Synthesis of 2-Amido-2,3-dihydro-1*H*-phenalene Derivatives as New Conformationally Restricted Ligands for Melatonin Receptors

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Tetrahydroanthracene, tetrahydrophenanthrene, and tetrahydrophenalene moieties were used to design novel constrained melatoninergic agents. Compounds **1** and **2** were synthesized from the cyclization of the aryl succinic acids **6a**,**b** followed by catalytic reduction, Curtius degradation to the amino derivatives, and acetylation. The phenalene derivatives **3** were prepared by cyclization of the aza lactones of the corresponding α-*N*-acetyl amino acids. The ketone derivatives were reduced directly by catalytic hydrogenation to produce the compounds **3**. The different compounds were evaluated *in vitro* in binding assays using 2-[^{125}I]iodomelatonin and chicken brain membranes. Melatonin and 2-acetamido-8-methoxytetralin were used as the reference compounds. The results showed the superiority of the dihydrophenalene framework **3** over those of tetrahydroanthracene and tetrahydrophenanthrene. **3a** had relatively good affinity for melatonin receptors (K_i = 28.7 nM). Introduction of an additional methoxy group gave a derivative (**3c**) with nanomolar affinity (K_i = 0.7 nM), confirming the existence of a secondary binding site in the receptor which has been described previously. An increase in the affinity was also observed with the propionamido derivative **3e** (K_i = 6.0 nM). The potential agonist properties of the compound **3e** were evaluated on the dermal melanocytes of *Xenopus laevis* tadpoles. At the concentration of 2.3 nM (5 × K_i), melatonin gave a melanophore index value of 1. Similarly to melatonin, **3e** was shown to be a potent agonist of the melanosome aggregation.

Introduction

Melatonin is a pineal hormone which regulates a variety of endocrinological, neurophysiological, and behavioral functions in vertebrates.[1] It plays a role in the regulation of circadian rhythms[2] and has been implicated in several disorders such as delayed sleep phase syndrome, anxiety, and seasonal depression.[3] More recently, some important new properties of melatonin have been described such as antitumoral activity, regulation of immune responsiveness,[4] and scavenging of hydroxy radicals.[5] Its effects are mediated through membrane receptors located in different structures of the brain, in particular in the suprachiasmatic nucleus (SCN)[6] and pars tuberalis[7] where it regulates reproductive function in photoperiodic species. Receptors are also present in peripheral tissues such as the intestine.[8] Recently, several melatonin receptors have been cloned;[9a−d] they are members of the G protein-coupled receptor family and appear to inhibit the synthesis of cyclic AMP.[9e] These receptors have been characterized by the high-affinity specific binding of 2-[^{125}I]iodomelatonin.[10] Moreover, melatonin has also been characterized as the natural ligand of some nuclear receptors.[11] Recently, considerable interest has evolved in the search for new molecules capable of mimicking or antagonizing responses to melatonin. Such compounds constitute important tools for the elucidation of the physiological roles of melatonin. Several indolic analogues[12a,b] of melatonin have been found to act as agonists, and structure−activity relationships have demonstrated the favorable role of 2-halogeno substitution in increasing the affinity for melatonin receptors. 5-Methoxy substitution seems to be implicated in agonist activity because *N*-acetyltryptamine has been described as a partial agonist[12a] and, more recently, antagonist activity at melatonin receptors has been demonstrated with 2-aryl-substituted derivatives.[13] Several recent reports have described the melatoninergic agonist properties of bioisosteric naphthalene derivatives,[14] and, in particular, we emphasized the important role played by the substituent located on the ortho position[14c] of the ethylamido chain on the affinity of such compounds for high-affinity 2-[^{125}I]iodomelatonin binding sites.

The determination of structural parameters implicated in molecular recognition by the receptor is important for the description of the melatoninergic pharmacophore. Conformational analysis[15] of melatonin demonstrated, as expected for such a molecule, a large number of permissible conformers and the impossibility of assigning the active conformation. The development of high-affinity, conformationally locked compounds is essential for this purpose. Several rigid analogues of melatonin have been reported by Garratt[13b] who demonstrated, in a series of tricyclic indoles, the superiority of the cis partially constrained conformer over the trans as an agonist in the pigment aggregation model. Copinga[16] also reported that 2-acetamido-8-methoxytetralin could be a useful framework for the design of new melatoninergic agents.

We present, herein, our approach to the design of new constrained melatoninergic agents using the 2-acet-

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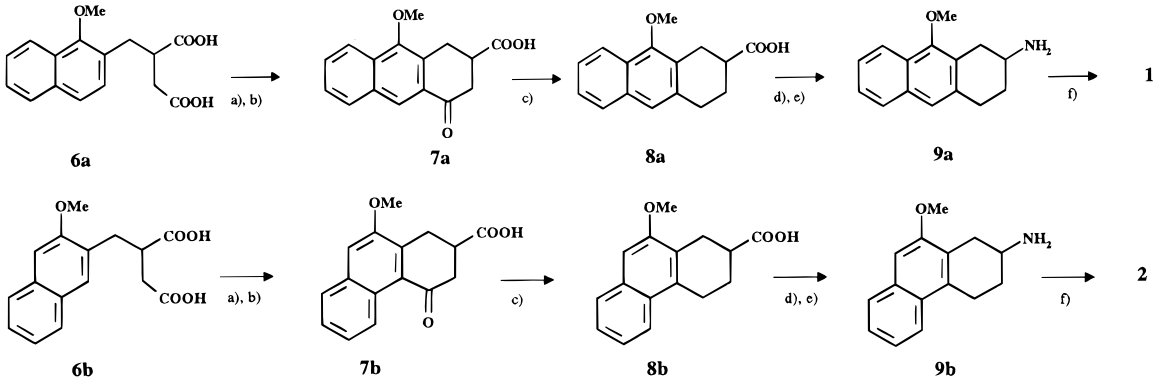
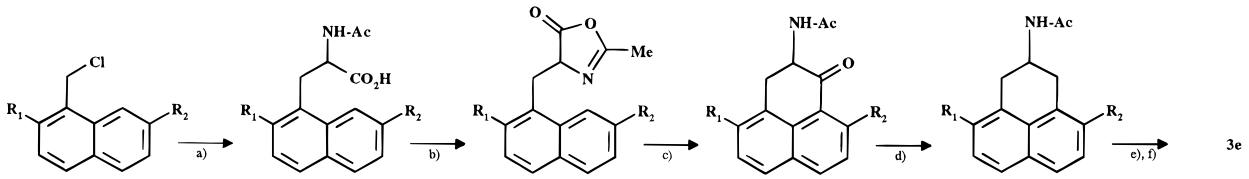
Scheme 1[a]



