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Synthesis and conformational study of 3,4-carbocyclic bridged indole melatonin and serotonin analogues

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Abstract—Tetrahydrobenz[*cd*]indole, has been usually assumed to be a rigid scaffold of arylethylamines of pharmaceutical interest, such as melatonin and serotonin. A series of molecules containing this scaffold has been synthesized and their conformation in solution has been determined by ^{1}H NMR. The values of the coupling constants show that the carbocycle fused with the indole ring is a mixture of the two conformers with substituent in equatorial or axial orientation. The molar fraction of the conformers appears to be sensibly affected by the bulkiness of the C-2 indole substituent. A pseudo-axial orientation of the C-3 alkylamido side chain is important for melatonin ligands to access the binding site and exhibit potent in vitro affinity, as illustrated for melatonin ligand **1** ($pK_i = 9.32$).

1. Introduction

Most compounds endowed with biological interest are molecules with a high degree of conformational freedom. The more flexible a drug is, the more likely it will interact with different receptors and produce multiple biological responses. This lack of selectivity could be responsible for undesired side effects. Flexible molecules adopt only one or a few pharmacophoric conformations, even though many structural possibilities exist. Understanding the conformational preferences of a molecule in its native environment is particularly important in the case of small biologically active substances (e.g., arylethylamines), whose functions arise from their ability to dock in a receptor site and bind via non-covalent interactions. Unfortunately, under physiological conditions this is often hampered by the coexistence of several conformational isomers, by the difficulty in assessing solvent effects and by the possibility of a rapid interconversion between conformers. Conformational restriction of bioactive molecules is a valuable tool for investigating the stereochemical features of small-molecule binding sites. This could also result in increasing receptor selectivity and activity, considering that the ligand locked in its active conformation is ready to fit its target receptor site. Furthermore, an analysis of the measured oral bioavailability in rats for over 1100 compounds studied at SmithKline Beecham has revealed a positive influence of increasing molecular rigidity.[1]

Melatonin (*N*-acetyl-5-methoxytryptamine, **MLT**) (Fig. 1), a neurohormone primarily secreted by the pineal

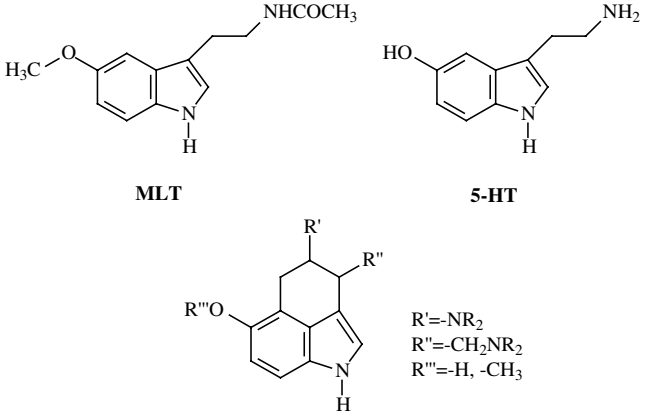

Figure 1. Melatonin (**MLT**), serotonin (**5-HT**), and tetrahydrobenz[*cd*]indoles.

Keywords: Melatonin; Serotonin; Conformational analysis; NMR coupling constants.
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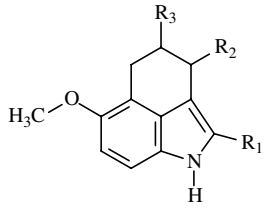
gland at night,[2] is an indole derivative with a flexible ethylamido chain linked at the C-3 position.

MLT is known to have a central role both in the regulation of daily and seasonal rhythms[3] and in the modulation of other endocrinological, neurophysiological, and behavioral functions.[4–6] These effects result from the activation of at least two high-affinity G-protein coupled receptors (named MT_1 and MT_2),[7–9] localized in the central nervous system and in peripheral tissues. Early SAR studies on melatonin analogues showed that suitably spaced methoxy and amido moieties are important for binding to melatonin receptors; various substituents on the 2-position of the indole ring enhance the binding affinity.[10]

The conformational flexibility of the C-3 ethylamido side chain is probably responsible for the broad spectrum of MLT biological activities. Till now, studies of the conformational preferences of MLT have been performed through experiments based on the structure–affinity relationship (SAR) of MLT and its conformational restricted analogues.[11] Usually, locking a conformation proceeds through the incorporation of the flexible fragment into a ring. For example, by the use of a design method based on conformational restriction, we have recently suggested the preferred orientation of the C-3 ethylamido side chain of melatonin. This corresponds to the side chain in an extended conformation perpendicular to the indole ring, as in the tetrahydrobenz[*cd*]indole melatonin derivative **1** (Fig. 2).[12]

The tetrahydrobenz[*cd*]indole moiety is present in a large number of substances possessing important biological activities. Some of them are selective antagonists of the glycine binding site associated to the NMDA receptor.[13] Other compounds have affinities and/or subtype selectivity for serotonin receptors[14–17] or represent an attractive therapeutic target for the treatment of Parkinson's disease and hyperprolactinemias.[18] Recently, a tetrahydrobenz[*cd*]indole derivative has been developed as inhibitor of tryptophanyl *t*RNA synthetase,[19] a target for antibacterial agents.



