2-Substituted 5-Methoxy-*N*-acyltryptamines: Synthesis, Binding Affinity for the Melatonin Receptor, and Evaluation of the Biological Activity

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A series of 2-substituted 5-methoxy-*N*-acyltryptamines was synthesized and their affinity for the melatonin receptor, isolated from whole quail brains, was tested in a succession of *in vitro* ligand–receptor binding experiments, using 2-[^{125}I]iodomelatonin as a labeled ligand. Optimization of the C_2 substituent and the *N*-acyl group resulted in compounds having picomolar affinity for the receptor (vs nanomolar affinity for melatonin). In two tests for evaluation of the biological activity (effects on the spontaneous firing activity of single neurons in the rabbit parietal cortex *in situ*, and the Syrian hamster gonadal regression model *in vivo*) most of the analogs behaved as agonists. Isopropyl substitution at C_2 alone, or concomitantly with cyclopropyl substitution at the *N*-acyl position, resulted in much lower affinity and weaker biological effect, or lack of activity in the latter case. Of interest are the compounds **4d** (R = phenyl, R_1 = CH_3) and **4g** (R = phenyl, R_1 = cyclopropyl), which expressed high affinity for the receptor and apparent antagonistic activity under the conditions of the experimental models employed, though the analog **4g** (R = phenyl, R_1 = cyclopropyl) seemingly was a weak antagonist and *in situ* expressed mixed activity in the higher concentration range. Cyclopropyl substitution at the *N*-acyl position inevitably resulted in lower affinity for the receptor and weaker biological activity. These data demonstrate that the *N*-acetyl group is important for both affinity and agonist biological activity. The substituents at C_2 are crucial for the affinity of the compound for the receptor and can be utilized to create putative high-affinity agonists or antagonists.

Melatonin (5-methoxy-*N*-acetyltryptamine) is a neurohormone synthesized and secreted by the vertebrate pineal gland on a circadian basis, with elevated peripheral blood levels at night. The interest in the sites and mechanism of action of this indole has been steadily growing since its isolation and synthesis in 1958.[1] Melatonin is now considered an important internal *Zeitgeber*, translating the photoperiodic information from the environment,[2] and the circadian melatonin signal is decoded by high-affinity binding sites in the central nervous system (CNS).[3] Melatonin plays a fundamental role in regulating the seasonal reproductive competence in most of the mammals living in the temperate zones and influences the circadian rhythmicity in reptiles, birds, and mammals.[4] The exact role of melatonin in human is less explicit, but recent research demonstrated presence of melatonin receptors in the human circadian clock,[5] as well as the ability of humans to respond to the melatonin signal with a well-defined circadian phase-response curve.[6] Changes in the melatonin rhythm and/or peripheral blood levels are now thought to be implicated in a number of patho/physiological conditions, such as the seasonal affective disorder,[7] regulation of the sleep–wake cycles,[8] puberty,[9] jet lag,[10] and reproduction.[11]

From the limited number of structure-activity studies with melatonin analogues performed in the last few years, it appeared that both the *N*-acetyl and 5-methoxy substituents are necessary for biological activity and binding affinity at the receptor level;[12,26] moreover, the indole nucleus apparently could be substituted with a naphthalenic ring.[13] Recently, we reported that substitution with bromine at the C_2 position of the indole nucleus yielded an agonist showing extremely high affinity for the melatonin receptor.[14] This compound was more potent *in vivo* and *in situ* than either melatonin or 2-iodomelatonin.

Recently reported experiments suggested that *N*-cyclopropanoyl substitution can lead to an increased affinity for the melatonin receptor expressed in the cells of pars tuberalis, isolated from ovine pituitaries.[13] Therefore, we investigated the consequences of a series of chemical modifications at C_2 and *N*-acyl positions, alone or in combination, on the binding affinity and the activity of the generated compounds, in order to acquire a better understanding of the structural requirements for binding to the melatonin receptor, and prerequisites in terms of biological activity.

