Bioorganic & Medicinal Chemistry 16 (2008) 7654–7661

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and pharmacological evaluation of pentacyclic 6a,7-dihydrodiindole and 2,3-dihydrodiindole derivatives as novel melatoninergic ligands

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ARTICLE INFO

Article history:
Received 9 May 2008
Revised 27 June 2008
Accepted 4 July 2008
Available online 10 July 2008

Keywords:
6a,7-Dihydrodiindole
Pentacyclic
Melatoninergic ligands
Nitrogen heterocycles

ABSTRACT

The synthesis of novel melatonin analogues **3a** and **4a–c** designed as melatonin receptor ligands is described. Among the newly synthesized ligands, 2-((*S*)-2-hydroxymethylindolin-1-ylmethyl)-melatonin **4b** displayed the highest affinity for MT_1 receptors (K_i = 9.8 nM) and for MT_2 subtype (K_i = 7.8 nM), whereas the rigid pentacyclic ligand **3** showed the highest selectivity towards the MT_2 receptor subtype (K_i = 319.3 nM for MT_1 and K_i = 65.2 nM for MT_2).

1. Introduction

Melatonin (*N*-acetyl-5-methoxytryptamine, MLT), the hormone mainly produced and secreted from the pinealocytes of the vertebrate pineal gland is synthesized in a circadian mode mainly at night.[1] MLT acts through activation of two G-protein-coupled receptors, designated as MT_1 and MT_2. In addition, a low-affinity putative MLT binding site called MT_3 has been recently characterized as a melatonin-sensitive form of the human enzyme quinone reductase 2.[2] Besides the well-known sleep-inducing properties of MLT, other effects described in the literature include its anti-inflammatory,[3] pain modulatory,[4] antitumor,[5] and antioxidant properties.[6] These diverse pharmacological effects of MLT result from activation of multiple signalling cascades that include cAMP-dependent, PLC-dependent and MAPK pathways via MT_1 and MT_2 receptors.[5a]

Since the identification of the MLT receptor subtypes, the synthesis of receptor subtype selective ligands is a valuable challenge. Furthermore, first pass metabolism and a short half life exhibited by exogenously administered deuterated MLT are responsible for its displayed low absolute bioavailability in man (1–37%)[7] leading to the search for metabolically stable synthetic analogues.

Herein, we report the synthesis and pharmacological evaluation of a hybrid structure **3** of our previously reported[8] melatoninergic ligands **1** and **2** (Fig. 1) to examine the importance of the methoxy group at the indoline moiety of compound **2** in the binding affinity. Flexible analogues **4a–c** of the rigid pentacyclic **2** were also prepared aiming to get entities with a favourable spatial orientation to occupy the binding pocket of MLT receptors and consequently improvement of the binding affinity.

2. Results and discussion

2.1. Synthesis of 3

The ring system of **1** and **2** was built through dimerization of the respective indoline-2-carboxylic acid using DCC as a condensing agent.[8] We used another synthetic strategy for the preparation of the non-symmetrical compound **3**. The crucial step involved coupling of the acid fluoride **6** with the indoline-2-carboxylic acid methyl ester **8** as a nucleophile. Thus, the commercially available 5-methoxyindole-2-carboxylic acid **5** was treated with cyanuric fluoride in acetonitrile at room temperature to afford acid fluoride **6**. Interestingly, the acyl fluoride **6** is a stable and storable solid in contrast to the self condensing and easily hydrolysed respective acyl chloride. Compound **6** was allowed to react with the weakly basic indoline-2-carboxylic acid derivative **8** at room temperature to obtain the amide **9** in an excellent yield (Scheme 1). The ring closure of **9** was accomplished with DMAP in THF at ambient temperature to furnish the dilactam **10**. Our attempts to reduce dilactam **10** to the diamine **11** using $LiAlH_4$ in THF and/or diethylether under different reaction conditions were unsuccessful and we obtained the alcohol **15c** instead. Elaboration of the alcohol **15c** to the

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doi:10.1016/j.bmc.2008.07.012

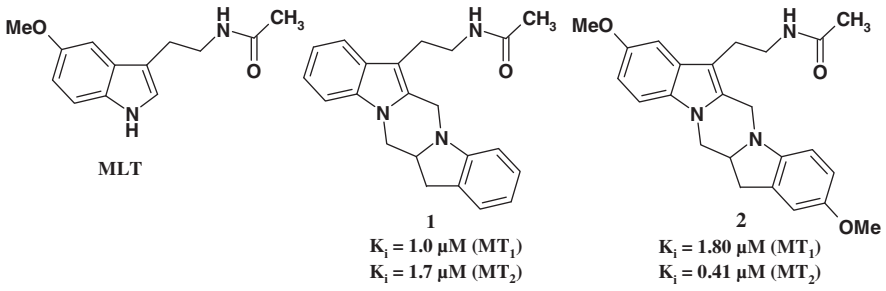
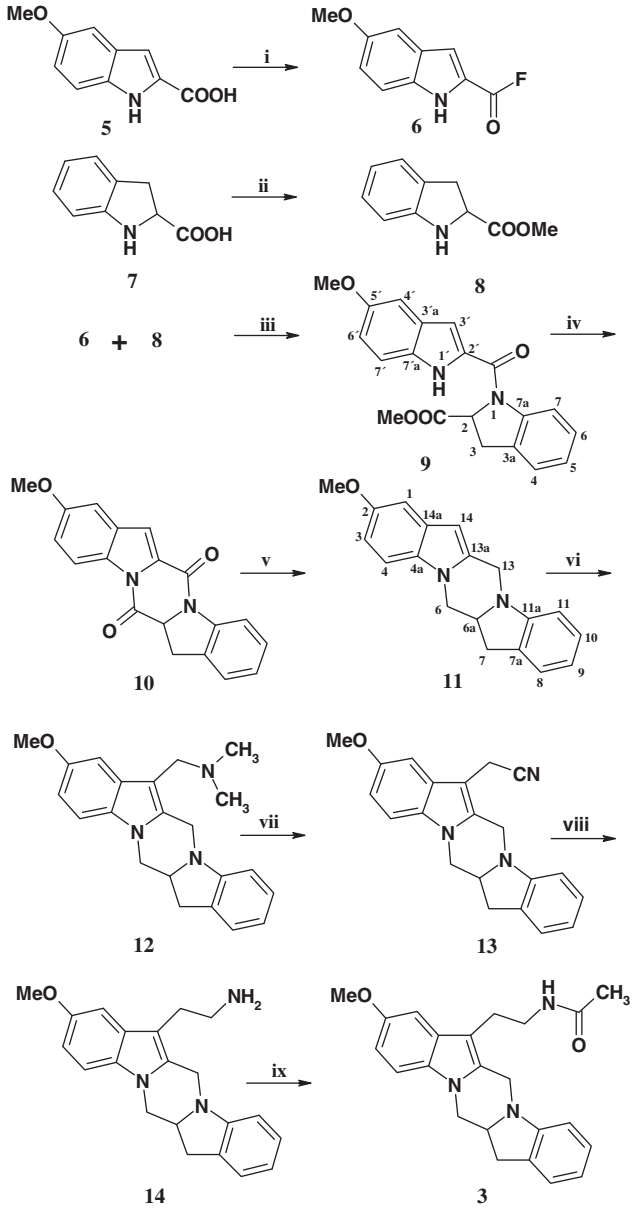


Figure 1.



Scheme 1. Reagents: (i) cyanuric fluoride, pyridine, MeCN (ii) $SOCl_2$, MeOH (iii) MeCN (iv) DMAP, THF (v) $LiAlH_4/AlCl_3$, THF/Et_2O (vi) $CH_2{=}NMe_2{}^+I^-$, CH_2Cl_2 (vii) 1. MeI, CH_2Cl_2, 2. KCN, Dicyclohexyl[18]-crown[6], MeCN (viii) $LiAlH_4/AlCl_3$, THF/Et_2O (ix) acetic anhydride, Et_3N, CH_2Cl_2.

pentacyclic compound **11** proved to be difficult. Thus, derivatization of the alcohol functionality of **15c** either through benzoylation[9] or tosylation[10] with subsequent base-catalyzed cyclization did not give the desired pentacyclic derivative **11** in any detectable yield. Interestingly, by adopting Apple reaction conditions[11] using *tris*-(dimethylamino)-phosphine and carbon tetrabromide on the alcohol **15c,** we obtained directly the desired pentacyclic ring system with simultaneous bromination at position 14 which could not be further transformed to our target compound **3**. Ultimately, we were fortunate to reduce dilactam **10** to the respective diamine **11** using $LiAlH_4/AlCl_3$ (3:1) in THF/diethyl ether solvent system for 18 h at room temperature.