	R_1	R_2	R_3
1	COOEt	$CH_2NHCOMe$	H
5	Me	COOMe	H
6	Me	$CONMe_2$	H
7	Me	CH_2NMe_2	H
8	COOEt	CN	H
9	H	NHCOMe	H
10	H	$CH_2NHCOMe$	H
11	H	H	NO_2
12	H	H	$NH_3^+ \cdot O_2C\text{-}CO_2H$

Figure 2. Structures of tetrahydrobenz[*cd*]indole derivatives.

The successful development of the tetrahydrobenz[*cd*]indole system as a structural unit of constrained melatonergic and serotoninergic ligands, prompted us to investigate this template for selective 5-HT_6 agents. Compounds **7** and **12** (Fig. 2), possessing the suitable pharmacophoric groups,[20] were synthesized and their affinity for the 5-HT_6 receptor subtype was evaluated.

The tetrahydrobenz[*cd*]indole is usually assumed to be a rigid scaffold of arylethylamines of pharmaceutical interest. However, the side chain incorporated in this tricyclic template retains considerable conformational freedom. The carbocycle fused with the indole system can adopt an envelope conformation with the substituent either equatorial or axial. This paper describes the synthesis and the NMR determination of the conformation of tetrahydrobenz[*cd*]indoles **1**, **5**–**12** (Fig. 2), having a substituent of varying degree of bulkiness in position 2, in order to investigate its role in determining the preferred conformation.

2. Results

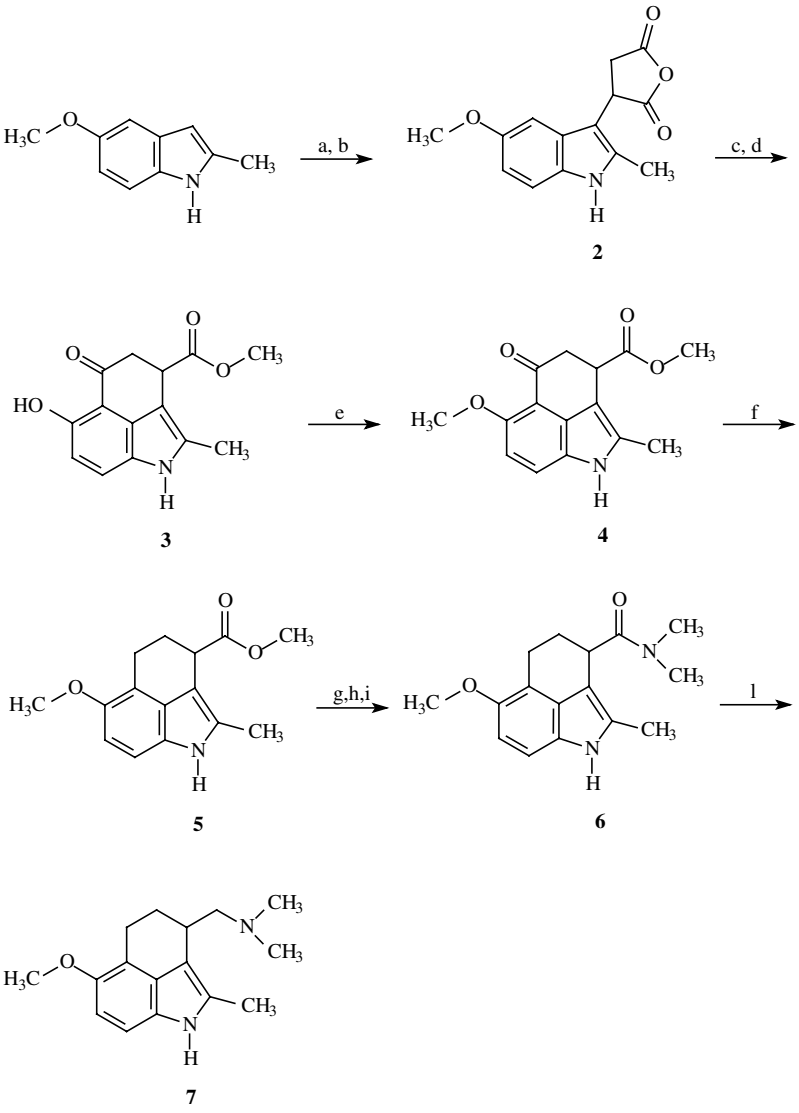
2.1. Chemistry

The synthesis of tetrahydrobenz[*cd*]indole derivatives **5**–**7** was carried out according to the synthetic pathway illustrated in Scheme 1.

