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Conformation by NMR of two tetralin-based receptor ligands

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Abstract

The conformation in solution of 1-phenyl-3-propionamido-1,2,3,4-tetrahydronaphthalene and 1-phenyl-3-(*N*,*N*-dimethylamino)-1,2,3,4-tetrahydronaphthalene has been determined by a combination of nuclear magnetic resonance measurements and molecular mechanics calculations. The results indicate that in the *cis* isomers the cyclohexene ring is in a locked conformation and the *trans* isomers correspond to a mixture of the two inverted half chairs. Moreover, the data allowed the identification of the two purposely-synthesized geometrical isomers of 1-phenyl-3-propionamidotetralin. Binding studies on melatonin receptor subtypes showed that the (±)-*cis*-1-phenyl-3-propionamido-1,2,3,4-tetrahydronaphthalene has higher affinity and selectivity ratio toward the MT_2 subtype than the (±)-*trans*-isomer.
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1. Introduction

A frequently encountered problem in drug design is to decide which conformation the drug molecule adopts at the binding site, i.e. the bioactive conformation. This is particularly true in the case of simple flexible molecules, such as arylethylamines, having a considerable rotational freedom. A method commonly used for reduce the flexibility has been the incorporation of the ethylamine fragment into a bicycle system. For instance, tetralins with appropriate substitution patterns have been used as convenient scaffolds for the preparation of molecules which act as ligands of pharmacological receptors [1–5]. The conformational analysis of the tetralin skeleton is a suitable starting point for the study of the pharmacological activity of these receptor ligands. The cyclohexene ring present in tetralin can in principle adopt half-chair conformation, with the substituent in position three being defined as pseudoequatorial or pseudoaxial and the substituent in position one being defined as equatorial or axial. Molecular mechanics calculations and crystallographic structure determinations can be used for describe the conformation of the molecules in vacuo and in the solid state, respectively. However, a spectroscopic method like nuclear magnetic resonance can give information relative to the solution state, which might be more appropriate for studies of biological activity. Recently, the tetralin skeleton has been successfully used as a rigid template for the synthesis of non-indolic melatonin-like agents [2]. Melatonin (*N*-acetyl-5-methoxytryptamine), a neurohormone mainly secreted by the pineal gland during the dark period, is an indole derivative with a flexible ethylamido chain attached at the C3 position. In mammals, melatonin modulates a variety of cellular, neuroendocrine and physiological processes through activation of at least two high-affinity G-protein coupled receptors, named MT_1 and MT_2 [6,7]. Recent studies have identified some compounds that are selective for melatonin MT_2 receptor binding [1,8,9]], suggesting some differences in the structure, or flexibility, of the MT_2 receptor compared to the MT_1 receptor. The most interesting compound, 1-phenyl-3-propionamido-1,2,3,4-tetrahydro-naphthalene [1], belongs to the tetralin series; however, despite the fact that it is a powerful and selective MT_2 melatonin receptor ligand, to our knowledge it has not been reported for it any *cis*/*trans* characterization.

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This short communication reports on the NMR determination of the conformation of both *cis* and *trans* diastereomers of 1-phenyl-3-propionamido-1,2,3,4-tetrahydro-naphthalene (**11**, **13**) and of 1-phenyl-3-(*N*,*N*-dimethylamino)-1,2,3,4-tetrahydronaphthalene (**10**, **12**). The conformational analysis has been carried out on the racemic mixture of the enantiomeric pair of each diastereoisomer, exploiting the property that the NMR spectrum is invariant to reflection.

An additional purpose of the present paper was the synthesis, the identification of the individual *cis* and *trans* isomers (**11**, **13**) and the characterization of their pharmacological profile. The latter information might be used for future studies, aimed to characterize the melatonin binding site.

2. Chemistry

(±)-*Cis* and (±)-*trans*-1-phenyl-3-aminotetralins (**8** and **9**) were prepared following the synthetic pathways outlined in Scheme 1 and according to previously described methods [5,10]. (±)-*Cis* and (±)-*trans*-1-phenyl-3-dimethylaminotetralins (**10**, **12**) and (±)-*cis*- and (±)-*trans*-1-phenyl-3-propionamidotetralins (**11**, **13**) were obtained by methylation of the corresponding primary ammines (**8**, **9**) with the Eschweiler–Clark method [11], and by acylation with propionic anhydride, respectively.