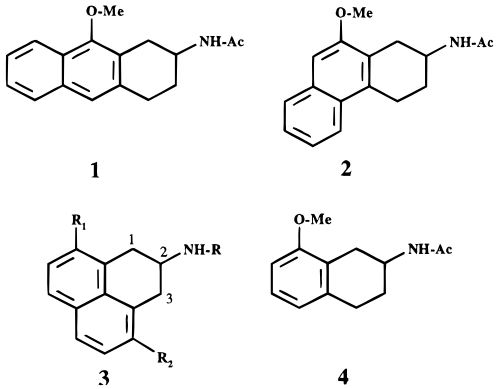
[a] (a) $(Ac)_2O$, reflux, 2 h; (b) $AlCl_3$, nitrobenzene, 0 °C, 5 min; (c) H_2, Pd/C (10%), AcOH, 70 °C or Pd/C (5%), MeOH, HCl, 50 °C, 19 h; (d) diphenyl phosphorazidate, Et_3N, *t*-BuOH, reflux, 18 h; (e) 4 N HCl, AcOEt, rt, 3 h; (f) AcCl, CH_2Cl_2, Et_3N.

Scheme 2[a]



[a] (a) Ac-NHCH(COOEt)$_2$, NaH/DMF or Na/EtOH, NaOH, EtOH, reflux; (b) $(Ac)_2O$, reflux; (c) $AlCl_3$, $CHCl_2$-$CHCl_2$ or CH_2Cl_2; (d) H_2, Pd/C (10%), AcOH; (e) **3b**, HCl (20%), reflux; (f) EtCOCl, Et_3N, CH_2Cl_2.

amido-8-methoxytetralin moiety as the main element of the pharmacophore and a naphthalene ring for its ability to increase the affinity. Consequently, compounds **1**−**3** were synthesized and evaluated in binding assays and compared to the tetralin derivative **4**. These derivatives should provide important structural information on the relative positions of the aromatic ring and the acetamido group of the active form of melatonin in the receptor site.

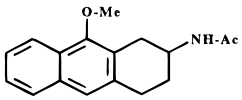
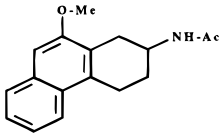
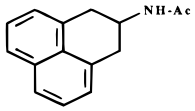
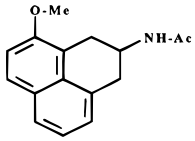
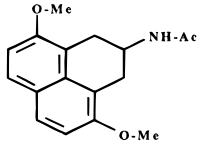
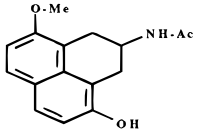
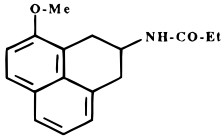


Chemistry

Compounds **1** and **2**, derived from tetrahydroanthracene and tetrahydrophenanthrene moieties, respectively, were prepared by the synthetic route shown in Scheme 1 according to the method reported by us for the synthesis of 8-OH-DPAT.[17] Briefly, (arylmethyl)-succinic acids **6a**,**b** were prepared by the condensation of diethylacetylsuccinate with 1-methoxy-2-(chloromethyl)naphthalene (**5a**) and 2-methoxy-3-(chloromethyl)-naphthalene (**5b**), respectively. These were then transformed to their corresponding anhydrides to be cyclized to the keto acids **7a**,**b** under Friedel−Craft conditions in nitrobenzene with $AlCl_3$. The ketone function was reduced by hydrogen in either acetic acid or methanol in the presence of 10% or 5% Pd/C, respectively, to provide the carboxylic acids **8a**,**b** which were reacted with diphenyl phosphorazidate (DPPA) in *tert*-butyl alcohol under reflux to give the corresponding carbamates. They were hydrolyzed directly by anhydrous HCl in EtOAc to the amino hydrochlorides **9a**,**b** and transformed to the acetamido derivatives **1** and **2** according to the process recently reported by us.[14c] **4** was synthesized by a previously described pathway.[17]

With the purpose of comparing the affinities of the phenalene derivatives **3** and the previously described naphthalene derivatives[14c] for melatonin receptors, the unsubstituted, monomethoxy, and dimethoxy derivatives **3a**−**c**, respectively, were synthesized. They were prepared via a pathway shown in Scheme 2 using cyclization of the α-*N*-acetyl amino acid derivatives **11**. Compounds **11a**−**c** were prepared from a condensation reaction between the diethyl *N*-acetamidomalonate and the corresponding arylmethyl chlorides **10a**−**c** followed by decarboxylation in a NaOH ethanolic solution. 1-Naphthylmethyl chloride (**10a**) was condensed easily with diethyl *N*-acetamidomalonate in ethanol under reflux in the presence of NaOEt to yield the corresponding derivative **11a**, while the 2-methoxy and 2,7-dimethoxy derivatives only reacted in DMF under reflux in the presence of NaH. The amino acids **11a**−**c** were transformed to the aza lactone derivatives **12** by reaction with acetic anhydride, and they were then cyclized to the tricyclic ketones **13a**−**c** in 1,1′,2,2′-tetrachloroethane in the presence of $AlCl_3$.[17] It was observed that the reaction could be run in methylene chloride instead of 1,1′,2,2′-tetrachloroethane. However, the yield from the reaction was poor (10−25%) in both cases. In the course of the cyclization reaction, demethylation of the

Table 1. Affinities of the Tricyclic Derivatives **1**−**3** for Melatonin Receptors[a]

No	Compound	[b] K_i ± SEM, nM
1		273 ± 34
2		168 ± 28
3a		195 ± 46
3b		28.7 ± 2.1
3c		0.7 ± 0.3
3d		31.9 ± 5
3e		6.0 ± 0.4
4	2-acetamido-8-methoxytetralin	161 ± 35
	Melatonin	0.46 ± 0.04
	N-acetyl-5-hydroxytryptamine	1640 ± 243

[a] K_i values were obtained *in vitro* by binding assays using 2-[^{125}I]iodomelatonin (0.05 nM) and chicken brain membranes (25 °C, 60 min). Seven concentrations of the compound under test were used, and each assay was performed in triplicate. [b] K_i values are expressed in nM ± the standard error of the mean (SEM) and were calculated using the Cheng−Prussof equation from the IC_{50} values obtained from competition curves; the data are the results of one or two separate determinations.

dimethoxy compound **13c** was observed to provide compound **13d.** The reduction of the different ketones **13a**−**d** took place in acetic acid in the presence of 10% Pd/C to give compounds **3a**−**d**.

Biological Evaluation 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[14c] and are reported in Table 1.