The already described indolesuccinic anhydride **2**[21] was prepared using an alternative route: commercially available 5-methoxy-2-methylindole was condensed with maleic acid[22] to yield the intermediate 3-indolesuccinic acid, which was transformed into the corresponding anhydride **2** by treatment with PPA at 80 °C. The 3,4-carbocyclic bridge was built by internal Friedel–Crafts acylation ($AlCl_3$)[22] of **2**, and the resulting 3-carboxylic acid was esterified (CH_2N_2) to yield the tricyclic keto ester **3**. During the cyclization step demethylation of the 6-methoxy group occurred, therefore **3** had to be realkylated with methyl iodide in the presence of sodium hydride to afford **4**; reduction of the ketone (Et_3SiH/TFA)[23] yielded the ester **5**. Hydrolysis of **5** to the corresponding carboxylic acid was easily performed under basic conditions, but the acid is somewhat unstable, so it was immediately converted to the dimethylamido derivative **6** by reaction with oxalyl chloride followed by treatment with aqueous dimethylamine. The desired amine **7** was obtained by reduction of the dimethylamido group with $LiAlH_4$. Compounds **1**,[12] **8**–**10**,[12] and **11**–**12**[24] were prepared as previously described.

2.2. Spectroscopy

The proton vicinal coupling constants of the carbocycle fused with the indole system are collected in Table 1, with the exception of $J_{3\text{–}4\alpha}$ and $J_{3\text{–}4\beta}$ of compounds **6** and **7**, which could not be determined because of the complexity of the NMR spectra.



Scheme 1. Reagents and conditions: (a) maleic acid, Δ; (b) PPA, 80 °C; (c) $AlCl_3$, DCE, reflux; (d) CH_2N_2, THF, 0 °C; (e) NaH, CH_3I, DMF; (f) TFA, Et_3SiH; (g) KOH, THF–MeOH; (h) oxalyl chloride, toluene; (i) aqueous 40% $NH(CH_3)_2$, 0 °C; (l) $LiAlH_4$, THF, reflux.

Table 1. Observed and calculated[a] J in 3-substituted ring

Compound	$J_{3-4\alpha}$	$J_{3-4\beta}$	$J_{4\beta5\alpha}$	$J_{4\beta5\beta}$	$J_{4\alpha5\beta}$	$J_{4\alpha5\alpha}$
1	4.93	2.00	2.33	4.65	13.41	4.87
	(4.64)	(2.09)	(2.38)	(4.02)	(13.03)	(4.62)
8	5.07	2.84	3.20	4.29	12.44	4.25
	(4.55)	(2.79)	(3.32)	(3.78)	(12.44)	(4.25)
7	n.d.	n.d.	3.80	4.40	11.84	4.80
			(3.85)	(3.86)	(11.70)	(4.39)
6	n.d.	n.d.	4.94	4.60	10.69	4.60
			(5.14)	(3.75)	(10.77)	(4.13)
5	5.04	4.38	4.96	4.47	10.40	4.71
	(4.16)	(5.09)	(5.09)	(3.80)	(10.74)	(4.18)
9	4.28	6.80	7.77	4.59	7.63	4.48
	(4.17)	(7.56)	(8.19)	(4.23)	(7.58)	(4.00)
10	4.56	8.49	8.81	4.51	6.51	4.64
	(4.41)	(8.41)	(9.15)	(4.13)	(6.57)	(4.64)

The data show a definite trend along the series of the examined compounds: while $J_{4\alpha5\beta}$ decreases from 13.4 toward 6.5 Hz and both $J_{4\beta5\alpha}$ and $J_{3-4\beta}$ increase from 2 toward 8.5 Hz, the other three couplings are steady around 4.6 Hz.

This behavior can be rationalized by assuming a fast exchange between the two envelope conformers with the substituent at position 3 in axial and equatorial orientation, respectively (Fig. 3).

Inspection of the Newman projections of the CH–CH_2 and CH_2–CH_2 fragments suggests that, as the equilibrium changes toward the equatorial conformer, $J_{4\alpha5\beta}$ should decrease and both $J_{4\beta5\alpha}$ and $J_{3-4\beta}$ should increase, while

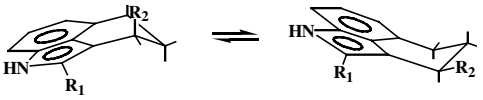
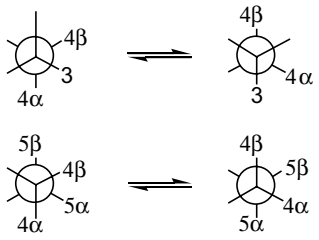


Figure 3.



