopulations. For example, during the past decade considerable efforts have been addressed, towards the development of κ selective opioid agonists because they are potential analgesics without the significant clinical side effects associated with morphine and other μ receptor selective analgesic drugs.[1–3] The development of ligands, which are highly selective for each receptor type, is thus an important goal, since these ligands could be very useful for investigating the biological effects produced by the involvement of different receptors and can be potential therapeutic agents.

Furthermore, apart from the direct activation of specific opiate receptors, antinociceptive effects can also derive either by an interaction with receptors having a stimulatory activity on an opioid neuron, such as Cholecystokinin[4] and neuropeptide FF analogue,[5] either by an inhibition at enzymatic level of opioid peptides degradation. There has been much research carried out to develop antagonists able to inactivate the enzymes responsible for degradation of these peptides.[6–9]

Our research in the past years has been focused on the design, synthesis and pharmacological evaluation of molecules that are useful for characterizing the mechanism of the analgesic activity and possibly to develop analgesic compounds as potential safe and effective drugs.

The results of our investigation on dialkylaminoalkylnaphthalenic and cycloaminoalkylnaphthalenic compounds have already been published. We showed that some compounds of both classes possess analgesic antinociceptive activity superior or comparable to that of morphine, as shown by AD_{50} values in the hot plate test.[10–12]

In particular, research on dialkylaminoalkylnaphthalenic compounds—(1*R*,2*R*/1*S*,2*S*)-1-ethyl-1-hydroxy-1-[2-(6-hydroxynaphthyl)]2-methyl-3-dimethylaminopropane **1** and its enantiomers (Fig. 1)—showed that (1*R*,2*R*)-**1** induces opioid-like analgesia with a relative potency that was 2.5-fold greater than that of (1*R*,2*R*/1*S*,2*S*)-**1**

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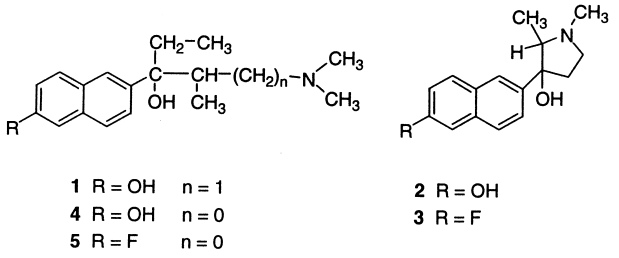


Figure 1.

and 4-fold than that of morphine, while the (1*S*,2*S*)-**1** appeared to have no activity.[10] These results agree with the stereospecificity of CNS opiate receptors.[13]

Furthermore our investigation on cycloaminoalkylnaphthalenes showed that *N*-methylpyrrolidinylnaphthalenic compounds (2*R*,3*S*/2*S*,3*R*)-**2**, (2*R*,3*S*/2*S*,3*R*)-**3** and their enantiomers possess antinociceptive activity depending on the involvement of the opiate receptor system, as evidenced by in vivo antagonist studies, showing that the antinociception is reversed by naloxone.[12] Slight and opposite stereoselectivity of the enantiomers was also evidenced.[11,12]

Owing to the superior activity of dialkylaminoalkylnaphthalenes with respect to cycloaminoalkylnaphthalenes, we proposed to acquire more information regarding the activity of compounds of the first class.

This paper reports the study of the structure–activity relationship of (1*R*,2*R*/1*S*,2*S*)-1-ethyl-1-[2-(6-hydroxynaphthyl)]-1-hydroxy-2-methyl-2-dimethylaminoethane [(1*R*,2*R*/1*S*,2*S*)-**4**] and its structural analogue (1*R*,2*R*/1*S*,2*S*)-1-ethyl-1-[2-(6-fluoronaphthyl)]-1-hydroxy-2-methyl-2-dimethylaminoethane [(1*R*,2*R*/1*S*,2*S*)-**5**] (Fig. 1), which allowed us to investigate the influence of the structural features of the aliphatic moiety as well as the role of the substituent (OH or F) of the naphthalene nucleus on the pharmacological properties.