3. Experimental

3.1. Radioligand binding assays

The binding affinity of compounds **11** and **13** was determined using 2-[^{125}I]-iodomelatonin (100 pM) as the labelled ligand in competition experiments on cloned human MT_1 and MT_2 receptor subtypes expressed in NIH3T3 rat fibroblast cells [12,13].

Their intrinsic activity was evaluated on [^{35}S]-guanosine-5′-*O*-(3-thiotriphosphate), ([^{35}S]GTPγS), binding in NIH3T3 rat fibroblast cells, transfected with human MT_1 and MT_2 receptors [13,14].

3.2. Chemistry

Melting points were determined on a Büchi SMP-510 capillary melting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a Bruker AC 200 spectrometer using $CDCl_3$ as solvent unless otherwise noted. Chemical shifts (δ scale) are reported in parts per million (ppm), coupling constants (J values) are given in hertz (Hz). EI MS spectra (70 eV) were taken on a Fisons Trio 1000. Only molecular ions (M^+) and base peaks are given. Infrared spectra were taken on a Bruker FT-48 spectrometer. Elemental analyses for C, H and N were performed on a Carlo Erba analyzer and are within ±4% of the theoretical value.

3.2.1. 1,4-Diphenyl-1-buten-3-one (*1*)

Compound **1** was prepared as described in the literature [15]. M.p. 69–73 °C (lit. [10] 69–73 °C); IR (cm^{-1}) (CCl_4 solution): 3061, 1720; MS (*m*/*z*): 222, 131; ^{1}H NMR: 7.75–7.25 (m, 10H), 6.82 (s, 1H), 6.78 (s, 1H), 3.97 (s, 2H).

3.2.2. 1-Phenyl-3-oxo-1,2,3,4-tetrahydronaphthalene (*2*)

Compound **2** was prepared as described previously by Wyrick et al. [5]. Gum, MS (*m*/*z*): 222, 131; IR (cm^{-1}) (CCl_4 solution): 3065, 1720; ^{1}H NMR: 7.5–7.0 (m, 9H), 4.5 (t, 1H), 3.65 (dd, 2H,), 2.78 (m, 2H).

3.2.3. (±)-cis-1-Phenyl-3-hydroxy-1,2,3,4-tetrahydronaphthalene (*3*)

Compound **3** was prepared as described previously [5]. White solid, m.p. 110–112 °C (lit. [5] 112–115 °C); MS (*m*/*z*): 224, 206; IR (cm^{-1}) (CCl_4 solution) 3623, 3057, 2966, 1613; ^{1}H NMR 7.4–7.0 (m, 8H), 6.77 (d, 1H), 4.18 (m, 2H), 3.22 (dd, 1H), 2.93 (dd, 1H), 2.4 (m, 1H), 1.9 (m, 1H).

3.2.4. (±)-trans-1-Phenyl-3-hydroxy-1,2,3,4-tetrahydronaphthalene (*5*)

Compound **5** was prepared as described previously [5]. White solid, m.p. 56–58 °C (lit. [5] as a gum); MS (*m*/*z*): 224, 206; IR (cm^{-1}) (CCl_4 solution) 3623, 3057, 2966, 1613; ^{1}H NMR: 7.4–7.0 (m, 8H), 6.85 (d, 1H), 4.4 (t, 1H), 4.3 (m, 1H), 3.28 (dd, 1H), 2.87 (dd, 1H), 2.3 (m, 2H).

3.2.5. (±)-cis-1-Phenyl-3-amino-1,2,3,4-tetrahydronaphthalene (*8*)