Chemistry

The synthesis of the novel melatonin analogues **4a–j** was achieved following the routes described in Scheme I. The modified Madelung synthesis[15] was adopted for the preparation of 2-substituted indoles **2a–d**, starting from *N*-(2-methyl-5-methoxyphenyl)alkanamides or -benzamides **1a–d**. The 2-substituted indoles **2a–d** were coupled with 1-(dimethylamino)-2-nitroethylene to give the required (nitrovinyl)indoles **3a–d** under the conditions previously described for related compounds.[16] The syntheses were completed by reducing the (nitrovinyl)indoles **3a–d** with lithium aluminum hydride ($LiAlH_4$) and acyl-

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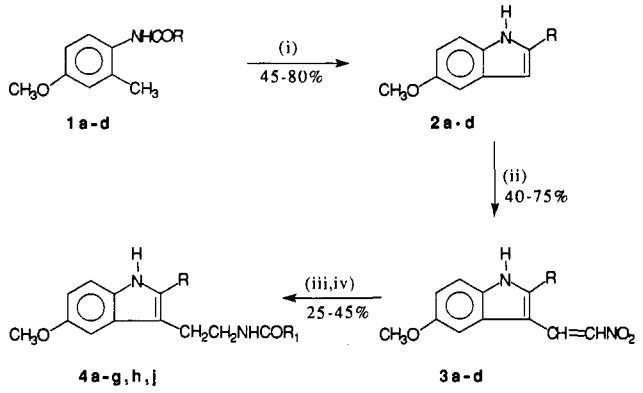
Scheme I [a]



4	R	R_1
a	CH_3	CH_3
b	isopropyl	CH_3
c	cyclohexyl	CH_3
d	C_6H_5	CH_3
e	CH_3	cyclopropyl
f	isopropyl	cyclopropyl
g	C_6H_5	cyclopropyl
h	Br	cyclopropyl
j	H	cyclopropyl

1,2,3	R
a	CH_3
b	isopropyl
c	cyclohexyl
d	C_6H_5

[a] Reagents: (i) *n*-BuLi, THF, rt, 16 h; (ii) 1-(dimethylamino)-2-nitroethylene, trifluoroacetic acid, 0 °C, 0.5 h; (iii) $LiAlH_4$, THF, reflux, 1 h; (iv) Ac_2O or cyclopropanecarbonyl chloride, THF, TEA, rt. For synthesis of **4h** and **4j**, see Chemistry section.

ating the resulting crude tryptamines with acetic anhydride or cyclopropanecarbonyl chloride in the presence of triethylamine (TEA).

Compound **4j** was obtained by acylating the 5-methoxytryptamine with cyclopropanecarbonyl chloride.[13] Compound **4h** was prepared by direct bromination of **4j** with *N*-bromosuccinimide according to a reported method.[14]

^1H NMR, IR, and elemental analysis data of the new compounds were found to be in accord with the assigned structures (see Tables I–III and the Experimental Section).

Biochemistry, Pharmacology and Physiology: Results and Discussion

The affinities of the synthesized molecules and their apparent biological activities, determined independently by using two experimental model systems, in comparison to those of previously developed compounds and the native indole are reported in Table IV.

The greater part of the new analogues expressed an increase in their apparent binding affinity. This increment ranged from average (**4a,e,g,h**) to very dramatic (**4d**). A number of compounds, however, expressed apparent loss of affinity, the worst decrement being registered in the case of **4f**. Among the substituted tryptamines examined, the affinity for the melatonin receptor decreased with the bulk and the lypophilicity of the alkyl substituents, as shown by the trend methyl (**4a**) > isopropyl (**4b**) > cyclohexyl (**4c**). From a careful analysis of the data, it became clear that only few substitutions at C_2 of the indole ring have resulted in increased affinity: halogenation, methylation, and introduction of aromatic ring (**4a,d**, 2-bromomelatonin, and 2-iodomelatonin). Moreover, only these substitutions (**4e,g,h**) were able to counteract to a great extent the decrease in the affinity resulting from introduction of cyclopropyl at R_1. In other words, introduction of cyclopropyl at the *N*-acyl of the lateral chain in all cases led to an average (2.2×10^{-9} for **4j** *vs* 1.1×10^{-9} for melatonin) or significant (2.1×10^{-10} for **4h** *vs* 5.8×10^{-11} for 2-bromomelatonin) decrease in the apparent binding affinity of the new compound. This is in clear contrast to the reported affinity value of this molecule (**4j**: R = H; R_1 = cyclopropyl) obtained in a different system, *i.e* crude membrane preparation enriched in melatonin receptors, isolated from ovine pars tuberalis.[13] This obvious discrepancy could possibly be due to a so far unsuspected diversity in the structural organization of the high-affinity melatonin receptor in the brain and the pars tuberalis, or to methodological differences in the evaluation of the affinity constants. The kinetic, pharmacological, and biochemical characteristics of the high-affinity melatonin receptor in the quail brain and the rabbit cortex are very similar;[18] a comparison between these receptor parameters in the sheep pars tuberalis and the avian brain has given essentially the same result.[19]

