2-[(2,3-Dihydro-1*H*-indol-1-yl)methyl]melatonin Analogues: A Novel Class of MT_2-Selective Melatonin Receptor Antagonists

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A novel series of 2-[(2,3-dihydro-1*H*-indol-1-yl)methyl]melatonin analogues has been prepared to probe the steric and electronic properties of the binding pocket of the MT_2 receptor accommodating the "out-of-plane" substituent of MT_2-selective antagonists. The acetamide (**6b**) bearing an ususbstituted indoline moiety displayed an excellent binding affinity and selectivity toward the MT_2-subtype (MT_2, $K_i = 1$ nM; MT_1, $K_i = 115$ nM), behaving as a competitive antagonist. 5-Me, 5-OMe, 5-Br, 6-NH_2, and 6-NO_2 substitution of the indoline moiety reduced both MT_2 affinity and selectivity, indicating that hydrophobic interactions play a decisive role in binding the out-of-plane substituent. The cyclobutanecarboxamide (**6e**) showed a biphasic binding pattern at MT_2 receptors, indicating the presence of two MT_2 binding sites, a high affinity ($K_i = 1$ pM) and a low affinity ($K_i = 148$ nM), while MT_1 binding affinity was very low ($K_i = 1.4$ μM). Functional analysis of **6e** revealed it to be an antagonist at MT_1 receptors and a partial agonist, at best, at MT_2 receptors.

Introduction

Melatonin (*N*-acetyl-5-methoxytryptamine, MLT[a], Figure 1) is a neurohormone mainly secreted by the pineal gland of mammals including humans.[1] The circadian pattern of MLT secretion is associated with the "biological clock", suggesting that MLT plays an important role in modulation of the sleep−wake cycle and of circadian rhythms in humans.[2] Other effects of MLT described in the literature include its antiinflammatory,[3] pain modulatory,[4] retinal,[5] vascular,[6] antitumor,[7] antioxidant,[8] stroke-protective,[9] and neuroprotective[10] properties. The physiological effects of MLT result mainly from the activation of the high affinity G-protein-coupled receptors MT_1 and MT_2. Both receptor subtypes have been found in mammals, including humans, and subsequently cloned.[11] While it is known that MT_1 and MT_2 receptors are expressed both centrally (suprachiasmatic nucleus, cortex, pars tuberalis, etc.) and peripherally (kidney, adipocytes, retina, blood vessels, etc.),[12] their physiological roles are not well defined. MT_1 receptors seem to be involved in the sleep promoting effects of MLT[13,14] and in mediating vasoconstriction,[15] whereas MT_2 receptors appear to play a major role in the resynchronizing activity of MLT[13,16,17] and in mediating vasodilation.

During the past 2 decades, a great number of melatonin receptor ligands have been reported in the literature.[18] The nonselective melatonin receptor agonist ramelteon has been successfully marketed in the U.S. for the treatment of insomnia.[19,20] Other nonselective agonists, such as LY-156735[21] or tasimelteon (VEC-162),[22] are undergoing clinical trials for the treatment of sleep disorders. Agomelatine, an MT_1/MT_2 agonist and 5-HT_{2c} antagonist, is under evaluation for the treatment of major depression.[23] Melatonin receptor antagonists have been only evaluated in preclinical studies, for instance, luzindole for its antidepressant-like effects,[24] S22153 in circadian rhythm entrainment experiments,[25] and ML-23 in the treatment of Parkinson's disease.[26] While the number of MT_1 and MT_2 selective agonists as well as MT_1-selective antagonists is still very limited,[18,27] several series of MT_2-selective antagonists have been reported.[18,28] The representative agents are displayed in Table 1.

A common structural feature in most of MT_2-selective antagonists is the presence of a lipophilic substituent located out of the plane of their core nucleus in a position corresponding to positions 1 and 2 of the indole ring in melatonin.[28] 3D-QSAR and docking within homology models of MT_1 and MT_2 receptors revealed that MT_2 receptors possess a hydrophobic pocket accommodating the lipophilic "out-of-plane" substituent of MT_2 antagonists.[37] However, there are only limited studies concerning the steric and electronic tolerance of this binding cavity.[30,33,35,37]

In order to probe the postulated pharmacophore for MT_2 antagonists, we have recently synthesized rigid pentacyclic ligands (**1a**,**b**) (Figure 2) possessing an indoline moiety attached to the positions 1 and 2 of melatonin.[38,39] The racemic compounds **1a**,**b** exhibited nanomolar affinity for MT_2 receptors (**1a**, $K_i = 65$ nM; **1b**, $K_i = 410$ nM) being 5-fold higher than for the MT_1 subtype (**1a**, $K_i = 320$ nM; **1b**, $K_i = 1.8$ μM). The most likely explanation for the poor selectivity and moderate binding of **1a**,**b** is the bulkiness and/or unfavorable spatial orientation of the indoline moiety, which is not able to occupy the lipophilic binding pocket of the MT_2 receptors because of the nearly planar geometry of the dihydropyrazinodiindole ring system (Figure 3).

In this paper, we report the synthesis and pharmacological evaluation of a novel class of MT_2-selective melatonin receptor antagonists formally obtained by opening the central six-membered ring of **1a** (Figure 2). In contrast to the rigid ring system of **1a**, the indoline moiety of the designed compounds is conformationally flexible and thus more likely to reach the lipophilic binding pocket of the MT_2 receptors. Additionally, the indoline moiety was substituted with groups of different electronic and steric properties, such as CH_3, OCH_3, Br, NH_2, and NO_2, in order to explore the binding behavior within the lipophilic pocket accommodating the "out-of-plane" substituent.

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[a] Abbreviations: MLT, melatonin; MT_1, melatonin receptor subtype 1, MT_2, melatonin receptor subtype 2.

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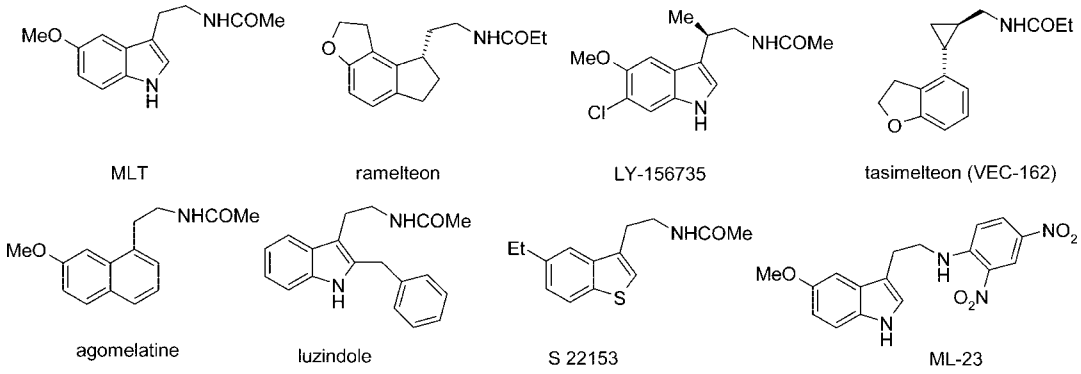


Figure 1. Melatonin and melatoninergic ligands in clinical and preclinical use.