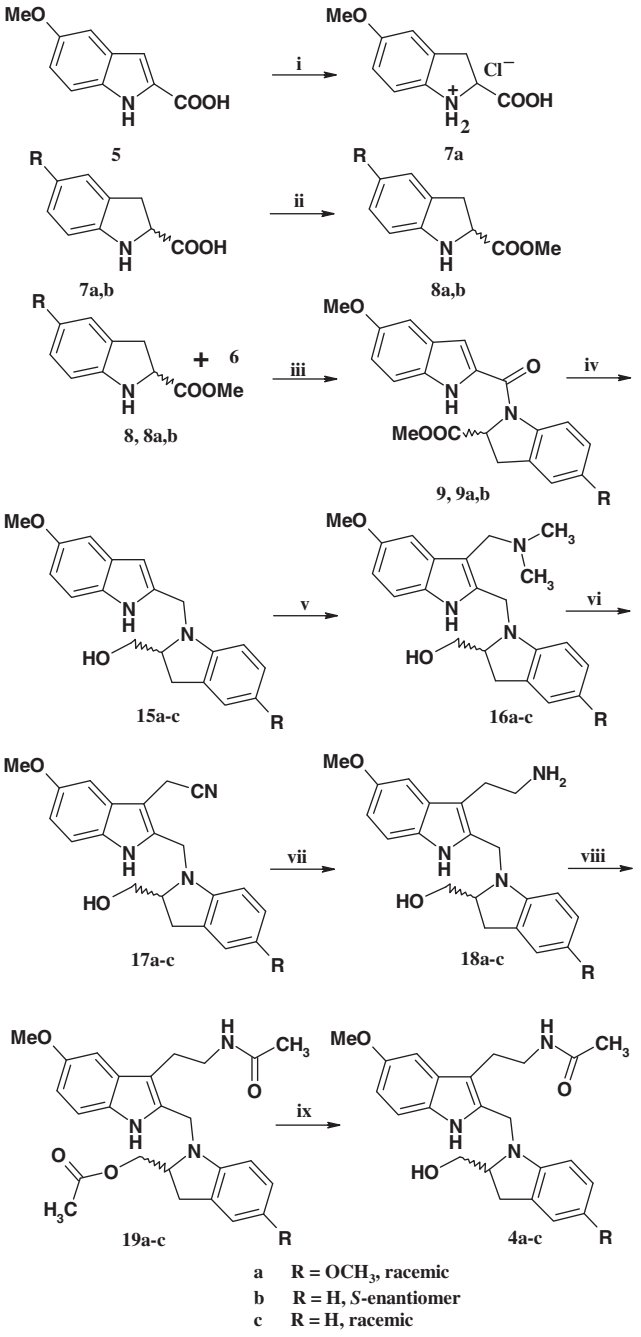
Introduction of the aminoethyl side chain into the diamine **11** at the position corresponding to C-3 of melatonin was achieved by applying our previously developed reaction sequence.[8,12] Briefly, compound **11** was subjected to Mannich reaction using dimethylmethyleneammonium iodide (Eschenmoser's salt) in refluxing dichloromethane for one hour to give the Mannich base **12** which was quaternized with methyl iodide in dichloromethane. The resulting trimethylammonium iodide derivative was substituted with potassium cyanide in acetonitrile in the presence of dicyclohexyl[18]-crown[6] to afford the cyano derivative **13** in 54% yield. Finally, the nitrile **13** was reduced to the amine **14** using $LiAlH_4/AlCl_3$ in THF/diethyl ether mixture with subsequent acetylation using acetic anhydride and triethylamine in dichloromethane to furnish the desired pentacyclic amide **3** in 14% overall yield based on 5-methoxyindole-2-carboxylic acid **5** (Scheme 1).

2.2. Synthesis of 4a–c

Synthesis of compounds **4a–c** was carried out according to the synthetic pathways depicted in Scheme 2. Thus, the amides **9, 9a,b** were obtained through the coupling of acyl fluoride **6** with the indoline methyl ester derivatives **8, 8a,b** which were prepared from the corresponding carboxylic acid by employing the reported reaction conditions and reagents.[13–15] The amide and ester functionalities of **8, 8a**,**b** were reduced using $LiAlH_4$ in THF/diethyl ether mixture at 40 °C for 18 h to give the respective amines **15a–c**. Compounds **15a–c** were transformed to the corresponding Mannich bases **16a–c** using Eschenmoser's salt followed by nucleophilic substitution using potassium cyanide with subsequent $LiAlH_4$ reduction as mentioned earlier in the synthesis of compound **3**. Acetylation of **18a–c** using acetic anhydride and triethylamine in dichloromethane gave the diacetyl derivatives **19a–c** which were selectively *O*-deacetylated using methanolic sodium hydroxide solution at room temperature to afford the target compounds **4a–c** in acceptable yields.

2.3. Pharmacology

The binding affinity for compounds **3** and **4a–c** were assessed by competition binding analysis as described previously.[16] Chinese hamster ovary cells, stably transfected with human MT_1 and MT_2 receptors, were grown to confluence in 10 cm dishes. Cells were lifted in buffer, pelleted by centrifugation (500*g* 10 min), re-sus-



Scheme 2. Reagents: (i) Ref. [8] (ii) $SOCl_2$, MeOH (iii) MeCN (iv) $LiAlH_4$, THF/Et_2O (v) $CH_2{=}NMe_2{}^+I^-$, CH_2Cl_2 (vi) 1—MeI, CH_2Cl_2, 2—KCN, Dicyclohexyl[18]-crown[6], MeCN (vii) $LiAlH_4$, THF/Et_2O (viii) acetic anhydride, Et_3N, CH_2Cl_2 (ix) NaOH, MeOH.

pended and lysed in Tris buffer 50 mM (pH 7.4). Cell lysates were then added to tubes containing 2-[^{125}I]-iodomelatonin (98–138 pM) and varying concentrations of either compounds **3**, **4a–c** (10^{-12} to 10^{-4} M) or melatonin (10^{-12} to 10^{-4} M). Samples were incubated at 25 °C for 1 h followed by rapid filtration on a Brandel Cell Harvester. Filters containing bound radioligand were read using a gamma counter. Curves were generated using non-linear regression analysis and K_i values were calculated using GraphPad Prism software (GraphPad Prism, Inc., San Diego, CA). As shown in Table 1, compounds **3, 4a–c** displayed nanomolar affinity for binding to human melatonin receptors. The affinity (K_i) of melatonin for human MT_1 or MT_2 melatonin receptors was similar to what has been reported previously[8] (Table 1).

Table 1
Pharmacological evaluation of binding affinities for compounds **3**, **4a–c** at human MT_1 and MT_2 melatonin receptors expressed in CHO cells

Compound	K_i (range of SEM)		K_i (MT_1)/K_i (MT_2)
	MT_1	MT_2	
3	319.3 nM (303.0–336.5)	65.2 nM (60.1–70.0)	4.89
4a	34.5 nM (30.3–39.1)	33.6 nM (30.5–37.0)	1.03
4b	9.8 nM (7.0–13.8)	7.8 nM (5.0–11.0)	1.25
4c	17.5 nM (16.8–18.3)	36.2 nM (33.4–39.2)	0.48
Melatonin	525 pM (417–661)	741 pM (562–977)	

The values represent the mean K_i values with the range of the standard error of the mean in parentheses.

2.4. Discussion

A consistent structural feature found in most melatonergic ligands is the presence of a bulky substituent in a position corresponding to positions 1 and 2 of the indole in MLT and located out of the plane from the indole ring. This arrangement confers selectivity for MT_2 receptors with a reduction in intrinsic activity. In addition, the methoxy group together with the amide side chain in MLT were claimed to mediate the appropriate orientation of MLT in the receptor binding pocket to trigger the biological response.

In search for subtype selective melatonin receptor ligands, we have recently synthesized the pentacyclic compound **2** which exhibited a nanomolar affinity for MT_2 (K_i = 410 nM) and a micromolar affinity for MT_1 receptors (K_i = 1.8 μM).[8] This relatively poor binding affinity could be due to the presence of the methoxy group in the indoline moiety of **2** which may interact with the receptor binding site accommodating the methoxy group of MLT and hence preventing the indolic methoxy group of **2** from binding. In order to examine this postulation, we have now synthesized compound **3** which is the non-methoxy analogue of **2**.

