Synthesis of Nitroindole Derivatives with High Affinity and Selectivity for Melatoninergic Binding Sites *MT$_3$*

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The aim of this study was to synthesize selective ligands for melatoninergic subtype receptors that could elucidate the physiological role of melatonin (*N*-acetyl-5-methoxytryptamine, **1**). So, we first investigated the role of a nitro substituent in the 4-, 6-, or 7-position of the indole heterocycle. Comparatively to melatonin, its analogues that nitrated in the 6- or 7-position (**6** and **22**) lose *MT$_3$* but retain good MT$_1$ and MT$_2$ affinities, whereas the 4-nitro isomer (**5**) shows very high affinity (nanomolar) and selectivity for the *MT$_3$* binding sites. *N*-Methylation of the indole nucleus of compound **5** potentiates these effects and affords the most potent and selective *MT$_3$* ligand (**17**). The 2-iodo derivatives (**12** and **10**) of compounds **5** and **17** have also been synthesized to evaluate their binding profile with a view to further develop *MT$_3$* selective radioligands.

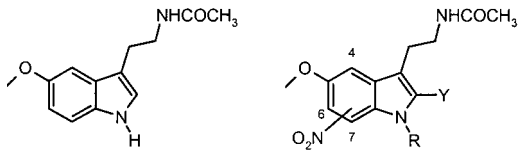
Introduction

Melatonin (*N*-acetyl-5-methoxytryptamine, **1**) (Chart 1) is a neurohormone produced by the pineal gland during the dark period, whatever the species considered, including humans.[1] The synthesis of melatonin is regulated by circadian and seasonal variations in day length through a polysynaptic neuronal pathway from the retina to the pineal gland.

Melatonin, which is released in the blood circulation and in the cerebrospinal fluid, transmits the information on the photoperiod to central and peripheral structures that express melatonin binding sites. Melatonin has been detected in numerous central and peripheral tissues using the specific radioligand 2-[^{125}I]-iodomelatonin.[2,3] Three melatonin receptors have been cloned. The first identified was the Mel$_{1C}$ receptor, which has been cloned from *Xenopus laevis*[4] and is not expressed in mammals.[5] The two others are expressed in human[5,6] and defined as MT$_1$ and MT$_2$.[7] These two receptors subtypes belong to the family of seven transmembrane G-protein coupled receptors, and they are involved in the chronobiotic properties of melatonin[8,9] and in the vasoconstrictor activities on animal vessels.[10,11]

Beside these two receptors that both present a high affinity for melatonin ($K_i \approx 0.1$ nM), another melatonin binding site with lower affinity ($K_i \approx 60$ nM) has been identified.[12−14] This binding site has been named *MT$_3$*, according to the IUPHAR nomenclature.[7] *MT$_3$* displays very fast kinetics of ligand association/dissociation,[12,15,16] which explain that binding studies are performed at 4 °C. Nevertheless, improvement of the filtration procedures allowed us to determine the pharmacological profile of *MT$_3$* at 20 °C.[17] *MT$_3$* presents a specific pharmacological profile as compared to MT$_1$/MT$_2$. Indeed, prazosin ($K_i \approx 8$ nM) and *N*-acetylserotonin ($K_i \approx 30$ nM, NAS) are good ligands;[14,15] 5-methoxycarbonylamino-*N*-acetyltryptamine (MCA-NAT)[15] has been described as a selective ligand ($K_i \approx 28$ nM).

Chart 1. Chemical Structures of Melatonin and Nitro Derivatives



The *MT$_3$* binding site has been mainly reported in hamster central and peripheral tissues using the selective radioligand 2-^{125}I-MCA-NAT, but it is also expressed in mice, rat, rabbit, chicken, pig, dog, and monkey.[15,18] The physiological function of this melatonin binding site has not been characterized to a great extent due to the lack of more selective *MT$_3$* ligands. The recent identification of *MT$_3$* as the quinone reductase 2 (QR$_2$)[18] opens new therapeutic perspectives although the physiological function of this enzyme is not well-characterized. Moreover, complementary experiments must be performed to determine if QR$_2$ represents only a part of *MT$_3$* binding sites. Some *MT$_3$* compounds have already been synthesized.[19]

The aim of the present study was to synthesize subtype selective melatonin receptor ligands to further elucidate the physiological role of melatonin and to provide highly effective therapeutic agents. Within the indolic series, a lot of melatonin analogues bearing various substituents in the 6- and/or in the 2-position have been studied whereas only a few derivatives substituted in the 4- or 7-position have been described. So, we decided to investigate the role of substituents in these later positions and we first chose the nitro (NO$_2$) group on account of its chemical accessibility and its interest as a starting material for many substitution