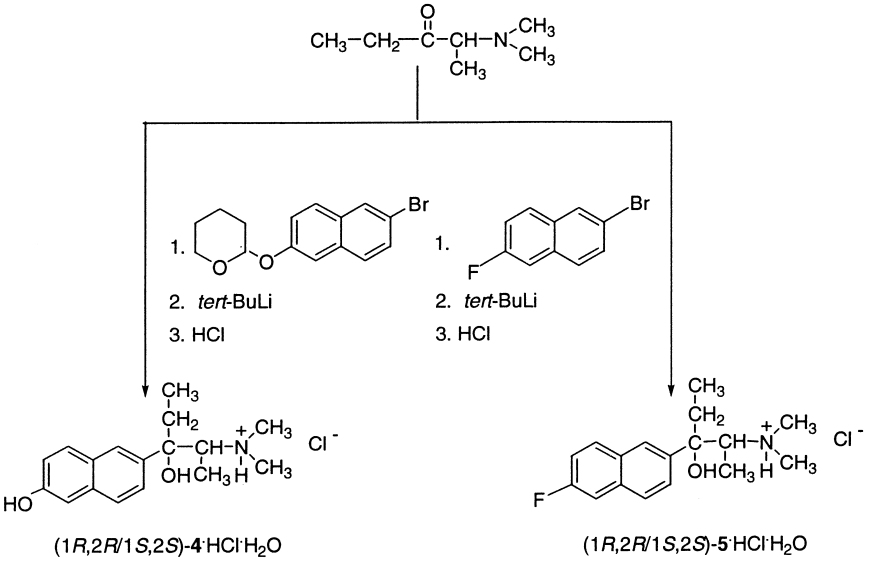
The F atom is only an acceptor of hydrogen bonding and not a donor and, furthermore, it influences the lipophilicity of the molecule, a factor that could have some relevance in the biological activities.

The synthesis, chemical characterization, configurational analysis and in vivo and in vitro studies of compounds (1*R*,2*R*/1*S*,2*S*)-**4** and (1*R*,2*R*/1*S*,2*S*)-**5** are reported. The configuration of the compounds was assigned by X-ray crystallographic analysis and ^{1}H NMR spectroscopy. Antinociceptive activity was evaluated by the hot plate test (HPT) and writhing test (WT). Rota-rod test (RRT) was performed to investigate the possible effects of the compounds on motor coordination. Binding affinity of the compounds versus μ, δ and κ opioid receptors was also tested by in vitro opioid binding assays. The involvement of the opioid system was investigated by the nonselective antagonist naloxone in the HPT.

Results and Discussion

Compounds **4** and **5** were synthesized following the procedure that we had previously used for the synthesis of **1**.[10] By reaction of (*R*,*S*)-2-dimethylamino-3-pentanone[14] with 2-lithium-6-(2-tetrahydropyranyloxy)naphthalene and 2-lithium-6-fluoronaphthalene,[12] respectively, the racemic couples (1*R*,2*R*/1*S*,2*S*)-**4** and -**5** were obtained (Scheme 1). That was proved by X-ray crystallographic analysis (Fig. 2) as well as by NOE experiments in ^{1}H NMR spectroscopy which allowed us to confirm what expected: the nucleophilic attack of the naphthalenic anion at the prochiral carbon atom of (*R*,*S*)-2-dimethylamino-3-pentanone occurs on the less hindered side of its chelate with Li^+ (the *re* side for the example of Figure 3).

The compounds were characterized by elemental analysis and IR and ^{1}H NMR spectroscopy. Differential scanning calorimetry (DSC) and thermogravimetry (TG)



Scheme 1.