Examination of the results showed the superiority of the dihydrophenalene framework over those of the tetrahydroanthracene and tetrahydrophenanthrene moieties for recognition by melatonin receptors. Compounds **1** and **2** had low activity compared to compound **3b** which had relatively good affinity (K_i = 28.7 nM) for these receptors. These results indicate that the relative position of the aromatic ring with regard to the acetamido group is an important structural parameter of the melatoninergic pharmacophore. The influence of the methoxy group on the affinity for the receptor was emphasized by these results as a marked decrease in affinity was observed with the unsubstituted compound **3a** (K_i = 195 nM), while the introduction of an additional methoxy group (compound **3c**) gave a derivative with nanomolar affinity (K_i = 0.7 nM), equipotent to melatonin (K_i = 0.46 nM). These data confirmed the results reported recently by us[14c] on the existence of a secondary binding site capable of productive interactions in the ortho position of the ethylamido chain of melatonin which is different from the binding site for the methoxy group mimicking that of melatonin. The unfavorable influence of the hydroxy group was noted with compound **3d** (K_i = 22.8 nM) compared to **3c**. However, its effect was less marked than in *N*-acetyl-5-hydroxytryptamine which was clearly less active (K_i = 1640 nM) than melatonin. The favorable role of the 10 π-electron system on the efficency of binding to the receptor was also confirmed by comparing the affinity of **3b** (K_i = 28.7 nM) with that of 2-acetamido-8-methoxytetralin (K_i = 161 nM). Moreover, the derivative **3e** was prepared for comparison with the propionamide naphthalene derivative previously reported by us[13c] to be a full melatoninergic agonist. A clear increase in affinity was observed with compound **3e** which was 5-fold more potent (K_i = 6 nM) than compound **3b**. These data again indicate that the structure−activity relationship for the dihydrophenalene derivatives seems to be similar to that observed with the naphthalene derivatives and, consequently, that the binding site could be similar for both series of compounds. Thus, dihydrophenalene derivatives provide essential information on the structural parameters implicated in recognition by melatonin receptors, particularly on the relative locations of the aromatic ring and the amido group of melatonin in the receptor site.

However, it was also important to examine the stereochemistry of the amido group with regard to the ring in compounds **3** to provide definitive confirmation of the pharmacophore structure. Analysis of the ^{1}H NMR spectra of compounds **3a**−**c** showed clearly that the pseudoaxial or pseudoequatorial stereochemistry of the amido group depended upon the solvent. In $CDCl_3$, the coupling values (Hz) of the hydrogen in the α positions of the amido function (J_{1-2} = 5.9, $J_{1'-2}$ = 3.9 for **3a**, J_{1-2} = 5.0, $J_{1'-2}$ = 4.3 for **3b**, and J_{1-2} = 5.9, $J_{1'-2}$ = 4.4 for **3c**) indicated an axial position of the amido group, while in DMSO-d_6 they were in favor of the equatorial position (J_{1-2} = 10.1, $J_{1'-2}$ = 4.2 for **3a**, J_{1-2} = 10.4, $J_{1'-2}$ = 3.4 for **3b**, and J_{1-2} = 10.5, $J_{1'-2}$ = 4.4 for **3c**). Conformational analysis of **3b** was performed with the Random Search program (Sybyl 6.03) and showed that the absolute minimum energy conformer had axial stereochemistry. However, an energy difference of only 0.6 kcal/mol was calculated for the corresponding equatorial stereoisomer, confirming the NMR data and indicating that either conformer could be considered as the biologically active form.

Comparison of the biological activity of compounds **3b**,**c** with the corresponding naphthalene derivatives[14c] showed a decrease of 1 order of magnitude in their potency for melatonin receptors. It is possible that the

loss of flexibility due to the phenalene framework decreased the fit with the receptor and might also have changed the pharmacological profile of these molecules. The potential agonist properties of the compound **3e**, the rigid analogue of the previously described[14c] naphthalene derivative, were evaluated on dermal melanocytes of *Xenopus laevis* tadpoles. It was previously demonstrated that melatonin brought about a contraction of the melanophores and, consequently, produced significant lightening of the skin.[19] The degree of the melanosome dispersion on the head and the dorsal surface of the tadpoles was examined under the microscope and evaluated using the melanophore index ranging from 1 to 5.[20] At a concentration of 30 nM (5 $\times$ K_i value for chicken brain receptors), the phenalene derivative **3e** gave a clear melanosome aggregation with a melanophore index value of 1. Melatonin used at a concentration of 2.3 nM (5 $\times$ K_i value for chicken brain receptors) gave a similar effect.

Phenalene derivatives would therefore appear to be promising tools to improve the understanding of the structural parameters implicated in molecular recognition by the receptor. Moreover they constitute a new class of melatonin receptor agonists. Further studies are in progress to evaluate their pharmacological potential.

Experimental Section

Melting points were determined on a METTLER FP 61 apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a BRUKER AC 200 or an AM 300 spectrometer with tetramethylsilane as the internal standard. Chemical shifts are reported in parts per million (ppm) in δ units. ^{1}H NMR multiplicity data are denoted by s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quadriplet), m (multiplet), and br (broad). Coupling constants are in hertz. Elemental analyses were performed at the CNRS analysis service in Vernaison (France) or at the Microanalysis Service in Châtenay-Malabry Faculty (France). Each compound gave an elemental analysis within $\pm$0.45% of the theoretical values. Diethyl acetylsuccinate, 2-methoxybenzyl alcohol, 1-methylnaphthyl chloride (**10a**), and the naphthoic acids were purchased from Aldrich-Chimie (Strasbourg).

Methyl 2-methoxy-3-naphthoate, methyl 1-methoxy-2-naphthoate, and methyl 2-methoxy-1-naphthoate were synthesized by esterification of the corresponding acids.[21] 2-Methoxy-3-(hydroxymethyl)naphthalene, 1-methoxy-2-(hydroxymethyl)-naphthalene, and 2-methoxy-1-(hydroxymethyl)naphthalene were prepared by the reduction of the above esters with $AlLiH_4$.[14c] 2,7-Dimethoxy-1-(hydroxymethyl)naphthalene was prepared by the reduction of 2,7-dimethoxy-1-naphthaldehyde.[14c] 2-Amino-8-methoxytetralin was synthesized according to the process already described.[17]

Preparation of Chlorides 5a,b and 10b,c: General Procedure. A solution of thionyl chloride (1.5 equiv) and pyridine (1 equiv) in anhydrous toluene (360 mL) was cooled to 0 °C in an ice bath. Alcohol (360 mmol, 1 equiv) was added slowly while stirring vigorously. The reaction mixture was stirred at room temperature overnight and then poured into ice. The solution was stirred for 1 h. The organic layer was separated and washed twice with water and then with a saturated aqueous solution of $NaHCO_3$ and brine. After drying over $MgSO_4$, the solvent was evaporated to give the crude chloride.