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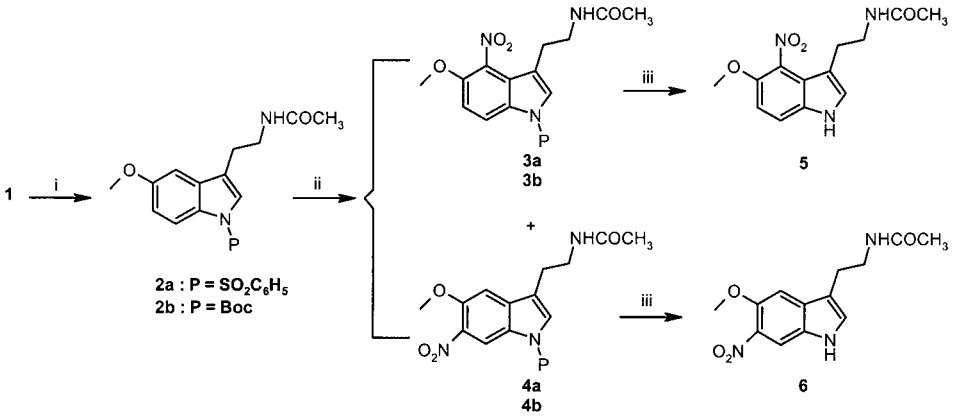
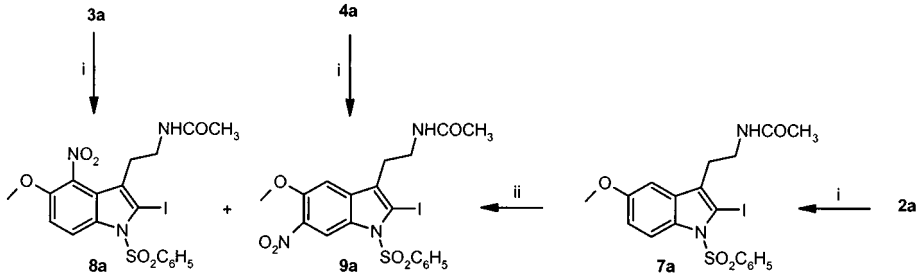
Scheme 1. Synthesis of 4- and 6-Nitro Melatonin (**5** and **6**)[a]



[a] Conditions: (i) NaOH, $Bu_4N^+HSO_4^-$, $C_6H_5SO_2Cl$ for **2a** or $(Boc)_2O$ for **2b**; (ii) HNO_3, Ac_2O; (iii) K_2CO_3, MeOH, THF, Δ for **3**−**4a** or NaOMe, MeOH, THF for **3**−**4b**.

Scheme 2. Synthesis of Compounds **8a** and **9a**[a]



[a] Conditions: (i) TMEDA, LDA, THF, I_2, −45 °C; (ii) HNO_3, Ac_2O.

reactions via its reduction and diazotation of the corresponding amine. Furthermore, the introduction of an electroattractive group such as nitro close to the methoxy pharmacophore should probably influence affinity and/or activity of the compounds and it was therefore interesting to test them on the three melatonin receptor subtypes. The first results obtained with these nitro analogues of melatonin led us to synthesize and study some other derivatives (1-methyl and/or 2-iodo) (Chart 1).

We report here the synthesis of new indolic derivatives that are very potent MT_3 ligands, with high selectivity toward the MT_1/MT_2 melatonin receptors.

Chemistry

Starting from melatonin **1**, compounds **2a** and **2b** were, respectively, prepared by action of benzenesulfonyl chloride and di-*tert*-butyl dicarbonate in the presence of sodium hydroxide and tetrabutylammonium hydrogensulfate in dichloromethane[20] (Scheme 1). Nitration of **2a** and **2b** using nitric acid in acetic anhydride led to the formation of the two isomers, 4-nitro (**3a**,**b**) and 6-nitro (**4a**,**b**). Analyses by ^1H nuclear magnetic resonance (NMR) indicated that the resulting mixtures were, respectively, composed of 66:33% of **3a**:**4a** and 50:50% of **3b**:**4b**. These isomers were separated by column chromatography with ethyl acetate/methanol as eluant. The *N*-deprotection was then realized in MeOH/tetrahydrofuran (THF) using basic conditions (potassium carbonate for benzenesulfonyl derivatives (**3a** and **4a**) or sodium methoxide for Boc derivatives (**3b** and **4b**)) and afforded the 4- and 6-nitro derivatives of melatonin (**5** and **6**, respectively).

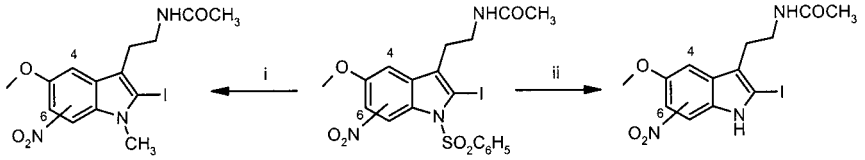
The synthesis of the corresponding 2-iodo derivatives (**12** and **13**) was first considered from the 4- or 6-nitro-*N*-protected compounds (**3a** and **4a**) (Scheme 2). Iodination[21] of **3a** by action of lithium diisopropylamide and I_2 at −45 °C in the presence of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine led to compound **8a** in good yield (68%), but the same conditions applied on **4a** only led to 17% of **9a**. So, we decided to reverse the synthetic pathway. Iodination of **2a** afforded **7a** in good yield (60%). Nitration of **7a** also led to the two isomers **8a** (4-nitro) and **9a** (6-nitro) in equal amounts.

N-Deprotection using the previously described conditions (potassium carbonate in MeOH/THF) did not give access to compounds **12** and **13** (Scheme 3) but to the corresponding *N*-methyl derivatives **10** and **11**. That methylation would be due to the action of in situ-formed methyl benzenesulfonate, which could come from the methylate ion attack on **8a** and **9a**. Indeed, compounds **12** and **13** were, respectively, obtained from **8a** and **9a** by action of sodium hydroxide in refluxing dioxane.