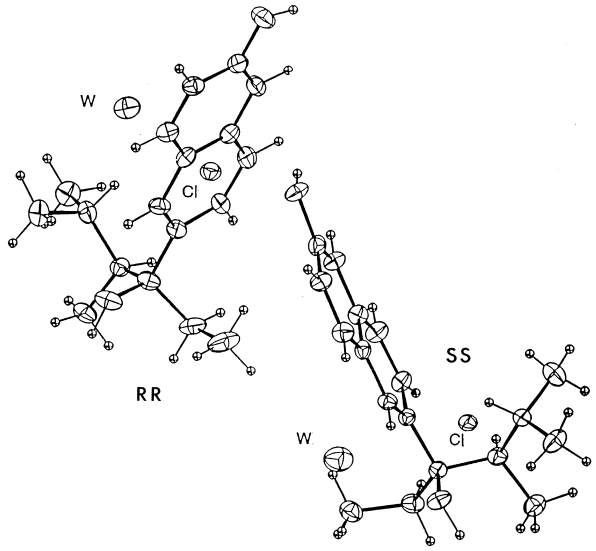


Figure 2. (1*R*,2*R*/1*S*,2*S*)-**4**·HCl·H_2O: ORTEP drawing of the *a* and *b* systems in the asymmetric unit with 30% probability ellipsoids.

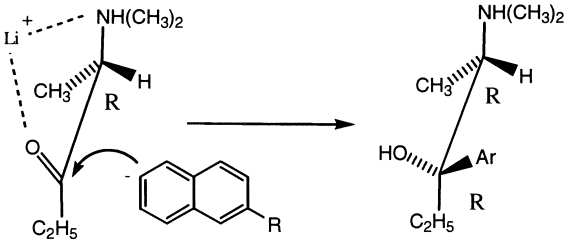


Figure 3. Conformation of the chelate of (*R*)-2-dimethylamino-3-pentanone with Li^+ and configuration expected for the new chiral center of the aminoalcohols **4** and **5**.

showed that (1*R*,2*R*/1*S*,2*S*)-**4** and -**5** hydrochlorides are monohydrates.

The NOE spectrum of compound **4** was accomplished and compared with that of the superior homologous **1.** The most evident effects are reported in Figure 4.

Owing to the presence of significative NOE effects corresponding to the interactions 5–7, 6–7, 5–9, 6–9 and 9–7, the absolute configuration *R*,*R* or *S*,*S* was confirmed for the compound **4**, in agreement with the results of crystallographic analysis. Analogously the absolute configuration *R*,*R* or *S*,*S* was shown for the compound **1** on the basis of the NOE effects 7–10 and 8–10.

The weaker NOE effects of compound **1** (with respect to **4**) suggest a superior mobility of the molecule, depending from the rotational freedom, around the C1–C2′ bond, which is confirmed by the presence of both the interactions 7–6 and 9–6.

Pharmacological profiles of (1*R*,2*R*/1*S*,2*S*)-**4** and -**5** were evaluated by in vivo and in vitro assays extending the investigation also to (1*R*,2*R*/1*S*,2*S*)-**1**, whose activity had already been tested with HPT[10] only. Analgesia was tested in animal models by monitoring behavioral responses subsequent to thermal (HPT) and chemical (WT) painful stimuli (Tables 1 and 2). Owing to the

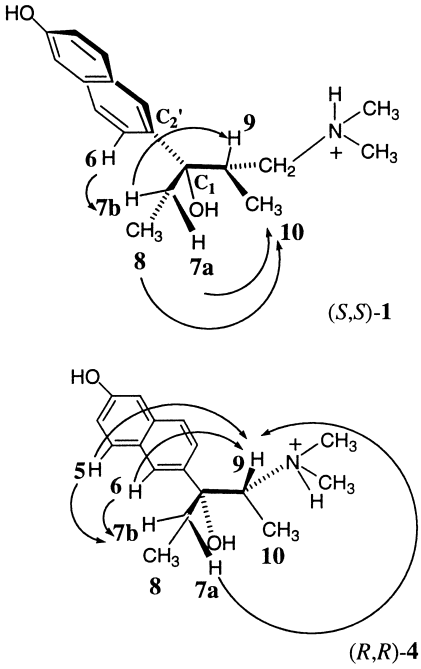


Figure 4. The most significative NOE-effects evidenced for (*S*,*S*)-**1** and (*R*,*R*)-**4**.

sensitivity of both HPT and WT to sedatives and muscle relaxant and, consequently, to the possibility of generating false positives, RRT was used in parallel to distinguish analgesia from drug-induced motor changes (Table 3).

