Brief Articles

Melatonergic Properties of the (+)- and (−)-Enantiomers of *N*-(4-Methoxy-2,3-dihydro-1*H*-phenalen-2-yl)amide Derivatives

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N-(4-Methoxy-2,3-dihydro-1*H*-phenalen-2-yl)amide derivatives, conformationally restricted ligands for melatonin receptors, were synthesized by an alternative synthetic method from the corresponding 1,8-naphthalic anhydride which was transformed into the phenalenecarboxylic acid **7**. A Curtius reaction on **7** gave the amino compound which was acylated to give compounds **4a**−**c**. The (+)- and (−)-**4a**−**c** enantiomers were separated by semipreparative chiral HPLC. Compounds were evaluated for their affinity for chicken brain melatonin receptors in binding assays using 2-[^{125}I]iodomelatonin and for their potency to lighten the skin of *Xenopus laevis* tadpoles. The butyramido derivative **4c** was the most potent ligand (K_i = 1.7 nM). No enantioselectivity was observed with the enantiomers which were equipotent to the racemic mixture. In contrast to the reference compounds, melatonin, agomelatine (S 20098), and *N*-[2-(2,7-dimethoxynaphth-1-yl)ethyl]acetamide, which were very potent at lightening the skin of *X. laevis* tadpoles, compounds **4a**−**c** were inactive or weakly active (EC_{50} > 1 μM). In this bioassay, compound **4a** was characterized as a putative antagonist of melatonin receptors.

Melatonin (*N*-acetyl-5-methoxytryptamine) is the vertebrate pineal gland hormone secreted during darkness.[1] It is now well-recognized that it regulates the circadian rhythm[2] in a large number of animals and in humans. It can be used to control diseases associated with circadian rhythm disorders. Melatonin alleviates jet lag, regulates delayed sleep phase syndrome,[3a,b] and induces sleep.[4] Conversely, it has been implicated in seasonal and winter depression.[5] Melatonin controls the breeding cycle in photoperiodic species and can be used to induce reproduction outside of the breeding season.[6] Melatonin has also been reported to have antiproliferative effects on mammary cell lines.[7] It has been demonstrated that a number of the effects of melatonin are mediated through G protein-coupled receptors[8] which have been cloned,[9] and coupling to one of the G_i family of G proteins appears to be the common signaling pathway for the receptors characterized to date.

Recently, considerable interest has evolved in the design of melatonin receptor ligands capable of mimicking or antagonizing the response to melatonin.[10,11] In particular, naphthalene derivatives were designed as bioisosteric melatonin compounds, and several of these have been demonstrated to be potent agonists for the melatonin receptors. The development of high-affinity, conformationally locked compounds has also constituted an important goal to obtain a clearer insight into the structural parameters implicated in the binding with the receptor. Recently, several constrained melatonergic ligands, where the melatonin pharmacophore groups were incorporated in a ring, have been reported. These were either derivatives of tricyclic indoles such as **2**[12a,b] and **3**[13] (Chart 1), where the ethylamido chain was partially constrained, or totally locked derivatives of 2-aminotetralin[14] and phenalene **4**.[15] To date, the enantioselectivity of the melatonin receptor has only been described for compound **2**[16,12b,] and 2-acetamido-8-methoxytetralin.[17]

We report herein a new synthetic method for the compounds **4** and the preparation of the corresponding (+)- and (−)-enantiomers. The compounds were evaluated for their affinity for chicken brain melatonin receptors in binding assays using 2-[^{125}I]iodomelatonin[18] and for their potency to lighten the skin of *Xenopus laevis* tadpoles.[19a,b]

Chemistry

The compounds **4** were initially synthesized by us[15] through a Friedel−Craft cyclization of *N*-acetyl amino acid derivatives such as 2-acetamido-3-(2-methoxynaphth-1-yl)propanoic acid, but the yield of the cyclization reaction was very poor and was not improved by any modification of the experimental conditions. Subsequently, an alternative synthetic pathway described in Scheme 1 was used. According to a process already reported,[20a,b] 2-methoxynaphthalene was condensed with bis(*N*-phenylchloromethylimine) to give the acenaphthenequinone derivative **5** with a 75% yield. Oxidation of **5** with H_2O_2 in naphthalic anhydride