1-Methoxy-2-(chloromethyl)naphthalene (5a). It was synthesized from 1-methoxy-2-(hydroxymethyl)naphthalene (26 g, 138.3 mmol) according to the general procedure. It was obtained as a pale yellow powder (27.95 g, 98%) and used without further purification: mp 134 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.0 (m, 1H), 7.7 (m, 1H), 7.3−7.5 (m, 4H), 4.75 (s, 2H), 3.9 (s, 3H); ^{13}C NMR (200 MHz, $CDCl_3$) δ 155.0, 134.9, 128.0, 127.6, 127.4, 126.6, 126.15, 125.9, 124.5, 122.3, 62.9, 40.9.

2-Methoxy-3-(chloromethyl)naphthalene (5b). It was synthesized from 2-methoxy-3-(hydroxymethyl)naphthalene (13.49 g, 71.8 mmol) according to the general procedure. It was obtained as a pale yellow powder (14.57 g, 98.3%) and used without further purification: mp 134 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.7 (m, 1H), 7.6 (m, 2H), 7.25−7.35 (m, 2H), 7.0 (s, 1H), 4.7 (s, 2H), 3.8 (s, 3H); ^{13}C NMR (200 MHz, $CDCl_3$) δ 155.2, 134.4, 129.7, 128.1, 127.5, 127.1, 126.6, 126.2, 123.8, 105.3, 55.3, 41.9.

2-Methoxy-1-(chloromethyl)naphthalene (10b). 2-Methoxy-1-(chloromethyl)naphthalene was synthesized from 2-methoxy-1-(hydroxymethyl)naphthalene (7.4 g, 39.31 mmol) according to the general procedure. The pale yellow powder obtained was pure 2-methoxy-1-(chloromethyl)naphthalene (7.2 g, 88%) and used without further purification: ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.05 (d, 1H), 7.84 (2d, 2H), 7.57 (t, 1H), 7.39 (t, 1H), 7.28 (d, 1H), 4.19 (s, 2H), 4.01 (s, 3H).

2,7-Dimethoxy-1-(chloromethyl)naphthalene (10c). 2,7-Dimethoxy-1-(chloromethyl)naphthalene was synthesized from 2,7-dimethoxy-1-(hydroxymethyl)naphthalene (4.0 g, 18 mmol) according to the general procedure; 4.08 g (94%) of the pure 2,7-dimethoxy-1-(chloromethyl)naphthalene was obtained and used without further purification: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.77 (d, J = 9 Hz, 1H), 7.70 (d, J = 9 Hz, 1H), 7.28 (d, J = 3 Hz, 1H), 7.11 (d, J = 9 Hz, 1H), 7.05 (dd, J = 9, 3 Hz, 1H), 5.16 (s, 2H), 3.99 (s, 3H), 3.97 (s, 3H).

Preparation of the (Arylmethyl)succinic Acids 6a,b: General Procedure. Sodium (265 mmol) was added in small pieces to a solution of diethyl acetylsuccinate (278 mmol) in 200 mL of dry toluene and heated at 80 °C. Arylmethyl chloride (316 mmol) was added slowly, and the reaction mixture was refluxed for 18 h. The cooled solution was acidified with acetic acid to pH 7 and evaporated. Water was added, and the solution was extracted with ether. The combined organic layers were dried over $MgSO_4$, and the solvent was removed to give a residue which was hydrolyzed by boiling with 1400 mL of 2 N NaOH for 18 h. The cold alkaline solution was washed with ether, and acidification with concentrated HCl produced the (arylmethyl)succinic acid as an oil which was extracted with ethyl acetate. The organic layer was dried ($MgSO_4$) and the solvent evaporated.

[(1-Methoxy-2-naphthyl)methyl]succinic Acid (6a). It was obtained from compound **5a** (27.95 g, 135.4 mmol) according to the general procedure. The crude product was recrystallized (acetone/toluene) to provide 6.51 g of the pure compound (17%): mp 161 °C; ^{1}H NMR (200 MHz, CD_3OD) δ 7.85 (d, J = 7.3 Hz, 1H), 7.6 (d, J = 7.3 Hz, 1H), 7.35 (d, J = 8.5 Hz, 1H), 7.2−7.3 (m, 2H), 7.1 (d, J = 8.5 Hz, 1H), 3.7 (s, 3H), 2.8−3.1 (m, 3H), 2.2−2.6 (m, 2H); ^{13}C NMR (200 MHz, CD_3OD) δ 178.2, 175.6, 155.3, 135.6, 129.2, 129.1, 129.0, 128.0, 126.9, 126.7, 125.1, 122.9, 62.3, 43.4, 35.0, 32.8. Anal. ($C_{16}H_{16}O_5$) C, H.

[(2-Methoxy-3-naphthyl)methyl]succinic Acid (6b). It was synthesized from compound **5b** (19.43 g, 94.1 mmol) according to the general procedure. The crude product was recrystallized (acetone/toluene) to provide 4.3 g of the pure compound (16%): mp 159 °C; ^{1}H NMR (200 MHz, CD_3OD) δ 7.75−7.8 (m, 2H), 7.6 (s, 1H), 7.3−7.5 (m, 2H), 7.3 (s, 1H), 4.0 (s, 3H), 3.2−3.4 (m, 2H), 3.0 (m, 1H), 2.4−2.75 (m, 2H); ^{13}C NMR (200 MHz, CD_3OD) δ 178.6, 175.9, 157.8, 135.6, 130.9, 130.2, 129.9, 128.3, 127.6, 127.1, 124.8, 106.3, 55.9, 42.9, 36.4, 34.2. Anal. ($C_{16}H_{16}O_5 \cdot 0.25H_2O$) C, H.

Preparation of the Carboxytetralone Derivatives 7a,b: General Procedure. The (arylmethyl)succinic acids (33.6 mmol) were converted to their corresponding anhydrides by refluxing for 2 h with acetic anhydride (80 g). The solvent was removed *in vacuo* to give a brown oil which was the crude anhydride used without further purification. It (25.5 mmol) was dissolved in 60 mL of nitrobenzene and added slowly to a cooled solution (0 °C) of $AlCl_3$ (75.5 mmol) in 60 mL of nitrobenzene. The reaction mixture was stirred for 5 min, and a solution of 40 g of ice with 40 mL of concentrated HCl was added. After standing overnight, the nitrobenzene was removed by steam distillation. The cooled solution was extracted with ethyl acetate, the organic layer was dried over $MgSO_4$, and the solvent was evaporated. The crude product was

purified by flash chromatography (silica gel, CH_2Cl_2–MeOH, 95:5) to give the pure carboxytetralone.