The *N*-methyl derivatives **16** and **17** were, respectively, obtained from 2-iodomelatonin (**14**) and 4-nitromelatonin (**5**) by action of sodium hydride and methyl iodide or dimethyl sulfate in the presence of sodium hydroxide (Scheme 4). *N*-Methylmelatonin (**15**) has been previously described.[22]

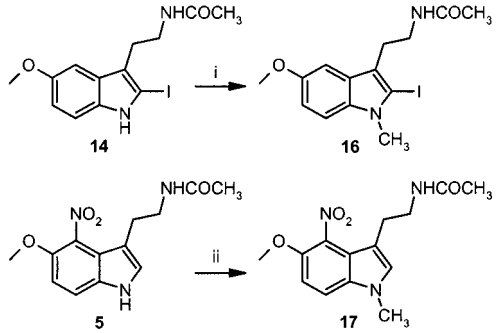
The 7-nitro derivative of melatonin (**22**) was obtained from 5-methoxy-7-nitroindole (**18**)[23,24] (Scheme 5).[20] A Mannich reaction with dimethylamine and formaldehyde in acetic acid gave **19**; methylation followed by displacement of the resulting ammonium ion with potassium cyanide afforded **20**. Reduction of the cyano group to the corresponding amine **21** was performed

Scheme 3. Synthesis of Nitrated and Iodinated Compounds **10**−**13**[a]



[a] Conditions: (i) K_2CO_3, MeOH, THF, Δ; (ii) NaOH, dioxane, Δ.

Scheme 4. Synthesis of Compounds **16** and **17**[a]



[a] Conditions: (i) NaH, CH_3I, THF, Δ; (ii) NaOH, $(CH_3O)_2SO_2$, $(CH_3)_2CO$.

with borane−THF complex. Finally, acetylation of **21** by acetyl chloride and potassium carbonate in a biphasic medium afforded the desired compound **22**.

Results and Discussion

The affinities of the compounds for the melatonin receptor subtypes were evaluated in vitro in binding assays using 2-[^{125}I]-iodomelatonin, human embryonic kidney cell line HEK293 stably expressing MT_1 or MT_2 human melatonin receptors, and hamster brain membrane preparations for the MT_3 binding site according to a previously described method.[15]

The results of the binding assays are presented in Table 1. Introduction of a NO_2 group on the indole heterocycle strongly influences affinity according to the receptor subtype and to the position of the substituent. Comparatively to melatonin **1**, the 6- and 7-nitro derivatives (**6** and **22**) lose MT_3 but retain good MT_1 and MT_2 affinities, the 6 isomer being slightly better than its 7 analogue. The most interesting result is obtained with the 4-nitro isomer (**5**), which shows a considerable loss of MT_1 and MT_2 binding affinity but a 60-fold higher affinity on the MT_3 subtype, leading to great selectivity ratios (964 toward MT_1 and 745 toward MT_2). It seems therefore that the presence of a nitro group in the 4-position of the indole heterocycle, in the proximity of the two pharmacophores, the methoxy group, and the acetamido ethyl side chain, is an essential element to get MT_3 ligands with very good affinity (∼nM) and high selectivity. Considering that the 6 and 7 isomers do not induce the same effects, we can assume that the steric parameters could be more important than the electronic ones.

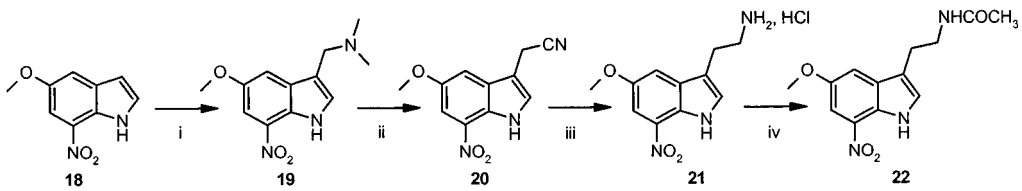
Because compound **5** was one of the best MT_3 selective ligands known at that time, we planned pharmacomodulations (i) to improve its binding profile and (ii) to obtain a new potential radioligand.

(i) Replacing the NH indolic group of melatonin (**1**) by a *N*-methylated one (**15**) leads to a 20-fold higher MT_3 affinity (1.1 nM) together with a 10-fold decrease on MT_1 and MT_2 affinities. It was therefore interesting to test the same pharmacomodulation with compound **5**. The resulting compound **17** shows a 3-fold higher affinity (0.31 nM) on the MT_3 subtype, an 8-fold lower affinity on the MT_1 receptor, but a 4-fold increased affinity on the MT_2 receptor. The selectivity ratios toward MT_1 and MT_2 subtypes are, respectively, 27 612 and 625.

(ii) It is well-known that introduction of an iodine atom in the 2-position of the indole heterocycle of melatonin leads to a 10 times higher affinity on all three receptor subtypes (**1** vs **14**); 2-[^{125}I]-iodomelatonin is the nonselective radioligand used for autoradiography and binding studies. The 2-iodo derivatives of compounds **5** and **17** could exhibit a binding profile for the development of potential MT_3 selective radioligands and were synthesized. Compound **12** and its *N*-methylated derivative **10** have the same and the greatest affinity on the MT_3 receptor (∼0.1 nM) but strongly differ in their MT_1 affinity and MT_1/MT_3 selectivity. The selectivity ratios MT_1/MT_3 and MT_2/MT_3, respectively, vary from 14 462 and 300 for **10** to 107 and 131 for **12**. These data highlight the role of both a 2-iodo and a 1-methyl substituent on the indole ring of melatonin.

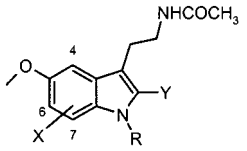
In conclusion, introducing a nitro substituent on the 4-position of the indole heterocycle of melatonin leads to a considerable increase of MT_3 binding affinity, which rises to the nanomolar range. This effect is accompanied by a loss of MT_1 and MT_2 binding affinity giving to very high selectivity ratios. Parallel *N*-methylation of the indole nucleus potentiates these effects and affords the most MT_3 potent and selective ligand. Finally, the

Scheme 5. Synthesis of 7-Nitro Melatonin (**22**)[a]



[a] Conditions: (i) $(CH_3)_2NH$, HCHO, AcOH; (ii) CH_3I, KCN, DMF, H_2O, THF, Δ; (iii) BH_3-THF, Δ; (iv) CH_3COCl, K_2CO_3, ethyl acetate, water.