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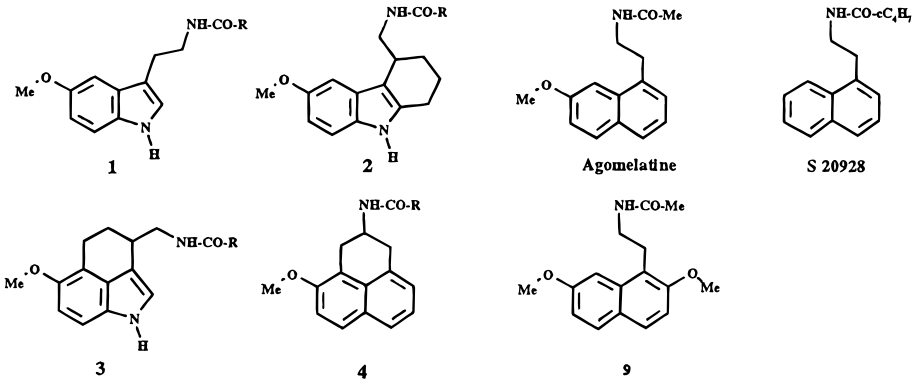
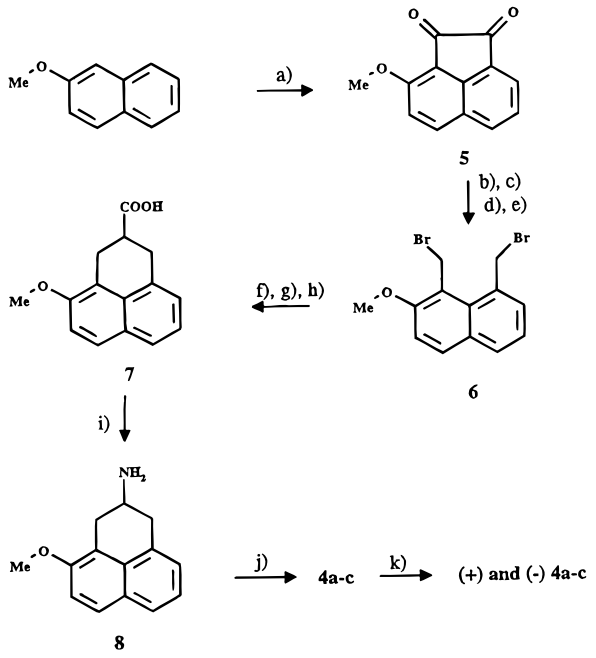
Chart 1



Scheme 1[a]



[a] (a) PhN=CCl−ClC=NPh, $AlCl_3$, CH_2Cl_2, −5 °C, rt; (b) NaOH, H_2O_2, dioxane; (c) HCl; (d) Me_2SO_4, K_2CO_3, Me_2CO, Δ, 48 h, $LiAlH_4$, THF, rt; (e) PBr_3, 0 °C, 40 min; (f) $CH_2(COOEt)_2$, NaOEt, Δ, 5 h; (g) NaOH, MeOH, Δ, 18 h, HCl; (h) Δ, 205−215 °C; (i) EtOCOCl, Et_3N, NaN_3, Δ, HCl, Δ, 3 h; (j) RCOCl, Et_3N, CH_2Cl_2; (k) HPLC, Chiros-Bond Cl column, heptane/EtOH.

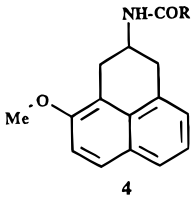
followed by reduction gave the diol which was transformed into the bromo derivative **6** to provide the acid **7** through a classical malonic condensation (50% yield). The hydrolysis and the decarboxylation gave the acid **7**. The amine **8** was prepared from **7** with a 75% yield by the Curtius reaction using thermal rearrangement of the acyl azide in the corresponding isocyanate followed by acid hydrolysis. Acetamido, propionamido, and butyramido derivatives (**4a−c)** were synthesized according to previous methods[15] from the amino compound **8**. The (+)- and (−)-enantiomers of compounds **4a−c** were isolated by semipreparative enantiomeric HPLC separation. The separation was made using a Chiros-Bond C1[21] column containing a chiral stationary phase made with a new chiral 3D-reticulated matrix. Enantiomeric separation was achieved using a heptane/EtOH mobile phase. The enantiomers, obtained with a high enantiomeric purity (>99%), were characterized by their optical rotations (α_D) and mass spectra.