Moreover, 2-(pyrrolidin-1-ylmethyl)melatonin, an analogue lacking a benzene ring, was prepared to complete the SARs.

Chemistry

The title compounds were prepared from the commercially available 5-methoxyindole-2-carboxylic acid according to two routes. The first synthetic sequence, displayed in Scheme 1, commenced with the condensation of 5-methoxyindole-2-carboxylic acid with 2-methylindoline and indoline using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI·HCl) in dry CH_2Cl_2 to give the amides **2a**,**b** in moderate yields. 2-Methylindoline was obtained from 2-methylindole via $NaCNBH_3$ reduction.[40] For the introduction of the ethylamine side chain we used the procedure previously applied for other ring systems.[38,41]

Our approach involved a sequence of a Mannich reaction, quaternization of the Mannich base, substitution of the trimethylamine moiety by a cyanide, and a final reduction of the cyanomethyl group to the ethylamine moiety. Thus, aminomethylation of **2a**,**b** using *N*,*N*-dimethylmethyleneiminium iodide in chloroform afforded the Mannich bases **3a**,**b**. Treatment of **3a**,**b** with methyl iodide in dichlormethane and heating of the resulting trimethylammonium iodides with potassium cyanide and dicyclohexyl-18-crown-6 in acetonitrile provided the nitriles **4a**,**b**. Simultaneous nitrile and amide reduction using $LiAlH_4$ in diethyl ether/THF afforded the ethylamines **5a**,**b**, which were converted to the desired melatoninergic ligands **6a**−**e** by N-acylation using acetic anhydride (**6a**,**b**), butyric anhydride (**6c**), propionic acid anhydride (**6d**), and cyclobutanecarboxylic acid chloride (**6e**).

The key intermediate for the more convenient route toward the target compounds **6f**−**k** was 3-(cynomethyl)-5-methoxyindole-2-carboxylic acid **9**, which was prepared from methyl 5-methoxyindole-2-carboxylate in three steps using standard procedures as outlined in Scheme 2. Briefly, the known Mannich base **7**,[42] obtained by aminomethylation of the starting material using *N*,*N*-dimethylmethyleneiminium chloride, was N-methylated and subsequently subjected to nucleophilic substitution using potassium cyanide. The resulting cyanomethyl ester **8** was hydrolyzed to the corresponding acid **9** using LiOH in THF/H_2O.

Starting from **9**, the target compounds **6f**−**i** were prepared in a reaction sequence already applied in the first route involving condensation with the appropriate amine, simultaneous nitrile and amide reduction using $LiAlH_4$, followed by N-acylation of the resulting amines (Scheme 3). The differently substituted indolines were obtained from the corresponding indoles via $NaCNBH_3$ reduction[40] except for the commercially available 6-nitroindoline. For the synthesis of 6′-nitroindoline substituted amine **5j** we used AlH_3 (created in situ from $LiAlH_4/AlCl_3$) as a reducing agent in order to avoid reduction of the nitro group. The 6′-amino substituted target compound **6k** was prepared from the nitroacetamide **6j** by catalytic transfer hydrogenation using ammonium formate and Pd/C (10%) (Scheme 4).

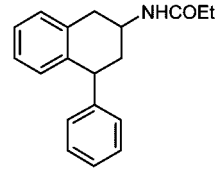
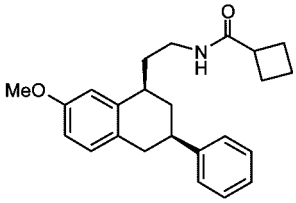
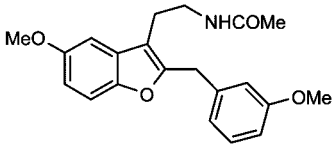
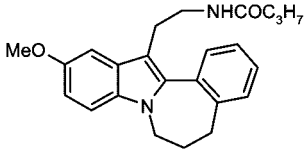
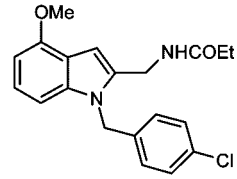
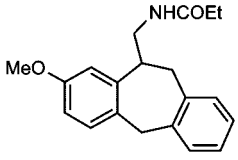
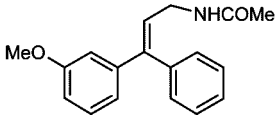
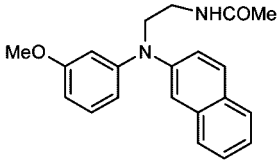
Results and Discussion

According to the existing pharmacophore, a structural characteristic of MT_2-selective antagonists is a lipophilic substituent located out of the plane of their core nucleus in a position corresponding to position 1 or 2 of melatonin. Docking of MT_2-selective antagonists to homology models of melatonin receptors indicated that MT_2 selectivity is due to a hydrophobic binding pocket accommodating this out-of-plane substituent that is only present in the MT_2 subtype.[37] Our previously reported pentacyclic ligands **1a**,**b** are derived from melatonin by attaching the indoline group to N1 and C2 via methylene groups. As shown in Figure 3, the indoline moiety in both enantiomers of **1b** is arranged nearly coplanar to the indole ring, being unable to reach the hydrophobic binding region of the MT_2 receptor.

Consequently, the binding affinity and MT_2 selectivity of **1b** are rather poor. In order to improve both affinity and MT_2 selectivity, we synthesized a series of compounds **6a**−**k** formally obtained from **1a**,**b** by breaking the C−N bond of the central six-membered ring as shown in Figure 2. The novel melatonin-derived agents bear differently substituted *N*-methylindoline moieties in position 2, which seem to be flexible enough to reach the lipophilic pocket of the MT_2 receptors. The binding affinities of **6a**−**k** for human MT_1 and MT_2 melatonin receptors measured by competition binding analysis using the radioligand 2-[^{125}I]-iodomelatonin[43] are reported in Table 2.

The data reveal a considerable increase of binding affinity at both receptor subtypes for all new derivatives when compared to compound **1b** (MT_1, K_i = 1800 nM; MT_2, K_i = 410 nM). However, the affinity improvement was strongly dependent on the substitution pattern of the indoline group. The 5-methoxyindoline derivative **6f** exerts the highest MT_1 binding affinity (MT_1, K_i = 5.8 nM) and is the only high-affinity ligand in this series showing no subtype selectivity (MT_2, K_i = 7.1 nM). Interestingly, this finding is not in agreement with affinity data obtained in a series of 2-benzyl-substituted benzofuran analogues with the 4-methoxybenzyl derivative being one of the most MT_2-selective agents (MT_1, K_i = 24 nM; MT_2, K_i = 0.50 nM).[31] The nonselective binding behavior of **6f** is probably caused by the competition of both methoxy groups for binding at the MT_2 receptor region accommodating the methoxy group of melatonin inducing the unfavorable ligand orientation. Removal of the methoxy group from the indoline moiety of **6f** generated a drastic 18-fold decrease of binding at MT_1 receptors, whereas the MT_2 affinity was increased by a factor of 6. The resulting