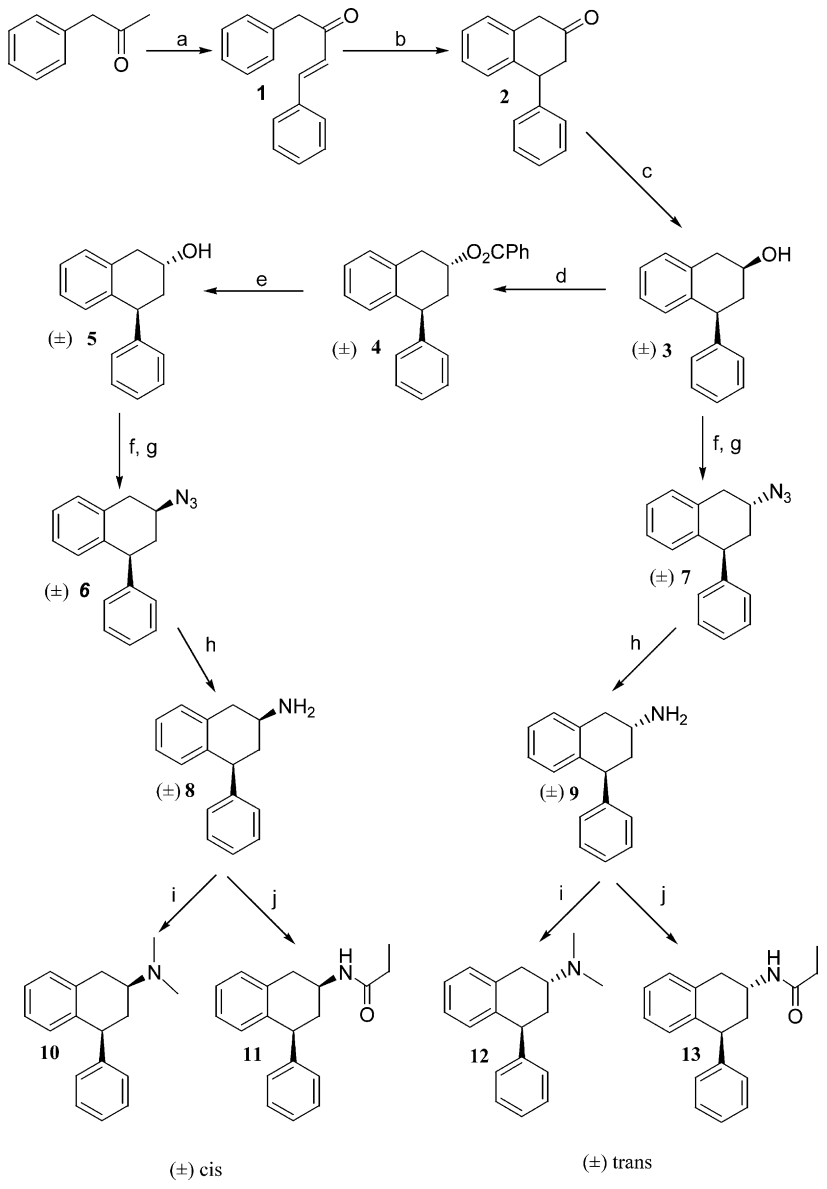
Compound **8** was prepared as previously described [5]. M.p. 79–81 °C (lit. [5] as a gum); MS (*m*/*z*): 206 ($M^+ - 17$) 179; IR (cm^{-1}) (nujol): 3364, 3287, 3060, 2936, 2860, 1613; ^{1}H NMR (CD_3COCD_3): 7.4–7.0 (m, 8H), 6.93 (d, 1H), 4.15 (m, 1H), 3.9 (bs, 2H), 3.10–2.85 (m, 3H), 2.4 (m, 1H), 1.8 (m, 1H).

3.2.6. (±)-trans-1-Phenyl-3-amino-1,2,3,4-tetrahydronaphthalene (*9*)

Compound **9** was prepared as described previously [5]. M.p. 57–59 °C; IR (cm^{-1}) (nujol) 3364, 3287, 3060, 2936, 2860 1613; MS (*m*/*z*): 223 (M^+), 206; ^{1}H NMR (CD_3COCD_3): 7.35–6.9 (m, 9H), 4.35 (m, 1H), 3.10 (m, 1H,), 3.5 (bs, 2H), 2.57–2.25 (m, 2H), 2.15 (m, 2H).

3.2.7. (±)-cis-1-Phenyl-3-(N,N-dimethylamino)-1,2,3,4-tetrahydronaphthalene (*10*)

The procedure of Eschweiler and Clark [11] was used. M.p. 232–234 °C; IR (cm^{-1}) (nujol) 3090, 2926, 2880,



Scheme 1. Reagents: (a) PhCHO, KOH, 18 h, 60 °C; (b) polyphosphoric acid, xilene, reflux; (c) $NaBH_4$, MeOH; (d) diethyl azodicarboxylate, $(C_6H_5)_3P$, PhCOOH; (e) NaOH; (f) TsCl, Py; (g) NaN_3, DMF/H_2O; (h) H_2, 10% Pd/C; (i) HCHO, HCOOH; (j) $(C_2H_5CO)_2O$ Et_3N, toluene, 24 h.