the other three couplings should remain nearly unchanged, in agreement with the general Karplus relation between a vicinal coupling constant and the torsional angle between the coupled protons. In quantitative terms, each observed vicinal coupling constant can be expressed as the molar average of the constants J_a (axial) and J_e (equatorial) of the individual conformers:

$$J_{obs} = xJ_a + (1 - x)J_e, \quad (1)$$

where x and $1 - x$ are the molar fractions of the axial and equatorial conformers, respectively. Rearranging gives the relation

$$x = (J_{obs} - J_e)/(J_a - J_e), \quad (2)$$

which allows us to estimate the molar fraction of the two conformers. In order to do so, we preliminarily evaluated the values of the coupling constants of the individual conformers by using the Karplus–Altona equations[25] for the CH–CH_2 and CH_2–CH_2 fragments, respectively, which correlate J to substituent electronegativity and interproton dihedral angles. The latter geometrical parameters were obtained by modeling the conformers with the use of Allinger's MM2 force field of molecular mechanics.[26] Then, by inserting in turn into relation (2) the observed values of the three coupling constants $J_{4\alpha5\beta}$, $J_{4\beta5\alpha}$, or $J_{3-4\beta}$ and the appropriate parameters of the individual conformers, average values of the molar fraction x were obtained, as reported in the first row of Table 2.

Finally, as a check of the reliability of the model, the complete set of the six vicinal coupling constants was estimated by means of relation (1), in order to compare it with the set of the experimentally observed values. From the results reported in parenthesis in Table 1 it appears that the model is reproducing the experimental data with a root mean square deviation of 0.4 Hz between estimated and experimental couplings, which, considering all the approximations involved in the computations, is quite satisfactory.

The molar fraction x was also estimated through the use of the relation

$$x = (e^{-\Delta E/RT})/(1 + e^{-\Delta E/RT}), \quad (3)$$

where ΔE is the energy difference between the axial and equatorial conformer as obtained from the MM2 calculations. The values of x are collected in the second row of Table 2 for comparison with the corresponding quantities derived by processing the NMR data. Both sets of data are without doubt affected by error, which can be conservatively estimated around 0.1, however, they show the same trend.

The interesting chemical consequence of these results is that the conformational equilibrium is very sensitive to the nature of the substituent at C-2. Inspection of conformer populations (Table 2) shows that compound **1** is present exclusively in the axial form, while, on the other side, compound **10** is mostly in the equatorial form. The remaining compounds are in an intermediate state. This reveals that the equatorial arrangement, which in monosubstituted six-membered rings is usually more stable than the axial one, can be considerably hampered in the examined tricyclic system, depending on the nature of the indole substituent at C-2.

The axial arrangement of **1** could be ascribed to the presence of an intramolecular H-bond between the amidic NH and the CO of the carboxyethyl substituent. However, the nitrile group of derivative **8** appears to be prevalently axial (92%) too even though, in this case, the hydrogen bond cannot be advocated. This observation indicates that the conformational arrangement of the tricyclic scaffold is definitely dependent on the bulkiness of the C-2 indole substituent. However, even when the latter is hydrogen, as in compounds **9** and **10**, the molar fraction of the equatorial form is restricted to less than around 60%, which could be ascribed to a residual steric interaction. With the intent of checking this hypothesis we have determined the conformation of compounds **11** and **12**, where the substituent at C-4, being faraway from the hydrogen at C-2, is expected to prefer favorably the equatorial orientation. The proton vicinal coupling constants of the carbocycle fused with the indole system are collected in Table 3.

Similarly to the procedure used above for the 3-substituted compounds, by inserting into relation (2) the observed values of the two coupling constants $J_{3\beta-4}$ or $J_{4-5\beta}$ and the parameters of the individual conformers, the values of the molar fraction of the axial conformer were obtained: 0.27 for **11** and 0.21 for **12**. As expected, the equatorial orientation is largely preferred.

2.3. Pharmacology

Melatonin receptor binding affinities of amido derivatives **1**, **9**, and **10** are reported in Table 4.[12] The most active compound **1** was also tested on cloned human MT_1 and MT_2 receptors.

Table 2. Molar fraction (x) of axial conformer

Compound	**1**	**8**	**7**	**6**	**5**	**9**	**10**
From NMR	1.00	0.92	0.89	0.77	0.77	0.47	0.39
From MM	1.00	0.98	0.93	0.57	0.68	0.56	0.35

Table 3. Coupling constants in 4-substituted ring

Compound	$J_{3\alpha-4}$	$J_{3\beta-4}$	$J_{4-5\alpha}$	$J_{4-5\beta}$
11	4.60	9.55	4.48	9.39
12	4.37	9.80	4.26	9.62

Table 4. Affinities[a] of compounds **1**, **9**, and **10** for melatonin receptors.