It is reasonable to suppose that the phenyl group can be more easily accomodated in a lipophilic pocket of the receptor site, covering the C_2 position, than the cyclohexyl group, which has larger dimensions. The increase in the affinity is probably due to a local negative, rather polarizable, charge around the C_2 position of the indole nucleus. A similar effect probably occurs with the C_2-halogen derivatives, where the affinity, increasing along the series Cl, Br, I, could be related to a polarizability effect: the more polarizable an atom, the more effectively it binds to the receptor (ref 19, this study). Of course, the size of the receptor pocket near C_2 plays a role in the complex formation, but the binding affinity to the receptor site can also be influenced by the electronic charge density around C_2, which may establish a receptor point interaction, by means of a secondary electrostatic interaction. These ideas are consistent with a recent QSAR study of a series of previoously synthesized melatonin analogues[20] which demonstrated a good correlation between the molecular polarizability and the affinity.

The extended sequences of experiments regarding the biological activity of the newly-synthesized compounds included two approaches, *in situ* and *in vivo*, commonly accepted as model systems (see Experimental Section). Both experimental series confirmed that the greater part of the compounds expressed agonist activity. Of all molecules, **4d** and **4g** only expressed apparent antagonist activity. The summary of the results is reported in Table IV. Figure 1 shows examples of the data obtained in the Syrian hamster gonadal regression model. As clearly seen, melatonin in doses of both 20 and 200 μg/animal/day induced complete gonadal regression within 6 weeks. The agonist–activity compounds generated the same response, and an excess dose of 200 μg, administered 30 min before melatonin (20 μg), was not able to prevent the melatonin-induced gonadal regression. On the contrary, two of the molecules tested (**4d** and **4g**) had no effect alone in a dose of 200 μg, but efficiently prevented the aftermath of 20 μg melatonin, injected 30 min later. Therefore, their effect was evaluated as clearly antagonistic. Compounds **4b**, **4e**, and **4j** given alone, induced partial gonadal regression, but were unable to prevent the impact of melatonin, and their behavior was evaluated as weakly agonistic. The only molecule that expressed no effect alone and did not influence the results of the subsequent melatonin administration was **4f**.

The iontophoretic administration of the drugs, under conditions of the rabbit parietal cortex model, were in accord with the *in vivo* data. The relative potency was

Table I. Chemical-Physical Data and Yields of the Amide 1c and the Indole 2c

compd	R	mp,[a] °C	% yield	formula[b]	IR (Nujol)	^{1}H NMR ($CDCl_3$)
1c	cyclohexyl	163	60	$C_{15}H_{21}NO_2$	3280, 1640	1.10–1.90 (complex m, 11 H, cyclohexyl), 3.35 (s, 3H, CH_3), 3.72 (s, 3H, OCH_3), 6.56–7.30 (m, $3H_{arom}$), 8.97 (br s, 1H, NH)
2c	cyclohexyl	113–114	40	$C_{15}H_{19}NO$	3460, 3400, 1620	1.20–2.10 (complex m, 11H, cyclohexyl), 3.80 (s, 3H, OCH_3), 6.10 (d, 1H, H-3), 6.60–7.15 (m, $3H_{arom}$), 7.70 (br s, 1H, NH)

[a] Recrystn solvent: CH_2Cl_2–hexane. [b] All compounds were analyzed for C, H, N; analytical results were within ±0.4% of the theoretical values.

Table II. Chemical-Physical Data and Yields of 2-Substituted 3-(2-Nitrovinyl)-5-methoxyindoles 3a–d

compd	R	mp, °C	% yield	formula[a] or reference	IR (Nujol)	^{1}H NMR (acetone-d_6)
3a	CH_3	189	58	186[24]		
3b	isopropyl	160	40	$C_{14}H_{16}N_2O_3$	3270, 1600, 1577, 1550, 1540	1.40 [d, 6H, $CH(CH_3)_2$], 3.52 [m, 1H, $CH(CH_3)_2$], 3.88 (s, 3H, OCH_3), 6.57–7.40 (m, $3H_{arom}$), 7.77 (d, 1H, J = 13Hz, =CH), 8.42(d, 1H, J = 13Hz, =CH)
3c	cyclohexyl	161–162	60	$C_{17}H_{20}N_2O_3$	3315, 1600, 1565, 1550, 1530	1.21–1.98 (complex m, 11H, cyclohexyl), 3.86 (s, 3H, OCH_3), 6.73–7.40 (m, $3H_{arom}$), 7.66 (d, 1H, J = 13Hz, =CH), 8.41 (d, 1H, J = 13Hz, =CH)
3d	phenyl	229–232	75	$C_{17}H_{14}N_2O_3$	3230, 1600, 1570	3.92 (s, 3H, OCH_3), 6.82–7.56 (m, $8H_{arom}$), 7.86 (d, 1H, J = 13Hz, =CH), 8.32 (d, 1H, J = 13Hz, =CH)