Results and Discussion

The affinities of the compounds for melatonin binding sites were evaluated in vitro in binding assays using 2-[^{125}I]iodomelatonin and chicken brain membranes according to the previously described method.[22] This assay was not predictive for the selectivity of the compounds as all the receptor subtypes mt_1, MT_2,[23] and Mel_{1C}[24] are present in the chicken brain.[9] Their functional activity on melatonin receptors was evaluated by examining the potency of the compounds to lighten the skin of *X. laevis* tadpoles as has been clearly demonstrated that melatonin mediates the aggregation of melanophores.[19a,b] The degree of the dispersion on the head and dorsal surface of the tadpoles observed under a microscope can be assessed using the melanophore index scale (1−5) of Hogben and Slome,[25] and a dose−effect curve can then be plotted. The agonist action was evaluated using EC_{50} values. It has been suggested that this effect is mediated by Mel_{1C} receptors.[12,13] It was important to compare the phenalene derivatives **4** to the reference compounds, melatonin, agomelatine,[26] and *N*-[2-(2,7-dimethoxynaphth-1-yl)ethyl]acetamide **(9)**.[22]

The results are presented in Table 1, and examination of the data showed an increase in affinity with the lengthening of the chain of the amido group. The butyramido compound **4c** (K_i = 1.7 nM) was the most potent as observed previously with the 7-MeO naphthalene derivatives.[26] On the other hand no or weak enantioselectivity was observed for the corresponding (+)- and (−)-enantiomers. These results were unexpected as the phenalene derivatives contain the 2-amidotetralin framework which has been shown to possess a marked enantioselectivity.[17] However, the existence of a secondary binding site, capable of productive interactions with the methoxy group located in the ortho position of the ethylamido chain, may explain the lack of enantioselectivity.[22] The existence of this site was demonstrated by us[15] with the symmetrical compound *N*-(4,9-dimethoxy-2,3-dihydro-1*H*-phenalen-2-yl)acetamide which was more potent than the monomethoxy derivative **4a**. Thus, it is possible that one of the enantiomers of **4** occupies the receptor site similarly to melatonin while the other one prefers to bind with a similar affinity in a symmetrical position involving the secondary binding site (Figure 1). Comparison of the affinities of the compounds **4** with those of the naphthalene derivatives, such as agomelatine and the 2-methoxy isomer, showed that the former were less potent. This decrease in affinity may be due to loss of the

Table 1. Melatoninergic Properties of Phenalene Derivatives **4**



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compd	R	binding assay K_i (nM)[a]	functional activitiy EC_{50} (nM)[b]
(±)-**4a**	Me	36.5 [29.6−44.7]	>1000 (0%)
(+)-**4a**	Me	38.3 [30.7−40.7]	>1000 (0%)
(−)-**4a**	Me	28.7 [24.1−36.6]	>1000 (0%)
(±)-**4b**	Et	8.6 [4.3−17.3]	>1000 (40%)
(+)-**4b**	Et	9 [7.6−16.7]	NT
(−)-**4b**	Et	7.6 [4.8−16.3]	NT
(±)-**4c**	Pr	1.7 [0.8−3.6][c]	>1000 (40%)
(+)-**4c**	Pr	3.2 [1.4−7.3]	NT
(−)-**4c**	Pr	10 [4.9−20]	NT
S20928		787 [480−1200]	>1000
agomelatine		0.54 [0.41−0.67]	10.1 [5.2−19.7] (100%)
melatonin		0.7 [0.41−1.2]	22.6 [12.5−41] (100%)
9		0.1 [0.05−0.2]	0.037 [0.02−0.05] (100%)

[a] 2-[^{125}I]Iodomelatonin was used as the radioligand, and the binding assays were carried out using membranes prepared from chicken brain (25 °C, 60 min). Each binding assay was performed in triplicate. Nonspecific binding was defined with 10 μM 2-iodomelatonin and represented about 10% of the total binding. K_i values are expressed in nM and were calculated using the Cheng-Prussof equation from the IC_{50} values with PRISM program; 95% confidence limits are in brackets. [b] *X. laevis* tadpoles were placed in groups of 5 in 100-mL beakers. The compounds under test (5 concentrations) were dissolved in a final volume of 5 mL and added to the beaker. After 15 min, the experiments was terminated with the addition of a 37% formaldehyde solution. The degree of the melanophore response was determined by the examination of the head and body surface using the melanophore index scale (1−5) of Hogben and Slome.[28] % represents the percentage of the maximum effect observed with regard to that of melatonin (100%). EC_{50} was determined with the PRISM program. EC_{50} values are the results of 2−5 separate experiments. [c] For (±)-**4c**, (−)-**4c**, and (+)-**4c** the Hill number of the competition experiments was <0.6 and K_i was calculated for one binding site.