Compounds **1**, **4** and **5** produce a dose-dependent antinociception in the mouse HPT yielding AD_{50} values (μmol/kg) of 13.4, 35.8 and 19.5, respectively; compounds **4** and **5** are less potent than analogue **1** and morphine (AD_{50} 14.3). These results suggest that the shortening of the carbon chain of compounds **4** and **5** in respect to **1** does not improve analgesic activity. On the other hand, the minor conformational freedom, evidenced in NOESY experiments, agrees with the hypothesis of deficient receptorial interaction for the compounds **4** and **5**.

The substitution of OH with F atom on naphthalene nucleus seems a positive modification because the activity of **5** is superior to that of **4**, as already shown for compounds **3** (AD_{50} 13.9) and **2** (AD_{50} 18.4). This could be attributable to the better pharmacokinetics of these molecules due to their higher lipophilicity.

It is noteworthy that the antinociceptive activity of compounds **1**, **4** and **5** was completely reversed by the nonspecific opioid antagonist naloxone, suggesting that they are able to promote an activation of the opioid system.

The WT confirmed the antinociceptive action of the examined compounds even though they were less potent than morphine, already noted for the cycloaminoalkylnaphthalenes **2** and **3**. Furthermore, the AD_{50} values are inferior to those obtained from HPT, in agreement with the data for morphine.

Table 1. Analgesic activity in the hot plate test

Compound	AD_{50} (mg/kg)	Conf. limits	AD_{50} (μmol/kg)	Relative potency (versus morphine)
Morphine·HCl·3H_2O	5.38	3.89–7.43	14.3	1.00
(1*R*,2*R*/1*S*,2*S*)-**1**·HCl	4.35	1.44–13.13	13.4	1.07
(1*R*,2*R*/1*S*,2*S*)-**4**·HCl·H_2O	11.75	9.06–15.25	35.8	0.40
(1*R*,2*R*/1*S*,2*S*)-**5**·HCl·H_2O	6.42	3.10–13.28	19.5	0.73

Table 2. Analgesic activity in the writhing test

Compound	AD_{50} (mg/kg)	Conf. limits	AD_{50} (μmol/kg)	Relative potency (versus morphine)
Morphine·HCl·3H_2O	0.44	0.06–3.40	1.2	1.00
(1*R*,2*R*/1*S*,2*S*)-**1**·HCl	1.01	0.71–1.42	3.1	0.39
(1*R*,2*R*/1*S*,2*S*)-**4**·HCl·H_2O	3.10	0.82–11.00	9.5	0.13
(1*R*,2*R*/1*S*,2*S*)-**5**·HCl·H_2O	1.87	0.08–4.00	5.7	0.21

Table 3. Locomotor activity in the rota-rod test: percentage of mice which remain on the bar after 30 or 120 s

Compound	Dose (mg/kg)	30 s (%)	120 s (%)
Morphine·HCl·3H_2O	4, 6, 8	65[a]	50[a]
(1*R*,2*R*/1*S*,2*S*)-**1**·HCl	2	100	87.5
	4, 6	75[a]	50[a]
(1*R*,2*R*/1*S*,2*S*)-**4**·HCl·H_2O	8, 12, 16	75[a]	50[a]
(1*R*,2*R*/1*S*,2*S*)-**5**·HCl·H_2O	4, 6, 8	75[a]	65[a]

[a] $P < 0.05\%$.