Compd	Quail optic tecta $pK_i \pm SEM$	Human MT_1 $pK_i \pm SEM$	Human MT_2 $pK_i \pm SEM$
MLT	9.215 ± 0.06	9.856 ± 0.058	9.654 ± 0.076
1	9.321 ± 0.078	9.485 ± 0.085	9.304 ± 0.041
9	<5	nt[b]	nt[b]
10	6.481 ± 0.092	nt[b]	nt[b]

[a] 2-[^{125}I]-iodomelatonin was used as the radioligand and the binding assays were carried out using membranes prepared from quail optic tecta[12] and from NIH3T3 cells lysates expressing the human MT_1 or MT_2 receptor. pK_i values were calculated from IC_{50} values, obtained from competition curves using the method of Cheng and Prusoff,[27] and are the mean ± SEM of at least three independent determinations performed in duplicate.

[b] nt = not tested.

The high binding affinity of compound **1** for melatonin receptors indicates that, at the binding site, MLT adopts a conformation with the ethylamido side chain perpendicular to the indole ring. It was not possible to ascertain the structural features responsible for the MT_1/MT_2 selectivity, due to the comparable affinity of compound **1** for the two subtypes.

The dimethylamino derivatives **7** and **12** tested on the 5-HT_6 receptor show a low binding affinity (IC_{50} = 613 nM for compound **12** and IC_{50} > 10,000 nM for **7**) and this precludes to define conformational preferences for this receptor subtype.

3. Conclusions

The results obtained in the present investigation can be condensed in the following points. The mono substituted carbocycle fused with the indole system is an equilibrium of axial and equatorial forms in fast exchange relative to the NMR time scale. The molar fraction of the two conformers can be determined from the values of the observed vicinal coupling constants, with the help of the Karplus–Altona equation together with molecular mechanics calculations based on the Allinger's MM2 force field. It appears that the substituent in position 4 adopts a prevailing equatorial conformation (compounds **11** and **12**). The orientation of the substituent in position 3 is sensibly affected by the nature of the substituent in position 2. Thus, by suitable choice of the latter, the synthetic chemist has the opportunity of generating either prevailing axial conformations like that of compound **1** or prevailing equatorial conformations like that of compound **10**. This kind of conformation-controlled synthesis could be usefully exploited for generating structures properly shaped to interact with specific receptor site. Finally, the NMR determination of the conformation of the powerful melatonin analog **1** confirms that the C-3 ethylamido side chain of MLT, in its active conformation, assumes an arrangement perpendicular to the indole ring, in agreement with our previous hypothesized pharmacophoric model.[12] Considering that compound **1** is chiral, one could expect a receptor binding stereoselectivity. We plan to carry on, in the future, a comprehensive investigation directed to clarify this crucial question.

4. Experimental

4.1. General methods

Melting points were determined on a Buchi B-540 capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AVANCE 200 or AVANCE 500 spectrometer, using $CDCl_3$ as solvent unless otherwise noted. Chemical shifts (δ scale) are reported in parts per million (ppm), coupling constants (J values) are given in hertz (Hz). EI-MS spectra (70 eV) were taken on a Fisons Trio 1000 instrument. Only molecular ions ($M^{+\cdot}$) and base peaks are given. Infrared spectra were obtained on a Nicolet Avatar 360 FT-IR spectrometer; absorbances are reported in ν (cm^{-1}). Elemental analyses for C, H, and N were performed on a Carlo Erba analyzer and the results are within 0.4% of the calculated values. Column chromatography purifications were performed under 'flash' conditions using Merck 230–400 mesh silica gel. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} plates. All chemicals were purchased from commercial suppliers and used directly without any further purification.

4.2. 3-(Dihydrofuran-2,5-dione-3-yl)-5-methoxy-2-methyl-1*H*-indole (2)

A mixture of 2-methyl-5-methoxyindole (1 g, 6.2 mmol) and maleic acid (0.72 g, 6.2 mmol) was heated until melting. After 5 min the mixture was cooled and the glassy solid was crushed and added to mechanically stirred preheated (80 °C) PPA (63 g). The resulting mixture was stirred at 80 °C for 1 h, then poured onto ice/water, and extracted 3× with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated to give a crude residue, which was purified by flash chromatography (cyclohexane–EtOAc 1:1 as eluent) and crystallization (EtOAc–cyclohexane): (63% yield), mp 168–170 °C (Lit. 146–147 °C[21]). MS (EI): *m*/*z* 259 ($M^{+\cdot}$), 187 (100). ^{1}H NMR ($CDCl_3$): δ, 2.42 (s, 3H), 3.2 (dd, 1H, J = 7.2, 19.1), 3.42 (dd, 1H, J = 10.5, 19.1), 3.82 (s, 3H), 4.51 (dd, 1H, J = 7.2, 10.5), 6.65 (d, 1H, J = 2.4) 6.82 (dd, 1H, J = 2.4, 8.9), 7.22 (d, 1H, J = 8.9), 7.89 (br s, 1H). IR (cm^{-1}, Nujol) 3349, 1860, 1787.