Pharmacological evaluation of compound **3** revealed that the removal of the indoline methoxy group in **2** led to retention of MT_2 selectivity and an approximately 6-fold increase of binding affinity at both MT_1 and MT_2 receptor subtypes (K_i = 319.3 and 65.2 nM for MT_1 and MT_2, respectively). Thus, the presence of methoxy group in the indoline moiety of compound **2** could influence its mis-orientation in the receptor binding site causing its low affinity.

Futhermore, the effect of rigidity in the pentacyclic ligand **2** on its receptor binding affinity was investigated through the synthesis and pharmacological evaluation of the flexible analogue **4a**. Compound **4a** displayed a higher affinity to MLT receptors (K_i = 34.5 and 33.6 nM for MT_1 and MT_2, respectively) as compared to **2**.

Removal of the methoxy group of the indoline moiety of **4a** to give **4c** led to 2-fold increase in binding affinity at MT_1 subtype (K_i = 17.5 nM) without any significant change in the binding affinity at MT_2 subtype (K_i = 36.2 nM. The *S*-enantiomer **4b** of the racemic mixture **4c** displayed the highest affinity at both MLT receptor subtypes (K_i = 9.8 and 7.8 nM for MT_1 and MT_2, respectively) and therefore the pharmacological profile exhibited by **4c** could be attributed to the *R*-enantiomer.

In general, the high binding affinity exhibited by compounds **4a–c** when compared to the rigid analogues **2** and **3** could be caused by the flexible indoline moiety. Therefore, further synthetic work on 2-(indolin-1-ylmethyl)-melatonin analogues is continued in our laboratory.

3. Conclusions

Briefly, a novel indole-indoline ring system, namely, 2-hydroxymethylindolin-1-ylmethyl)-melatonin derivatives **4a–c** were prepared in eight steps from the commercially available 5-methoxyindole-2-carboxylic acid. The newly developed indole–indoline

scaffold **4a**–**c** displayed a high affinity to MLT receptors as compared to the conformationally restricted pentacyclic analogues **2** and **3**. The spatial orientation of the rigid pentacyclic compounds **2** and **3** is not the optimum conformation to bind in the MLT receptors with a high affinity. Moreover, the presence of two methoxy groups and only one ethylamido side chain in the melatoninergic ligands seems to be unfavourable for high affinity ligands.

4. Experimental

4.1. General

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, respectively. The NMR resonances were assigned by means of HH-COSY, and HMQC experiments. EI mass spectra were determined on a Finnigan MAT 8200 and on a ESI-microTOF spectrometers. IR spectra, recorded as ATR, were obtained by using a Biorad PharmalyzIR FT-IR instrument. Optical rotations were determined with a Perkin-Elmer polarimeter 241 at 589 nm. 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.

4.1.1. 5-Methoxy-1*H*-indole-2-carbonyl fluoride (6)

A solution of cyanuric fluoride (0.35 g, 2.59 mmol) in dry acetonitrile (5 ml) was added dropwise to a stirred suspension of 5-methoxyindole-2-carboxylic acid **5** (1.0 g, 5.23 mmol) in dry acetonitrile (70 ml). Immediately, dry pyridine (4.3 ml, 5.23 mmol) was added dropwise to the reaction mixture and stirring was continued for 18 h at room temperature. The solvent was evaporated under reduced pressure and the residue was suspended in water (50 ml) and extracted with ethyl acetate (3× 30 ml). The combined organic layer was washed with 1 N hydrochloric acid, dried (Na_2SO_4) and evaporated under vacuum to give 0.77 g (76%) of **6** as a light brown solid which was pure enough to be used in the next step. Analytical sample of **6** was obtained by silica gel chromatography (*n*-pentane/diethyl ether, 1:1) to yield **6** as a white solid mp 126–127 °C. FTIR (ATR) ν = 3378, 1755, 1525, 1209 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 3.78 (s, 3H, OCH_3), 6.99–7.03 (m, 2H, H-3, H-6), 7.24–7.27 (m, 2H, H-4, H-7), 8.83 (br, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 55.7 (OCH_3), 102.4 (C-3), 113.2 (C-4), 113.5 (C-7), 119.7 (C-6), 127.5 (C-2), 133.9, 134.0 (C-3a, C-7a), 154.1 (C-5), 155.3 (C=O). MS (EI): *m/z* (%) = 193 (M^+, 48), 173 (100), 158 (50), 130 (27). Anal. Calcd for $C_{10}H_8FNO_2$: C, 62.18; H, 4.17; N, 7.25. Found: C, 62.16; H, 4.49; N, 7.24.

4.1.2. 2,3-Dihydro-1*H*-indole-2-carboxylic acid methyl ester (8)

Compound **8** was prepared according to the literature procedure.[14]

4.1.3. 1-(5́-Methoxy-1́*H*-indole-2́-carbonyl)-2,3-dihydro-1*H*-indole-2-carboxylic acid methyl ester (9)

A solution of **6** (0.58 g, 3 mmol) in dry acetonitrile (10 ml) was added dropwise to a stirred solution of **8** (1.06 g, 6 mmol) in dry acetonitrile (5 ml) at room temperature. The resulting reaction mixture was stirred for 18 h at room temperature and the solvent was evaporated under vacuum. The residue was dissolved in ethyl acetate (60 ml), extracted with 5 N hydrochloric acid (3× 30 ml), washed with water (2× 20 ml) and dried (Na_2SO_4). The organic layer was evaporated in vacuo and the residue was recrystallized from isopropanol to afford 0.89 g (85%) of **9** as a pale yellow solid mp 181–183 °C. FTIR (ATR) ν = 3403, 1735, 1620, 1401, 1394, 1205, 857, 755 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 3.22 (dd, 1H, *J* = 16.4, 2.5 Hz, H^a-3), 3.58–3.64 (m, 1H, H^b-3), 3.62 (s, 3H, O=C–OCH_3), 3.75 (s, 3H, OCH_3), 5.44 (dd, 1H, *J* = 10.6, 2.5 Hz, H-2), 6.69 (d, 1H, *J* = 1.5 Hz, H-3́), 6.88 (dd, 1H, *J* = 8.8, 2.5 Hz, H-6́), 6.96 (d, 1H, *J* = 2.5 Hz, H-4́), 6.99 (ddd, 1H, *J* = 7.6, 7.3, 0.8 Hz, H-5), 7.14 (d, 1H, *J* = 7.3 Hz, H-4), 7.17–7.19 (m, 1H, H-6), 7.23 (d, 1H, *J* = 8.8 Hz, H-7́), 8.11 (m, 1H, H-7), 9.77 (br, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 34.2 (C-3), 52.9 (O = C-OCH_3), 55.7 (OCH_3), 62.0 (C-2), 102.4 (C-4́), 105.0 (C-3́), 112.9 (C-7́), 116.7 (C-6́), 117.8 (C-7), 124.5 (C-5), 124.6 (C-4), 127.9 (C-6), 128.1, 130.2, 131.5, 143.1, 154.7 (ArC), 161.2 (Amide O=C), 172.2 (ester). MS (EI): *m/z* (%) = 350 (M^+, 37), 318 (49), 292 (10), 174 (100), 118 (42). Anal. Calcd for $C_{20}H_{18}N_2O_4$: C, 68.56; H, 5.18; N, 8.00. Found: C, 68.21; H, 5.14; N, 7.89.

4.1.4. 2-Methoxy-6a,7-dihydro-6*H*,13*H*-pyrazino[1,2-*a*;4,5-*á*]diindole (11)