1595, 1224; MS (*m*/*z*): 251 (M^+) 178; ^{1}H NMR 7.4–7.0 (m, 8H), 6.77 (d, 1H), 4.10 (dd, 1H), 3.15–2.85 (m, 3H), 2.45 (s, 6H) 2.37 (m,1H), 1.76 (ddd,1H).

*3.2.8. (±)-trans-1-Phenyl-3-(N,N-dimethylamino)-1,2,3,4-tetrahydronaphthalene (**12**)*

The procedure of Eschweiler and Clark [11] was used. M.p. 60–62 °C; MS (*m*/*z*): 251 (M^+) 178; IR (cm^{-1}) (nujol) 3090, 2926, 2880, 1595; ^{1}H NMR 7.4–7.0 (m, 8H), 6.95 (d,1H), 4.38 (t, 1H), 3.06 (dd, 1H,), 2.87 (dd, 1H), 2.65 (m,1H), 2.3 (s, 6H), 2.15 (m, 2H).

*3.2.9. (±)-cis-1-Phenyl-3-propionamido-1,2,3,4-tetrahydronaphthalene (**11**)*

Propionic anhydride (0.115 ml, 0.9 mmol) was added to a cooled and stirred mixture of (±)-*cis*-phenyl-3-aminotetralin (**8**) (200 mg, 0.9 mmol) and triethylamine (0.15 ml, 1.07 mmol) in toluene (10 ml) under argon. The mixture was slowly warmed to room temperature and stirred for 24 h. Triethylamine HCl was removed by filtration and washed with toluene (10 ml). The filtrate was concentrated under reduced pressure to give a solid residue which was purified by flash cromatography using cyclohexane–ethyl acetate 7/3 as eluent. Crystal-

lization from acetone/hexane gave 88 mg (35%) of the desired compound; m.p. 170–172 °C; MS (*m/z*): 279 (M^+) 206; IR (cm^{-1}) (nujol): 3289, 3089, 3024, 2938, 1670; ^1H NMR 7.4–7.0 (m, 8H), 6.90 (d, 1H), 5.4 (bd,1H), 4.39 (m,1H), 4.25 (dd, 1H), 3.22 (dd, 1H,), 2.80 (dd, 1H), 2.42 (m,1H), 2.15 (q, 2H), 1.8 (ddd,1H), 1.15 (t, 3H).

3.2.10. (±)-trans-1-Phenyl-3-propionamido-1,2,3,4-tetrahydronaphthalene (*13*)

Compound **13** was prepared as described above starting from *trans*-4-phenyl-2-aminotetralin (**9**). M.p. 120–122 °C; MS (*m/z*): 279 (M^+), 206; IR (cm^{-1}) (nujol) 3289, 3024, 2938, 1686; ^1H NMR 7.4–7.0 (m, 8H), 6.90 (d, 1H), 5.5 (bd, 1H), 4.38 (m, 1H), 4.20 (t, 1H), 3.36 (dd, 1H), 2.75 (dd, 1H), 2.25 (m, 4H), 1.15 (t, 3H).

3.3. Spectroscopy

NMR spectra of samples **10**–**13** were measured at 600 MHz on a Bruker Avance spectrometer, at the probe-head temperature. Samples were dissolved in 0.01 M concentration in deuterated chloroform containing TMS as internal reference. Spectra were processed off-line by using the WINNMR Bruker software for personal computers. Digital resolution of the experimental spectra was 0.05 Hz. Simulations and iterations were performed by using the WINDAISY Bruker software for personal computers. *R*-factors better than 2% were considered acceptable.

Molecular mechanics calculations were performed with the MM2 force field by using the HYPERCHEM software for personal computers.

Table 1
Binding affinity [a] and intrinsic activity of **11** and **13** for the human MT_1 and MT_2 melatonin receptors

Compound	Human MT_1		Human MT_2	
	pK_i	IAr [b]	pK_i	*IAr*
Melatonin	9.63	1	9.43	1
(±)-**11**	6.98	0.07	10.80 [c]	0.37
(±)-**13**	6.62	0.05	8.45	0.08

[a] pK_i values were calculated from IC_{50} values obtained from competition curves by the method of Cheng and Prusoff [19] and are the means of at least three independent determinations performed in duplicate.

[b] The relative intrinsic activity values (*IAr*) were obtained by dividing the maximal analogue-induced G-protein activation by that of melatonin.

[c] pIC_{50} value. Compound **11** competed with 2-[^{125}I]-iodomelatonin binding in $NIH3T3_{MT2}$ membranes with a slope of −0.58 (significantly different from unity), thus not allowing K_i calculation.