10-Methoxy-3-carboxy-1,2,3,4-tetrahydroanthracen-1-one (7a). It was prepared from the 1-succinic acid derivative **6a** (7.79 g, 27 mmol) according to the general procedure to give 3.38 g of the pure tetralone **7a** (46.3%): mp 202 °C; ^{1}H NMR (200 MHz, CD_3OD) δ 8.3 (s, 1H), 8.0 (d, J = 8.4 Hz, 1H), 7.95 (d, J = 8.2 Hz, 1H), 7.5 (td, J = 8.2, 6.9 Hz, 1H), 7.4 (td, J = 8.4, 6.91 Hz, 1H), 3.9 (s, 3H), 2.8–3.5 (m, 5H); ^{13}C NMR (200 MHz, CD_3OD) δ 197.0, 170.0, 153.7, 136.3, 130.2, 128.7–127.2–126.4–124.6–121.7 (7C), 62.0, 40.8, 39.3, 25.3. Anal. ($C_{16}H_{14}O_4 \cdot 0.3H_2O$) C, H.

10-Methoxy-2-carboxy-1,2,3,4-tetrahydrophenanthren-4-one (7b). It was synthesized from the succinic acid derivative **6b** (5.09 g, 17.7 mmol) according to the general procedure to give 2.77 g of the pure tetralone **7b** (58%): mp 196 °C; ^{1}H NMR (200 MHz, CD_3OD) δ 9.1 (m, 1H), 7.6 (m, 1H), 7.35 (m, 2H), 7.2 (s, 1H), 3.8 (s, 3H), 2.8–3.4 (m, 5H); ^{13}C NMR (200 MHz, CD_3OD) δ 200.6, 177.0, 155.7, 138.8, 135.2, 129.4, 128.9, 128.4, 127.7, 127.5, 127.2, 112.7, 56.4, 43.4, 40.2, 27.7. Anal. ($C_{16}H_{14}O_4$) C, H.

Preparation of the 2-Acetamidopropanoic Acid Derivatives 11a–c: General Procedure. A suspension of 60% sodium hydride in oil (1.2 equiv) was added in small portions to a solution of diethyl acetamidomalonate (1.2 equiv) in 80 mL of dry DMF under an inert atmosphere. The solution was stirred until no more gaseous emissions were observed. The (chloromethyl)naphthalene derivative (50.57 mmol, 1.0 equiv), dissolved in 40 mL of dry DMF, was added dropwise, and the reaction mixture was refluxed with stirring for 20 h. The solvent was removed *in vacuo* and the residue taken up in CH_2Cl_2 and washed with water. The organic layer was dried over $MgSO_4$ and evaporated to give the acetamidomalonate derivative which was used without further purification. It (1.0 equiv) was dissolved in 44 mL of ethanol. A solution of 2 N aqueous sodium hydroxide (3 equiv) was added, and the reaction mixture was refluxed for 6 h and then concentrated. After an initial extraction with diethyl ether, the aqueous solution was acidified to pH 1 with a solution of 1 N $KHSO_4$. The suspension obtained was extracted with ethyl acetate. The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure to give the pure acetamido acid derivative.

2-Acetamido-3-(1-naphthyl)propanoic Acid (11a). It was prepared from 1-methylnaphthyl chloride (**10a**) (13.5 mL, 91 mmol) according to the general procedure to give diethyl (1-methylnaphthyl)acetamidomalonate (24.5 g, 95%) as a pure product: mp 107 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.93–7.88 (m, 1H), 7.75–7.43 (m, 2H), 7.38–7.24 (m, 3H), 7.09 (d, 1H), 6.36 (s, 1H), 4.18–4.12 (m, 4H), 4.07 (s, 2H), 1.82 (s, 3H), 1.26 (t, 6H).

It was converted into **11a** according to the general procedure. The product was recrystallized (acetone/petroleum ether) to give 11.26 g (61%) of a white powder: mp 169 °C; ^{1}H NMR (200 MHz, CD_3OD) δ 7.98 (d, 1H), 7.68 (dd, 1H), 7.58 (t, 1H), 7.38–7.19 (m, 4H), 4.65–4.60 (m, 1H), 3.63–3.53 (dd, 1H), 3.19–3.08 (dd, 1H), 1.68 (s, 3H). Anal. ($C_{15}H_{15}NO_3 \cdot 0.25H_2O$) C, H, N.

2-Acetamido-3-(2-methoxynaphthyl)propanoic Acid (11b). It was prepared from the chloride derivative **10b** (10.45 g, 50.57 mmol) according to the general procedure to give the diethyl acetamidomalonate derivative (12.63 g, 64.5%) as a pure product: mp 110 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.89 (d, 1H), 7.71 (d, 1H), 7.68 (d, 1H), 7.36 (t, 1H), 7.24 (t, 1H), 7.16 (d, 1H), 6.19 (s, 1H), 4.16 (q, 4H), 4.05 (s, 2H), 3.78 (s, 3H), 1.63 (s, 3H), 1.22 (t, 6H).

It (8.1 g, 21.38 mmol) was converted into **11b** (5.34 g, 87%) by hydrolysis according to the general procedure: mp 201 °C; ^{1}H NMR (200 MHz, CD_3OD) δ 8.12 (d, 1H), 7.87 (2d, 2H), 7.56 (t, 1H), 7.45–7.36 (m, 2H), 4.78–4.74 (q, 1H), 4.04 (s, 3H), 3.77–3.52 (m, 2H), 1.88 (s, 3H). Anal. ($C_{16}H_{17}NO_4$) C, H, N.

2-Acetamido-3-(2,7-dimethoxynaphthyl)propanoic Acid (11c). It was prepared from the chloride derivative **10c** (4.08 g, 17 mmol) according to the general procedure to give the diethyl acetamidomalonate derivative as a pure compound (4.01 g, 55%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.69 (d, J = 9 Hz, 1H), 7.63 (d, J = 9 Hz, 1H), 7.27 (d, J = 2.3 Hz, 1H), 7.04 (d, J = 9 Hz, 1H), 6.96 (dd, J = 9, 2.3 Hz, 1H), 6.27 (br s, 1H), 4.38–4.17 (m, 4H), 4.07 (s, 2H), 3.94 (s, 3H), 3.82 (s, 3H), 1.65 (s, 3H), 1.29 (t, 6H).