4.3. Methyl 6-hydroxy-2-methyl-5-oxo-1,3,4,5-tetrahydrobenz[*cd*]indole-3-carboxylate (3)

$AlCl_3$ (7 g, 52 mmol) was added portionwise to an ice cooled stirred suspension of **2** (2.75 g, 10.6 mmol), in dry DCE (50 mL) under N_2 atmosphere. The resulting mixture was refluxed for 1 h, then poured onto ice/water mixture containing concd HCl (7 mL), and extracted 3× with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated to give a crude residue, which was purified by flash chromatography with cyclohexane–EtOAc–AcOH 50:50:5 as eluent. The intermediate acid was dissolved in THF (40 mL) and treated with an ethereal solution of CH_2N_2 at 0 °C until the disappearance of the starting material. Removal of the solvent and purification of

the residue by flash chromatography (cyclohexane–EtOAc 1:1 as eluent) and crystallization (EtOAc–cyclohexane) gave the desired product: (22% yield), mp 179–180 °C. MS (EI): *m/z* 259 ($M^{+\cdot}$), 200 (100). ^{1}H NMR ($CDCl_3$): δ 2.48 (s, 3H), 3.00 (dd, 1H, J = 6.7, 18.0), 3.12 (dd, 1H, J = 3.4, 18.0), 3.66 (s, 3H), 4.22 (dd, 1H, J = 3.7, 6.9), 6.67 (d, 1H, J = 8.7), 7.33 (d, 1H, J = 8.7), 7.88 (br s, 1H), 9.95 (br s, 1H).

4.4. Methyl 6-methoxy-2-methyl-5-oxo-1,3,4,5-tetrahydrobenz[*cd*]indole-3-carboxylate (4)

Sodium hydride (110 mg of an 80% dispersion in mineral oil, 3.6 mmol) was added to a stirred ice-cooled solution of the ester **3** (0.58 g, 2.24 mmol) in dry DMF (8.7 mL) under N_2. CH_3I (0.22 mL, 3.6 mmol) was added and the resulting mixture was stirred at room temperature for 5 h. Water was added and the desired solid was collected by filtration, washed with water, and diethyl ether and used for the next step without any further purification: (62% yield), mp 239 °C (dec). MS (EI): *m/z* 273 ($M^{+\cdot}$), 214 (100). ^{1}H NMR ($CDCl_3$): δ 2.48 (s, 3H), 2.90 (dd, 1H, J = 6.6, 16.0), 3.11 (dd, 1H, J = 3.7, 16.0), 3.64 (s, 3H), 3.97 (s, 3H), 4.18 (dd, 1H, J = 3.9, 6.6), 6.79 (d, 1H, J = 8.8), 7.38 (d, 1H, J = 8.8), 7.88 (br s, 1H).

4.5. Methyl 6-methoxy-2-methyl-1,3,4,5-tetrahydrobenz[*cd*]indole-3-carboxylate (5)

Triethylsilane (0.76 mL, 4.76 mmol) was added to a solution of ester **4** (0.45 g, 1.65 mmol) in trifluoroacetic acid (4.2 mL) and the mixture was stirred at room temperature for 5 h. The reaction mixture was concentrated, diluted with water, and extracted 3× with EtOAc. The combined organic phases were washed with brine, dried (Na_2SO_4), and concentrated to give a residue, which was purified by flash chromatography (cyclohexane–EtOAc 75:25 as eluent) and crystallization (diethyl ether–cyclohexane): (92% yield), mp 114–115 °C. MS (EI): *m/z* 259 ($M^{+\cdot}$), 200 (100). ^{1}H NMR ($CDCl_3$): δ 2.01 (m, 1H), 2.38 (s, 3H), 2.44 (m, 1H), 2.99 (m, 2H), 3.68 (s, 3H), 3.85 (s, 3H), 3.88 (dd, 1H, J = 4.8), 6.76 (d, 1H, J = 8.6), 7.00 (d, 1H, J = 8.6), 7.56 (br s, 1H).

4.6. *N*,*N*-dimethyl 6-methoxy-2-methyl-1,3,4,5-tetrahydrobenz[*cd*]indole-3-carboxamide (6)

A solution of methyl ester **5** (0.38 g, 1.47 mmol) in THF (3 mL), MeOH (3.5 mL), and 3 N KOH (1.47 mL, 4.41 mmol) was stirred at room temperature for 24 h. The solvents were removed in vacuo, the residue was dissolved in water, and washed twice with EtOAc. The aqueous phase was acidified with 2 N HCl and extracted 3× with EtOAc. The combined organic phases were washed with brine, dried (Na_2SO_4), and concentrated to give the acid, which was used directly for the next step: (80% yield), MS (EI): *m/z* 245 ($M^{+\cdot}$), 200 (100).