4-(Dimethylamino)-pyridine (0.25 g, 2 mmol) was added to a stirred solution of **9** (0.35 g, 1 mmol) in dry THF (20 ml) at ambient temperature. Stirring was continued for 5 h at the same temperature and the precipitated solid was filtered off and washed with THF (5 ml) to give 0.24 g (74%) of 2-methoxy-6a,7-dihydropyrazino[1,2-*a*;4,5-*á*]diindole-6,13-dione (**10**). Crude **10** (0.14 g, 0.44 mmol) was dissolved in dry THF (5 ml) and was added dropwise to a cooled (0 °C) suspension of $LiAlH_4/AlCl_3$ in dry diethyl ether (prepared by a slow addition of $AlCl_3$ (0.18 g, 1.34 mmol) to a suspension $LiAlH_4$ (0.15 g, 3.96 mmol) in dry diethyl ether (15 ml) at 0 °C). The resulting reaction mixture was stirred at room temperature for 18 h and the reaction was quenched by a slow addition of a saturated sodium sulphate solution. The solids were removed by filtration, washed with chloroform (20 ml) and the combined organic phase was dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by silica gel chromatography (chloroform/ethyl acetate, 8:2) to give 0.1 g (79%) of **11** as a white solid mp 202–204 °C. FTIR (ATR) ν = 2838, 2348, 1481, 1240, 1028, 842, 725 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 2.77 (dd, 1H, *J* = 14.9, 10.8 Hz, H^a-7), 3.12 (dd, 1H, *J* = 14.9, 7.6 Hz, H^b-7), 3.55–3.58 (m, 1H, H-6a), 3.76 (s, 3H, OCH_3), 3.77–3.84 (m, 1H, H^a-6), 4.06 (d, 1H, *J* = 15.0 Hz, H^a-13), 4.34 (dd, 1H, *J* = 10.7, 3.7 Hz, H^b-6), 4.81 (d, 1H, *J* = 15.0 Hz, H^b-13), 6.18 (s, 1H, H-14), 6.52 (d, 1H, *J* = 8.8 Hz, H-11), 6.69–6.73 (m, 1H, H-9), 6.76 (dd, 1H, *J* = 8.8, 2.3 Hz, H-10), 6.96 (d, 1H, *J* = 2.3 Hz, H-1), 7.06–7.11 (m, 3H, H-3, H-4, H-8). ^{13}C NMR ($CDCl_3$): δ 32.1 (C-7), 43.6 (C-13), 46.2 (C-6), 54.9 (OCH_3), 60.5 (C-6a), 95.6 (C-14), 101.3 (C-1), 106.4 (C-11), 108.2 (C-4), 109.8 (C-3), 118.4 (C-9), 123.7 (C-10), 126.7 (C-8), 127.8, 127.9, 130.2, 132.2, 149.8, (C-4a, C-7a, C-11a, C-13a, C-14a), 153.4 (C-2). MS (EI): *m/z* (%) = 290 (M^+, 55), 173 (100), 130 (24). Anal. Calcd for $C_{19}H_{18}N_2O$: C, 78.59; H, 6.25; N, 9.65. Found: C, 78.19; H, 6.16; N, 9.42.

4.1.5. 2-Methoxy-14-(dimethylaminomethyl)-6a,7-dihydro-6*H*,13*H*-pyrazino[1,2-*a*;4,5-*á*]diindole (12)

Dimethylmethyleniminium iodide (0.27 g, 1.46 mmol) was added to a solution of **11** (0.35 g, 1.19 mmol) in dry CH_2Cl_2 (150 ml). The reaction mixture was refluxed for one hour, allowed to cool and basified with 25% ammonia. The organic layer was separated, washed with water (2× 30 ml), dried (Na_2SO_4) and evaporated under reduced pressure to afford 0.37 g (90%) of **12** as a light brown solid mp 140–142 °C. The crude Mannich base **12** was used in the next step without further purification. FTIR (ATR) ν = 2927, 2355, 1480, 1226, 1037, 797, 753 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 2.19 (s, 6 H, NMe_2), 2.76 (dd, 1H, *J* = 14.6, 10.9 Hz, H^a-7), 3.12 (dd, 1H, *J* = 14.6, 7.6 Hz, H^b-7), 3.42 (d, 1H, *J* = 13.1 Hz, -*H*CH-NMe_2), 3.48 (d, 1H, *J* = 13.1 Hz, -HC*H*-NMe_2), 3.64–3.72 (m, 1H, H-6a), 3.78 (s, 3H, OCH_3), 3.79–3.83 (m, 1H, H^a-6), 3.99 (d, 1H, *J* = 15.2 Hz, H^a-13), 4.33 (dd, 1H, *J* = 10.6, 3.6 Hz, H^b-6), 4.83 (d, 1H, *J* = 15.2 Hz,

H^b-13), 6.58 (d, 1H, *J* = 8.1, Hz, H-11), 6.68–6.72 (m, 1H, H-9), 6.76 (dd, 1H, *J* = 8.6, 2.0 Hz, H-3), 7.04 (d, 1H, *J* = 2.0 Hz H-1), 7.06–7.09 (m, 3H, H-4, H-8, H-10). ^{13}C NMR ($CDCl_3$): δ 33.2 (C-7), 43.6 (C-13), 45.5 (NMe_2), 47.2 (C-6), 53.6 (CH_2–NMe_2), 56.0 (OCH_3), 61.5 (C-6a), 101.3 (C-1), 107.5 (C-11), 109.1 (C-4), 110.8 (C-3), 119.3 (C-9), 124.7 (C-10), 127.8 (C-8), 106.7, 128.9, 129.2, 131.1, 131.6, 150.9 (C-4a, C-7a, C-11a, C-13a, C-14, C-14a), 154.4 (C-2). MS (EI): *m/z* (%) = 347 (M^+, 0.14), 302 (100), 186 (13), 151 (11).

4.1.6. (2-Methoxy-6a,7-dihydro-6*H*,13*H*-pyrazino[1,2-*a*;4,5-*á*]diindol-14-yl)-acetonitrile (13)

Methyl iodide (0.1 ml) was added to a solution of **12** (0.08 g, 0.23 mmol) in dry CH_2Cl_2 (50 ml). The reaction mixture was stirred at room temperature for one hour. The volatiles were removed under reduced pressure and the residual ammonium salt was dissolved in dry acetonitrile (100 ml). Dicyclohexyl[18]-crown[6] (0.10 g) and potassium cyanide (0.20 g) were added and resulting reaction mixture was heated at reflux for 2 h. The solvent was evaporated under vacuum and the residue was subjected to silica gel chromatography (ethyl acetate/chloroform, 8:2) to yield **13** (0.041 g, 54%) as a brown solid, mp 180–182 °C. FTIR (ATR) ν = 2924, 2827, 2361, 1484, 1229, 794, 750 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 2.76 (dd, 1H, *J* = 14.6, 11.1 Hz, H^a-7), 3.13 (dd, *J* = 14.6, 7.6 Hz, H^b-7), 3.62–3.70 (m, 3H, H-6a, CH_2–CN), 3.78 (s, 3H, OCH_3), 3.96–4.05 (m, 2H, H^a-6, H^a-13), 4.32 (dd, 1H, *J* = 10.9, 3.5 Hz, H^b-6), 4.78 (d, 1H, *J* = 14.9 Hz, H^b-13), 6.57 (d, 1H, *J* = 8.4 Hz, H-11), 6.69–6.75 (m, 1H, H-9), 6.77 (dd, 1H, *J* = 8.6, 2.3 Hz, H-3), 6.69–6.75 (m, 1H, H-8), 6.80 (dd, 1H, *J* = 8.4, 2.2 Hz, H-10), 6.92 (d, 1H, *J* = 2.3 Hz, H-1), 7.10 (d, 1H, *J* = 8.6 Hz, H-4). ^{13}C NMR ($CDCl_3$): δ 12.8 (CH_2CN), 33.1 (C-7), 43.2 (C-13), 47.2 (C-6), 55.9 (OCH_3), 61.2 (C-6a), 97.1 (CN), 99.7 (C-1), 107.6 (C-11), 109.8 (C-4), 111.9 (C-3), 119.7 (C-9), 124.8 (C-10), 127.9 (C-8), 117.7, 127.2, 128.8, 130.7, 130.8, 150.5 (C-4a, C-7a, C-11a, C-13a, C-14, C-14a), 154.9 (C-2). MS (EI): *m/z* (%) = 329 (M^+, 56), 289 (16), 212 (100), 172 (34). Anal. Calcd for $C_{21}H_{19}N_3O$: C, 76.57; H, 5.81; N, 12.76. Found: C, 76.38; H, 5.41; N, 12.48.