4. Results and discussion

The affinities of (±)-*cis*- and (±)-*trans*-1-phenyl-3-propionamidotetralin (**11**, **13**) for both MT_1 and MT_2 human melatonin receptors are shown in Table 1. Concerning the structural aspects of tetralins **10**–**13**, the problem has been approached with the following procedure. The ^1H NMR spectra of the cyclohexene moiety have been analyzed for connectivity by standard decoupling technique. Then the first-order parameters, chemical shifts and interproton coupling constants, obtained by inspection, have been refined by simulation of the spectrum and by fitting to the observed frequencies and intensities. A representative example of the quality of the fitting is reproduced in Fig. 1. The results of the treatment are collected in Table 2. Here the labelling a and b of the methylene resonances refers to the position in the spectrum, meaning high and low frequency, respectively. The interpretation of the spectra in terms of configuration and conformation has then been achieved with the following procedure. The interproton vicinal coupling constants of the cyclohexene ring have been correlated with the respective torsion angles by using the empirical relationship proposed by Altona and coworkers [16]. For this purpose the torsion angles were estimated by modeling the molecules in vacuo with the MM2 force field of Burkert and Allinger [17], assuming that solvent effects could be neglected. For the *cis* isomers the observed trend in the coupling constants resulted very close to that expected for the half chair conformation, with the phenyl substituent in position 1-pseudoequatorial, and the nitrogen atom in position 3-equatorial, as shown in Fig. 2. Slight adjustments of some of the torsion angles predicted by the MM2 model produced the coupling constants reported in Table 3 (where the labeling α or β of the hydrogen atoms refers to their *cis* or *trans* position, respectively, relative to the nitrogen substituent). In addition, this assignment allowed identifying the geometrical configuration of propionamido–tetralin, which, differently from the dimethylamino–tetralin case, was not described in the literature.

Concerning the two *trans* isomers, the experimental values of the interproton vicinal coupling constants of the cyclohexene ring could be interpreted as arising from a mixture of two half chair conformations **I** (with the phenyl substituent being pseudoaxial and the NHCOEt substituent equatorial) and **II** (with phenyl pseudoequatorial and NHCOEt axial), inverting rapidly on the NMR time scale, as shown in Fig. 2. The average coupling constants were best calculated with a molar ratio **I/II** equal to 50/50 for the case of the propionamido–tetralin and 75/25 for the dimethylamino–tetralin and are reported in Table 3. In all cases the agreement with the experimental values,

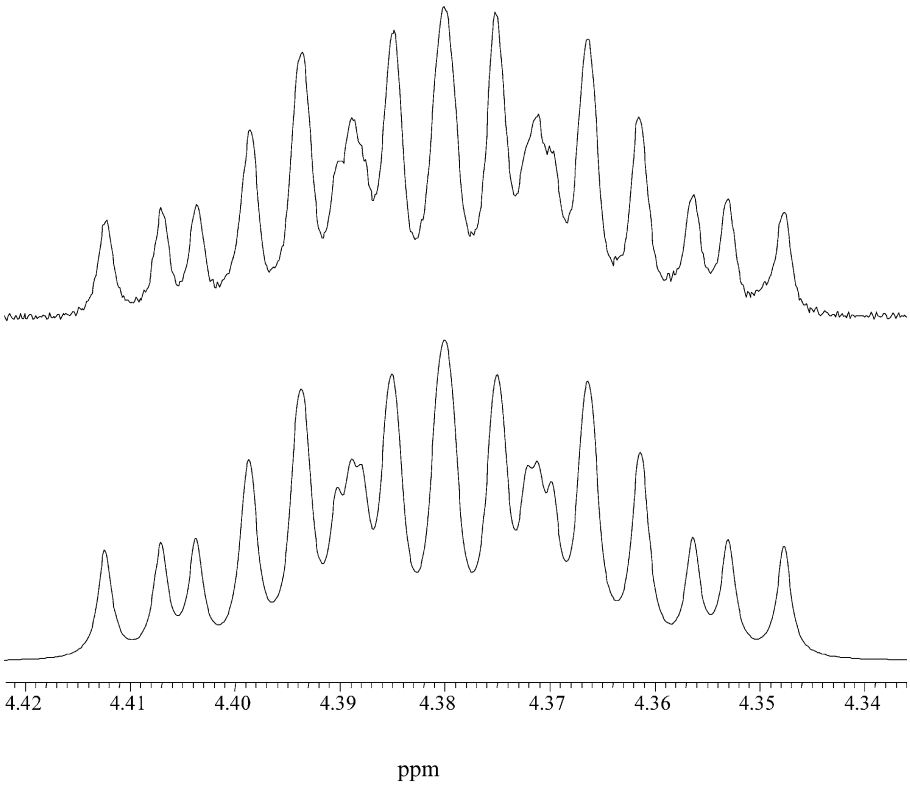


Fig. 1. Observed (upper trace) and calculated (lower trace) spectrum of H_3 of *trans*-1-phenyl-3-propionamidotetralin.