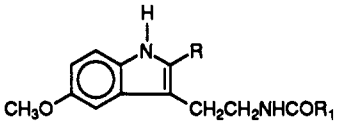
[a] All compounds were analyzed for C, H, N; analytical results were within ±0.4% of the theoretical values.

Table III. Yields, Melting Points, and Elemental Analyses of 2-Substituted *N*-Acyl-5-methoxytryptamines 4a–j

compd	R	R_1	mp, °C	recrystn solvent	% yield	formula[a] or reference
4a	CH_3	CH_3	122	EtOAc/hexane	44	viscous oil[25]
4b	isopropyl	CH_3	amorphous solid		30	$C_{16}H_{22}N_2O_2$
4c	cyclohexyl	CH_3	amorphous solid		42	$C_{19}H_{26}N_2O_2$
4d	phenyl	CH_3	amorphous solid		48	$C_{19}H_{20}N_2O_2$
4e	CH_3	cyclopropyl	102–103	EtOAc/hexane	45	$C_{16}H_{20}N_2O_2$
4f	isopropyl	cyclopropyl	142–143	Et_2O/pet. ether	25	$C_{16}H_{24}N_2O_2$
4g	phenyl	cyclopropyl	amorphous solid		45	$C_{21}H_{22}N_2O_2$
4h	Br	cyclopropyl	82–83	$CHCl_3$/hexane	20[b]	$C_{15}H_{17}BrN_2O_2$
4j	H	cyclopropyl	102–103	CH_2Cl_2/hexane	90[b]	101–102[13]

[a] All compounds were analyzed for C, H, N; analytical results were within ±0.4% of the theoretical values. [b] Overall yield starting from 5-methoxytriptamine.

Table IV. Relative Binding Affinities and Biological Activity of 2-Substituted 5-Methoxy-*N*-acyltryptamines for the Melatonin Receptor[a]



compound	R	R_1	K_a ± SEM	K_i ($1/K_a$)	SHGRM	RPCM	INH
4a	CH_3	CH_3	$(2.3 \pm 0.77) \times 10^9$	4.3×10^{-10}	agonist	agonist	60
4b	isopropyl	CH_3	$(2.4 \pm 0.17) \times 10^8$	4.3×10^{-9}	weak agonist	weak agonist	10
4c	cyclohexyl	CH_3	$(1.9 \pm 0.71) \times 10^8$	5.3×10^{-9}	agonist	agonist	35
4d	phenyl	CH_3	$(1.8 \pm 0.56) \times 10^{10}$	5.7×10^{-11}	antagonist	mixed activity*	
4e	CH_3	cyclopropyl	$(1.5 \pm 0.41) \times 10^9$	6.3×10^{-10}	weak agonist	weak agonist	20
4f	isopropyl	cyclopropyl	$(5.5 \pm 0.71) \times 10^7$	1.8×10^{-8}	no effect	no effect	
4g	phenyl	cyclopropyl	$(4.1 \pm 0.51) \times 10^9$	2.4×10^{-10}	antagonist	mixed activity**	
4h	Br	cyclopropyl	$(4.6 \pm 0.22) \times 10^9$	2.1×10^{-10}	agonist	agonist	60
4j	H	cyclopropyl	$(4.5 \pm 0.90) \times 10^8$	2.2×10^{-9}	weak agonist	weak agonist	15
melatonin	H	CH_3	$(8.5 \pm 0.71) \times 10^8$	1.1×10^{-9}	agonist	agonist	50
6-chloromelatonin	H	CH_3	$(4.5 \pm 0.31) \times 10^8$	2.2×10^{-9}	agonist	agonist	46
2-bromomelatonin	Br	CH_3	$(1.7 \pm 0.59) \times 10^{10}$	5.8×10^{-11}	agonist	agonist	80
2-iodomelatonin	I	CH_3	$(4.6 \pm 0.59) \times 10^{10}$	2.1×10^{-11}	agonist	agonist	76