4.1.7. *N*-[2-(2-Methoxy-6a,7-dihydro-6*H*,13*H*-pyrazino[1,2-*a*;4,5-*á*]diindol-14-yl)-ethyl]-acetamide (3)

$AlCl_3$ (0.089 g, 0.67 mmol) was added slowly to a stirred suspension of $LiAlH_4$ (0.075 g, 1.98 mmol) in dry diethyl ether (15 ml) at 0 °C. A solution of **13** (0.15 g, 0.44 mmol) in dry THF (5 ml) was added dropwise to the cooled (0 °C) reaction mixture and stirring was further continued for one hour at room temperature. 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 vacuum to afford 0.14 g (91%) of 2-(2-methoxy-6a,7-dihydro-6 H,13 H-pyrazino[1,2-*a*;4,5-*á*]diindol-14-yl)-ethylamine (**14**) as a pale yellow viscous oil. A stirred solution of **14** (0.14 g, 0.42 mmol) in dry CH_2Cl_2 (15 ml) was treated with triethylamine (0.21 ml, 1.47 mmol) and acetic anhydride (0.20 ml, 2.11 mmol) at 0–5 °C. The reaction mixture was stirred at ambient temperature for 18 h. The solvent was evaporated under reduced pressure and the residue was purified by silica gel chromatography (chloroform/ethyl acetate/methanol, 8:1:1) to give **3** (0.09 g, 56%) as a red solid mp 103–105 °C. FTIR (ATR) ν = 3307, 2937, 1617, 1498, 1226, 750 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.83 (s, 3H, CH_3), 2.73–2.89 (m, 3H, H^a-7, CH_2-CH_2-N), 3.12 (dd, 1H, *J* = 14.8, 7.5 Hz, H^b-7), 3.33–3.50 (m, 2H, CH_2-CH_2-N), 3.65–3.69 (m, 1H, H-6a), 3.76 (s, 3H, OCH_3), 3.83 (d, 1H, *J* = 10.9 Hz, H^a-6), 3.93 (d, 1H, *J* = 14.9 Hz, H^a-13), 4.33 (dd, 1H, *J* = 10.9, 3.6 Hz, H^b-6), 4.73 (d, 1H, *J* = 14.9 Hz, H^b-13), 5.66 (br, 1H, NH), 6.57 (d, 1H, *J* = 7.8 Hz, H-11), 6.69–6.73 (m, 1H, H-9), 6.77 (dd, 1H, *J* = 8.7, 1.1 Hz, H-3), 6.87–7.09 (m, 4H, H-1, H-4, H-8, H-10). ^{13}C NMR ($CDCl_3$): δ 22.4 (CH_3), 23.1 (CH_2-CH_2-N), 32.1 (C-7), 38.8 (CH_2-CH_2-N), 42.4 (C-13), 46.2 (C-6), 54.9 (OCH_3), 60.6 (C-6a), 99.4 (C-1), 106.5 (C-11), 108.4 (C-4), 109.9 (C-3), 118.5 (C-9), 123.7 (C-10), 126.8, (C-8), 105.1, 127.4, 127.9, 129.4, 130.2, 149.8 (C-4a, C-7a, C-11a, C-13a, C-14, C-14a), 153.4 (C-2), 169.3 (C = O). MS (EI): *m/z* (%) = 375 (M^+, 32), 330 (31), 289 (100), 186 (36). Anal. Calcd for $C_{23}H_{25}N_3O_2$: C, 73.58; H, 6.71; N, 11.19. Found: C, 73.39; H, 6.52; N, 11.22.

4.1.8. 5-Methoxy-2,3-dihydro-1*H*-indole-2-carboxylic acid methyl ester (8a)[15]

Compound **8a** (1.6 g, 96%) was obtained from **7a** (2.0 g) following the above procedure described for **8** as a light red powder mp 54–56 °C. ^{1}H NMR ($CDCl_3$): δ 3.22 (dd, 1H, *J* = 16.2, 5.3 Hz, H^a-3), 3.29 (dd, 1H, *J* = 16.2, 10.1 Hz, H^b-3), 3.65 (s, 3H, OCH_3), 3.67 (s, 3H, O = C-OCH_3), 4.30 (dd, 1H, *J* = 10.1, 5.3 Hz, H-2), 6.55 (dd, 1H, *J* = 8.4, 2.3 Hz, H-6), 6.59 (d, 1H, *J* = 8.4 Hz, H-7), 6.64 (d, 1H, *J* = 2.3 Hz, H-4). ^{13}C NMR ($CDCl_3$): δ 34.2 (C-3), 52.5 (O=C–OCH_3), 55.9 (OCH_3), 60.3 (C-2), 110.9 (C-7), 111.2 (C-4), 112.8 (C-6), 128.4, 143.5 (C-3a, C-7a), 154.2 (C-5), 174.7 (ester).

4.1.9. (*S*)-2,3-Dihydro-1*H*-indole-2-carboxylic acid methyl ester (8b)

Compound **8b** was prepared according to the literature procedure.[13]

4.1.10. 1-(5́-Methoxy-1́*H*-indole-2́-carbonyl)-5-methoxy-2,3-dihydro-1*H*-indole-2-carboxylic acid methyl ester (9a)

Compound **9a** (1.8 g, 95%) was obtained from **6** (0.96 g) following the above procedure described for **9** as a light brown powder mp 172–174 °C (isopropanol). FTIR (ATR) ν = 3320, 1746, 1610, 1403, 1214, 836, 754 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 3.31 (dd, 1H, *J* = 16.4, 2.0 Hz, H^a-3), 3.68–3.73 (m, 1H, H^b-3), 3.74 (s, 3H, O = C-OCH_3), 3.82 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 5.55 (dd, 1H, *J* = 10.1, 2.0 Hz, H-2), 6.75 (br, 1H, H-3́), 6.81–6.83 (m, 2H, H-4, H-6), 6.99 (dd, 1H, *J* = 8.8, 2.3 Hz, H-6́), 7.08 (d, 1H, *J* = 2.3 Hz, H-4́), 7.35 (d, 1H, *J* = 8.8 Hz, H-7́), 8.15–8.17 (m, 1H, H-7), 9.63 (br, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 34.4 (C-3), 53.0 (O = C-OCH_3), 55.6 (OCH_3), 55.7 (OCH_3), 62.1 (C-2), 102.4 (C-4́), 104.6 (C-3́), 110.7 (C-6), 112.6 (C-4), 112.7 (C-7́), 116.5 (C-6́), 118.6 (C-7), 128.3, 130.3, 131.2, 136.6, 154.7, 157.0 (ArC), 160.4 (Amide O = C), 172.2 (ester). MS (EI): *m/z* (%) = 380 (M^+, 23), 348 (85), 207 (71), 173 (100), 148 (87). Anal. Calcd for $C_{21}H_{20}N_2O_5$: C, 66.31; H, 5.30; N, 7.36. Found: C, 66.00; H, 5.28; N, 7.35.

4.1.11. (*S*)-1-(5́-Methoxy-1́*H*-indole-2́-carbonyl)-2,3-dihydro-1*H*-indole-2-carboxylic acid methyl ester (9b)

Compound **9b** (1.64 g, 90%) was obtained from **6** (1.0 g) following the above procedure described for **9** as a light brown powder mp 173–175 °C (isopropanol). $[\alpha]_D^{22} = -36.1$ (*c* = 0.4, $CHCl_3$). Anal. Calcd for $C_{20}H_{18}N_2O_4$: C, 68.56; H, 5.18; N, 8.00. Found: C, 68.35; H, 5.37; N, 7.91. The spectral data of **9b** are identical with the corresponding data of the racemic mixture **9**.

4.1.12. [5-Methoxy-1-(5́-methoxy-1́*H*-indol-2́-ylmethyl)-2,3-dihydro-1*H*-indol-2-yl]-methanol (15a)

A solution of **9a** (1.0 g, 2.83 mmol) in dry THF (10 ml) was added dropwise to a stirred suspension of $LiAlH_4$ (1.06 gm, 27.80 mmol) in dry diethyl ether (30 ml) at 0–5 °C. The reaction mixture was heated at 40 °C for 18 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 and the residue

was purified by silica gel chromatography (chloroform/ethyl acetate, 9:1) to give **15a** (0.44 g, 49%) as light red viscous oil. FTIR (ATR) ν = 3344, 2938, 1452, 1285, 1228, 837, 731 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.26 (br, 1H, OH), 2.84–2.93 (m, 2H, H-3), 3.55–3.58 (m, 2H, C*H*$_2$-OH), 3.64 (s, 3H, OCH_3), 3.68–3.72 (m, 1H, H-2), 3.74 (s, 3H, OCH_3), 4.23 (d, 1H, J = 15.2 Hz, *H*CH–N), 4.34 (d, 1H, J = 15.2 Hz, HC*H*-N), 6.23 (s, 1H, H-3́), 6.40 (d, 1H, J = 8.1 Hz, H-7), 6.51 (dd, 1H, J = 8.1, 1.8 Hz, H-6), 6.62 (s, 1H, H-4), 6.69 (dd, 1H, J = 8.6, 2.5 Hz, H-6́), 6.93 (d, 1H, J = 2.5 Hz, H-4́), 7.05 (d, 1H, J = 8.6 Hz, H-7́), 8.54 (br, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 31.6 (C-3), 47.9 (CH_2-N), 55.8 (OCH_3), 55.9 (OCH_3), 62.9 (CH_2-OH), 66.7 (C-2), 100.8 (C-3́), 102.2 (C-4́), 109.2 (C-7), 111.7 (C-7́), 111.8 (C-6́), 111.9 (C-6), 112.3 (C-4), 128.8, 130.6, 131.4, 136.8, 146.1, 153.9, 154.2 (ArC). MS (EI): *m/z* (%) = 338 (M^+, 22), 179 (75), 160 (100), 148 (68), 117 (20). Anal. Calcd for $C_{20}H_{22}N_2O_3$: C, 70.99; H, 6.55; N, 8.28. Found: C, 70.64; H, 6.21; N, 8.25.