Table 1. Binding Affinity of Derivatives **1**, **5**, **6**, **10**−**17**, and **22**



				$K_i \pm$ SEM (nM)			selectivity	
compd	X	R	Y	MT_3 [a]	MT_1 [b]	MT_2 [b]	MT_1/MT_3	MT_2/MT_3
1		H	H	56.9 ± 0.400	0.12 ± 0.020	0.31 ± 0.050	0.0020	0.005
15	H	CH_3	H	2.8 ± 0.241	2.6 ± 0.253	3.1 ± 0.196	0.9286	1.100
14		H	I	6.5 ± 0.031	0.013 ± 0.000	0.16 ± 0.014	0.0020	0.020
16		CH_3	I	0.088 ± 0.004	0.08 ± 0.001	0.047 ± 0.002	0.9091	0.530
5		H	H	1.1 ± 0.071	1060 ± 14.919	820 ± 25.156	964	745
17	4-NO_2	CH_3	H	0.31 ± 0.003	8560 ± 427.075	194 ± 2.820	27612	625
12		H	I	0.18 ± 0.014	19.2 ± 0.191	23.7 ± 2.133	107	131
10		CH_3	I	0.13 ± 0.006	1880 ± 67.353	39 ± 0.008	14462	300
6		H	H	∼1000	14.6 ± 1.037	5.3 ± 0.354	∼0.0140	∼0.005
13	6-NO_2	H	I	70 ± 2.311	0.3 ± 0.026	0.17 ± 0.005	0.0043	0.002
11		CH_3	I	6.1 ± 0.527	1.1 ± 0.038	0.77 ± 0.047	0.1803	0.120
22	7-NO_2	H	H	∼1000	49 ± 0.215	29 ± 0.192	∼0.049	∼0.029

[a] Syrian brain. [b] HEK-human.

1-methyl-2-iodo-4-nitro derivative of melatonin could be radiomarked with [^{125}I]iodine and so could serve as a novel and selective radioligand for the characterization of MT_3 binding sites in both central and peripheral tissues.

Experimental Section

Chemistry. Melting points were determined using a Büchi SMP-535 apparatus. Column chromatography was carried out using silica gel (silica gel 60, 70−230 Mesh, ASTM, Merck) with an appropriate solvent. IR spectra were recorded on Perkin-Elmer 297 or BRUKER Vector 22 spectrometers, using KBr pellets. ^{1}H NMR spectra were recorded on a BRUKER AC 300 P (300 MHz) spectrometer using dimethyl sulfoxide (DMSO)-d_6 or $CDCl_3$ as solvents. Chemical shifts are expressed downfield from the internal standard tetramethylsilane. Coupling constants (J) are expressed in Hertz (key: br = broad, d = doublet, dd = double doublet, m = multiplet, s = singlet, t = triplet). Elemental analysis (C, H, I, N) was determined by the CNRS center of analysis, in Vernaison (France), and agrees with the proposed structures within ±0.4% of the theoretical values.

***N*-[2-(1-Benzenesulfonyl-5-methoxy-1*H*-indol-3-yl)ethyl]-acetamide (2a).** A solution of melatonin **1** (10 g, 43 mmol) in dichloromethane (500 mL) was cooled to 0 °C. NaOH (6.89 g, 172.2 mmol) and tetrabutylammonium hydrogensulfate (0.7 g, 2.1 mmol) were added. The mixture was stirred at 0 °C for 30 min, and then, benzenesulfonyl chloride (8.24 mL, 64.5 mmol) was added dropwise. The solution was kept at 0 °C for a further 1 h and allowed to warm to room temperature overnight. The medium was filtered, and the precipitate was twice washed with dichloromethane. The organic filtrate was washed with 3 N HCl and water, dried ($MgSO_4$), filtered, and evaporated under reduced pressure to give a crude solid that was triturated in diethyl ether and collected by filtration. Recrystallization from 2-propanol yielded **2a** as a white solid (14.4 g, 88%); mp 139.5−140.5 °C. ^{1}H NMR (DMSO-d_6): δ 1.81 (s, 3H), 2.77 (t, J = 6.7 Hz, 2H), 3.35 (m, 2H), 3.77 (s, 3H), 6.95 (dd, J = 2.4 and 9.0 Hz, 1H), 7.12 (d, J = 2.4 Hz, 1H), 7.54−7.60 (m, 3H), 7.67 (m, 1H), 7.82 (d, J = 9.0 Hz, 1H), 7.92 (m, 2H), 8.01 (br t, J = 5.5 Hz, 1H).

***N*-[2-(1-*tert*-Butoxycarbonyl-5-methoxy-1*H*-indol-3-yl)-ethyl]acetamide (2b).** The procedure was the same described above for the preparation of **2a** except that di-*tert*-butyl dicarbonate (14.38 g, 64.6 mmol) was used instead of benzenesulfonyl chloride. After it was stirred overnight, the mixture was filtered, the precipitate was washed twice with dichloromethane, and the organic filtrate was washed only with water, dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The crude solid was recrystallized from ethanol to give **2b** (11.45 g, 80%) as a white solid; mp 133−134.5 °C. ^{1}H NMR ($CDCl_3$): δ 1.67 (s, 9H), 2.01 (s, 3H), 2.89 (t, J = 6.6 Hz, 2H), 3.59 (m, 2H), 3.89 (s, 3H), 5.62 (br s, 1H), 6.95 (dd, J = 2.4 and 9.1 Hz, 1H), 7.00 (d, J = 2.4 Hz, 1H), 7.40 (s, 1H), 8.02 (d, J = 9.1 Hz, 1H).