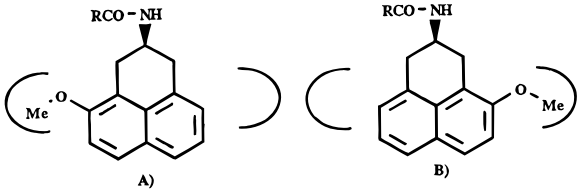

Figure 1. Binding of the enantiomers of **1** to the melatonin receptor site: (A) melatonin-like position; (B) binding with the accessory binding site.

flexibility of the ethylamido chain, leading to a less good fit with the receptor site. However, the introduction of constraints in the agonist structure might also modify the efficiency of the molecule in the transduction process. Therefore the pharmacological profile of these compounds was evaluated on the dermal melanocytes of *X. laevis* tadpoles. An agonist effect on the lightening of tadpole skin was described for the compound **4b** in preliminary experiments[15] using a single dose. The determination of EC_{50}, using a large range of concentrations (0.1 × K_i − 100 × K_i value of chicken brain receptors), did not confirm this result. For the compounds **4a**−**c**, it was not possible to determine EC_{50} values. **4a**, tested as a racemic mixture or enantiomers, was totally inactive up to 1 μM. **4b**, initially described as an agonist,[27] and **4c** were weakly active and were

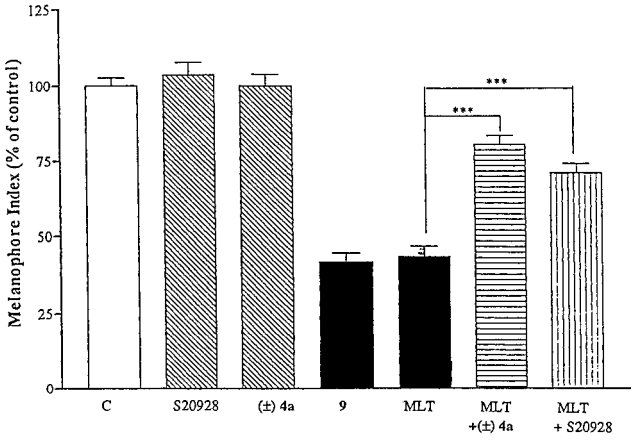

Figure 2. Evaluation of the antagonist effect of compound **4a** on the lightening-induced effects of melatonin (MLT) at 50 nM on the melanophores. The data are represented in melanophore index (% of control): C, control; MLT, melatonin alone (50 nM), **9** was used at the 0.3 nM concentration for the comparison to melatonin; compound **4a** (1 μM) and S 20928 (1 μM) were added 30 min prior to the addition of melatonin. The data are the results of three experiments and represent the mean ± SEM 15−45 animals. MLT and MLT + **4a** (P < 0.01); MLT and MLT + S 20928 (P < 0.01).

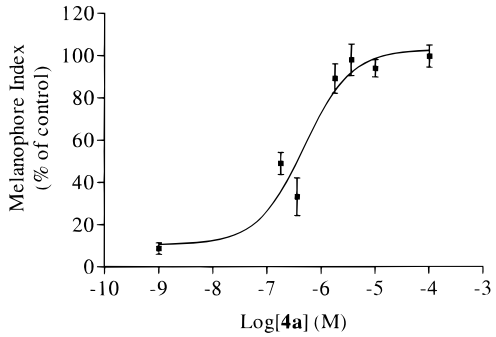

Figure 3. Dose-dependent inhibition by **4a** of the lightening of the *X. laevis* melanophores induced by 50 nM melatonin.

characterized as partial agonists, while the reference compounds, melatonin, agomelatine, and *N*-[2-(2,7-dimethoxynaphth-1-yl)ethyl]acetamide **(9)**, were particularly potent in this bioassay, with EC_{50} values of 22.6, 10.7, and 0.037 nM, respectively. The contraction of the melanophores is thought to be mediated through the Mel_{1c} receptor, and the lack of a clear effect of compounds **4** could be due to the low affinity of these compounds for this receptor subtype. However, preliminary experiments (n = 3) showed that **4a** used at 1 μM concentration inhibited the lightening-induced effects of melatonin **(**50 nM) on the melanophores, indicating an antagonist profile for this compound (Figure 2). Similar effect was obtained with S 20928, an already described melatonin antagonist.[28] It was possible to obtain with **4a** a dose-dependent inhibition of the melatonin effect (50 nM), and the IC_{50} value of 487 nM (246−964) was calculated (Figure 3). The interest in **4a** for the characterization of the cloned human receptors mt_1 and MT_2 was demonstrated by the potent affinity of this compound for the receptors expressed in HEK 293 cells[29] and calculated by binding experiments (IC_{50} = 12.3 and 2.9 nM, respectively).