[a] K_i's are in mol/L and are the means of five to nine independent determinations, derived from calculations using nonlinear fitting strategies (LIGAND). 2-[^{125}I]iodomelatonin was used as a labeled ligand in the series of saturation and competition experiments. The K_d was calculated simultaneously from the series of saturation experiments, and the mean value was 20 pM. The protocols for the in vitro ligand–receptor binding, using the quail brain as a source of melatonin receptors, were as described previously.[18] SHGRM, Syrian hamster gonadal regression model;[22] RPCM, rabbit parietal cortex model;[25] INH, percent inhibition of the spontaneous firing activity of single cortical neurons in the RPCM, with concentrations of 1×10^{-6} M of the tested compounds. Behaves as antagonist in *80% and **60% of the tested neurons.

determined on the basis of a large series of dose–response studies, evaluating the repercussion on the spontaneous neuronal firing rates and the duration of the observed effects. Most compounds (**4a–c,e,h,j**), similarly to melatonin, 2-iodomelatonin and 2-bromomelatonin expressed clearly benzodiazepine-like properties and administration of nano- to micromolar quantities always led to slight or significant inhibition of the spontaneous firing rates of the tested cortical neurons. The antagonistic action of **4d** was most clear; **4g** expressed dose-dependent mixed antagonist/agonist properties; **4f** was without effect. A summary of the results is reported in Table IV; examples of the original experimental data are given in Figure 2. As clearly seen, iontophoretic administration of 2-methyl-

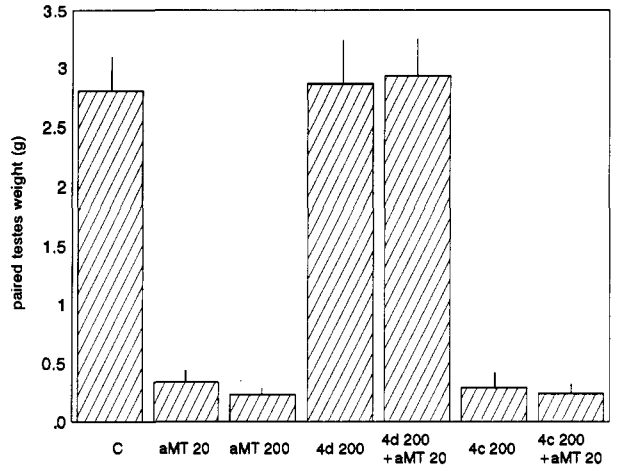


Figure 1. Examples of the effects of late-afternoon treatment with melatonin (aMT), an antagonist (**4d**), and an agonist (**4c**) alone, or followed by melatonin, for 6 weeks, on the paired testes weights of Syrian hamsters, kept under constant photoperiod conditions of 14:10 light:dark (lights off at 20:00). C, saline-treated controls. The numbers following the abbreviations of compounds denote the doses (μg/animal/day) applied. The results are summarized in Table IV. See the text for details.