General Procedure for the Synthesis of the Nitro Compounds (3a−4a, 3b−4b, and 8a−9a). The method adopted for the synthesis of *N*-[2-(1-benzenesulfonyl-5-methoxy-4 and 6-nitro-1*H*-indol-3-yl)ethyl]acetamides (**3a** and **4a**) is described. To a suspension of **2a** (5 g, 13.4 mmol) in glacial acetic acid (25 mL) at −5 °C was added dropwise 1.1 equiv of a 68% solution of nitric acid (0.97 mL, 14.8 mmol). The medium was progressively allowed to warm to room temperature. After it was stirred for 2 days, the solution was poured into ice-water and a precipitate containing a mixture of **3a** and **4a** was isolated by filtration. The two isomers were separated by column chromatography (silica gel), first with ethyl acetate as eluant to yield **3a** (3.14 g, 58%) and then with ethyl acetate/methanol (9/1) to give **4a** (1.68 g, 30%).

***N*-[2-(1-Benzenesulfonyl-5-methoxy-4-nitro-1*H*-indol-3-yl)ethyl]acetamide (3a).** Yellow solid; mp 177−178 °C (2-propanol or ethanol). ^{1}H NMR (DMSO-d_6): δ 1.78 (s, 3H), 2.50 (m, 2H), 3.30 (m, 2H), 3.90 (s, 3H), 7.37 (d, J = 9.5 Hz, 1H), 7.62 (m, 2H), 7.74 (m, 1H), 7.84 (s, 1H), 7.95 (br t, J = 5.5 Hz, 1H), 8.01 (m, 2H), 8.13 (d, J = 9.5 Hz, 1H).

***N*-[2-(1-Benzenesulfonyl-5-methoxy-6-nitro-1*H*-indol-3-yl)ethyl]acetamide (4a).** Yellow solid; mp 209−210 °C (2-propanol). ^{1}H NMR (DMSO-d_6): δ 1.77 (s, 3H), 2.80 (t, J = 6.9 Hz, 2H), 3.35 (m, 2H), 3.93 (s, 3H), 7.51 (s, 1H), 7.60 (m, 2H), 7.71 (m, 1H), 7.91 (s, 1H), 7.98−8.02 (m, 3H), 8.40 (s, 1H).

***N*-[2-(1-*tert*-Butoxycarbonyl-5-methoxy-4-nitro-1*H*-indol-3-yl)ethyl]acetamide (3b).** Yield, 43% from **2b**; yellow solid; mp 155−156 °C (2-propanol). ^{1}H NMR ($CDCl_3$): δ 1.67 (s, 9H), 1.98 (s, 3H), 2.71 (t, J = 7.1 Hz, 2H), 3.47 (m, 2H), 3.96 (s, 3H), 5.67 (br s, 1H), 7.05 (d, J = 9.1 Hz, 1H), 7.51 (s, 1H), 8.30 (d, J = 9.1 Hz, 1H).

***N*-[2-(1-*tert*-Butoxycarbonyl-5-methoxy-6-nitro-1*H*-indol-3-yl)ethyl]acetamide (4b).** Yield, 45% from **2b**; yellow solid; mp 152−153 °C (diisopropyl ether). ^{1}H NMR ($CDCl_3$): δ 1.69 (s, 9H), 2.00 (s, 3H), 2.92 (t, J = 7.1 Hz, 2H), 3.56 (m, 2H), 4.02 (s, 3H), 5.66 (br s, 1H), 7.22 (s, 1H), 7.61 (s, 1H), 8.67 (s, 1H).

***N*-[2-(1-Benzenesulfonyl-2-iodo-5-methoxy-4-nitro-1*H*-indol-3-yl)ethyl]acetamide (8a).** Yield, 41% from **7a**; yellow solid; mp 177−178 °C (ethanol). ^{1}H NMR (DMSO-d_6): δ 1.71 (s, 3H), 2.56 (t, J = 6.8 Hz, 2H), 2.97 (m, 2H), 3.93 (s, 3H),

7.36 (d, J = 9.3 Hz, 1H), 7.64 (m, 2H), 7.76 (m, 1H), 7.84−7.87 (m, 3H), 8.39 (d, J = 9.3 Hz, 1H).

***N*-[2-(1-Benzenesulfonyl-2-iodo-5-methoxy-6-nitro-1*H*-indol-3-yl)ethyl]acetamide (9a).** Yield, 42% from **7a**; yellow solid; mp 191−193 °C (ethanol). ^{1}H NMR (DMSO-d_6): δ 1.70 (s, 3H), 2.81 (t, J = 7.0 Hz, 2H), 3.18 (m, 2H), 3.96 (s, 3H), 7.49 (s, 1H), 7.62 (m, 2H), 7.74 (m, 1H), 7.87 (m, 2H), 7.94 (br t, J = 5.6 Hz, 1H), 8.65 (s, 1H).

General Procedure for the Synthesis of the 2-Iodo Compounds (7a, 8a, and 9a). The method adopted for the synthesis of *N*-[2-(1-benzenesulfonyl-2-iodo-5-methoxy-1*H*-indol-3-yl)ethyl]acetamide (7a) is described. To a solution of **2a** (10 g, 26.8 mmol) in dry THF (60 mL) was added under nitrogen *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (10.2 mL, 67.1 mmol) with a syringe, and then, the mixture was cooled to −22 °C and stirred at this temperature for 30 min. A solution of lithium diisopropylamide (2 M in THF, 40.3 mL, 80.5 mmol) was then added dropwise with a syringe. After the mixture was stirred for a further 1 h at −22 °C, it was cooled to −45 °C and then a solution of iodine (20.5 g, 80.5 mmol) in dry THF (30 mL) was slowly added so that the temperature of the medium did not exceed −35 °C. The mixture was stirred at this temperature for a further 1 h, allowed to warm to room temperature for 4 h, and poured into ice-water. The precipitate was collected by filtration and recrystallized from ethanol to yield **7a** (7.76 g, 58%). White solid; mp 143−145 °C. ^{1}H NMR (DMSO-d_6): δ 1.73 (s, 3H), 2.74 (t, J = 6.9 Hz, 2H), 3.13 (m, 2H), 3.79 (s, 3H), 6.91 (dd, J = 2.5 and 9.2 Hz, 1H), 7.11 (d, J = 2.5 Hz, 1H), 7.57 (m, 2H), 7.68 (m, 1H), 7.76 (m, 2H), 7.96 (br t, J = 5.6 Hz, 1H), 8.03 (d, J = 9.2 Hz, 1H).