It (4.01 g, 10 mmol) was converted into **11c** by hydrolysis according to the general procedure to give 2.3 g (76%) of the pure product: ^{1}H NMR (200 MHz, CD_3OD) δ 7.68 (d, 1H), 7.63 (d, 1H), 7.32 (d, 1H), 7.13 (d, 1H), 6.84–6.78 (dd, 1H), 4.67 (t, 1H), 3.90 (s, 6H), 3.67–3.40 (m, 2H), 1.83 (s, 3H). Anal. ($C_{17}H_{19}NO_5$) C, H, N.

Preparation of the 2,3-Dihydro-1*H*-phenalen-1-one Derivatives 13a–c: General Procedure. 2-Acetamidopropanoic acid derivatives **11a–c** (13.92 mmol) were converted into their corresponding azalactones **12a–c** by refluxing for 40 min in acetic acid (14 mL). The solvent was removed *in vacuo* to give a brown-yellow product as an oil which was used without purification in the next step. It was dissolved in dry 1,1′,2,2′-tetrachloroethane (90 mL) or CH_2Cl_2 and added dropwise to a suspension of $AlCl_3$ (3 equiv) in the same solvent (50 mL). The reaction mixture was heated to 60 °C for 2 h, poured into a mixture of ice (17 g) and concentrated HCl (1.1 mL), and stirred vigorously for 1 h. The organic layer was separated and the aqueous phase extracted twice with dichloromethane. The combined organic layers were dried over $MgSO_4$ and removed *in vacuo*. The residue was chromatographed (silica gel, CH_2Cl_2–MeOH, 99:1) to give the pure product.

2-Acetamido-2,3-dihydro-1*H*-phenalen-1-one (13a). It was synthesized from the corresponding acetamido acid **11a** (6 g, 23 mmol) according to the general procedure in 1,1′,2,2′-tetrachloroethane to give 1.47 g (26%) of the desired product **13a**: mp 180 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.17–8.09 (m, 2H), 7.86–7.79 (m, 1H), 7.64–7.49 (m, 3H), 6.89 (br s, 1H), 5.09–4.96 (m, 1H), 4.13–4.02 (dd, J = 15, 6.5 Hz, 1H), 3.21–3.08 (dd, J = 15, 13 Hz, 1H), 2.15 (s, 3H). Anal. ($C_{15}H_{13}NO_2 \cdot 0.25\ H_2O$) C, H, N.

4-Methoxy-2-acetamido-2,3-dihydro-1*H*-phenalen-1-one (13b). It was synthesized from the corresponding acetamido acid **11b** (4 g, 13.92 mmol) according to the general procedure in 1,1′,2,2′-tetrachloroethane to give 0.93 g (25%) of the pure compound **13b**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.83 (d, 1H), 7.72 (2d, 2H), 4.72 (t, 1H), 7.18–7.3 (m, 2H), 4.41–4.51 (q, 1H), 3.88 (s, 3H), 3.55–3.60 (m, 2H), 2.1 (s, 3H). Anal. ($C_{16}H_{15}NO_3 \cdot 0.25H_2O$) C, H, N.

4,9-Dimethoxy- and 4-Methoxy-9-hydroxy-2-acetamido-2,3-dihydro-1*H*-phenalen-1-one (13c,d). They were synthesized from the corresponding acetamido acid **11c** (4 g, 13.92 mmol) according to the general procedure in dichloromethane. The compounds were obtained as solids. **13c** (0.19 g, 10%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.96 (d, J = 9 Hz, 1H), 7.71 (d, J = 9 Hz, 1H), 7.18–7.12 (q, 2H), 6.97 (br s, 1H), 4.88 (m, 1H), 4.39–4.19 (dd, J = 16, 7 Hz, 1H), 4.04 (s, 3H), 3.94 (s, 3H), 2.74–2.59 (m, 1H), 2.11 (s, 3H). Anal. ($C_{17}H_{17}NO_4$) C, H, N. **13d** (0.2 g, 12%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 12.35 (s, 1H), 7.90 (d, J = 9 Hz, 1H), 7.67 (d, J = 9 Hz, 1H), 7.12 (d, J = 9 Hz, 1H), 6.97 (d, J = 9 Hz, 1H), 6.52 (br s, 1H), 4.97–4.94 (m, 1H), 4.22–4.17 (dd, J = 16, 8 Hz, 1H), 3.94 (s, 3H), 2.74–2.67 (m, 1H), 2.15 (s, 3H).

Preparation of the 2-Carboxytetralin Derivatives 8a,b and 2-Acetamido-2,3-dihydro-1*H*-phenalene Derivatives 3a–d: General Procedure. The ketone derivative (11.1 mmol) was reduced with H_2 under atmospheric pressure using palladium on activated carbon as the catalyst. For the tetralin derivatives **7a,b**, the mixture was stirred overnight with a 10% weight of Pd/C (10%) in AcOH or with Pd/C (5%) with a few drops of concentrated HCl in MeOH. For the phenalene derivatives **13a–d**, a 50% weight of Pd/C (10%) was used at room temperature for 28 h. The catalyst was filtered, and the solution was concentrated under reduced pressure. The residue was purified by chromatography on silica gel to give the pure compound.

9-Methoxy-2-carboxy-1,2,3,4-tetrahydroanthracene (8a). It was synthesized from **7a** (2.81 g, 9.8 mmol) according to the general procedure in MeOH at 50 °C. After filtration the oil was hydrolyzed by boiling with 2 N NaOH for 30 min and acidified with concentrated HCl to pH 1 (1.53 g, 61%): mp 192 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.9 (m, 1H), 7.6 (m, 1H), 7.3 (s, 1H), 7.3–7.2 (m, 2H), 3.8 (s, 3H), 3.3 (m, 1H), 3.0–2.5 (m, 5H), 2.1 (m, 1H), 1.8 (m, 1H); ^{13}C NMR (200 MHz, $CDCl_3$)

δ 178.4, 153.1, 135.3, 133.1, 127.1, 125.4, 124.9, 122.6, 121.4 (11C), 61.1, 39.5, 28.8, 25.9, 25.6. Anal. ($C_{16}H_{16}O_3 \cdot 0.25H_2O$) C, H.

10-Methoxy-2-carboxy-1,2,3,4-tetrahydrophenanthrene (8b). It was synthesized from **7b** (2.5 g, 9.3 mmol) according to the general procedure in AcOH at 70 °C to give the pure compound (1.96 g, 83%): mp 235 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.8 (dd, J = 7.3, 2.2 Hz, 1H), 7.6 (dd, J = 7.2, 2 Hz, 1H), 7.3 (m, 2H), 6.9 (s, 1H), 3.8 (s, 3H), 2.5−3.3 (m, 5H), 2.25 (m, 1H), 1.8 (m, 1H); ^{13}C NMR (200 MHz, $CDCl_3$) δ 178.4, 155.6, 132.8, 132.0, 127.4, 126.9, 125.3, 125.2, 123.3, 122.5, 102.5, 54.9, 39.0, 26.2, 25.2, 25.1; low-resolution CIMS m/z 257 $(M + 1)^+$.