Thus, phenalene derivatives constitute an interesting class of compounds for the study of melatonin receptors. The equipotency of the enantiomers, regardless of the

alkyl chain of the amido group, confirmed the existence of the putative secondary accessory binding site in the receptor for the 2-methoxy group in the naphthalene melatonin derivatives. The lack or the weak effect of these compounds on the aggregation of the melanophores in *X. laevis* tadpoles and the clear antagonist profile observed with **4a** suggest that these compounds can constitute a class of melatonin receptor antagonists. They confirmed the interest of the introduction of the steric constraints in the agonist structure for the design of antagonist derivatives as **4a** can be considered a rigid analogue of agomelatine.

Experimental Section

Melting points were determined on a Kofler 7841 apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 200 or an AM 400 spectrometer. Chemical shifts are reported in parts per million (δ) relative to tetramethylsilane as the internal standard. ^{1}H NMR multiplicity data are denoted by s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quadruplet), m (multiplet), and br (broad). Coupling constants are in hertz. TLC was performed on silica gel 60 F_{254} (Merck) with detection by UV light. Preparative chromatography was performed under pressure with SDS chromagel silica 60, 35−70 mesh. All solvents and reagents were reagent grade unless otherwise noted. Elemental analyses were performed at the CNRS microanalysis service in Châtenay-Malabry (France). Oxanilide was purchased from Lancaster; other products or reactants were purchased from Aldrich-Chimie (Strasbourg). 2-Methoxy-1,8-(bromomethyl)naphthalene was synthesized from 2-methoxynaphthalene in five steps according to the process already described.[20a,b]

4-Methoxy-2,3-dihydro-1*H*-phenalene-2-carboxylic Acid (7). Sodium (2.3 g) was added in small pieces to absolute ethanol (60 mL); 20.4 mL of this solution was added to diethylmalonate (2.55 mL, 16.8 mmol) and ethanol (20 mL). The mixture was heated at 60 °C for 30 min. The dibromide compound **6**[20a] (4.87 g, 14.2 mmol) was added at 55 °C in small fractions, and the mixture was heated at reflux for 5 h. The cooled solution was hydrolyzed and extracted with diethyl ether, and the combined organic layers were washed with water and brine and dried over $MgSO_4$. The solvent was removed to give a residue which was purified by column chromatography on silica gel with petroleum ether/ethyl acetate (90/10). 4-Methoxy-2,2-(ethoxycarbonyl)-2,3-dihydro-1*H*-phenalene was obtained as a colorless oil (3.5 g, 72%): ^{1}H NMR ($CDCl_3$) δ 1.15 (t, 6H, J = 7.1 Hz, CH_2CH_3), 3.56 (s, 4H, CH_2Ar), 3.95 (s, 3H, OCH_3), 4.09 (q, 4H, J = 7.1 Hz, CH_2CH_3), 7.17−7.25 (m, 3H), 7.59 (m, 1H), 7.67 (d, 1H, J = 9.0 Hz); ^{13}C NMR ($CDCl_3$) δ 13.95, 29.63, 36.08, 53.47, 56.28, 61.39, 113.15, 117.36, 123.41, 124,90, 126.38, 127.29, 128.45, 129.50, 131.15, 153.29, 170.98. Anal. ($C_{20}H_{22}O_5$) C, H.

A solution of potassium hydroxide (7.2 g, 128 mmol) in water (20 mL) was added to a solution of the previous diester (2.30 g, 6.72 mmol) in methanol (22 mL) and refluxed for 18 h. The cooled solution was filtered and evaporated in vacuo. The residue was dissolved in water and extracted with diethyl ether, and the aqueous phase was acidified with concentrated HCl. The yellow precipitate was filtered, washed with cold water, and dried in vacuo to give 4-methoxy-2,3-dihydro-1*H*-phenalene-2,2-dicarboxylic acid (1.55 g, 81%): mp 208 °C; ^{1}H NMR (DMSO-d_6) δ 3.37, 3.40 (2s, 4H, CH_2Ar), 3.91 (s, 3H, OCH_3), 7.18−7.26 (m, 2H), 7.38 (d, 1H, J = 9.0 Hz), 7.61−7.67 (m, 1H), 7.76 (d, 1H, J = 9.0 Hz), 8.43 (br s, 2H, COO*H*); ^{13}C NMR (DMSO-d_6) δ 29.18, 35.49, 52.50, 56.23, 113.49, 117.47, 123.33, 124.76, 125.96, 126.98, 128.00, 129.17, 131.82, 152.90, 172.33. Anal. ($C_{16}H_{14}O_5 \cdot 0.25H_2O$) C, H.