The influence of the examined compounds on motor coordination was also investigated. RRT data, at 30 s from the beginning of the test, showed that motor coordination was slightly less influenced by the examined compounds than by morphine. Furthermore, after 120 s, the control of locomotor activity for the fluoro-derivative **5** decreased slightly with respect to both analogue **4** and morphine. The dose–effect relationship was not significant because the percentage of the mice that fell from the rod was the same for all compounds at all doses tested, except for **1** (time 30 s: 0% at 2 mg and 25% at 4 and 6 mg; time 120 s: 12.5% at 2 mg and 50% at 4 and 6 mg). Finally, when the test was repeated 20 and 40 min after the first control, the results were the same.

As regards the in vitro assays (Table 4), there is evidence that all compounds showed very poor affinity for opiate receptors (μmolar order of K_i values), even if the analgesic activity (HPT) is completely reversed by naloxone. Therefore the data suggest that the compounds produce antinociceptive effects by indirect activation of the opioid system. Additional studies aimed at assessing the mechanism of action of the examined compounds will be performed.

Table 4. Radioligand binding test

Compound	K_i (μM)		
	μ	κ	δ
Morphine·HCl·3H_2O	0.003	0.151[a]	0.456[a]
(1*R*,2*R*/1*S*,2*S*)-**1**·HCl	0.02	> 1.00	1.00
(1*R*,2*R*/1*S*,2*S*)-**4**·HCl·H_2O	> 1.00	> 1.00	> 1.00
(1*R*,2*R*/1*S*,2*S*)-**5**·HCl·H_2O	> 1.00	> 1.00	> 1.00

[a]Experiments performed on guinea pig brain membranes.

Experimental

Chemistry

Reagents and chemicals were obtained from commercial suppliers and used without further purification except 2-bromo-6-naphthol which was purified by recrystallization from 65% aqueous MeOH.

Melting points (mp) were determined with a Büchi apparatus. The elemental analyses were carried out with a Perkin–Elmer 240 C, H, N analyzer and were within ±0.4% of the theoretical values. Analytical results are given in Table 5. IR spectra were obtained on a Perkin–Elmer 682 spectrophotometer and ^{1}H NMR spectra (TMS as internal standard (δ = 0.00)) on a Bruker AMX 400 apparatus. Experiments of ^{1}H NMR spectroscopy by NOESY Phase Sensitive with a mixing time of 750 ms were performed. Differential scanning calorimetry and thermogravimetry were carried out with a Mettler TA 4000 apparatus equipped with cells DSC 25 and TG 50. TLC were effected on Merck silica gel 60 F_{254}.

(1*R*,2*R*/1*S*,2*S*)-1-Ethyl-1-[2-(6-hydroxynaphthyl)]-1-hydroxy-2-methyl-2-dimethylaminoethane hydrochloride monohydrate ((1*R*,2*R*/1*S*,2*S*)-4·HCl·H_2O). The compound was prepared essentially according to the procedure that we had already reported for the synthesis of (1*R*,2*R*/1*S*,2*S*)-**1**·HCl,[10] by employing 2-bromo-6-tetrahydropiranyloxynaphthalene (65 mmol), *tert*-butyllithium (130 mmol), and (*R*,*S*)-2-dimethylamino-3-pentanone[14] (65 mmol). The crude product was crystallized from 87.5% tetrahydrofurane to give pure (1*R*,2*R*/1*S*,2*S*)-**4**·HCl·H_2O with mp (127–130°) 189–190 °C (57.2% yield). R_f 0.42 (60 ethyl acetate, 40 *n*-hexane, 3 methanol, 4 diethylamine). ^{1}H NMR (400 MHz, CD_3OD) δ 0.8 (t, 3H, CH_3CH_2), 1.72 (d, 3H, CH_3CH), 2.15 (q, 2H, CH_2CH_3), 2.93 (d, 6H, $(CH_3)_2N$), 4.18 (q, 1H, $CHCH_3$), 7.32 (dd, 1H, aromatic, H7, $J_{7,5} = 2.48$), 7.35 (d, 1H, aromatic, H5), 7.68 (dd, 1H, aromatic, H3,