2-Acetamido-2,3-dihydro-1*H*-phenalene (3a). Compound **3a** was synthesized from the corresponding phenalen-1-one derivative **13a** (1.47 g, 6 mmol) according to the general procedure and purified by chromatography (silica gel, CH_2Cl_2−MeOH, 98:2) to give 0.96 g (70%) of the desired product: mp 186 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.72 (d, J = 8 Hz, 2H), 7.41 (t, J = 8 Hz, 2H), 7.25 (d, J = 7 Hz, 2H), 5.54 (br s, 1H), 4.7−4.69 (m, 1H), 3.4−3.35 (dd, J = 16, 4 Hz, 2H), 3.15−3.09 (dd, J = 16, 6 Hz, 2H), 1.86 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 169.87, 133.52, 132.63 (2C), 129.47, 126.6 (2C), 125.9 (2C), 125.5 (2C), 44.18, 36.54 (2C), 23.49. Anal. ($C_{15}H_{15}NO \cdot 0.25H_2O$) C, H, N.

4-Methoxy-2-acetamido-2,3-dihydro-1*H*-phenalene (3b). Compound **3b** was synthesized from the corresponding phenalen-1-one derivative **13b** (1.0 g, 3.71 mmol) according to the general procedure and purified by chromatography (silica gel, CH_2Cl_2−MeOH, 98:1) to give the pure product (76%): mp 196−197 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.73 (d, J = 9 Hz, 1H), 7.65 (dd, J = 7 Hz, 1H), 7.3−7.19 (m, 3H), 5.5 (br s, 1H), 4.71−4.62 (m, 1H), 3.93 (s, 3H), 3.34−3.01 (m, 4H), 1.86 (s, 3H). Anal. ($C_{16}H_{17}NO_2$) C, H, N.

4,9-Dimethoxy-2-acetamido-2,3-dihydro-1*H*-phenalene (3c). Compound **3c** was synthesized from the corresponding phenalen-1-one derivative **13c** (0.17 g, 0.6 mmol) according to the general procedure and purified by chromatography (silica gel, CH_2Cl_2−MeOH, 98:2) to give 0.07 g of the pure product (43%): mp 254 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.99 (d, 1H), 7.71 (dd, 2H), 7.22 (d, 2H), 3.95−3.89 (m, 1H), 3.86 (s, 6H), 3.28−3.23 (dd, J = 16, 4 Hz, 2H), 2.6−2.53 (dd, J = 16, 10 Hz, 2H), 1.83 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 169.83, 153.07, 130.7, 127.05, 123.7, 116.74, 11.12, 55.98, 43.64, 29.02, 22.77. Anal. ($C_{17}H_{19}NO_3 \cdot 0.25H_2O$) C, H, N.

4-Methoxy-9-hydroxy-2-acetamido-2,3-dihydro-1*H*-phenalene (3d). Compound **3d** was synthesized from the corresponding phenalen-1-one derivative **13d** (0.17 g, 0.6 mmol) according to the general procedure and purified by chromatography (silica gel, CH_2Cl_2−MeOH, 98:2) to give 0.07 g of the pure product (37%): mp 245°C, ^{1}H NMR (200 MHz, CD_3COCD_3) δ 7.62 (d, J = 9 Hz, 1H), 7.50 (d, J = 9 Hz, 1H), 7.25 (br s, 1H), 7.12 (d, J = 9 Hz, 1H), 7.01 (d, J = 9 Hz, 1H), 4.32−4.18 (m, 1H), 3.89 (s, 3H), 3.41−3.33 (dd, 1H), 2.99 (br s, 1H), 2.79−2.67 (q, 2H), 1.86 (s, 3H).

Preparation of the 2-Aminotetralin Hydrochlorides 9a,b. A solution of 2-carboxytetralin **8a**,**b** (7.5 mmol), diphenyl phosphorazidate (1 equiv), and triethylamine (1.1 equiv) in 35 mL of *tert*-butyl alcohol was refluxed for 18 h. The reaction mixture was evaporated, 220 mL of toluene was added, and this solution was successively washed with a 5% citric acid solution (20 mL), water (20 mL), a saturated aqueous $NaHCO_3$ solution (40 mL), and brine (20 mL). The solvent was removed *in vacuo* to provide the carbamate which was used without further purification.

To a solution of the carbamate (1.1 mmol) in 10 mL of ethyl acetate was added 10 mL of a 4 N HCl solution in dry ethyl acetate. The reaction mixture was stirred for 3 h and then evaporated to give the crude hydrochloride which was purified by dissolution in ethanol and precipitation by the addition of diethyl ether. The precipitate was filtered and dried to give the pure amino hydrochloride.

9-Methoxy-2-amino-1,2,3,4-tetrahydroanthracene Hydrochloride (9a). It was synthesized from the corresponding acid **8a** (1.45 g, 5.7 mmol) according to the general procedure (45%): mp 285 °C; ^{1}H NMR (200 MHz, CD_3OD) δ 7.8 (m, 1H), 7.6 (m, 1H), 7.3 (s, 1H), 7.2−7.3 (m, 2H), 3.8 (s, 3H), 3.4 (m, 1H), 3.0 (m, 2H), 2.8 (m, 1H), 2.5 (m, 1H), 2.1 (m, 1H), 1.8 (m, 1H); ^{13}C NMR (200 MHz, CD_3OD) δ 154.8, 135.15, 135.1, 128.6, 127.6, 127.1, 126.4, 124.0, 123.5, 122.6, 61.6, 49.0, 29.3, 28.2, 26.3. Anal. ($C_{15}H_{18}ClNO$) C, H, N.

10-Methoxy-2-amino-1,2,3,4-tetrahydrophenanthrene Hydrochloride (9b). It was obtained from the corresponding acid **8b** (1.18 g, 4.5 mmol) according to the general procedure (77%): mp 272 °C; ^{1}H NMR (200 MHz, CD_3OD) δ 7.6 (d, J = 7.3 Hz, 1H), 7.5 (d, J = 8.7 Hz, 1H), 7.2−7.1 (m, 2H), 6.9 (s, 1H), 3.7 (s, 3H), 3.3 (m, 1H), 3.2−3.0 (m, 2H), 3.0−2.8 (m, 1H), 2.5 (m, 1H), 2.15 (m, 1H), 1.8−1.7 (m, 1H); ^{13}C NMR (200 MHz, CD_3OD) δ 156.5, 134.7, 132.3, 128.5, 128.2, 126.8, 124.8, 123.6, 123.5, 104.4, 55.7, 48.9, 29.3, 27.7, 25.0. Anal. ($C_{15}H_{18}ClNO \cdot 0.5H_2O$) C, H, N, Cl.