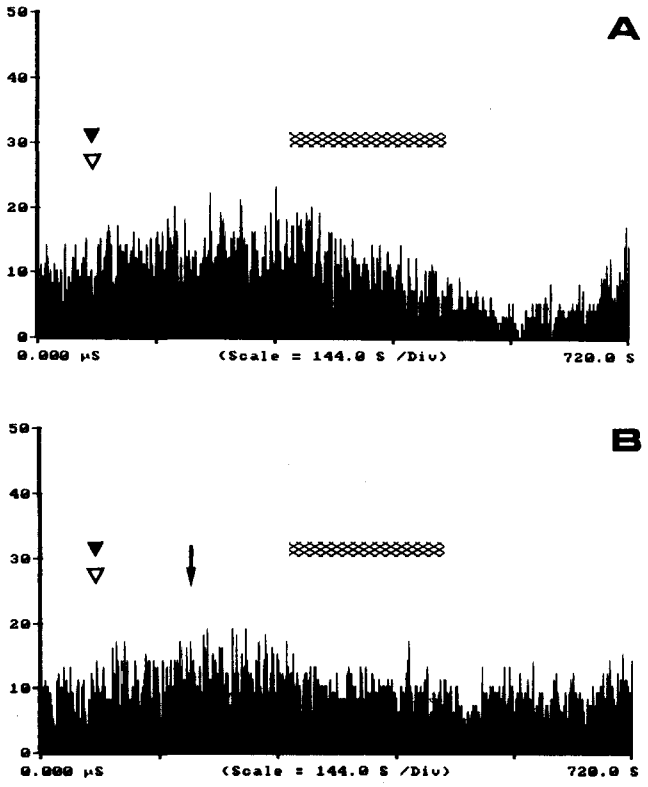


Figure 2. Examples of the effects of an agonist (**4a**, hatched bar, 1×10^{-6} M concentration in the electrode) applied by iontophoresis, on the spontaneous firing activity of single cortical neurons in the rabbit parietal cortex. Triangles denote the beginning of the application of phaclofen and bicuculline 1×10^{-4} M and 5×10^{-4} M, corresponding concentrations in the electrodes). The arrow denotes the beginning of administration of the antagonist (**4d**, 1×10^{-6} M concentration in the electrode) (B). Note that the prolonged firing rate inhibition, induced by **4a** (A) is completely blocked by preceding coadministration of **4d** (B).

melatonin (**4a**) leads to strong, prolonged inhibition of the firing rate of the cortical neurons (Figure 2A). Application of **4d** (2-phenylmelatonin) has no significant influence *per se*, but is able to virtually block the effect of the subsequent administration of **4a** (Figure 2B). These structure–activity data were well in line with the results of the structure–affinity evaluation experiments, though further analysis of the compounds in other model systems [(i) forskolin-stimulated cAMP accumulation *in vitro*, (ii) induction of locomotor activity phase-shifts *in vivo*] will be necessary for the full characterization of the new molecules.

Two important points emerge from the present study. First, the *N*-acetyl group is important for both affinity for the receptor and the biological activity. Clearly, cyclopropyl substitution has always led to a decreased affinity and somewhat weaker activity, suggesting that there is probably a small hydrophobic pocket in the receptor site, limiting the size of the *N*-acyl group. Second, methylation, halogenation, and substitution with an aromatic ring at C_2 of the indole nucleus has led to a dramatic increase in the binding affinity; the biological activity, however, varied greatly, and clear agonist properties were obtained in the cases of methylation or halogenation. Isopropyl substitution at C_2 has brought a decrease in both affinity and agonist biological activity.

Experimental Section

Chemistry. Melting points were determined on a Büchi apparatus in glass capillary tubes and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker 200 or a Varian EM 360L spectrometer; chemical shifts are reported in parts per million (ppm, δ) using chloroform or tetramethylsilane as internal standards, and signals are quoted as s (singlet), d (doublet), t (triplet), q (quartet), br s (broad singlet), or m (multiplet). IR spectra were determined with a Perkin–Elmer 257 spectrophotometer; values are reported in reciprocal centimeters (cm^{-1}).

Satisfactory elemental analyses (±0.4%) for C, H, and N were obtained using a Perkin–Elmer CHN analyzer 240C.

Materials. Tetrahydrofuran (THF) was distilled from sodium/benzophenone. Flash chromatography was carried out on Merck silica gel 60 (230–400 mesh). Compounds **1b**, **2b**,[17] **1d**, **2d**,[15] and **4j**[13] were prepared according to previously described procedures. The chemical physical properties of the compounds **1c** and **2c** are summarized in Table I. The indole **2a** is commercially available (Aldrich Chemical Co.).

General Method for the Synthesis of 2-Substituted 3-(2-Nitrovinyl)-5-methoxyindoles (3a–d). To a stirred ice-cooled solution of 1-(dimethylamino)-2-nitroethylene (1.16 g, 10 mmol) in trifluoroacetic acid (6 mL) was added the appropriate 2-substituted 5-methoxyindole **2a–d** (10 mmol). The mixture was stirred under N_2 at room temperature for 0.5 h and then poured into ice water. The aqueous solution was extracted with ethyl acetate; the organic phase was washed with a saturated $NaHCO_3$ solution and with water and then dried (Na_2SO_4). Evaporation of the solvent and crystallization from CH_2Cl_2/hexane gave the required compounds **3a–d**. Yields and chemical-physical data on compounds **3a–d** are given in Table II.

General Method for the Synthesis of 2-Substituted *N*-Acyl-5-methoxytryptamines (4a–g). To a stirred suspension of $LiAlH_4$ (1.14 g, 30 mmol) in THF (45 mL) under a N_2 atmosphere was added dropwise a solution of the suitable (nitrovinyl)indole **3a–d** (5 mmol) in THF (20 mL). After the addition, the reaction was refluxed for 1 h and then allowed to stand overnight at room temperature. After cooling at 0 °C, water was added dropwise to destroy the excess hydride. The mixture was filtered on Celite and the filtrate concentrated in vacuo and partitioned between water and ethyl acetate. The organic layer was washed with NaCl solution, dried (Na_2SO_4), and concentrated in vacuo to give crude tryptamines which were then used without further purification.