Decarboxylation of the previous diacid (2.2 g) was realized by heating the crude solid at 200−215 °C in a metallic bath until all the gas has been removed. The resulting brown oil was recrystallized from diisopropyl ether to give **7** as a yellow solid (1.42 g, 91%): mp 181 °C; ^{1}H NMR ($CDCl_3$) δ 2.93−3,09 (m, 2H, CH_2Ar), 3.17−3.41 (m, 2H, CH_2Ar), 3.61 (dd, 1H, H_2), 3.94 (s, 3H, OCH_3), 7.22−7.29 (m, 3H), 7.64 (m, 1H), 7.71 (d, 1H, J = 9.0 Hz); ^{13}C NMR ($CDCl_3$) δ 26.89, 33.55, 39.66, 57.00, 113.67, 119.29, 124.00, 125.34, 126.95, 127.92, 129.30, 130.78, 133,36, 153.73, 182.04. Anal. ($C_{15}H_{14}O_3$) C, H.

4-Methoxy-2-amino-2,3-dihydro-1*H*-phenalene (8). To a cooled solution of compound **7** (500 mg, 2.06 mmol) in an acetone/water (9 mL/0.5 mL) mixture were added triethylamine (330 μL, 2.37 mmol, 1.15 equiv) and ethyl chloroformate (250 μL, 2.61 mmol, 1.3 equiv). After 45 min at 0 °C, sodium azide (175 mg, 2.61 mmol, 1.3 equiv) in water (0.9 mL) was added at 0 °C, and the reaction mixture was stirred at 0 °C for 1 h and then poured onto ice and extracted with diethyl ether. The organic layers were washed with water, dried over Na_2SO_4, and evaporated in vacuo without heating. The resulting azide, diluted with 10 mL of dry toluene, was heated at 80 °C until the gas has been removed. After evaporation of the toluene, the resulting oil, corresponding to the isocyanate, was heated at 100 °C with 20% HCl (4 mL) for 3 h. After stirring at room-temperature overnight, dilution with water, and filtration, the liquid phase was extracted with diethyl ether. The aqueous phase was made alkaline with sodium carbonate and then extracted with dichloromethane. The organic phases were washed with water and dried over K_2CO_3. After evaporation in vacuo, the amine **8** was obtained as an oil which was used without purification (340 mg, 77%): ^{1}H NMR ($CDCl_3$) δ 1.55 (br s, 2H, NH_2), 2.64−2.90 (m, 2H, CH_2CH), 3.12−3.39 (m,_3H, CH_2CH), 3.89 (s, 3H, OCH_3), 7.15−7.28 (m, 3H), 7.59−7.69 (2*d, 2H); ^{13}C NMR ($CDCl_3$) δ 33.65, 40.23, 46.09, 56.18, 113.00, 119.14, 123.40, 124.96, 125.94, 126.88, 128.58, 130.40, 133.46, 153.25. Anal. ($C_{14}H_{15}NO$) C, H, N.

***N*-(4-Methoxy-2,3-dihydro-1*H*-phenalen-2-yl)acetamide (4a).** The free amine **8** (300 mg, 1.14.mmol, 1 equiv) was taken up in a biphasic system of CH_2Cl_2/water (17 mL/17 mL) with sodium carbonate (854 mg, 796 mmol, 7 equiv). Acetic anhydride (110 μL, 1.17 mmol, 1 equiv) was slowly added at 0 °C. The reaction mixture was stirred for 30 min at 0 °C and then for 10 min at room temperature. It was washed with water, saturated $NaHCO_3$, and brine and then dried over $MgSO_4$, and the solvent was removed. The crude acetamido derivative **4a** was purified by column chromatography (silica gel, CH_2Cl_2/MeOH: 99/1). Recrystallization from hexane/ethyl acetate mixture gave a white solid for **4a** (227 mg, 74%): mp 196−197 °C; ^{1}H NMR ($CDCl_3$) δ 1.86 (s, 3H, CH_3CO), 3.01−3.34 (m, 4H, CH_2CH), 3.93 (s, 3H, OCH_3), 4.62−4.71 (m, 1H, CH_2CH), 5.55 (br s, 1H, N*H*), 7.19−7.30 (m, 3H), 7.65 (dd, 1H, J = 7.4 Hz), 7.73 (d, 1H, J = 9.1 Hz); ^{13}C NMR ($CDCl_3$) δ 23.55, 29.59, 36.03, 43.40, 56.19, 112.96, 117.08, 123.51, 125.90, 126.37, 127.38, 128.50, 130.25, 131.36, 153.91, 169.65. Anal. ($C_{16}H_{17}NO_2$) C, H, N.