graphically summarized in Fig. 3, is of the order usually expected for such kind of treatment.

As a check of the assumption of a conformational equilibrium between forms **I** and **II**, it was measured the 2D-ROESY spectrum of the *trans*-1-phenyl-3-propionamido-1,2,3,4-tetrahydronaphthalene. The cross peak observed between protons 4b and 2a and the cross peak observed between 4a and 2b, having the same intensity, must be accounted for by a proximity of the involved protons. However, MM2 models show that in each individual form **I** and **II** the two interproton distances are extremely different, 2.5 and 4.3 Å, and this fact rules out the presence of a single conformer, which indeed should show cross peaks with different intensity. Instead, the 50/50 mixture hypothesis agrees with the observed equal cross peaks, resulting from the averaging of internuclear distances. An additional information contained in the ROESY spectrum is the lack of any

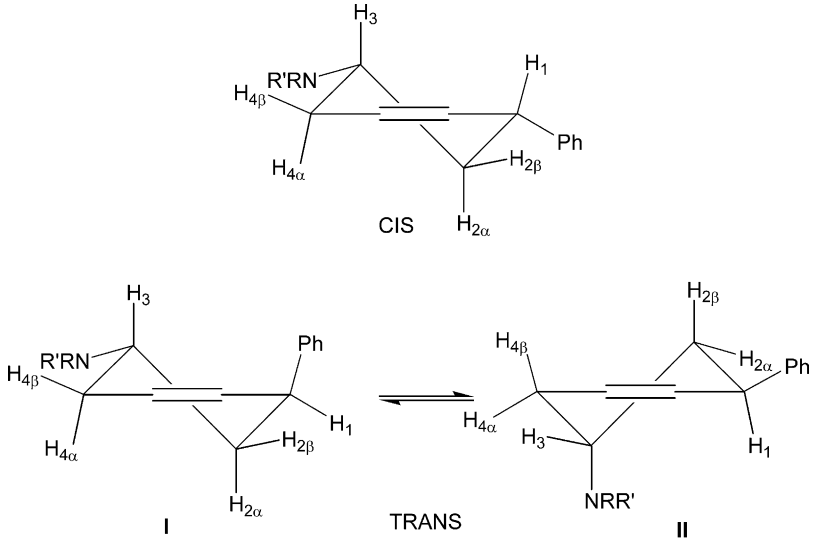


Fig. 2. Cyclohexene ring conformation of tetralins (only one enantiomer shown).

dipolar coupling between protons in position 1 and 4: this observation shows that the high energy boat form is not detectable by NMR in solution.

In order to reduce the rate of conformational interconversion and thus ascertain the presence of the conformational equilibrium, proton spectra were measured at low temperatures (consistently with the freezing point of the solvent). Indeed, a considerable broadening of the signals was observed, however, it appears that the energy barrier to interconversion is not enough to reach the coalescence temperature.

Moreover, an evidence of the conformation across the CH–NH bond in the propionamido–tetralin could be obtained from the measured value of the corresponding coupling constant. In the approximation of three rotational isomers, one with anti and two with gauche arrangement of the involved protons, this parameter is the molar average of the coupling constants of the individual rotamers, $J = xJ_{anti} + (1-x)J_{gauche}$, where x is the molar fraction of the anti form and J_{anti} and J_{gauche} are the coupling constants of the corresponding rotamers. The latter parameters were estimated by a Karplus type relationship [18] and this allowed to evaluate the molar fraction x. The result is 75% of the *anti* form and 25% of the *gauche* forms.

Finally, concerning the binding to melatonin receptors, the conformation of the *cis*-isomer of 1-phenyl-3-propionamido-1,2,3,4-tetrahydro-naphthalene appears to be more suitable for the interaction with the MT_2 receptor. Indeed, the two racemates (±)-*cis*-**11** and (±)-*trans*-**13** showed comparable MT_1 binding affinity, but the *cis* isomer has a considerably higher MT_2 affinity leading to a better MT_1/MT_2 selectivity ratio than the *trans* form.