Table 5. Analytical data

Compound	Formula	Calcd (%)			Found (%)		
		C	H	N	C	H	N
(1*R*,2*R*/1*S*,2*S*)-**4**·HCl·H_2O	$C_{17}H_{24}NO_2Cl·H_2O$	62.28	7.99	4.27	62.18	8.36	4.21
(1*R*,2*R*/1*S*,2*S*)-**5**·HCl·H_2O	$C_{17}H_{23}NOClF·H_2O$	61.90	7.64	4.25	61.72	7.82	4.20

$J_{1,3}$ = 1.90 Hz), 7.95 (d, 1H, aromatic, H4, $J_{3,4}$ = 8.68 Hz), 7.99 (d, 1H, aromatic, H8, $J_{7,8}$ = 8.69 Hz), 8.23 (d, 1H, aromatic, H1). IR in Nujol (cm^{-1}) 3360, 3140, 2720, 1638, 1605, 1504, 1290, 1260, 1180, 902, 861, 826, 816.C, H, N analysis ($C_{17}H_{26}ClNO_3$ (327.85)).

(1*R*,2*R*/1*S*,2*S*)-1-Ethyl-1-[2-(6-fluoronaphthyl)]-1-hydroxy-2-methyl-2-dimethylaminoethane hydrochloride monohydrate [(1*R*,2*R*/1*S*,2*S*)-5·HCl·H_2O]. The compound was prepared starting from 2-bromo-6-fluoronaphthalene,[12] by the above mentioned procedure. After crystallization from 99.5% tetrahydrofurane pure (1*R*,2*R*/1*S*,2*S*)-**5**·HCl·H_2O with mp (125–130°) 193–194 °C was obtained (58.8% yield). R_f 0.75 (60 ethyl acetate, 40 *n*-hexane, 3 methanol, 4 diethylamine). ^1H NMR (400 MHz, CD_3OD) δ 0.78 (t, 3H, CH_3CH_2), 1.74 (d, 3H, CH_3CH), 2.16 (q, 2H, CH_2CH_3), 2.98 (d, 6H, $(CH_3)_2N$], 4.18 (q, 1H,$CHCH_3$), 7.56 (dt, 1H, aromatic, H7, $J_{7,5}$ = 2.48 Hz), 7.77 (dd, 1H, aromatic, H8, $J_{8,7}$ = 8.69 Hz), 7.84 (dd, 1H, aromatic, H3, $J_{3,1}$ = 1.90 Hz), 8.16 (d, 1H, aromatic, H5, $J_{5,F}$ = 8.68 Hz), 8.23 (dd, 1H, aromatic, H4, $J_{4,5}$ = 1.90 Hz), 8.38 (d, 1H, aromatic, H1). IR in Nujol (cm^{-1}) 3480, 3430, 3335, 2725, 1630, 1606, 1413, 1250, 1168, 1076, 980, 898, 866, 810. C, H, N analysis ($C_{17}H_{25}ClFNO_2$ (329.84)).

Differential scanning calorimetry (DSC) revealed an endothermic process with biphasic profile at a temperature range near to 133 °C and thermogravimetry (TG) (10 °C/min) showed a decrease of weight (5.6% for (1*R*,2*R*/1*S*,2*S*)-**4** and 5.8% for (1*R*,2*R*/1*S*,2*S*)-**5**) in the same range of temperature. Theoretical value for one water mol: 5.5% (for both compounds).

X-ray crystallography

Crystals of (1*R*,2*R*/1*S*,2*S*)-**4**·HCl·H_2O for X-ray diffraction were grown from water. A crystal was used for data collection.