2-Methylmelatonin (4a). To a cold solution in THF (15 mL) of the crude 2-methyl-5-methoxytryptamine, from the step above were added TEA (0.7 mL) and 0.47 mL of Ac_2O. The ice bath was removed and the solution stirred for 8 h. The solvent was evaporated in vacuo, and the residue was taken up in ethyl acetate and washed with a saturated aqueous solution of $NaHCO_3$

followed by saturated NaCl solution. The organic phase was dried (Na_2SO_4) and concentrated under vacuum, and the crude oil residue was purified by flash chromatography (silica gel; ethyl acetate–cyclohexane, 7:3) and crystallization. The overall yield of **4a** was 0.541 g (44%), mp 122 °C. ^{1}H NMR data were identical with that reported in the literature.[25]

2-Isopropylmelatonin (4b). Acetylation of the crude 2-isopropyl-5-methoxytryptamine in the above procedure gave 0.411 g (30% yield) of **4b** as white amorphous solid. IR ν_{max}: 3450, 3300, 1655. ^{1}H NMR: 1.31 [d, 6H, CH(CH_3)$_2$], 1.92 (s, 3H, Ac), 2.90 (t, 2H, β-CH_2), 3.21 [m, 1H, CH(CH_3)$_2$], 3.51 (q, 2H, α-CH_2), 3.85 (s, 3H, OCH_3), 5.65 (br s, 1H, NH), 6.82–7.23 (m, 3H$_{arom}$), 8.06 (br s, 1H, NH$_{indole}$).

2-Cyclohexylmelatonin (4c). Acetylation of the crude 2-cyclohexyl-5-methoxytryptamine in the above procedure produced 0.660 g (42% yield) of **4c** as amorphous solid. IR ν_{max}: 3450, 3400, 1655. ^{1}H NMR: 1.39–1.91 (complex m, 11H, cyclohexyl), 1.93 (s, 3H, Ac), 2.91 (t, 2H, β-CH_2), 3.50 (q, 2H, α-CH_2), 3.86 (s, 3H, OCH_3), 5.51 (br s, 1H, NH), 6.77–7.23 (m, 3H$_{arom}$), 7.79 (br s, 1H, NH$_{indole}$).

2-Phenylmelatonin (4d). Acetylation of the crude 2-phenyl-5-methoxytryptamine in the above procedure afforded 0.740 g (48% yield) of **4d** as amorphous solid. IR ν_{max}: 3450, 3300, 1660, 1620. ^{1}H NMR: 1.77 (s, 3H, Ac), 3.08 (t, 2H, β-CH_2), 3.52 (q, 2H, α-CH_2), 3.89 (s, 3H, OCH_3), 5.51 (br s, 1H, NH), 6.88–7.51 (m, 8H$_{arom}$), 8.25 (br s, 1H, NH$_{indole}$).

2-Methyl-*N*-cyclopropanoyl-5-methoxytryptamine (4e). A solution of the crude 2-methyl-5-methoxytryptamine (from 5 mmol of **3a**), in THF (15 mL) and TEA (0.7 mL), was acylated with cyclopropanecarbonyl chloride (0.45 mL) at room temperature for 1.5 h using the procedure described for **4a**. The desired product **4e** was obtained as white solid (0.615 g, 45% overall yield), mp 102–103 °C. IR ν_{max}: 3460, 3300, 1650. ^{1}H NMR: 0.70 (m, 2H, $CH_{2\ cyclopropyl}$), 0.97 (m, 2H, $CH_{2\ cyclopropyl}$), 1.24 (m, 1H, cyclopropyl-CH), 2.36 (s, 3H, 2-CH_3), 2.90 (t, 2H, β-CH_2), 3.51 (q, 2H, α-CH_2), 3.85 (s, 3H, OCH_3), 5.75 (br s, 1H, NH), 6.75–7.25 (m, 3H$_{arom}$), 8.00 (br s, 1H, NH$_{indole}$).