5. Conclusion

In summary we have shown that a complete analysis of the NMR spectrum of tetralins, combined with force field calculations, describes the conformation in solution of the 1,3-disubstituted tetralins. It results that the *cis* isomer corresponds to the cyclohexene ring conformation in which the phenyl group in position one is pseudoequatorial and the amido or the dimethylamino group in three is equatorial. However, the *trans* isomer is a mixture of the two conformers **I** and **II** with the inverted half chair cyclohexene ring. The preference of the dimethylamino group for the equatorial orientation appears to be larger than that of the propionamido group, and this observation could be ascribed to a strong steric 1–3 interaction of the dimethylamino group with the pseudoaxial H_1 in form **II**.

The data show that the reduction in flexibility attainable through the use of a bicycle substructure is easily achieved in the case of *cis* isomer, where the cyclohexene ring appears to be in a locked conformation, but not in the *trans* case, which resulted as a mixture of ring conformations. Moreover, the composition of the mixture resulted to depend on the nature of the ring substituent.

Concerning the conformation across the CH–NH bond in the propionamido–tetralins, the same predominant *anti* orientation of the two hydrogen atoms was observed for both *cis* and *trans* isomers, showing that this local conformation is not related to the cyclohexene ring arrangement.

Finally, the above synthesis and analysis allowed, for the first time, the unambiguous identification of the geometrical isomers of 1-phenyl-3-propionamidotetralin. Moreover, the observed higher binding affinity for MT_2 receptor of the *cis* isomer relative to the *trans* can

Table 2
^{1}H NMR spectrum parameters of tetralins

Chemical shifts ppm from internal TMS	Coupling constants (Hz)	
(±)-cis-1-Phenyl-3-propionamido-tetralin		
δ (NH) = 5.4134	J (1,2a) = 5.73 ± 0.001	J (3,4a) = 5.21 ± 0.001
δ (3) = 4.3799	J (1,2b) = 11.56 ± 0.002	J (3,4b) = 10.89 ± 0.001
δ (1) = 4.2475	J (2a,2b) = −12.46 ± 0.001	J (4a,4b) = −15.79 ± 0.001
δ (4a) = 3.2278	J (2a,3) = 3.19 ± 0.001	
δ (4b) = 2.7891	J (2b,3) = 11.55 ± 0.002	
δ (2a) = 2.4213	J (2a,4a) = 2.13 ± 0.001	
δ (2b) = 1.7850	J (NH,3) = 8.04 ± 0.002	
(±)-trans-1-Phenyl-3-propionamido-tetralin		
δ (NH) = 5.3963	J (1,2a) = 6.19 ± 0.001	J (3,4a) = 5.07 ± 0.001
δ (3) = 4.3287	J (1,2b) = 7.64 ± 0.001	J (3,4b) = 6.37 ± 0.001
δ (1) = 4.1358	J (2a,2b) = −13.12 ± 0.001	J (4a,4b) = −16.60 ± 0.001
δ (4a) = 3.2614	J (2a,3) = 7.92 ± 0.001	
δ (4b) = 2.6424	J (2b,3) = 2.97 ± 0.001	
δ (2a) = 2.1960	J (NH,3) = 7.79 ± 0.003	
δ (2b) = 1.9670		
(±)-cis-1-Phenyl-3-(N,N-dimethylamino)tetralin		
δ (1) = 4.0357	J (1,2a) = 5.45 ± 0.001	J (3,4a) = 4.88 ± 0.003
δ (4a) = 3.0195	J (1,2b) = 12.32 ± 0.002	J (3,4b) = 11.78 ± 0.003
δ (4b) = 2.9202	J (2a,3) = 2.81 ± 0.002	J (4a,4b) = −15.67 ± 0.002
δ (3) = 2.8709	J (2b,3) = 11.78 ± 0.001	
δ (2a) = 2.3227	J (2a,2b) = −12.47 ± 0.001	
δ (2b) = 1.7060	J (2a,4a) = 2.17 ± 0.002	
(±)-trans-1-Phenyl-3-(N,N-dimethylamino)tetralin		
δ (1) = 4.3847	J (1,2a) = 4.77 ± 0.001	J (3,4a) = 4.95 ± 0.001
δ (4a) = 3.0423	J (1,2b) = 5.85 ± 0.001	J (3,4b) = 9.62 ± 0.001
δ (4b) = 2.8762	J (2a,2b) = −12.90 ± 0.001	J (4a,4b) = −16.25 ± 0.001
δ (3) = 2.6843	J (2a,3) = 2.90 ± 0.001	
δ (2a) = 2.1577	J (2b,3) = 10.20 ± 0.001	
δ (2b) = 2.1308		