Crystal data. A colorless, needle-shaped crystal (0.50×0.34×0.55 mm) was used for data collection with a Philips Pw 1100 computer-controlled four circle diffractometer, graphite-monochromated Mo K_α radiation, ω scan technique (±h, ±k,l), scan width 2.4, scan speed 0.06 Θ range 3–23°. The compound $C_{17}H_{24}NO_2Cl·H_2O$ is monoclinic, M_r = 327.85, V = 3576.22 (2) $Å^3$, D_x = 1.21 g cm^{-3}, μ = 2.21 cm^{-1}, F(000) = 956.31. Cell dimension by least-squares refinement of the setting angles of 60 reflections was found to be: a = 16.940 (5), b = 26.515 (9), c = 8.029 (2) Å, β = 97.41 (3)°, space group $P2_1/a$ from systematic absences h0l, 0k0 for h,k odd, respectively. The asymmetric unit of the crystal contains two independent systems. The measured reflections were 10765, unique reflections 4983 of which 2143 considered as observed ($I > 3\sigma(I)$). Lorentz and polarization corrections were applied. The crystal structure was solved by direct method using MULTAN 80.[15] A local modified version of program ORFLS[16] and the program PARST[17] were used for the crystal structure refinement. Figures 2 and 5 were drawn with ORTEP II.[18] The H atoms were obtained by geometrical considerations using XANADU program.[19] They were not varied during refinement. The H atoms of the Wa and Wb water molecules could not be identified. The non-hydrogen atoms were refined anisotropically on F by full-matrix least square. The refinement converged at R = 0.0513 ($I > 3\sigma(I)$), R_{all} = 0.1393 for 682 parameters.

Figure 2 shows the structure of the enantiomers of **4**·HCl·H_2O and Figure 5 the projection along [c] axis of the equivalent molecules in the crystal structure. The packing shows that all inter-molecular bonds are mediated through Cl atoms and water molecules.

Pharmacology

In vivo assays were effected by hot plate test (HPT), writhing test (WT), and Rota Rod Test (RRT) on male

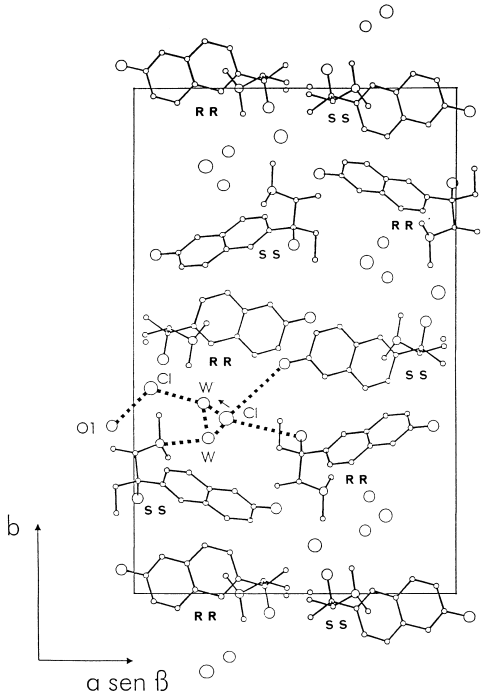


Figure 5. (1*R*,2*R*/1*S*,2*S*)-**4**·HCl·H_2O: ORTEP view along [c] axis of the equivalent molecules.

adult swiss mice weighting 30 ± 5 g. Compounds were dissolved in saline solution and administered within 1 h.

Hot plate test (HPT)

The response to a thermal stimulus was evaluated using a copper plate heated at 55 °C. The reaction time was measured in seconds. The response of the mouse included the sitting on its hind legs and licking.[20] Once the basal animal reaction time had been determined, groups of 10 mice were treated (via ip injection) with increasing doses of compound. Control animals received the same volume of saline solution. The reaction time to the pain stimulus was measured 20 min after ip injection. The reaction time of the control animals was 23 ± 2 s. AD_{50} values were determined using a computerized program.[21] HPT data are reported in Table 1. Antagonist activity was investigated by using the opioid antagonist naloxone at high dose (10 mg/kg) as nonselective opioid inhibitor.