Table 1. Structures of the Representative MT_2-Selective Antagonists and Their Binding Constants K_i at the MT_1 and MT_2 Receptors

	MT_1 [nM]	MT_2 [nM]	Lit.
	59	0.46	29
	797	0.90	30
	27	0.25	31
	66	0.50	32
	1290	9	33
UCM454 	1.4	0.16	34
	230	2.5	35
	132	0.11	36

ligand **6b** is characterized by an outstanding pharmacological profile possessing the highest binding affinity for the MT_2 receptor ($K_i = 1.1$ nM) and an excellent affinity ratio ((K_i MT_1)/(K_i MT_2)) of 91. Introduction of a methyl group into the position adjacent to the indoline nitrogen atom of **6b**, to give **6a**, decreased MT_2 and MT_1 affinity 12.5 and 1.5 times, respectively, leading to a considerably reduced MT_2 selectivity ((K_i MT_1)/(K_i MT_2)) of approximately 11. Removal of the benzene ring from the indoline group of **6b**, to yield the *N*-methylpyrrolidine

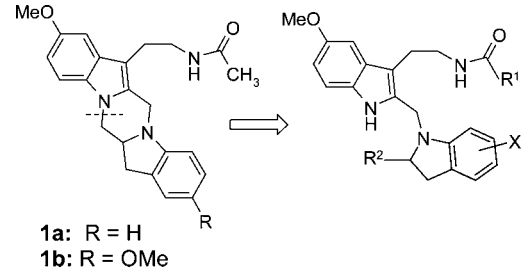


Figure 2. Design of a novel class of melatoninergic ligands.

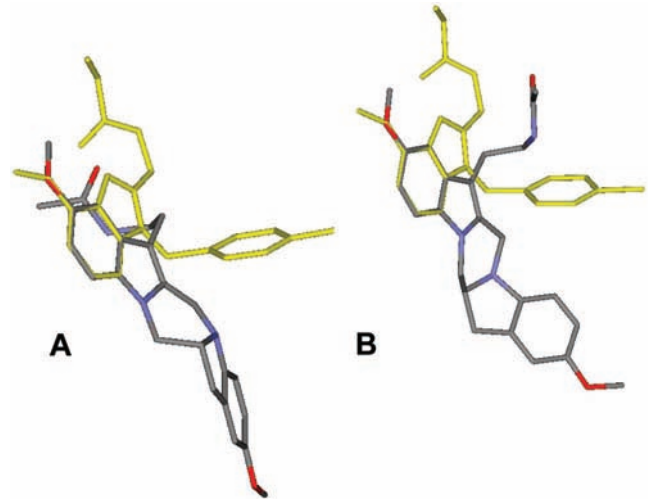


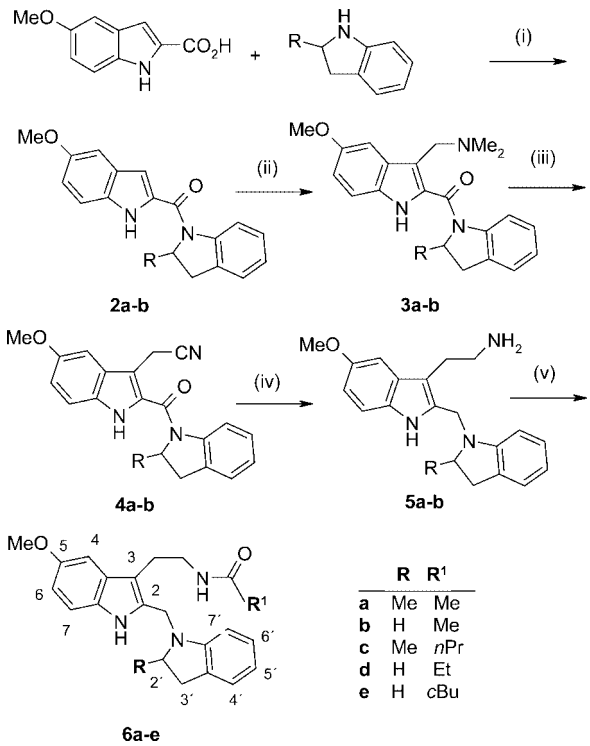
Figure 3. Superposition of (*R*)-**1b** (A) and (*S*)-**1b** (B) onto the selective MT_2 antagonist UCM454 (yellow structure, Table 1) in a conformation adopted in the MT_2 binding pocket.[37]

analogue **6i**, considerably decreased MT_2 binding ($K_i = 25$ nM) while the MT_1 affinity was unaffected ($K_i = 121$ nM), indicating the importance of the presence of aromatic rings for ligand−receptor interactions within the hydrophobic pocket of MT_2 receptors. This is consistent with the structures of other MT_2-selective ligands, all of them having an aromatic ring in a corresponding position (see Table 1).

In order to further probe the steric, lipophilic, and electronic requirements of the MT_2 binding pocket accommodating this aromatic substituent, we substituted the indoline benzene ring with 5-Me, 5-Br, 6-NO_2, and 6-NH_2 groups. The resulting methyl, bromo, and amino substituted agents **6g**, **6h**, and **6k** are nonselective ligands exhibiting similar MT_1 binding constants (41, 85, and 108 nM, respectively) and drastically reduced affinity for the MT_2 receptors (15, 30, and 118 nM, respectively) when compared to the nonsubstituted parent compound **6b** (MT_1 $K_i = 101$ nM, MT_2 $K_i = 1.1$ nM). The 6-nitro derivative **6j** is a moderate MT_2-selective ligand (MT_1, $K_i = 302$ nM; MT_2, $K_i = 26.5$ nM) having a selectivity ratio of 11.5. These findings indicate that the hydrophobic binding pocket accommodating the out-of-plane substituent is sterically restricted. Morover, the ligand receptor interactions within this binding site are likely to be of a pure hydrophobic nature, as polar groups, such as NO_2 and NH_2, reduce binding.

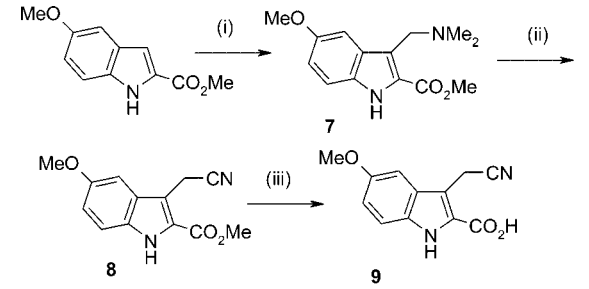
In order to further optimize the pharmacological profile of the most MT_2-selective agent **6b**, we modulated the acyl chain by replacing the methyl group with substituents that are often present in other MT_2-selective ligands, such as ethyl and cyclobutyl (see Table 1). Surprisingly, the propionamide **6d** displayed a 12-fold lower binding affinity for MT_2 receptors ($K_i = 14.5$ nM) and 3-fold higher affinity for MT_1 receptors ($K_i = 38$ nM) than the acetamide **6b**, resulting in decreased

Scheme 1[a]



[a] Reagents and conditions: (i) EDCI·HCl, CH_2Cl_2, room temp; (ii) $(CH_2{=}NMe_2)^+I^-$, $CHCl_3$, reflux; (iii) (1) MeI, CH_2Cl_2, room temp, (2) KCN, dicyclohexyl-18-crown-6, MeCN, reflux; (iv) $LiAlH_4$, Et_2O, THF, 0 °C to room temp; (v) respective acylation agent, Et_3N, CH_2Cl_2, room temp.