Table 3
Calculated and observed vicinal coupling constants of cyclohexene ring in tetralins

Hydrogen atoms	Torsion angle (°)	J coupling constant		$J_{calc}-J_{obs}$
		Calculated	Observed	
(±)-cis 1-Phenyl-3-propionamido-tetralin, 1ψeq–3eq form				
1–2α	169	12	11.6	+0.4
1–2β	47	5.3	5.7	−0.4
2α–3	175	11.8	11.6	+0.2
2β–3	68	2.6	3.2	−0.6
3–4α	175	11.6	10.9	+0.7
3–4β	51	5.0	5.2	−0.2
(±)-cis 1-Phenyl-3-(N,N-dimethylamino)tetralin, 1ψeq–3eq form				
1–2α	172	12.2	12.3	−0.1
1–2β	49	4.9	5.5	−0.6
2α–3	177	11.8	11.8	0
2β–3	64	3.0	2.8	−0.2
3–4α	175	11.2	11.8	−0.6
3–4β	55	5.3	4.9	+0.4

Hydrogen atoms	Torsion angle (°)		J coupling constant				$J_{calc}-J_{obs}$
			Calculated			Observed	
	I	II	I	II	Mixture		
(±)-trans 1-Phenyl-3-(N,N-dimethylamino)tetralin							
1–2α	48	50	4.9	4.8	4.9	4.8	+0.1
1–2β	62	167	2.6	11.9	5.0	5.8	−0.8
2α–3	178	56	11.8	4.0	9.9	10.2	−0.3
2β–3	64	60	3.1	2.6	3.0	3.0	−0.1
3–4α	163	63	10.8	3.0	9.2	9.7	−0.5
3–4β	45	50	5.9	3.8	5.4	5.0	+0.4
(±)-trans 1-Phenyl-3-propionamido-tetralin							
1–2α	45	50	5.4	4.8	5.2	6.2	−1.0
1–2β	67	167	2.1	11.9	7.0	7.6	−0.6
2α–3	175	56	11.8	4.0	7.9	7.9	0
2β–3	68	60	2.6	2.6	2.6	3.0	−0.4
3–4α	169	67	11.3	2.5	6.9	6.4	+0.5
3–4β	52	50	4.8	3.8	4.3	5.1	−0.8

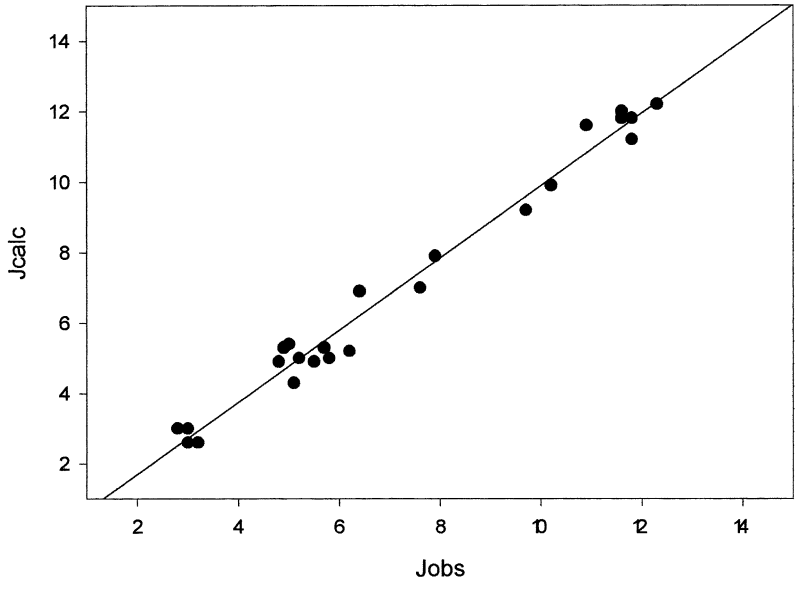


Fig. 3. Calculated versus observed vicinal proton-proton coupling constants of cyclohexene ring.

provide further insights for the characterization of the melatonin binding sites.

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