Oxalyl chloride (1.18 mL, 13.5 mmol) was added to an ice-cooled solution of the crude acid (0.29 g, 1.18 mmol) in toluene (12 mL) at 0 °C; the mixture was stirred at room temperature for 2 h. The solvent and the excess of oxalyl chloride were removed under reduced pressure and the residue was diluted with toluene and treated dropwise with aqueous 40% dimethylamine (6 mL, 47.4 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 2 h then extracted 3× with EtOAc; the combined organic phases were washed with brine, dried (Na_2SO_4), and concentrated to give a residue, which was purified by flash chromatography (EtOAc as eluent) and crystallization (EtOAc): (60% yield), mp 177–178 °C. MS (EI): *m/z* 272 ($M^{+\cdot}$), 200 (100). ^{1}H NMR ($CDCl_3$): δ 2.17 (m, 2H), 2.24 (s, 3H), 2.79 (ddd, 1H, J = 16.6, 10.7, 4.9), 3.11 (s, 6H), 3.13 (ddd, 1H, J = 16.6, 4.9, 4.6), 3.84 (s, 3H), 4.15 (m, 1H), 6.75 (d, 1H, J = 8.6), 7.00 (d, 1H, J = 8.6), 7.50 (br s, 1H).

4.7. 3-Dimethylaminomethyl-6-methoxy-2-methyl-1,3,4,5-tetrahydrobenz[*cd*]indole (7)

A solution of **6** (0.18 g, 0.66 mmol) in dry THF (10 mL) was added dropwise to a suspension of $LiAlH_4$ (0.206 g, 5.42 mmol) in dry THF (15 mL) under N_2 atmosphere. Upon completion of the addition, the mixture was refluxed 3 h, then it was cooled to 0 °C and the excess of hydride decomposed by slow addition of EtOAc and water. The mixture was vacuum filtered through a Celite pad and the filtrate was extracted 3× with EtOAc, the combined organic phases were washed with brine, dried (Na_2SO_4), and concentrated to give a residue, which was purified by crystallization (diethyl ether–petroleum ether): (63% yield), mp 128 °C. MS (EI): *m/z* 258 ($M^{+\cdot}$), 58 (100). ^{1}H NMR ($CDCl_3$): δ 1.82 (m, 1H), 2.16 (m, 1H), 2.30 (s, 6H), 2.38 (s, 3H), 2.47 (m, 2H), 2.76 (ddd, 1H, J = 17.0, 11.8, 4.4), 3.01 (ddd, 1H, J = 17.0, 4.8, 3.8), 3.13 (m, 1H), 3.86 (s, 3H), 6.75 (d, 1H, J = 8.6), 7.01 (d, 1H, J = 8.6), 7.45 (br s, 1H). IR (cm^{-1}, Nujol) 3163, 1510. Anal. Calcd $C_{16}H_{22}N_2O$: C, 74.38; H, 8.58; N, 10.84. Found: C, 74.62; H, 8.64; N, 10.45.

4.8. Receptor binding experiments

The binding affinity of compounds **1**, **9**, and **10** for melatonin receptors was evaluated in competition experiments, using 2-[^{125}I]iodomelatonin as the labeled ligand, on membranes obtained from quail optic tecta.[12] Binding to h-MT_1 and h-MT_2 receptors was determined using 2-[^{125}I]iodomelatonin in competition experiments on cloned human MT_1 and MT_2 receptors stably expressed in NIH3T3 rat fibroblast cells according to the method already described.[28]

Displacement experiments were carried out by Cerep[29] in order to determine the affinity of compounds **7**, **12** for the serotonin 5-HT_6 receptor, according to a published method.[30] The binding assay has been performed employing rat 5-HT_6 receptor stably transfected to HEK293 (human embrionic kidney cells) with [^{3}H]-LSD (lysergic acid diethylamide) as radioligand. Each compound was dissolved in DMSO to prepare 10 mM stock solution, and then dissolved in H_2O to a final concentration of 0.1 mM. After serial dilutions, eight different concentrations (from 100 μM to 0.01 nM) in duplicate were employed to obtain a competition curve

by which to evaluate binding affinity for the 5-HT_6 receptor of each test compound. Non-specific binding was defined by 100 μM serotonin creatinine sulfate. Following incubation (60 min at 37 °C), the membranes were rapidly filtered under vacuum through glass fiber filters (GF/B, Packard). Bound radioactivity was measured with scintillation counter (Topcount, Packard) using a liquid scintillation cocktail (Microscint 0, Packard). The IC_{50} of each compound were determined by non-linear regression analysis of the competition curves using Hill equation curve fitting. Standard errors were typically 20% of the mean value.