Scheme 2[a]



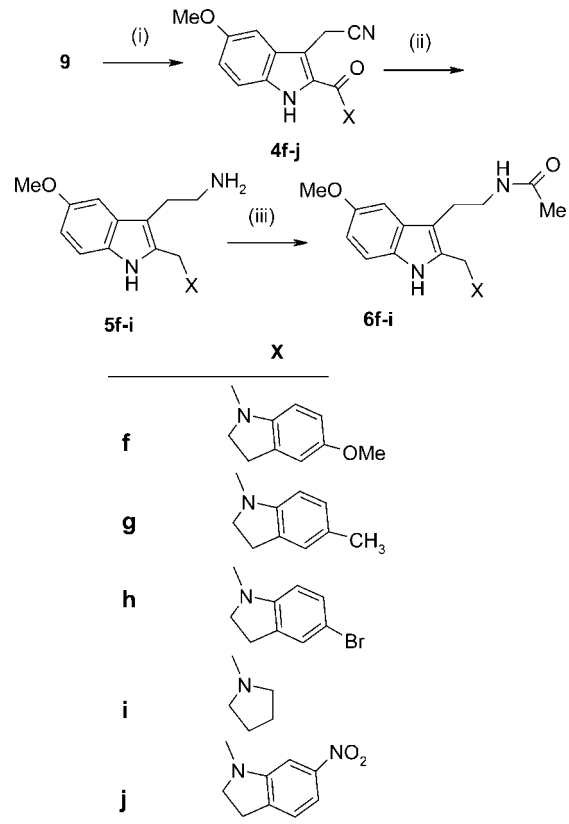
[a] Reagents and conditions: (i) $(CH_2{=}NMe_2)^+Cl^-$, $CHCl_3$, reflux; (ii) (1) MeI, CH_2Cl_2, room temp, (2) KCN, dicyclohexyl-18-crown-6, MeCN, reflux; (iii) LiOH, H_2O, THF, room temp.

MT_2 selectivity. A similar reduction of MT_2 selectivity was observed in the 2-methylindoline substituted agents **6a** and **6c**. While the acetamide **6a** was 11 times more selective for MT_2 (K_i = 14 nM) than for MT_1 receptors (K_i = 148 nM), the corresponding butyramide **6c** displayed an affinity ratio of 5 (MT_1, K_i = 41 nM; MT_2, K_i = 8 nM). Interestingly, replacement of the methyl group in **6b** with a cyclobutyl, to produce **6e**, generated a biphasic pharmacological profile for MT_2 receptors (K_{iHigh} = 1 pM and K_{iLow} = 148 nM) but not for MT_1 receptors (K_i = 1.4 μM), as shown in Figure 4.

The existence of a high-affinity and a low-affinity state has been reported for the MT_1 receptor only,[43] and to the best of our knowledge, this is the first time such pharmacological behavior could be detected for the MT_2 receptor.

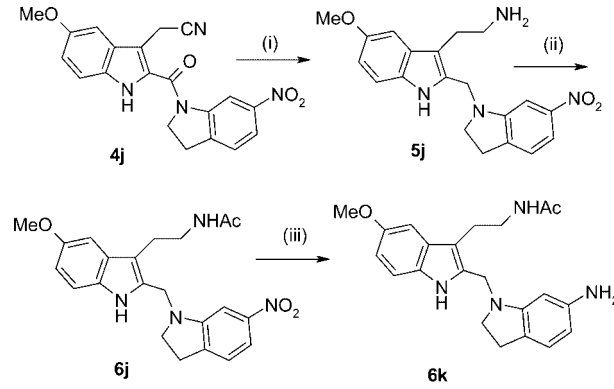
Functional analysis of **6b** showed it to be a competitive antagonist. As shown in Figure 5, the addition of **6b** along with melatonin (0.01 pM to 10 μM) produced a rightward shift in the melatonin curves for both MT_1 and MT_2 receptors, suggestive of competitive antagonism at both receptors. The potency of melatonin

Scheme 3[a]



[a] Reagents and conditions: (i) EDCI·HCl, respective indoline, CH_2Cl_2, room temp; $CHCl_3$, reflux; (ii) $LiAlH_4$, Et_2O, THF, 0 °C to room temp; (iii) respective acylation agent, Et_3N, CH_2Cl_2, room temp.

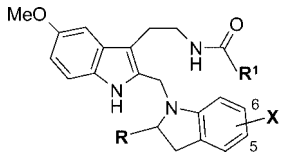
Scheme 4[a]



[a] Reagents and conditions: (i) $LiAlH_4$, $AlCl_3$, Et_2O, THF, 0 °C to room temp; (ii) Ac_2O, Et_3N, CH_2Cl_2, room temp; (iii) HCO_2NH_4, 10% Pd/C, EtOH.

to inhibit forskolin-induced cAMP accumulation was 0.23 pM for the high affinity state of the MT_1 receptor and 88 pM for the low affinity state of the MT_1 receptor. The addition of 10 or 100 nM of **6b** produced a potency value of 100 and 79 pM, respectively. As for the MT_2 receptor, the potency of melatonin to inhibit forskolin-induced cAMP accumulation was 61 pM, which was lowered to 263 pM and 5.5 nM in the presence of 10 or 100 nM **6b**, respectively. No concentration-dependent inhibition of forskolin-induced cAMP accumulation occurred when **6b** (0.1 pM to 10 nM) was added alone, suggesting that it displays no intrinsic activity at either the MT_1 or MT_2 receptor (data not shown). Functional analysis of **6e** showed it to lack intrinsic activity at MT_1 receptors, as no concentration-dependent inhibition of forskolin-induced cAMP accumulation occurred at MT_1 recep-

Table 2. Binding Affinity[a] of Compounds **1**−**6k** for the Human MT_1 and MT_2 Receptors Expressed in CHO Cells Obtained in Competition Radioligand Binding Assays Using 2-[^{125}I]Iodomelatonin



				pK_i ± SEM	
compd	R	R^1	X	MT_1	MT_2
MLT				9.34 ± 0.10	9.02 ± 0.09
1a				6.52 ± 0.02	7.22 ± 0.03
1b				5.75 ± 0.21	6.39 ± 0.27
6a	Me	Me	H	6.83 ± 0.04	7.85 ± 0.19
6b	H	Me	H	6.94 ± 0.02	8.93 ± 0.05
6c	Me	*n*-Pr	H	7.39 ± 0.02	8.09 ± 0.05
6d	H	Et	H	7.42 ± 0.05	7.84 ± 0.19
6e	H	*c*-Bu	H	5.85 ± 0.03	12.00 ± 0.58 6.83 ± 0.03
6f	H	Me	5-OMe	8.24 ± 0.16	8.15 ± 0.04
6g	H	Me	5-Me	7.39 ± 0.02	7.83 ± 0.04
6h	H	Me	5-Br	7.07 ± 0.04	7.52 ± 0.02
6i	H	Me		6.92 ± 0.03	7.61 ± 0.23
6j	H	Me	6-NO_2	6.51 ± 0.04	7.58 ± 0.07
6k	H	Me	6-NH_2	6.96 ± 0.06	6.93 ± 0.06

[a] pK_i values were calculated from IC_{50} values obtained from competitive curves according to the method of Cheng and Prusoff[44] and are the mean of three determinations.

tors. Regarding its actions at MT_2 receptors, it may display partial agonist activity, at best, at MT_2 receptors at concentrations higher than 10 nM (Figure 5).