4.1.13. [(*S*)-1-(5́-Methoxy-1́*H*-indol-2́-ylmethyl)-2,3-dihydro-1*H*-indol-2-yl]-methanol (15b)

Compound **15b** (0.88 g, 70%) was obtained from **9b** (1.0 g) following the above procedure described for **15a** as a light brown viscous oil. Purification was done by silica gel chromatography (chloroform/ethyl acetate, 9:1). $[\alpha]_D^{21}$ = +1.1 (c = 1, $CHCl_3$). FTIR (ATR) ν = 3307, 3047, 1604, 1458, 1386, 1233, 838, 733 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.18 (br 1H, OH), 2.83 (dd, 1H, J = 15.9, 9.3 Hz, H^a-3), 2.98 (dd, 1H, J = 15.9, 9.3 Hz, H^b-3), 3.55–3.59 (m, 1H, *H*CH-OH), 3.62–3.69 (m, 1H, H-2), 3.73 (s, 3H, OCH_3), 3.71–3.75 (m, 1H, HC*H*-OH), 4.30 (d, 1H, J = 16.0 Hz, *H*CH-N), 4.38 (d, 1H, J = 16.0 Hz, HC*H*-N), 6.23 (d, 1H, J = 0.8 Hz, H-3́), 6.49 (d, 1H, J = 7.8 Hz, H-7), 6.63–6.67 (m, 1H, H-5), 6.69 (dd, 1H, J = 8.8, 2.5 Hz, H-6́), 6.92 (d, 1H, J = 2.5 Hz, H-4́), 6.96–6.98 (m, 2H, H-4, H-6), 7.04 (d, 1H, J = 8.8 Hz, H-7́), 8.58 (br, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 31.3 (C-3), 46.8 (CH_2-N), 55.9 (OCH_3), 63.2 (CH_2-OH), 66.1 (C-2), 100.8 (C-3́), 102.2 (C-4́), 108.3 (C-7), 111.7 (C-7́), 111.8 (C-6́), 119.3 (C-5), 124.7 (C-4), 127.8 (C-6), 128.8, 128.9, 131.4, 136.7, 151.9, 154.2 (ArC). MS (EI): *m/z* (%) = 308 (M^+, 14), 160 (100), 118 (89). Anal. Calcd for $C_{19}H_{20}N_2O_2$: C, 74.00; H, 6.54; N, 9.08. Found: C, 74.32; H, 6.34; N, 9.01.

4.1.14. [1-(5́-Methoxy-1́*H*-indol-2́-ylmethyl)-2,3-dihydro-1*H*-indol-2-yl]-methanol (15c)

Compound **15c** (0.75 g, 60%) was obtained from **9** (1.0 g) following the above procedure described for **15a** as a light brown viscous oil. Purification was done by silica gel chromatography (chloroform/ethyl acetate, 9:1). Anal. Calcd for $C_{19}H_{20}N_2O_2$: C, 74.00; H, 6.54; N, 9.08. Found: C, 74.25; H, 6.28; N, 9.09. The spectral data of **15c** are identical with the corresponding data of the stereoisomer **15b**.

4.1.15. [1-(3́-Dimethylaminomethyl-5́-methoxy-1́*H*-indol-2́-ylmethyl)-5-methoxy-2,3-dihydro-1*H*-indol-2-yl]-methanol (16a)

Dimethylmethyleniminium iodide (0.46 g, 2.49 mmol) was added to a solution of **15a** (0.69 g, 2.04 mmol) in dry CH_2Cl_2 (100 ml). The reaction mixture was stirred for two hours at room temperature and the precipitated solid was filtered off and washed with CH_2Cl_2 (20 ml). The collected solid was suspended in water (30 ml), basified with 25% ammonia and extracted with chloroform (3× 25 ml). The combined organic layer was washed with water (2× 30 ml), dried (Na_2SO_4) and evaporated under reduced pressure to afford 0.57 g (71%) of **16a** as a light red viscous oil. The crude **16a** was pure enough to be used in the next step without further purification. FTIR (ATR) ν = 3196, 3056, 2937, 1454, 1283, 1214, 796, 729 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.21 (s, 6 H, NMe_2), 2.68 (dd, 1H, J = 16.2, 9.7 Hz, H^a-3), 2.89 (dd, 1H, J = 16.2, 9.7 Hz, H^b-3), 3.31 (d, 1H, J = 12.9 Hz, *H*CH-NMe_2), 3.49–3.56 (m, 2H, C*H*$_2$-OH), 3.67 (s, 3H, OCH_3), 3.69–3.75 (m, 2H, H-2, HC*H*-NMe_2), 3.77 (s, 3H, OCH_3), 4.49 (s, 2H, CH_2-N), 6.36 (d, 1H, J = 8.6 Hz, H-7), 6.54 (dd, 1H, J = 8.6, 2.3 Hz, H-6), 6.64 (d, 1H, J = 2.3 Hz, H-4), 6.71 (dd, 1H, J = 8.6, 2.3 Hz, H-6́), 6.94 (d, 1H, J = 2.3 Hz, H-4́), 7.06 (d, 1H, J = 8.6 Hz, H-7́), 8.31 (br, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 31.8 (C-3), 44.8 (NMe_2), 47.1 (CH_2-N), 52.5 (*C*H$_2$–NMe_2), 55.9 (OCH_3), 56.0 (OCH_3), 65.1 (CH_2-OH), 66.7 (C-2), 102.6 (C-4́), 107.9 (C-7), 109.3 (C-3́), 111.4 (C-7́), 111.6 (C-6́), 112.1 (C-6), 112.2 (C-4), 129.7, 130.2, 130.7, 136.0, 147.3, 153.5, 154.3 (ArC). HRMS (ESI, pos.) $C_{23}H_{29}N_3O_3{\cdot}H^+$: *m/z* calcd 396.2289, *m/z* found 396.2282.

4.1.16. [(*S*)-1-(3́-Dimethylaminomethyl-5́-methoxy-1́*H*-indol-2́-ylmethyl)-2,3-dihydro-1*H*-indol-2-yl]-methanol (16b)

Compound **16b** (0.47 g, 63%) was obtained from **15b** (0.63 g) following the above procedure described for **16a** as a light brown solid mp 170–172 °C. $[\alpha]_D^{21}$ = -7.6 (c = 0.5, CH_3OH). FTIR (ATR) ν = 3028, 2923, 1483, 1339, 1245, 1029, 754 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.17 (s, 6 H, NMe_2), 2.66–2.72 (m, 1H, H^a-3), 2.88–2.94 (m, 1H, H^b-3), 3.28 (d, 1H, J = 12.9 Hz, *H*CH-NMe_2), 3.45–3.58 (m, 2H, H-2, *H*CH-OH), 3.65–3.73 (m, 2H, HC*H*-OH, HC*H*–NMe_2), 3.76 (s, 3H, OCH_3), 4.46 (d, 1H, J = 16.2 Hz, *H*CH-N), 4.54 (d, 1H, J = 16.2 Hz, HC*H*-N), 6.41 (d, 1H, J = 7.8 Hz, H-7), 6.58–6.62 (m, 1H, H-5), 6.68 (dd, 1H, J = 8.7, 2.3 Hz, H-6́), 6.94 (d, 1H, J = 2.3 Hz, H-4́), 6.96–7.01 (m, 3H, H-4, H-6, H-7́), 8.43 (br, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 31.5 (C-3), 45.0 (NMe_2), 46.2 (CH_2–N), 52.5 (*C*H$_2$–NMe_2), 56.0 (OCH_3), 65.1 (CH_2–OH), 66.3 (C-2), 100.7 (C-4́), 107.3 (C-7), 109.6 (C-3́), 111.4 (C-7́), 111.6 (C-6́), 118.6 (C-5), 124.8 (C-4), 127.7 (C-6), 128.8, 129.8, 130.2, 135.8, 153.1, 154.2 (ArC). HRMS (ESI, pos.) $C_{22}H_{27}N_3O_2{\cdot}H^+$-2: *m/z* calcd 364.2026, *m/z* found 364.2030.

4.1.17. [1-(3́-Dimethylaminomethyl-5́-methoxy-1́*H*-indol-2́-ylmethyl)-2,3-dihydro-1*H*-indol-2-yl]-methanol (16c)

Compound **16c** (0.55 g, 73%) was obtained from **15c** (0.63 g) following the above procedure described for **16a** as a light brown solid mp 136–138 °C. HRMS (ESI, pos.) $C_{22}H_{27}N_3O_2{\cdot}H^+$: *m/z* calcd 366.2183, *m/z* found 366.2176. The spectral data of **16c** are identical with the corresponding data of the stereoisomer **16b**.