This procedure allowed us to obtain **8a** and **9a**, respectively, from **3a** and **4a** with 63 and 17% yields.

General Procedures for the *N*-Deprotection of the Indole Nucleus (5, 6, and 10−13). Methods adopted for the synthesis of *N*-[2-(5-methoxy-4-nitro-1*H*-indol-3-yl)ethyl]acetamide (**5**) are described from **3a** (method A) and from **3b** (method B).

Method A. To a solution of **3a** (7 g, 16.8 mmol) in a mixture (300 mL) of THF/MeOH (1/1) was added 100 mL of an aqueous solution of K_2CO_3 (10.89 g, 78.8 mmol). The mixture was refluxed overnight, and after it was cooled, the mixture was concentrated under reduced pressure and then poured into water. The resulting precipitate was filtered and recrystallized from ethyl acetate to afford **5** (3.3 g, 71%). Yellow solid; mp 187−189 °C. ^{1}H NMR (DMSO-d_6): δ 1.78 (s, 3H), 2.58 (t, J = 7.5 Hz, 2H), 3.22 (m, 2H), 3.87 (s, 3H), 7.11 (d, J = 8.9 Hz, 1H), 7.38 (s, 1H), 7.56 (d, J = 8.9 Hz, 1H), 7.92 (br t, J = 5.4 Hz, 1H), 11.41 (br s, 1H). Anal. ($C_{13}H_{15}N_3O_4$) C, H, N. This method allowed us to obtain the *N*-methyl derivatives **10** and **11**, respectively, from **8a** and **9a**.

Method B. To a solution of **3b** (0.5 g, 1.32 mmol) in dry THF (10 mL) under nitrogen was added a methanolic solution of NaOMe (0.15 g in 0.35 mL of MeOH, 2.65 mmol). After it was stirred for 1 h, the mixture was hydrolyzed and then the precipitate was isolated by filtration to afford 66% of **5** after recrystallization.

***N*-[2-(5-Methoxy-6-nitro-1*H*-indol-3-yl)ethyl]acetamide (6).** Yields, 49 and 65%, respectively, from **4a** (method A) and from **4b** (method B); yellow solid; mp 197−198 °C (2-propanol). ^{1}H NMR (DMSO-d_6): δ 1.79 (s, 3H), 2.84 (t, J = 7.3 Hz, 2H), 3.32 (m, 2H), 3.91 (s, 3H), 7.33 (s, 1H), 7.50 (s, 1H), 7.95−7.99 (m, 2H), 11.26 (br s, 1H). Anal. ($C_{13}H_{15}N_3O_4$) C, H, N.

***N*-[2-(2-Iodo-5-methoxy-1-methyl-4-nitroindol-3-yl)ethyl]acetamide (10).** Yield, 90% from **8a** (method A); yellow solid; mp 215−217 °C (ethanol). ^{1}H NMR (DMSO-d_6): δ 1.77 (s, 3H), 2.59 (t, J = 7.2 Hz, 2H), 3.00 (m, 2H), 3.81 (s, 3H), 3.89 (s, 3H), 7.16 (d, J = 9.1 Hz, 1H), 7.76 (d, J = 9.1 Hz, 1H), 7.87 (br t, J = 5.6 Hz, 1H). Anal. ($C_{14}H_{16}IN_3O_4$) C, H, I, N.

***N*-[2-(2-Iodo-5-methoxy-1-methyl-6-nitroindol-3-yl)ethyl]acetamide (11).** Yield, 65% from **9a** (method A); yellow solid; mp 200−202 °C (ethanol). ^{1}H NMR (DMSO-d_6): δ 1.76 (s, 3H), 2.82 (t, J = 6.8 Hz, 2H), 3.20 (m, 2H), 3.79 (s, 3H), 3.92 (s, 3H), 7.35 (s, 1H), 7.96 (br t, J = 6.1 Hz, 1H), 8.20 (s, 1H). Anal. ($C_{14}H_{16}IN_3O_4$) C, H, I, N.

Method C. The method adopted for the synthesis of *N*-[2-(2-iodo-5-methoxy-4-nitro-1*H*-indol-3-yl)ethyl]acetamide (**12**) is described. A solution of **8a** (3 g, 5.5 mmol) was refluxed for 5 h in dioxane (60 mL) with 5 equiv of aqueous NaOH 10% (11 mL). After it was cooled, the mixture was hydrolyzed and the precipitate was collected by filtration and recrystallized to afford **12** (1.54 g, 69%). Yellow solid; mp 191−193 °C (ethanol). ^{1}H NMR (DMSO-d_6): δ 1.77 (s, 3H), 2.55 (t, J = 6.9 Hz, 2H), 2.99 (m, 2H), 3.87 (s, 3H), 7.08 (d, J = 9.0 Hz, 1H), 7.48 (d, J = 9.0 Hz, 1H), 7.85 (br t, J = 5.3 Hz, 1H), 12.08 (br s, 1H). Anal. ($C_{13}H_{14}IN_3O_4$) C, H, I, N.