2-Isopropyl-*N*-cyclopropanoyl-5-methoxytryptamine (4f). Acylation of the crude 2-isopropyl-5-methoxytryptamine with cyclopropanecarbonyl chloride in the above procedure gave 0.375 g (25% yield) of **4f** as white solid, mp 142–143 °C. IR ν_{max}: 3460, 3440, 3300, 1650. ^{1}H NMR: 0.69 (m, 2H, $CH_{2\ cyclopropyl}$), 0.98 (m, 2H, $CH_{2\ cyclopropyl}$), 1.22 (m, 1H, cyclopropyl-CH), 1.33 [d, 6H, CH(CH_3)$_2$], 2.92 (t, 2H, β-CH_2), 3.23 [m, 1H, CH(CH_3)$_2$], 3.52 (q, 2H, α-CH_2), 3.87 (s, 3H, OCH_3), 5.72 (br s, 1H, NH), 6.78–7.27 (m, 3H$_{arom}$), 7.82 (br s, 1H, NH$_{indole}$).

2-Phenyl-*N*-cyclopropanoyl-5-methoxytryptamine (4g). Acylation of the crude 2-phenyl-5-methoxytryptamine with cyclopropanecarbonyl chloride in the above procedure gave 0.755 g (45% yield) of **4g** as amorphous solid. IR ν_{max}: 3440, 3300, 1650. ^{1}H NMR: 0.66 (m, 2H, $CH_{2\ cyclopropyl}$), 0.91 (m, 2H, $CH_{2\ cyclopropyl}$), 1.12 (m, 1H, cyclopropyl-CH), 3.10 (t, 2H, β-CH_2), 3.59 (q, 2H, α-CH_2), 3.89 (s, 3H, OCH_3), 5.69 (br s, 1H, NH), 7.26–7.55 (m, 8H$_{arom}$), 8.17 (br s, 1H, NH$_{indole}$).

2-Bromo-*N*-cyclopropanoyl-5-methoxytryptamine (4h). *N*-Bromosuccinimide (0.89 g, 5 mmol) was added to a solution of **4j** (1.29 g, 5 mmol) in acetic acid (20 mL). The reaction mixture was stirred under N_2 at room temperature for 4 h, then cooled at 0 °C, neutralized with a 50% solution of NaOH, and extracted with ethyl acetate. The combined organic layers were washed with NaCl solution, dried (Na_2SO_4) and concentrated. Purification by flash chromatography (silica gel; ethyl acetate–cyclohexane 6:4) and crystallization gave 0.490 g (29% yield) of **4h** as white solid, mp 82–83 °C. IR ν_{max}: 3460, 3300, 1650. ^{1}H NMR: 0.72 (m, 2H, $CH_{2\ cyclopropyl}$), 0.99 (m, 2H, $CH_{2\ cyclopropyl}$), 1.27 (m, 1H, cyclopropyl-CH), 2.93 (t, 2H, β-CH_2), 3.56 (q, 2H, α-CH_2), 3.85 (s, 3H, OCH_3), 5.72 (br s, 1H, NH), 6.80–7.25 (m, 3H$_{arom}$), 8.21 (br s, 1H, NH$_{indole}$).

Binding Assays. The source of the animals, the characterization of the melatonin receptor in the quail brain, the isolation of the crude membrane preparation, enriched with melatonin receptors, used in the present study has been described in detail elsewhere.[18,21]

Determination of the Biological Activity *in Vivo*. For the *in vivo* studies, the Syrian hamster gonadal regression model[14,22] was employed. Briefly, male sexually mature Syrian hamsters (five per group), held under constant photoperiod conditions of 14:10 light:dark (lights off at 20:00), were treated with the tested compounds alone, in a dose of 200 μg/animal/day at 17:00, or 200 μg at 16:30, followed by 20 μg of melatonin at 17:00, for 6 weeks. The negative controls were given equal volumes of saline; the positive controls received 200 μg or 20 μg melatonin at 16:30 and 17:00, respectively. At the end of the experimental period, the combined testes and seminal vesicle weights were recorded. Analogs that induced partial gonadal regression (testes and seminal vesicle weights equal to 50% or less, compared to the respective positive control) were considered weak agonists. Compounds that had no influence *per se*, but were able to block the effect of subsequent melatonin treatment, were evaluated as antagonists.

Determination of the Biological Activity *in Situ*. The *in situ* electrophysiological studies employed the rabbit parietal cortex as a model system and the basic approach has been described in detail elsewhere.[23] Additionally, in a separate series of experiments, saturating concentrations of GABA antagonists phaclofen and bicuculline (1×10^{-4} M) were applied, starting before and continuing during the drugs application, in order to block the possible effects of the compounds on the GABA–receptor complex and allow for a delineation of their principal activities at the level of the melatonin receptor.

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