4.1.18. [2-(2-Hydroxymethyl-5-methoxy-2,3-dihydro-1*H*-indol-1-ylmethyl)-5́-methoxy-1́*H*-indol-3́-yl]-acetonitrile (17a)

Methyl iodide (0.1 ml) was added to a solution of **16a** (0.09 g, 0.23 mmol) in dry CH_2Cl_2 (50 ml). The reaction mixture was stirred at room temperature for one hour. The volatiles were removed under vacuum and the residual ammonium salt was dissolved in dry acetonitrile (100 ml). Dicyclohexyl[18]-crown[6] (0.10 g) and potassium cyanide (0.20 g) were added and resulting reaction mixture was heated at reflux for one hour. The solvent was evaporated under reduced pressure and the residue was subjected to silica gel chromatography (ethyl acetate/chloroform, 1:1) to afford 0.055 g (63%) of **17a** as a red viscous oil. FTIR (ATR) ν = 3346, 3056, 2125, 1485, 1285, 1213, 796, 731 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.36 (br, 1H, OH), 2.79–2.85 (m, 1H, H^a-3), 2.89–3.04 (m, 1H, H^b-3), 3.60–3.65 (m, 2H, H-2, *H*CH-OH), 3.63 (s, 3H, OCH_3), 3.69–3.76 (m, 2H, CH_2-CN, HC*H*-OH), 3.78 (s, 3H, OCH_3), 4.29 (d, 1H, J = 16.2 Hz, *H*CH-N), 4.35 (d, 1H, J = 16.2 Hz, HC*H*-N), 6.23 (d, 1H, J = 8.6 Hz, H-7), 6.49 (dd, 1H, J = 8.6, 1.8 Hz, H-6), 6.63 (s, 1H, H-4), 6.74 (dd, 1H, J = 8.8, 2.3 Hz, H-6́), 6.92 (d, 1H, J = 2.3 Hz, H-4́), 7.07 (d, 1H, J = 8.8 Hz, H-7́), 8.89 (br, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 12.9 (*C*H$_2$-CN), 31.7 (C-3), 46.7 (CH_2-N), 55.9 (OCH_3), 56.0 (OCH_3), 63.5 (CH_2-OH), 67.2 (C-2), 99.6 (C-4́), 100.0 (CN), 109.0 (C-7), 112.0 (C-7́), 112.2 (C-6́), 112.4 (C-6), 112.6 (C-4), 118.1 (C-3́), 127.6, 130.3, 130.7, 134.4, 145.6, 154.5 (ArC). MS (EI): *m/z* (%) = 377 (M^+, 22), 199 (78), 179 (100), 133 (22). Anal. Calcd for $C_{22}H_{23}N_3O_3$: C, 70.01; H, 6.14; N, 11.13. Found: C, 70.32; H, 6.43; N, 11.18.

4.1.19. [2-((*S*)-2-Hydroxymethyl-2,3-dihydro-1*H*-indol-1-ylmethyl)-5́-methoxy-1́*H*-indol-3́-yl]-acetonitrile (17b)

Compound **17b** (0.28 g, 56%) was obtained from **16b** (0.52 g) following the above procedure described for **17a** as a light red solid mp 77–79 °C. Purification was done by silica gel chromatography (chloroform/ethyl acetate, 1:1). $[\alpha]_D^{21}$ = +44.6 (*c* = 1.0, $CHCl_3$). FTIR (ATR) *ν* = 3973, 3855, 2323, 1483, 1247, 1216, 799, 747 cm^{-1}. ^1H NMR ($CDCl_3$): *δ* 2.18 (br, 1H, OH), 2.84 (dd, 1H, *J* = 15.9, 8.8 Hz, H^a-3), 3.05 (dd, 1H, *J* = 15.9, 8.8 Hz, H^b-3), 3.64–3.68 (m, 2H, H-2, *H*CH-OH), 3.69–3.75 (m, 2H, CH_2-CN, HC*H*–OH), 3.78 (s, 3H, OCH_3), 4.39 (s, 2H, CH_2-N), 6.32 (d, 1H, *J* = 7.8 Hz, H-7), 6.64–6.68 (m, 1H, H-5), 6.74 (dd, 1H, *J* = 8.8, 2.3 Hz, H-6́), 6.92 (d, 1H, *J* = 2.3 Hz, H-4́), 6.94–7.01 (m, 2H, H-4, H-6), 7.06 (d, 1H, *J* = 8.8 Hz, H-7́), 8.87 (br, 1H, NH). ^{13}C NMR ($CDCl_3$): *δ* 12.9 (*C*H_2-CN), 31.5 (C-3), 45.3 (CH_2-N), 55.9 (OCH_3), 63.8 (CH_2-OH), 66.4 (C-2), 99.6 (C-4́), 99.9 (CN), 107.8 (C-7), 112.2 (C-7́), 112.6 (C-6́), 118.1 (C-3́), 119.4 (C-5), 124.8 (C-4), 127.9 (C-6), 127.7, 128.8, 130.2, 134.5, 152.0, 154.5 (ArC). MS (EI): *m/z* (%) = 347 (M^+, 11), 199 (100), 149 (24), 118 (51). Anal. Calcd for $C_{21}H_{21}N_3O_2$: C, 72.60; H, 6.09; N, 12.09. Found: C, 72.38; H, 5.89; N, 12.07.

4.1.20. [2-(2-Hydroxymethyl-2,3-dihydro-1*H*-indol-1-ylmethyl)-5́-methoxy-1́*H*-indol-3́-yl]-acetonitrile (17c)

Compound **17c** (0.28 g, 57%) was obtained from **16c** (0.52 g) following the above procedure described for **17a** as a light brown viscous oil. Purification was done by silica gel chromatography (chloroform/ethyl acetate, 1:1). HRMS (ESI, pos.) $C_{21}H_{21}N_3O_2{\cdot}Na^+$: *m/z* calcd 370.1533, *m/z* found 370.1526. The spectral data of **17c** are identical with those of the stereoisomer **17b**.

4.1.21. 2-(2-Hydroxymethyl-5-methoxyindolin-1-ylmethyl)-melatonin (4a)