***N*-[2-(2-Iodo-5-methoxy-6-nitro-1*H*-indol-3-yl)ethyl]acetamide (13).** Yield, 75% from **9a** (method C); yellow solid; mp 209−211 °C (ethanol 70°). ^{1}H NMR (DMSO-d_6): δ 1.77 (s, 3H), 2.77 (t, J = 7.0 Hz, 2H), 3.20 (m, 2H), 3.91 (s, 3H), 7.33 (s, 1H), 7.85 (s, 1H), 7.95 (br t, J = 5.7 Hz, 1H), 11.98 (br s, 1H). Anal. ($C_{13}H_{14}IN_3O_4$) C, H, I, N.

***N*-[2-(2-Iodo-5-methoxy-1-methylindol-3-yl)ethyl]acetamide (16).** To a solution of **14** (0.5 g, 1.4 mmol) and methyl iodide (0.11 mL, 1.8 mmol) in dry THF (5 mL) was added portionwise NaH (0.07 g, 1.8 mmol). The mixture was stirred for 4 h at room temperature, and then, it was hydrolyzed. The precipitate that was formed in water was filtered and recrystallized from toluene to afford **16** (0.29 g, 56%). White solid; mp 129−131 °C. ^{1}H NMR (DMSO-d_6): δ 1.78 (s, 3H), 2.77 (t, J = 7.1 Hz, 2H), 3.18 (m, 2H), 3.69 (s, 3H), 3.77 (s, 3H), 6.75 (dd, J = 2.0 and 8.9 Hz, 1H), 7.04 (d, J = 2.0 Hz, 1H), 7.36 (d, J = 8.9 Hz, 1H), 7.96 (br t, J = 5.6 Hz, 1H). Anal. ($C_{14}H_{17}IN_2O_2$) C, H, I, N.

***N*-[2-(5-Methoxy-1-methyl-4-nitroindol-3-yl)ethyl]acetamide (17).** To a solution of **5** (0.5 g, 1.8 mmol) in acetone (10 mL) was added a solution of sodium hydroxide 10% (1.44 mL, 3.6 mmol) and then dropwise dimethyl sulfate (0.26 mL, 2.7 mmol). The mixture was stirred for 2 h and then hydrolyzed. After acidification, the medium was filtered, and the precipitate was recrystallized from toluene to afford **17** (0.4 g, 70%). Yellow solid; mp 144−146 °C. ^{1}H NMR ($CDCl_3$): δ 1.95 (s, 3H), 2.76 (t, J = 7.0 Hz, 2H), 3.44 (m, 2H), 3.77 (s, 3H), 3.95 (s, 3H), 5.65 (br s, 1H), 6.99 (d, J = 8.9 Hz, 1H), 7.04 (s, 1H), 7.37 (d, J = 8.9 Hz, 1H). Anal. ($C_{14}H_{17}N_3O_4$) C, H, N.

3-Dimethylaminomethyl-5-methoxy-7-nitro-1*H*-indole (19). A solution of acetic acid (35 mL), dimethylamine (1.4 mL, 12.5 mmol), and formaldehyde (0.83 mL, 10 mmol) was stirred at 0 °C for 30 min. To this solution was added 5-methoxy-7-nitroindole **18** (1.71 g, 8.9 mmol). After it was stirred for 3 days at room temperature, the mixture was hydrolyzed with a solution of sodium hydroxide 15% (180 mL) and then extracted with dichloromethane. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The oily residue was eluted on silica gel chromatography with ethyl acetate and then with a mixture of acetone/toluene/cyclohexane/triethylamine (5/3/2/1) to afford **19** as a yellow-orange-colored solid (1.9 g, 86%); mp 155−156 °C. ^{1}H NMR (DMSO-d_6): δ 2.22 (s, 6H), 3.68 (s, 2H), 3.87 (s, 2H), 7.44 (s, 1H), 7.66 (d, J = 2.0 Hz, 1H), 7.73 (d, J = 2.0 Hz, 1H), 11.65 (br s, 1H).

(5-Methoxy-7-nitro-1*H*-indol-3-yl)acetonitrile (20). Compound **19** (1.9 g, 7.6 mmol) was dissolved in a mixture of dimethylformamide (DMF; 3.2 mL), water (3.2 mL), THF (150 mL), and methyl iodide (2.4 mL, 38 mmol). This mixture was refluxed for 10 min, and then, potassium cyanide (4.0 g, 61 mmol) was added and the reflux kept for 5 h. After it was cooled, the mixture was filtered and the filtrate was evaporated under reduced pressure to give a crude residue that was triturated with methanol and collected by filtration. Recrystallization from toluene yielded **20** (1.19 g, 68%). Yellow solid; mp 188−189 °C. ^{1}H NMR (DMSO-d_6): δ 3.89 (s, 3H), 4.13 (s, 2H), 7.52 (s, 1H), 7.72 (s, 1H), 7.75 (s, 1H), 11.77 (br s, 1H).

***N*-2-(5-Methoxy-7-nitro-1*H*-indol-3-yl)ethylamine hydrochloride (21).** To a solution of nitrile **20** (1 g, 4.3 mmol) in dry THF (20 mL) was added a solution 1 M of borane−THF complex. The mixture was warmed at reflux under nitrogen

for 24 h. After the medium was cooled, an aqueous solution of 3 N HCl (17 mL) was added dropwise. The mixture was evaporated under reduced pressure. The crude solid was recrystallized in DMF to give yellow-green crystals (0.46 g, 39%); mp >250 °C. ^{1}H NMR (DMSO-d_6): δ 3.01−3.07 (m, 4H), 3.90 (s, 3H), 7.44 (s, 1H), 7.67 (d, J = 2.1 Hz, 1H), 7.78 (d, J = 2.1 Hz, 1H), 8.04 (br s, 3H), 11.65 (br s, 1H).