In summary, a novel series of 2-[(2,3-dihydro-1*H*-indol-1-yl)methyl]melatonin analogues has been prepared in order to probe the steric and electronic properties of the lipophilic binding pocket of MT_2 receptors accommodating the "out-of-plane" substituent of MT_2-selective antagonists. The best tolerated substituent attached to position 2 of melatonin is the 2-(indoline-1-yl)methyl group. Increasing the bulkiness of the methylindoline moiety by a methyl group in positions 2 and 5 as well as changing the electronic, lipophilic, and hydrogen bond donor and acceptor properties by introduction of 5-OMe, 5-Br, 6-NH_2, and 6-NO_2 substituents is detrimental to MT_2 affinity and selectivity. The results indicate a sterically restricted binding pocket with hydrophobic interactions playing a decisive role in binding the out-of-plane substituent of MT_2-selective antagonists. The acetamide **6b** bearing an usubstituted indoline moiety displayed an excellent binding affinity and selectivity toward the MT_2 subtype (MT_2, K_i = 1 nM; MT_1, K_i = 115 nM), behaving as a competitive antagonist. The cyclobutane amide **6e** showed the most interesting pharmacological profile. Its biphasic binding pattern at MT_2 receptors indicated the presence of two MT_2 binding sites, the high affinity one (K_i = 1 pM) and the low affinity one (K_i = 148 nM), while MT_1 binding affinity was very low (K_i = 1.4 μM) resulting in the highest MT_2 selectivity of the whole series. These findings help to define the steric and electronic requirements of the hydrophobic binding pocket of MT_2 receptors accommodating the out-of-plane substituent of MT_2-selective melatonin receptor antagonists.

Experimental Section

General Methods. Melting points were determined using a capillary melting point apparatus (Gallenkamp, Sanyo) and are uncorrected. Column chromatography was carried out on silica gel 60 (0.063−0.200 mm) obtained from Merck. A Bruker AV-400 spectrometer was used to obtain ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra. Proton chemical shifts are refereneced to $CHCl_3$ (7.24 ppm) and DMSO-d_6 (2.55 ppm). Coupling constants (*J*) are given in hertz (Hz). Carbon chemical shifts are referenced to $CDCl_3$ (77.00 ppm) and DMSO-d_6 (39.50 ppm). The NMR resonances were assigned by means of HH-COSY, HMQC, and HMBC experiments. EI mass spectra were determined on Finnigan MAT 8200 and on ESI-microTOF spectrometers. IR spectra, recorded as ATR, were obtained by using a Biorad PharmalyzIR FT-IR instrument. Elemental analyses were performed by the Microanalytical Section of the Institute of Inorganic Chemistry, University of Würzburg. All reactions were carried out under an argon atmosphere. All chemicals were purchased from commercial suppliers and used directly without any further purification. The radioligand 2-[^{125}I]iodomelatonin was purchased from Perkin-Elmer (Shelton, CT).

General Procedure for the Synthesis of 2-(2,3-Dihydro-1*H*-indol-1ylcarbonyl)-5-methoxy-1*H*-indoles 2a,b. A solution of the appropriate amine (1 equiv) in dry CH_2Cl_2 (5 mL) was added to a stirred solution of 5-methoxyindole-2-carboxylic acid (1 equiv) and EDCI·HCl (1.5 equiv) in dry CH_2Cl_2 (15 mL). The reaction mixture was stirred for 18 h at room temperature, extracted with 5 N hydrochloric acid (3 × 5 mL), washed with water (2 × 10 mL), and dried (Na_2SO_4). The organic layer was evaporated in vacuo, and the residue was recrystallized from isopropanol.

5-Methoxy-2-[(2-methyl-2,3-dihydro-1*H*-indol-1yl)carbonyl]-1*H*-indole (2a). Compound **2a** (1.02 g, 86%) was obtained from 5-methoxyindole-2-carboxylic acid (0.74 g) and 2-methylindoline as a pale-yellow powder, mp 174−176 °C. MS (EI): *m*/*z* (%) = 306 (M^+, 48), 174 (55), 133 (100), 118 (49). Anal. ($C_{19}H_{18}N_2O_2$) C, H, N.

2-[(2,3-Dihydro-1*H*-indol-1yl)carbonyl]-5-methoxy-1*H*-indole (2b). Compound **2b** (0.45 g, 87%) was obtained from 5-methoxyindole-2-carboxylic acid (0.34 g) and indoline as a pale-yellow powder, mp 218−220 °C. MS (EI): *m*/*z* (%) = 292 (M^+, 41), 174 (50), 119 (100). ^{1}H NMR (DMSO-d_6): δ 3.28 (t, 2H, *J* = 8.2), 3.82 (s, 3H), 4.54 (t, 2H, *J* = 8.2), 6.95 (dd, 1H, *J* = 8.8, 2.3), 7.08−7.12 (m, 2H), 7.12 (d, 1H, *J* = 2.3), 7.27 (dd, 1H, *J* = 7.6, 7.6), 7.35 (d, 1H, *J* = 7.3), 7.44 (d, 1H, *J* = 8.8), 8.22 (d, 1H, *J* = 7.8), 11.65 (br, 1H). ^{13}C NMR (DMSO-d_6): δ 28.2, 49.6, 55.2, 102.1, 105.2, 113.1 (C-7), 115.3, 116.9, 123.8, 124.8, 126.9, 127.5, 131.0, 131.5, 132.3, 143.3, 153.8, 160.2. IR (cm^{-1}) ν = 3277, 3249, 1614, 1577. Anal. ($C_{18}H_{16}N_2O_2$) C, H, N.

General Procedure for the Synthesis of 1-[(2,3-Dihydro-1*H*-indol-1ylcarbonyl)-5-methoxy-1*H*-indole-3yl]-*N*,*N*-dimethylmethanamines 3a,b. Dimethylmethyleniminium iodide (1.3 equiv) was added to a solution of **2a**,**b** (1.0 equiv) in dry $CHCl_3$ (50 mL). The reaction mixture was refluxed for 18 h, allowed to cool, and basified with 25% ammonia. The organic layer was separated, washed with water (4 × 15 mL), dried (Na_2SO_4), and evaporated under reduced pressure to afford the Mannich bases **3a**,**b**. The crude products were pure enough to be used in the next step without further purification as indicated by ^{1}H NMR.