A solution of **17a** (0.20 g, 0.53 mmol) in dry THF (5 ml) was added dropwise to a stirred suspension of $LiAlH_4$ (0.20 g, 5.20 mmol) in dry diethyl ether (25 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 vacuum to afford 0.19 g (92%) of 2-[2-(2-hydroxymethyl-5-methoxy-2,3-dihydro-1*H*-indol-1-ylmethyl)-5́-methoxy-1́*H*-indol-3́-yl]-ethylamine (**18a**) as a pale yellow viscous oil. ^1H NMR ($CDCl_3$): *δ* 2.68–2.74 (m, 2H, H^a-3, *H*CH-CH_2-NH_2), 2.86–2.97 (m, 4H, H^b-3, HC*H*–C*H*$_2$–NH_2), 3.09 (br, 2H, –NH_2), 3.53–3.59 (m, 2H, H-2, *H*CH–OH), 3.62 (s, 3H, OCH_3), 3.64–3.71 (m, 1H, HC*H*–OH), 3.74 (s, 3H, OCH_3), 4.29 (d, 1H, *J* = 15.6 Hz, *H*CH–N), 4.37 (d, 1H, *J* = 15.6 Hz, HC*H*–N), 6.24 (d, 1H, *J* = 8.6 Hz, H-7), 6.47 (dd, 1H, *J* = 8.6, 2.3 Hz, H-6), 6.60 (s, 1H, H-4), 6.69 (dd, 1H, *J* = 8.8, 2.3 Hz, H-6́), 6.89 (d, 1H, *J* = 2.3 Hz, H-4́), 7.04 (d, 1H, *J* = 8.8 Hz, H-7́), 8.48 (br, 1H, NH). ^{13}C NMR ($CDCl_3$): *δ* 26.4 (*C*H_2-CH_2-NH_2), 31.8 (C-3), 41.2 (CH_2–*C*H_2–NH_2), 46.2 (CH_2-N), 55.9 (OCH_3), 56.0 (OCH_3), 63.8 (CH_2–OH), 66.8 (C-2), 100.8 (C-4́), 108.4 (C-7), 109.0 (C-3́), 111.4 (C-7́), 111.7 (C-6́), 111.9 (C-6), 112.2 (C-4), 128.6, 130.6, 130.7, 134.7, 147.1, 153.6, 153.9 (ArC). A stirred solution of **18a** (0.19 g, 0.49 mmol) in dry CH_2Cl_2 (15 ml) was treated with triethylamine (0.24 ml, 1.72 mmol) and acetic anhydride (0.20 ml, 2.11 mmol) at 0–5 °C. The reaction mixture was stirred at ambient temperature for 18 h. The solvent was evaporated under vacuum and the residue containing the ester **19a** was dissolved directly in methanol (10 ml), treated with sodium hydroxide (0.18 g, 4.5 mmol) and the resulting solution was stirred at room temperature for 2 h. The solvent was removed under reduced pressure and the residue was purified by silica gel chromatography (chloroform/methanol/25% ammonia, 10:1:0.1) to afford 0.13 g (59%) of **4a** as a red solid mp 78–80 °C. FTIR (ATR) *ν* = 3279, 2830, 1627, 1485, 1284, 1213, 797, 732 cm^{-1}. ^1H NMR ($CDCl_3$): *δ* 1.73 (s, 3H, CH_3), 2.77–2.92 (m, 3H, H^a-3, C*H*$_2$–CH_2–N), 3.31–3.46 (m, 3H, H^b-3, CH_2-C*H*$_2$-N), 3.62–3.76 (m, 6 H, OCH_3, H-2, C*H*$_2$–OH), 3.77 (s, 3H, OCH_3), 4.28 (s, 2H, CH_2–N), 5.81 (t, *J* = 5.0 Hz, NH), 6.23–6.32 (m, 1H, H-7), 6.46–6.52 (m, 1H, H-6), 6.62 (s, 1H, H-4), 6.70 (dd, 1H, *J* = 8.8, 2.3 Hz, H-6́), 6.87 (d, 1H, *J* = 2.3 Hz, H-4́), 7.06 (d, 1H, *J* = 8.8 Hz, H-7́), 8.77 (br, 1H, NH). ^{13}C NMR ($CDCl_3$): *δ* 23.2 (CH_3), 24.2 (C*H*$_2$–CH_2–N), 31.8 (C-3), 40.3 (CH_2–*C*H_2–N), 46.7 (CH_2-N), 55.9 (OCH_3), 56.0 (OCH_3), 63.6 (CH_2-OH), 67.3 (C-2), 100.4 (C-4́), 109.1 (C-7), 109.3 (C-3́), 111.7 (C-7́), 111.9 (C-6́), 112.1 (C-6), 112.3 (C-4), 128.8, 130.7, 130.8, 133.9, 146.9, 153.9, 154.1 (ArC), 170.6 (C=O). MS (EI): *m/z* (%) = 423 (M^+, 13), 392 (5), 148 (100), 133 (38). Anal. Calcd for $C_{24}H_{29}N_3O_4$: C, 68.07; H, 6.90; N, 9.92. Found: C, 67.99; H, 6.64; N, 9.91.

4.1.22. 2-((*S*)-2-Hydroxymethylindolin-1-ylmethyl)-melatonin (4b)

2-[2-((*S*)-2-Hydroxymethyl-2,3-dihydro-1*H*-indol-1-ylmethyl)-5́-methoxy-1́*H*-indol-3́-yl]-ethylamine (**18b**) was obtained in 98% yield from **17b** (0.19 g) following the above procedure described for **18a** as a pale yellow viscous oil and was used directly without any purification. ^1H NMR ($CDCl_3$): *δ* 2.68–2.79 (m, 2H, H^a-3, *H*CH–CH_2–NH_2), 2.81–2.96 (m, 4H, H^b-3, HC*H*–C*H*$_2$–NH_2), 3.09 (br, 2H, –NH_2), 3.53–3.68 (m, 3H, H-2, C*H*$_2$-OH), 3.72 (s, 3H, OCH_3), 4.37 (s, 2H, CH_2–N), 6.31 (d, 1H, *J* = 7.8 Hz, H-7), 6.56–6.60 (m, 1H, H-5), 6.67 (dd, 1H, *J* = 8.8, 2.0 Hz, H-6́), 6.86 (d, 1H, *J* = 2.0 Hz, H-4́), 6.89–6.95 (m, 2H, H-4, H-6), 7.01 (d, 1H, *J* = 8.8 Hz, H-7́), 8.79 (br, 1H, NH). ^{13}C NMR ($CDCl_3$): *δ* 26.8 (*C*H_2–CH_2–N), 31.5 (C-3), 41.5 (CH_2-*C*H_2–N), 45.1 (CH_2–N), 56.1 (OCH_3), 63.8 (CH_2–OH), 66.2 (C-2), 100.8 (C-4́), 107.5 (C-7), 109.2 (C-3́), 111.4 (C-6́), 111.6 (C-7́), 118.7 (C-5), 124.6 (C-4), 127.7 (C-6), 128.6, 128.8, 130.7, 134.1, 152.9, 153.7 (ArC). Compound **4b** (0.15 g, 72%) was obtained from **18b** (0.19 g) following the above procedure described for **4a** as a pale yellow solid mp 65–67 °C. Purification was done by silica gel chromatography (chloroform/methanol/25% ammonia, 10:1:0.1). $[\alpha]_D^{21}$ = +8.7 (*c* = 0.25, $CHCl_3$). FTIR (ATR) *ν* = 3282, 2927, 1632, 1482, 1243, 1214, 746 cm^{-1}. ^1H NMR ($CDCl_3$): *δ* 1.68 (s, 3H, CH_3), 2.76–2.89 (m, 3H, H^a-3, C*H*$_2$–CH_2–N), 2.97 (dd, 1H, *J* = 16.2, 8.8 Hz, H^b-3), 3.29–3.40 (m, 2H, CH_2-C*H*$_2$-N), 3.55–3.68 (m, 3H, H-2, C*H*$_2$–OH), 3.72 (s, 3H, OCH_3), 4.29 (s, 2H, CH_2–N), 5.88 (t, *J* = 5.7 Hz, NH), 6.26 (d, 1H, *J* = 7.8 Hz, H-7), 6.55–6.59 (m, 1H, H-5), 6.65 (dd, 1H, *J* = 6.8, 2.3 Hz, H-6́), 6.85 (d, 1H, *J* = 7.8 Hz, H-6), 6.89 (d, 1H, *J* = 2.3 Hz, H-4́), 6.93 (d, 1H, *J* = 7.4 Hz, H-4), 7.02 (d, 1H, *J* = 6.8 Hz, H-7́), 8.96 (br, 1H, NH). ^{13}C NMR ($CDCl_3$): *δ* 23.1 (CH_3), 24.2 (C*H*$_2$–CH_2–N), 31.5 (C-3), 40.3 (CH_2–*C*H_2–N), 45.3 (CH_2–N), 56.0 (OCH_3), 63.8 (CH_2–OH), 66.5 (C-2), 100.5 (C-4́), 107.9 (C-7), 108.6 (C-3́), 111.5 (C-6́), 111.8 (C-7́), 118.9 (C-5), 124.6 (C-4), 127.7 (C-6), 128.8, 128.9, 130.7, 134.3, 152.6, 153.9 (ArC), 170.8 (C=O). MS (EI): *m/z* (%) = 393 (M^+, 1), 245 (3), 149 (12), 118 (100). Anal. Calcd for $C_{23}H_{27}N_3O_3$: C, 70.21; H, 6.92; N, 10.68. Found: C, 69.95; H, 6.81; N, 10.29.

4.1.23. 2-(2-Hydroxymethylindolin-1-ylmethyl)-melatonin (4c)

2-[2-(2-Hydroxymethyl-2,3-dihydro-1*H*-indol-1-ylmethyl)-5́-methoxy-1́*H*-indol-3́-yl]-ethylamine (**18c**) was obtained in 95% yield from **17c** (0.19 g) following the above procedure described for **18a** as a pale yellow viscous oil and was used directly without any purification. The spectral data of **18c** are identical with those of the stereoisomer **18b**. Compound **4c** (0.17 g, 78%) was obtained from **18c** (0.19 g) following the above procedure described for **4a** as a pale yellow solid mp 62–64 °C. Purification was done by silica gel chromatography (chloroform/methanol/25% ammonia, 10:1:0.1). Anal. Calcd for $C_{23}H_{27}N_3O_3$: C, 70.21; H, 6.92; N, 10.68. Found: C, 70.56; H, 7.14; N, 10.45. The spectral data of **4c** are identical with the corresponding data of the stereoisomer **4b.**

Acknowledgments

Thanks are due to PD Dr. D.P. Zlotos, Pharmaceuitical Institute, University of Würzburg for scientific discussion and to Mrs. A. Betz for Technical Assistance.

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