***N*-(4-Methoxy-2,3-dihydro-1*H*-phenalen-2-yl)propanamide (4b).** The free amine **8** (170 mg, 0.797 mmol, 1 equiv) was dissolved in dry CH_2Cl_2 (8 mL) with triethylamine (167 μL, 1.20 mmol, 1.5 equiv). Propionyl chloride (70 μL, 0.797 mmol, 1 equiv) was added at 0 °C, and the reaction was stirred for 40 min. According to the previous procedure, column chromatography and recrystallization under the same conditions gave the pure compound **4b** (110 mg, 51%): mp 184−186 °C; ^{1}H NMR ($CDCl_3$) δ 1.07 (t, 3H, J = 7.6 Hz, CH_3CH_2), 2.03 (q, 2H, J = 7.6 Hz, CH_3CH_2), 3.03−3,35 (m, 4H, CH_2CH), 3.93 (s, 3H, OCH_3), 4.58−4.65 (m, 1H, CH_2CH), 5.45 (br s, 1H, N*H*), 7.18−7.29 (m, 3H), 7.65 (d, 1H, J = 7.5 Hz), 7.73 (d, 1H, J = 9.0 Hz); ^{13}C NMR ($CDCl_3$) δ 9.79, 29.66, 29.74, 36.12, 43.28, 56.16, 112.91, 117.24, 123.44, 125.72, 126.26, 127.28, 128.44, 130.20, 131.52, 153.78, 177.22. Anal. ($C_{17}H_{19}NO_2$) C, H, N.

***N*-(4-Methoxy-2,3-dihydro-1*H*-phenalen-2-yl)butanamide (4c).** According to the previous procedure, the free amine **8** (320 mg, 1.5 mol, 1 equiv), dissolved in dry CH_2Cl_2 (15 mL) with triethylamine (314 μL, 2.25 mmol 1.5 equiv) and butyryl chloride (156 μL, 1.5 mmol, 1 equiv), gave **4c** after column chromatography and recrystallization under the same condi-

tions (343 mg, 87%): mp 170 °C; ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3H, J = 7.4 Hz, CH_3CH_2), 1.54−1.63 (m, 2H, CH_3CH_2), 2.02 (t, 2H, J = 7.3 Hz, $COCH_2CH_2$), 3.04−3.33 (m, 4H, CH_2CH), 3.94 (s, 3H, OCH_3), 4.56−4.71 (m, 1H, CH_2CH), 5.42 (br s, 1H, NH), 7.20−7.22 (d, 1H, J = 6.9 Hz), 7.24−7.28 (m, 2H), 7.65 (dd, 1H, J = 8.0 Hz), 7.74 (d, 1H, J = 9.0 Hz); ^{13}C NMR ($CDCl_3$) δ 13.62, 19.16, 29.69, 36.17, 38.79, 43.29, 56.22, 112.99, 117.27, 123.52, 125.81, 126.33, 127.35, 128.52, 130.26, 131.54, 153.88, 172.57. Anal. ($C_{18}H_{21}NO_2$) C, H, N.

Separation of the Enantiomers of the Derivatives 4a−c. A direct chromatographic method using a semipreparative chiral HPLC column (Chirose-Bond C1) 10 μm, 250 × 10 mm (Chiralsep, Parc d'activités de la Boissière, La Frenaye, 76170 France), gave the pure enantiomers of **4a**−**c**. The conditions for the semipreparative separations were estimated by analytical runs using 20 μL of a 0.5 mg/mL solution on an analytical chiral HPLC Chirose-Bond C1 column (5 μm, 250 × 4.6 mm). A 500-μL loop was used to introduce the samples which were filtered through HV filters (pore size 0.45 μm) (Millipore Corp., Bedford, MA). The concentrations chosen varied between 4 and 12 mg/mL in a mixture of ethanol/heptane (1/3), depending on the resolution of the compound. The mobile phase consisted of heptane/ethanol (9/1) which was degassed by sonication. The solvent delivery system was a Varian 5000 HPLC pump set at a flow rate of 4 mL/min. Detection at 254 nm was carried out using a Varian 2050 tunable absorbance detector. The chromatograms were recorded using a Varian 4270 integrator.