1-{5-Methoxy-2-[(2-methyl-2,3-dihydro-1*H*-indol-1ylcarbonyl)-1*H*-indol-3yl]}-*N*,*N*-dimethylmethanamine (3a). Compound **3a** (0.64 g, 82%) was obtained from **2a** (0.66 g).

1-[2-(2,3-Dihydro-1*H*-indol-1ylcarbonyl)-5-methoxy-1*H*-indol-3yl]-*N*,*N*-dimethylmethanamine (3b). Compound **3b** (0.38 g, 79%) was obtained from **2b** (0.40 g). ^{1}H NMR ($CDCl_3$): δ 2.18 (s, 6H), 3.01 (t, 2H, *J* = 8.2), 3.59 (s, 2H), 3.83 (s, 3H), 4.08 (t, 2H, *J* = 8.2), 6.86 (dd, 1H, *J* = 8.8, 2.5), 6.95−7.03 (m, 2H), 7.16−7.19 (m, 3H), 7.19 (d, 1H, *J* = 2.5), 9.23 (br, 1H). ^{13}C NMR ($CDCl_3$): δ 27.7, 45.2, 49.6, 53.8, 55.7, 101.9, 112.5, 113.9, 114.8, 115.8, 123.9, 125.0, 127.2, 128.2, 131.2, 130.9, 132.8, 141.9, 154.3, 162.7.

General Procedure for the Synthesis of [(2,3-Dihydro-1*H*-indol-1ylcarbonyl)-5-methoxy-1*H*-indole-3yl]acetonitriles 4a,b. Methyl iodide (1.2 equiv) was added to a solution of **3a**,**b** (1.00 equiv) in dry CH_2Cl_2 (20 mL). The reaction mixture was stirred at room temperature for 1 h. The volatiles were removed under reduced pressure, and the residual ammonium salt was dissolved in dry acetonitrile (30 mL). Dicyclohexyl-18-crown-6 (0.10 g) and potassium cyanide (5 equiv) were added, and resulting reaction mixture

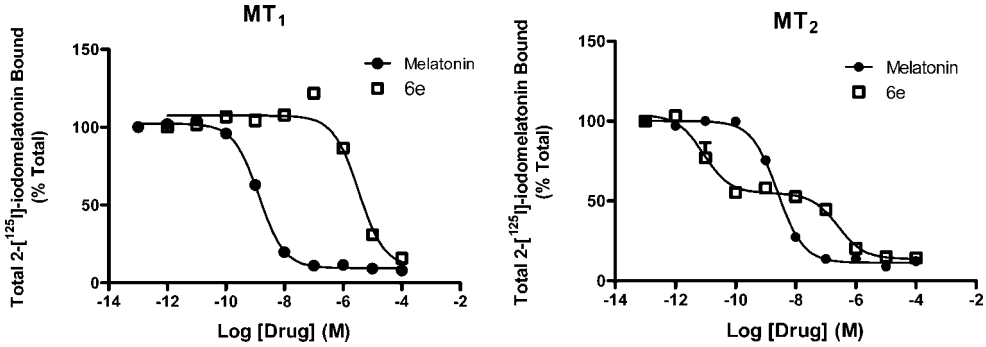


Figure 4. Competition of melatonin or **6e** for 2-[^{125}I]iodomelatonin binding to MT_1 or MT_2 receptors expressed in CHO cells. Each data point represents the mean ± standard error of three independent experiments performed in duplicate. Curves were fit by nonlinear regression analysis by one- or two-site fit to obtain affinity (K_i) values using the GraphPad Prism software.

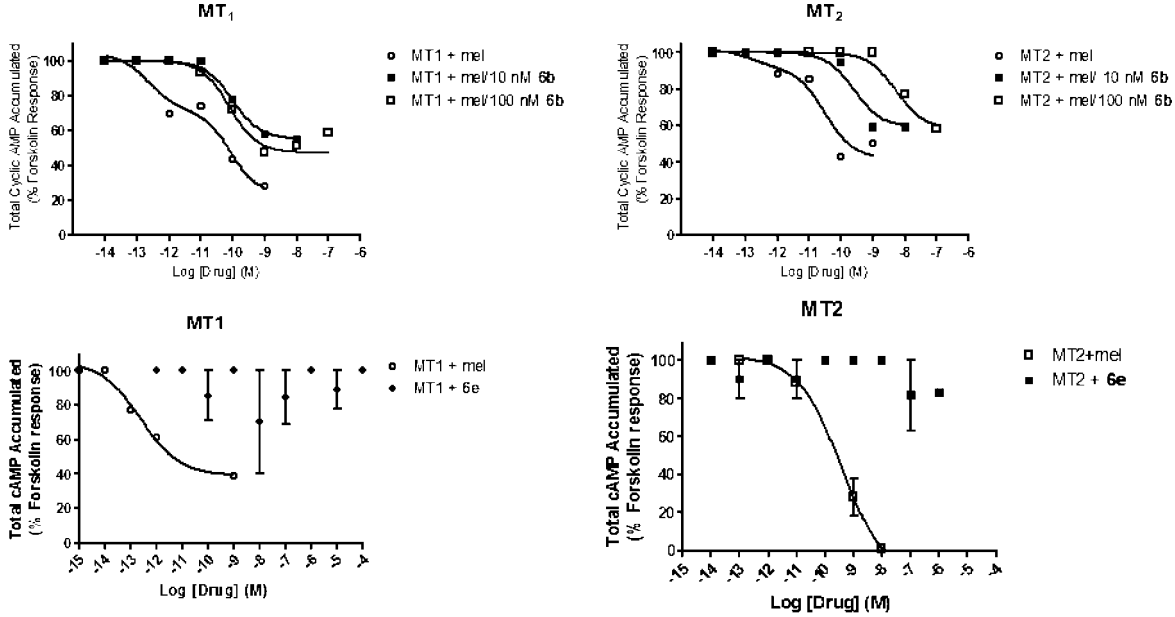


Figure 5. Functional analysis of melatonin, **6b** (top graphs), and **6e** (bottom graphs) on forskolin-stimulated cAMP formation in CHO cells expressing either the MT_1 receptor or the MT_2 receptor. Each data point represents the mean ± standard error of two to three independent experiments performed in duplicate. Curves were fit by nonlinear regression analysis by one- or two-site fit to obtain potency (IC_{50}) values using the GraphPad Prism software.

was heated at 80 °C for 1 h. The solvent was evaporated under vacuum and the residue was subjected to silica gel chromatography to give **4a**,**b**.

{5-Methoxy-2-[(2-methyl-2,3-dihydro-1*H*-indol-1ylcarbonyl)-1*H*-indol-3-yl]}acetonitrile (4a). Compound **4a** (0.40 g, 84%) was obtained from **3a** (0.50 g) as a light-brown solid, mp 95−98 °C. Eluent: chloroform/ethyl acetate, 1:1. MS (ESI): *m*/*z* (%) = 346 ($M + H^+$, 100). Anal. ($C_{21}H_{19}N_3O_2$) C, H, N.