***N*-[2-(5-Methoxy-7-nitro-1*H*-indol-3-yl)ethyl]acetamide (22).** The hydrochloride salt of amine **21** (0.46 g, 1.7 mmol) was dissolved in water (55 mL). Ethyl acetate (55 mL) and potassium carbonate (0.7 g, 5 mmol) were then added. Acetyl chloride (0.18 mL, 2.55 mmol) was added dropwise, and stirring was continued for 2 h. The organic layer was dried over $MgSO_4$, filtered, and evaporated. The crude residue was recrystallized from ethyl acetate to afford **22** as a yellow-orange solid (0.31 g, 67%); mp 155−156 °C. ^{1}H NMR (DMSO-d_6): δ 1.79 (s, 3H), 2.84 (t, J = 7.3 Hz, 2H), 3.32 (m, 2H), 3.89 (s, 3H), 7.33 (s, 1H), 7.65 (d, J = 2.1 Hz, 1H), 7.69 (d, J = 2.1 Hz, 1H), 7.97 (br t, J = 5.4 Hz, 1H), 11.51 (br s, 1H). Anal. ($C_{13}H_{15}N_3O_4$) C, H, N.

Cell Culture. Human embryonic kidney cell line HEK293 stably expressing MT_1 or MT_2 human melatonin receptors (provided by A. D. Strosberg, Paris, France) were grown as monolayers at 37 °C (95% O_2/5% CO_2) in Dulbecco's modified Eagle's medium glutamax-1 (Gibco 31966-036; Gibco Laboratories, Grand Island, NY) supplemented with 10% fetal calf serum, penicillin, and streptomycin (1%) in the presence of the selection agent Geneticin G-418 (4%) (Gibco 11811-031). Then, the cells were washed twice with phosphate-buffered saline (PBS), harvested in MatriSperse (Becton Dickinson, Le Pont-de-Claix, France), pelleted at 4 °C at 1000 rpm, and suspended in PBS. The cells were homogenized with a polytron tissue disrupter, and the resulting homogenate was centrifuged at 20 000*g* for 30 min. The pellet was suspended in buffer, and the protein concentration was measured by the method of Bradford,[25] with bovine serum albumin (BSA) as standard. The membranes were stored at −80 °C at a concentration of 5 mg/mL.

Hamster Organ Membrane Preparations. Hamster frozen tissues were prepared by Charles River Breeding Laboratories, Inc. (Saint Aubin les Elbeuf, France) from male Syrian hamsters weighing 120−130 g. The tissues were thawed and homogenized in 15 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.4) containing 2 mM ethylenediaminetetraacetic acid and 1 mM phenylmethylsulfonyl fluoride with a Polytron (Kinematica GmbH; Lucerne; Switzerland) set at 4−5 for 15 s. The homogenate was centrifuged at 45 000*g* for 20 min. Pellets were washed by repeating the homogenization and centrifugation procedure. Membrane pellets were suspended by passing back and forth through a 26 gauge needle connected to a syringe and finally adjusted to a concentration of approximately 5 mg/mL in homogenization buffer. The membrane fractions were filtered through cheesecloth, flash frozen in dry ice, and stored at −80 °C until use.

Binding Assays. In saturation experiments, membrane suspensions of MT_1 (0.04 mg/mL) and MT_2 (0.04 mg/mL) were incubated for 2 h at 37 °C in 0.25 mL (final volume) of 50 mM Tris-HCl containing 5 mM of $MgCl_2$, at pH 7.40, with varying concentrations of 2-[^{125}I]iodomelatonin (2200 Ci/mmol) from 0.005 to 1.5 nM for MT_1 and from 0.02 to 3 nM for MT_2 in the absence or presence of melatonin (10 μM), which determines the nonspecific binding. Competition studies for 2-[^{125}I]iodomelatonin binding (radioligand concentration, 0.025 nM for MT_1 studies and 0.200 nM for MT_2 studies) were performed in the presence of reference substances to determine their affinities on the two human melatonin subtype receptors.

Binding assay conditions were essentially as previously described.[14] Briefly, membranes (50−100 μg) were incubated at 4 °C in 250 μL of total volume per sample with 96 well assay blocks (Corning-Costar, Corning, NY). Unless otherwise stated, the binding of 2-[^{125}I]iodomelatonin (0.2 nM) was routinely measured in 50 mM Tris-HCl buffer (pH 7.4) and was initiated by addition of 200 μL of membrane preparations. After incubation at 4 °C for 30 min, reactions were terminated by filtration with a cell harvester (Brandel M-48; Gaithersburg, MD) connected to a vacuum pump (Edwards 18; Gennevilliers, France) through glass-fiber filters (GF/B; Brandel) soaked in 0.5% (v/v) polyethylenimine. Filters were washed three times with 1 mL of ice-cold 50 mM Tris-HCl buffer. Total filtration time was less than 5 s. Radioactivity was measured in γ-counter (Auto-Gamma 5000 series, Packard). Nonspecific binding was estimated as binding in the presence of 30 μM melatonin.[15,26] For competition studies, 2-[^{125}I]iodomelatonin was incubated in the presence of increasing concentrations of our compounds from 10^{-11} to 10^{-6} M. For saturation binding assays, 0.125−20 nM of 2-[^{125}I]iodomelatonin was used. Protein contents were determined with Coomassie blue dye reagent (Bio-Rad Laboratories, Inc., Richmond, CA) as previously described,[25] with BSA as standard.

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