Writhing test (WT)

The WT was effected after intraperitoneal administration of phenylquinone, which causes a syndrome, characterized by intermittent contractions of the abdomen and extension of the hind limbs persisting for more than 1 h within 10 min of administration. The compounds were administered to groups of 10 mice, at different doses. A group of untreated animals was used as control.

Animals were treated with increasing doses of the compound solution and 10 min after administration all mice received 0.25 mL of a 0.02% phenylquinone alcoholic solution.[22] The typical abdominal contractions (writhing) were monitored for 20 min following the injection. For each animal the percentage of inhibition of contractions (contr. inhib. %) was calculated by means of the following formula:

$$\text{contr. inhib.}\% = \frac{\text{contractions of control animals} - \text{contractions of treated animals}}{\text{contractions of control animals}} \times 100$$

After recording the highest percentages of inhibition induced in each animal, the average values for each group and for every dose were calculated. AD_{50} values were calculated from the percentage of writhing inhibition. WT data are reported in Table 2.

Rota-rod test (RRT)

To assess the possible nonspecific muscle-relaxant or sedative effects of the investigated compounds, the integrity of motor coordination of the mice was tested on a rota-rod apparatus using a slightly modified method of Vaught et al.[23]

The apparatus consisted of a platform equipped with a rotating rod (3 cm diameter) with a non-slippery surface. The rod was placed at a 15 cm height from the base and was divided, by six disks, into five equal sections in order to test up to five mice simultaneously. The rotating speed was fixed at 17 rpm. The animals were selected randomly and divided in groups of 10 mice for any dose.

Before carrying out of the test the mice were trained to remain on the rod for a fixed maximum time (120 s) and animals that did not remain on the bar during this time were rejected. Performance time was monitored 15 min after treatment with the tested substances. A range of doses was chosen on the basis of AD_{50} values from HPT. The integrity of motor coordination was evaluated counting the number of mice of each group that fell from the rod at 30 and 120 s. The assay was also repeated at 20 and 40 min after the first control. In all sets of experiments, one group of mice was treated with morphine, as standard antinociceptive agent, to compare results with the tested compounds. Results are expressed as percentage of mice which remain on the bar for the fixed time and are given as the mean ± MSE. Statistical analysis was performed by Student's *t* test for grouped data. *P* values of less than 0.05 were considered significant. RRT data are reported in Table 3.

Radioligand binding test

Opioid receptor binding experiments were carried out using membranes prepared by hypotonic lysis from CHO (μ and δ) or HEK-293 (κ) cells expressing cloned human opioid receptors.[24] [^{3}H]-U-69593, [^{3}H]-DADLE (D-Ala^2, D-Leu^5 enkephalin) and [^{3}H]-DAMGO (D-Ala^2, MePhe^4, Gly-ol^5 enkephalin) were used as the radioligands to label κ, δ and μ receptors, respectively.

The assay was carried out in 50 mM Tris buffer, pH 7.4 at a final volume of 1 or 2 mL. The assay tubes were incubated at 25 °C for 60 min. Nonspecific receptor binding was evaluated in the presence of 10 μM Naloxone. Bound ligand was separated from free ligand by filtration through Whatman GF/B filters using a Brandell Cell Harvester. The radioactivity on the filters was measured by liquid scintillation counting. The data obtained from competition experiments were analyzed using non linear fitting analysis according to Benfenati and Guardabasso[25] using the RS/1 software. K_i values were determined from IC_{50} using the Cheng and Prusoff equation. Opioid receptor binding data are reported in Table 4.

Supplementary material available

Atomic coordinates and equivalent isotropic displacement parameters, bond distances and angles, and torsion angles for compound (1*R*,2*R*/1*S*,2*S*)-**4**·HCl·H_2O (four tables).

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