[2-(2,3-Dihydro-1*H*-indol-1ylcarbonyl)-5-methoxy-1*H*-indol-3-yl]acetonitrile (4b). Compound **4b** (0.15 g, 61%) was obtained from **3b** (0.26 g) as a pale-yellow solid, mp 199−201 °C. Eluent: chloroform/ethyl acetate, 1:9. MS (ESI): *m*/*z* (%) = 332 (MH^+, 100). ^{1}H NMR ($CDCl_3$): δ 3.07 (t, 2H, J = 8.1), 3.85 (s, 5H), 4.14 (t, 2H, J = 8.1), 6.95 (dd, 1H, J = 8.8, 2.3), 7.00−7.03 (m, 2H), 7.11 (d, 1H, J = 2.3), 7.19−7.24 (m, 3H), 9.00 (br, 1H). ^{13}C NMR ($CDCl_3$): δ 13.8, 27.9, 50.1, 55.8, 99.9, 106.4, 113.2, 115.7, 116.5, 117.3, 124.6, 125.4, 127.5, 126.7, 129.2, 130.8, 132.9, 141.6, 155.1, 161.1. IR (cm^{-1}) ν = 3280, 2832, 2197, 1614. Anal. ($C_{20}H_{17}N_3O_2$) C, H, N.

General Procedure for the Synthesis of Amides 6a−i. A solution of **4a**−**i** (1.0 equiv) in dry THF (5 mL) was added dropwise to a stirred suspension of $LiAlH_4$ (10.0 equiv) in dry diethyl ether (30 mL) at 0−5 °C. The reaction mixture was heated at 40 °C for 4 h. The reaction was quenched by a slow addition of saturated sodium sulfate solution at 0−5 °C. The formed precipitate was filtered off and washed with THF (10 mL). The combined filtrate and washings were dried (Na_2SO_4), filtered, and evaporated under reduced pressure to give amines **5a,b,f**−**i** as viscous oils. The crude amines (1 equiv) were dissolved in dry CH_2Cl_2 (15 mL) and treated with triethylamine (3.5 equiv) and the appropriate acid anhydride (5.0 equiv for **6a**−**d** and **f**−**i**) or acid chloride (1 equiv for **6e**) at 0−5 °C. The reaction mixture was stirred at ambient temperature for 18 h under an inert atmosphere. The solvent was evaporated under reduced pressure and the residue was purified by silica gel chromatography to give **6a**−**i**.

***N*-(2-{5-Methoxy-2-[(2-methyl-2,3-dihydro-1*H*-indol-1yl)methyl)]-1*H*-indole-3-yl}ethyl)acetamide (6a).** Compound **6a** (0.24 g, 75%) was obtained from **4a** (0.29 g) as a yellow solid, mp 63−65 °C. Eluent: chloroform/methanol/ammonia, 10:1:0.1. MS (EI): *m*/*z* (%) = 377 (M^+, 59), 291 (17), 245 (100), 186 (96), 174 (43), 118 (50). Anal. ($C_{23}H_{27}N_3O_2$) C, H, N.

***N*-(2-{5-Methoxy-2-[(2,3-dihydro-1*H*-indol-1yl)methyl)]-1*H*-indol-3-yl}ethyl)acetamide (6b).** Compound **6b** (0.095 g, 58%) was obtained from **4b** (0.15 g) as a pale-yellow solid, mp 62−64 °C. Eluent: chloroform/ethyl acetate, 1:1. ^{1}H NMR ($CDCl_3$): δ 1.77 (s, 3H), 2.93−2.97 (m, 4H), 3.25−3.32 (m, 2H), 3.48 (m, 2H), 3.84 (s, 3H), 4.29 (s, 2H), 5.86 (br, 1H), 6.52 (d, 1H, J = 7.8), 6.75 (dd, 1H, J = 7.6, 7.3), 6.82 (dd, 1H, J = 8.8, 2.5), 7.00 (d, 1H, J = 2.3), 7.04 (dd, 1H, J = 7.8, 7.6), 7.12 (d, 1H, J = 7.3), 7.18 (d, 1H, J = 8.8), 8.45 (br, 1H). ^{13}C NMR ($CDCl_3$): δ 23.1, 24.0, 28.5,

40.2, 46.2, 54.3, 55.9, 100.4, 108.3, 109.7, 111.7, 112.0, 114.9, 124.8, 127.5, 128.8, 130.5, 130.6, 132.8, 151.8, 154.1, 170.1. IR (cm^{-1}) ν = 3254, 2924, 1633 cm^{-1}. HRMS (ESI, pos) $C_{22}H_{25}N_3O_2 \cdot Na^+$: *m/z* calcd 386.1844, *m/z* found 386.1839. Anal. ($C_{22}H_{25}N_3O_2$) C,H,N.

***N*-{2-[5-Methoxy-2-[(2,3-dihydro-1*H*-indol-1yl)methyl)]-1*H*-indol-3-yl}ethyl)butanamide (6c).** Compound **6c** (0.15 g, 64%) was obtained from **4a** (0.20 g) as a light-brown solid, mp 54−56 °C. Eluent: chloroform/methanol/ammonia, 10:1:0.1. HRMS (ESI, pos) $C_{25}H_{31}N_3O_2 \cdot Na^+$: *m/z* calcd 328.2314, *m/z* found 328.2309. Anal. ($C_{25}H_{31}N_3O_2$) C, H, N.

***N*-{2-[5-Methoxy-2-[2,3-dihydro-1*H*-indol-1yl)methyl)]-1*H*-indol-3-yl}ethyl)propanamide (6d).** Compound **6d** (0.11 g, 88%) was obtained from **4b** (0.11 g) as a pale-yellow solid, mp 55−57 °C. Eluent: chloroform/ethyl acetate, 1:1. MS (EI): *m/z* (%) = 377 (M^+, 54), 259 (90), 203 (87), 186 (100), 174 (56). Anal. ($C_{23}H_{27}N_3O_2$) C, H, N.