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References and notes

1. Veber, D. F.; Johnson, S. R.; Cheng, H. Y.; Smith, B. R.; Ward, K. W.; Kopple, K. *J. Med. Chem.* **2002**, *45*, 2615.
2. Reiter, R. J. *Best Pract. Res. Clin. Endocrinol. Metab.* **2003**, *17*, 273.
3. Pevet, P.; Bothorel, B.; Slotten, H.; Saboureau, M. *Cell Tissue Res.* **2002**, *309*, 183.
4. Cardinali, D. P.; Brusco, L. I.; Lloret, S. P.; Furio, A. M. *Neuro Endocrinol. Lett.* **2002**, *23*, 9.
5. Zisapel, N. *CNS Drugs* **2001**, *15*, 311.
6. Maestroni, G. J. *Expert Opin. Invest. Drug* **2001**, *10*, 467.
7. Reppert, S. M.; Weaver, D. R.; Goodson, C. *Trends Pharmacol. Sci.* **1996**, *17*, 100.
8. Dubocovich, M. L.; Cardinali, D. P.; Delagrange, P.; Krause, D. N.; Strosberg, A. D.; Sugden, D.; Yocca, F. D. The IUPHAR compendium of receptor characterization and classification. *IUPHAR Media*, London; 2000, pp 271–277.
9. Von Gall, C.; Stehle, J. H.; Weaver, D. R. *Cell Tissue Res.* **2002**, *309*, 151.
10. Mathé-Allainmat, M.; Andrieux, J.; Langlois, M. *Exp. Opin. Ther. Patents* **1997**, *7*, 1447.
11. Mor, M.; Plazzi, P. V.; Spadoni, G.; Tarzia, G. *Curr. Med. Chem.* **1999**, *6*, 501.
12. Spadoni, G.; Balsamini, C.; Diamantini, G.; Di Giacomo, B.; Tarzia, G.; Mor, M.; Plazzi, P. V.; Rivara, S.; Lucini, V.; Nonno, R.; Pannacci, M.; Fraschini, F.; Stankov, B. M. *J. Med. Chem.* **1997**, *40*, 1990.
13. Katayama, S.; Ae, N.; Kodo, T.; Masumoto, S.; Hourai, S.; Tamamura, C. T.; Nagata, R. *J. Med. Chem.* **2003**, *46*, 691.
14. Taylor, E. W.; Nikam, S.; Weck, B.; Martin, A.; Nelson, D. *Life Sci.* **1987**, *41*, 1961.
15. Chambers, J. J.; Parrish, J. C.; Jensen, N. H.; Kurrasch-Orbaugh, D. M.; Marona-Lewicka, D.; Nichols, D. E. *J. Med. Chem.* **2003**, *46*, 3526.
16. Foreman, M.; Fuller, R. W.; Rasmussen, K.; Nelson, D.; Calligaro, D. O.; Zhang, L.; Barrett, J. E.; Booher, R. N.; Paget, C. J., Jr.; Flaugh, M. E. *J. Pharmacol. Exper. Ther.* **1994**, *270*, 1270.
17. Flaugh, M. E.; Mullen, D. L.; Fuller, R. W.; Mason, N. R. *J. Med. Chem.* **1988**, *31*, 1746.
18. Boissier, J. R.; Nedelec, L.; Oberlander, C.; Labrie, F. *Acta Pharma Suecica* **1983**, 120.
19. Witty, D. R.; Walker, G.; Bateson, J. H.; O'Hanlon, P. J.; Eggleston, D. S.; Haltiwanger, R. C. *Tetrahedron Lett.* **1996**, *37*, 3067.
20. Pullagurla, M. R.; Westkaemper, R. B.; Glennon, R. A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4569.
21. Agbalyan, S. G.; Grigoryan, G. V. *Chem. Heterocycl. Compd. (Engl. Transl.); EN* **1972**, *8*, 1377.
22. Smuszkovicz, J. *J. Org. Chem.* **1964**, *29*, 843.
23. West, C. T.; Donnelly, S. J.; Kooistra, D. A.; Doyle, M. P. *J. Org. Chem.* **1973**, *38*, 2675.
24. Kruse, L. I.; Meyer, M. D. *J. Org. Chem.* **1984**, *49*, 4761.
25. Haasnoot, C. A. G.; De Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *38*, 2783.
26. Burkert, U.; Allinger, N. L. *Molecular Mechanics, ACS Monograph 177*; American Chemical Society: Washington, DC, 1982.
27. Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
28. Nonno, R.; Lucini, V.; Pannacci, M.; Mazzucchelli, C.; Angeloni, D.; Fraschini, F.; Stankov, B. M. *Br. J. Pharmacol.* **1998**, *124*, 485.
29. Cerep, Le Bois l'Eveque-B. P. 1, 86600 Celle L'Evescault, FRANCE.
30. Monsma, F. J., Jr.; Shen, Y.; Ward, R. P.; Hamblin, M. W.; Sibley, D. R. *Mol. Pharmacol.* **1993**, *43*, 320.