Preparation of the 2-Acetamido Derivatives 1, 2, and 4. The free amine (1 equiv) was dissolved in dry dichloromethane in the presence of triethylamine (1.2 equiv). Acetyl chloride (1.2 equiv) was added at 0 °C, and the reaction mixture was stirred for 1 h at room temperature. The organic solution was washed with water, dried over $MgSO_4$, and evaporated. The crude residue was purified by chromatography (silica gel, CH_2Cl_2−MeOH, 98:5) to give the expected acetamide.

8-Methoxy-2-acetamidotetralin (4). Compound **4** was synthesized from the corresponding amine (0.35 g, 2 mmol) according to the general procedure to give 0.24 g (56%) of the pure product: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.0 (t, J = 7.87 Hz, 1H), 6.61 (d, J = 7.66 Hz, 1H), 6.55 (d, J = 8.14 Hz, 1H), 6.05 (d, 1H), 4.16−4.08 (m, 1H), 3.68 (s, 3H), 3.03−2.92 (q, J = 17.26, 5.5 Hz, 1H), 2.74−2.72 (m, 2H), 2.40−2.27 (q, J = 17.24, 8.4 Hz, 1H), 1.99−1.7 (m, 1H), 1.87 (s, 3H), 1.67−1.56 (m, 1H). Anal. ($C_{13}H_{17}NO_2 \cdot 0.25H_2O$) C, H, N.

9-Methoxy-2-acetamido-1,2,3,4-tetrahydroanthracene (1). Compound **1** was synthesized from the corresponding amine **9a** (0.18 g, 0.79 mmol) to give 0.18 g (85%) of pure product: mp 161−162 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.0 (m, 1H), 7.74 (m, 1H), 7.44−7.40 (m, 3H), 5.55 (d, 1H), 4.38 (m, 1H), 3.90 (s, 3H), 3.38−3.27 (q, 1H), 3.10−3.03 (t, 2H), 2.86−2.73 (q, 1H), 2.17−2.10 (m, 1H), 1.99 (s, 3H), 1.97−1.74 (m, 1H). Anal. ($C_{17}H_{19}NO_2$) C, H, N.

10-Methoxy-2-acetamido-1,2,3,4-tetrahydrophenanthrene (2). Compound **2** was synthesized from the corresponding amine **9b** (0.11 g, 0.5 mmol) according to the general procedure to give 0.24 g (56%) of the pure product: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.81 (d, 1H), 7.64 (d, 1H), 7.38−7.26 (m, 2H), 6.9 (s, 1H), 5.34 (d, 1H), 4.33−4.2 (m, 1H), 3.83 (s, 3H), 3.1−3.04 (m, 2H), 2.67−2.44 (m, 2H), 2.11−2.01 (m, 1H), 1.90 (s, 3H), 183−1.69 (m, 1H). Anal. ($C_{17}H_{19}NO_2 \cdot 0.25H_2O$) C, H, N.

4-Methoxy-2-propionamido-2,3-dihydro-1*H*-phenalene (3e). To a solution of compound **3b** (1.52 g, 5.95 mmol) in ethanol (20 mL) was added 54 mL of a 10% aqueous HCl solution. The reaction mixture was stirred for 20 h at 90 °C and then cooled and made basic using 10% NaOH. The aqueous solution was extracted with dichloromethane. The organic layer was dried over $MgSO_4$ and removed *in vacuo* to afford the free amine which was used without further purification.

The free amine (0.120 g, 0.56 mmol) was dissolved in dry dichloromethane (5 mL) with triethylamine (0.118 mL, 0.84 mmol). Propionyl chloride (0.05 g, 0.56 mmol) was added at 0 °C, and the reaction mixture was stirred for 40 min at room temperature. It was washed with water and dried over $MgSO_4$ and the solvent removed. The crude propionamido derivative was purified by column chromatography (silica gel, CH_2Cl_2−MeOH, 95:5) to give the pure compound **3e** (66%): mp 184−186 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.73 (d, J = 9 Hz, 1H), 7.65 (d, J = 7.5 Hz, 1H), 7.29−7.18 (m, 3H), 5.45 (d, 1H), 4.68−4.65 (m, 1H), 3.93 (s, 3H), 3.35−3.03 (m, 4H), 2.03 (q, J = 7.6 Hz, 2H), 1.07 (t, J = 7.6 Hz, 3H); ^{13}C NMR (200 MHz, $CDCl_3$) δ 177.22, 153.78, 131.52, 130.20, 128.44, 127.28, 126.26, 125.72, 123.44, 117.24, 112.91, 56.16, 43.28, 36.12, 29.74, 29.66, 9.79. Anal. ($C_{17}H_{19}NO_2$) C, H, N.

Melatonin Receptor Binding Assay. Chickens (Red Brook, 4 months, 3−4 kg; Cellubio, France) were decapitated at 12 a.m. The brains were quickly removed and stored at −80 °C. They were homogenized (Polytron) in 10 vol of ice-

cold Tris-HCl buffer (50 mM, pH 7.4) and washed twice by centrifugation (44000*g*, 25 min, 4 °C). The resulting pellet was resuspended in 10 vol of the same buffer to a final concentration of 5 or 6 mg of protein/mL as determined by the method of Lowry et al. (1951). The membrane aliquots were stored at −80 °C until subsequent use. 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 in a γ-counter (Crystal-Packard). Nonspecific binding was defined with 10 μM 2-iodomelatonin and represented 10% of the total binding.

Melanophore Contraction in *X. laevis* Tadpoles. The *X. laevis* larvae (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 at 22 °C in the laboratory 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 18 h before the experiments which were performed at midday. The compound under test was dissolved in DMF and water mixture in a final volume of 5 mL and added to the liquid in the beaker (45 mL) to a final concentration of 5 × K_i, the K_i value having been determined in binding assays on chicken brain membranes. After 15 min, the experiment was terminated by the addition to the test beaker of an equal volume of 30% formaldehyde solution. The degree of the melanophore response in each tadpole was determined by examination of the melanosome configuration on the head and dorsal surface under a microscope (magnification ×4) and evaluated according to the melanophore index scale (1−5) of Hogben and Slome.[19] The data are the result of the mean of individual determinations of the melanophore index and of two consecutive assays. In the control assays, the value of the melanophore index was 4.

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