***N*-{2-[5-Methoxy-2-[(2,3-dihydro-1*H*-indol-1yl)methyl)]-1*H*-indol-3-yl}ethyl)cyclobutanecarboxamide (6e).** Compound **6e** (0.04 g, 22%) was obtained from **4b** (0.15 g) as a beige solid, mp 66−68 °C, and the reaction time was only 1 h at 0 °C. Eluent: chloroform/ethyl acetate, 1:1. MS (EI): *m/z* (%) = 403 (M^+, 34), 285 (61), 203 (65), 186 (66), 118 (100). ^{1}H NMR ($CDCl_3$): δ 1.70−1.91 (m, 2H), 1.95−2.03 (m, 2H), 2.13−2.23 (m, 2H), 2.73−2.82 (m, 1H), 2.93−2.97 (m, 4H), 3.28 (t, 2H, J = 8.1), 3.49 (m, 2H), 3.84 (s, 3H), 4.31 (s, 2H), 5.58 (br, 1H), 6.49 (d, 1H, J = 7.8), 6.73 (dd, 1H, J = 7.6, 7.3), 6.81 (dd, 1H, J = 8.8, 2.5), 6.99 (d, 1H, J = 2.3), 7.04 (dd, 1H, J = 7.8, 7.6), 7.12 (d, 1H, J = 7.3), 7.19 (d, 1H, J = 8.8), 8.26 (br, 1H). ^{13}C NMR ($CDCl_3$): δ 18.1, 24.2, 25.3, 28.6, 39.9, 40.0, 45.9, 54.3, 56.0, 100.5, 107.8, 109.5, 111.6, 111.8, 119.0, 124.8, 127.5, 128.9, 130.4, 130.5, 133.2, 152.2, 154.1, 174.9. IR (cm^{-1}) ν = 3265, 2931, 1635, 1484. Anal. ($C_{25}H_{29}N_3O_2$) C, H, N.

***N*-{2-[5-Methoxy-2-[(5-methoxy-2,3-dihydro-1*H*-indol-1-yl)methyl)]-1*H*-indole-3-yl}ethyl)acetamide (6f).** Compound **6f** (0.08 g, 37%) was obtained from **4f** (0.20 g) as a yellow solid, mp 158−160 °C, Eluent: chloroform/methanol/ammonia, 10:1:0.1. MS (EI): *m/z* (%) = 393 (M^+, 17), 245 (24), 186 (25), 149 (79), 134 (100). Anal. ($C_{23}H_{27}N_3O_3$) C, H, N.

***N*-{2-[5-Methoxy-2-[(5-methyl-2,3-dihydro-1*H*-indol-1yl)methyl)]-1*H*-indol-3-yl}ethyl)acetamide (6g).** Compound **6g** (0.15 g, 49%) was obtained from **4g** (0.28 g) as a pale-yellow solid, mp 65−68 °C. Eluent: ethyl acetate. MS (EI): *m/z* (%) = 377 (M^+, 41), 245 (68), 203 (43), 186 (63), 132 (100). Anal. ($C_{23}H_{27}N_3O_2$) C, H, N.

***N*-{2-{2-[(5-Bromo-2,3-dihydro-1*H*-indol-1yl)methyl)]-5-methoxy-1*H*-indole-3-yl}ethyl)acetamide (6h).** Compound **6h** (0.13 g, 80%) was obtained from **4h** (0.15 g) as a pale-yellow solid, mp 149−150 °C. Eluent: ethyl acetate. MS (EI): *m/z* (%) = 442, 444 ($M + H^+$, 100). Anal. ($C_{22}H_{24}BrN_3O_2$) H. C: calcd, 59.74; found, 59.10. N: calcd, 9.50; found, 9.05.

***N*-{2-[5-Methoxy-2-(pyrrolidin-1ylmethyl)-1*H*-indol-3-yl]ethyl}acetamide (6i).** The reaction time for the acetylation of **5i** was reduced to 30 min. Subsequently, 2 M NaOH (5 mL) was added and stirring was continued for 3 h. After addition of water (100 mL) the reaction mixture was extracted with CH_2Cl_2 (2 × 30 mL). The combined organic layers were washed with water and dried over $MgSO_4$. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel chromatography (ethyl acetate). Compound **6i** (0.13 g, 39%) was obtained from **4i** (0.30 g) as a colorless solid, mp 155−156 °C. MS (EI): *m/z* (%) = 316 ($M + H^+$, 100). Anal. ($C_{18}H_{25}N_3O_2$) C, H, N.

Molecular Modeling. The 3D structures of both enantiomers of **1b** were obtained by conformational analysis using the Cartesian method of the steric energy minimization program GLOBAL-MMX implemented in Pcmodel, version 9.0,[45] and further optimized by means of semiempirical PM3 calculations of HyperChem, version 7.1.[46] For the superposition of **1b** onto UCM454 (HyperChem), only the benzene rings bearing the methoxy substituent were considered.

Competition Binding Analysis. All synthesized compounds were tested for their binding affinity and selectivity for each of the melatonin receptor subtypes MT_1 and MT_2 using competition binding analysis. Briefly, cells expressing the human MT_1 or MT_2 melatonin receptor (MT_1-CHO, MT_2-CHO) were grown to confluence on 10 cm cell culture plates until they reached approximately 80% confluence. Next, cells were washed, lifted, and added to tubes containing 80−100 pM 2-[^{125}I]iodomelatonin in the absence (total binding) or presence of melatonin (1 fM to 1 μM) or the test compounds (1 pM to 1 μM). The mixtures were incubated for 1 h at 25 °C, and then the reaction was terminated following the addition of cold Tris-HCl solution (50 mM, pH 7.4). The mixtures were filtered through glass fiber filters (Schleicher and Schuell, Keene, NH) saturated in polyethylenimine 0.5% solution (v/v). Radioactive counts were counted using a γ counter. Data points were fit by one- or two-site nonlinear regression analysis based on the lowest residual sum of squares (GraphPad Prism), and affinity constants (K_i) were calculated. The affinity values (K_i) of each test compound were compared between receptors to obtain selectivity profiles of each. Compounds whose affinity values were in the subnanomolar range and whose selectivity profile was greater than 50 were subjected to functional analysis as described below.

Cyclic AMP Assays. The cAMP accumulation assays were carried out using Enzyme Immuno Antibody (EIA) kit according to the manufacturer's directions. Stable CHO cell lines expressing human MT_1 or human MT_2 receptors were cultured on 10 cm plates in F-12 media containing 10% FBS and 1% pen/strep until they were 70−80% confluent, after which the cells were lifted and plated in 24-well plates. The following day, the cells were incubated in serum-free media containing one of the following treatment groups for 20 min at 37 °C: (a) 30 μM rolipram alone (basal); (b) 30 μM rolipram and 100 μM forskolin (maximal accumulation); (c) 30 μM rolipram, 100 μM forskolin, and melatonin (in concentrations ranging from 10^{-14} to 10^{-7} M); (d) 30 μM rolipram, 100 μM forskolin, and **6b** (in concentrations ranging from 10^{-13} to 10^{-8} M) or **6e** (in concentrations ranging from 10^{-14} to 10^{-4} M). Co-incubation of melatonin (from 10^{-14} to 10^{-7} M) along with 10 or 100 nM **6b** was also performed to determine the competitive nature of **6b** at MT_1 or MT_2 receptors. Cyclic AMP accumulation was expressed as a percentage of forskolin response within each group. Curves were fit using nonlinear regression analysis (one-site or two-site), and potency (IC_{50}) values were calculated using the commercially available software (GraphPad Prism; GraphPad Prism, Inc., San Diego, CA).

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Supporting Information Available: Experimental data for compounds **7**−**9**, **4f**−**j**, and **6j**,**k**; ^{1}H NMR, ^{13}C NMR, and IR data for compounds **7**−**9**, **2a**, **3a**, **4a**,**f**−**j**, **6a**,**c**,**d**,**f**−**k**; elemental analysis for all novel compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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