The enantiomers of the phenalene derivatives were separated on a milligram scale, and their purity was controlled by analytical HPLC (>99%). They were characterized by their melting points: **4a**(−) and **4a**(+), mp 223 °C; **4b**(−) and **4b**(+), mp 199 °C; **4c**(−) and **4c**(+), mp 187 °C.

Each enantiomer was tested by positive chemical ionization of NH_4^+: ($M + H^+$) for enantiomers **4a**(−) and **4a**(+), 256; ($M + H^+$) for enantiomers **4b**(−) and **4b**(+), 270; ($M + H^+$) for enantiomers **4c**(−) and **4c**(+), 284.

Optical rotations α_D were measured on a Polartronic C-Schimdt-Haensch instrument for each enantiomer at 2 mg/mL in chloroform at 589 nm. They were uncorrected and all the (+)- and (−)-enantiomers of **4a**−**c** had an optical rotation $[\alpha]^{20}_D = 15°$ at 20 °C.

Melatonin Receptor Binding Assay in Chicken Brain. Chickens (Redbrook, male or female, 4 months (3−4 kg); Cellubio, France) were decapitated at 12 a.m. The brains were quickly removed and stored at −80 °C. The brains were homogenized (Polytron) in 10 volumes of ice-cold Tris-HCl buffer (50 mM, pH 7.4) and washed twice by centrifugation (44000g, 25 min, 4 °C). The resulting pellet was resuspended in 10 volumes of the same buffer to a final concentration of 5 or 6 mg of protein/mL as determined by the method of Lowry.[30] Membrane aliquots (30 μL) were incubated in a total volume of 0.25 mL of Tris-HCl buffer (50 mM, pH 7.4) with 0.05 nM 2-[^{125}I]iodomelatonin and seven concentrations of the compound under test. Each binding assay was performed in triplicate. The incubation (25 °C, 60 min) was stopped by the addition of 3 mL of ice-cold buffer and immediate vacuum filtration through glass fiber filters (GF/B Whatman strips) presoaked in 0.1% poly(ethylenimine) using a Brandel cell harvester. The filters were washed (3 × 4 mL) with buffer, dried, and counted on a γ-counter (Crystal-Packard). Nonspecific binding was defined with 10 μM 2-iodomelatonin and represented 10% of the total binding.

Melatonin Receptor Binding Assay with mt_1 and MT_2 Receptors. Competitive displacements were performed according to the methods already described[29] with the receptors expressed in HEK 293 cells with 2-[^{125}I]iodomelatonin as radioligand.

Melanophore Contraction in *X. laevis* Tadpoles. The *X. laevis* tadpoles (stage 41) used in this study were obtained from the Laboratoire de Biologie Cellulaire et Reproduction CNRS (Rennes, France). They were maintained in an aquarium in the laboratory at 22 °C under natural illumination for 8 days and fed daily with powdered fish food. Prior to the bioassay, tadpoles of uniform stage, size, and color, were selected, removed from the aquarium, and placed in groups of 5 in 100-mL beakers placed on a dark background and filled with 45 mL of pool water, 18 h before the experiments. They were lightened with the artificial light (60 W) for 3 h before the experiment which was performed at midday. The compound under test was dissolved in a DMF and water mixture in a final volume of 5 mL and added to the liquid in the beaker (45 mL) to achieve the final selected concentration. After 15 min, the experiment was terminated by the addition of 37% formaldehyde solution. The degree of the melanophore response in each tadpole was determined by examination of the melanophore configuration under a microscope (Leitz, magnification ×4) and evaluated according to the melanophore index scale (1−5) of Hogben and Slome.[25] The data are the results of the sum of the determinations of the melanophore index on the body and the dorsal surface of the tadpole. EC_{50} values of the compounds were determined from the concentration−response curve obtained with five concentrations in the range of 0.1−100 × K_i values for chicken brain receptors. The mean of the data control (no treated animals with vehicle) represented 100%. In experiments in the presence of antagonist, the compound (1 μM) was added 30 min prior to the addition of melatonin (50 nM). IC_{50} of **4a** was determined with six doses (0.01−10 μM), and it was the result of two separate experiments. The different data (2−5 different experiments) were calculated using the PRISM program (Graphpad).

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