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# Synthesis and pharmacological evaluation of 6a,7-dihydro-6*H*,13*H*-pyrazino[1,2-*a*;4,5-*a*′]diindole analogs as melatonin receptor ligands

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**Abstract**—The synthesis of two melatonin-derived analogs of the novel 6a,7-dihydro-6*H*,13*H*-pyrazino[1,2-*a*;4,5-*a*′]diindole ring system is described. The non-methoxy and methoxy analogs, **4a** and **4b** were prepared in seven steps starting from indoline-2-carboxylic acid **5a** and 5-methoxyindoline-2-carboxylic acid **5b**, respectively. While **4a** exhibited micromolar affinities for both melatonin receptors, the methoxy analog **4b** displayed moderate affinity for $MT_2$ receptors ($K_i$=0.41 μM) being 4.4-fold higher than for the $MT_1$ subtype.


## 1. Introduction

Melatonin (**1**) is a hormone exerting its multiple pharmacological actions through two G-protein-coupled receptors $MT_1$ and $MT_2$.[1] Exploring the physiological role of each of these subtypes requires subtype selective $MT_1$ and $MT_2$ ligands. Although several $MT_1$ and $MT_2$ selective agonists and antagonists are known to date,[2] conformationally restricted melatoninergic agents are still needed in order to explore the structural differences between $MT_1$ and $MT_2$ binding pockets. 3D-QSAR analysis of subtype selective melatoninergic ligands revealed that $MT_2$ binding affinity could be enhanced by occupying the out-of-plane region surrounding positions 1 and 2 of melatonin as well as the area corresponding to the methoxy substituent.[3] This pharmacophore hypothesis could be exemplified by the rigid tetracyclic indole analogs (**2**) and (**3**), respectively, which are among the most $MT_2$ selective melatoninergic ligands described to date (Fig. 1).[4] In this paper, we report the synthesis of two novel pentacyclic non-methoxy and methoxy melatonin analogs (**4a**) and (**4b**), respectively, in which an indoline moiety is attached to the positions 1 and 2 of melatonin. These novel agents could help probing the existing pharmacophore for potent $MT_2$ selective ligands.

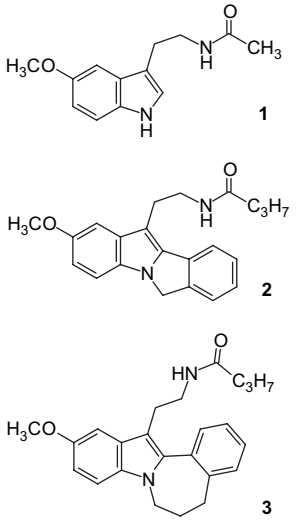


**Figure 1**.

## 2. Results and discussion

### 2.1. Synthesis of the non-methoxy compound 4a

We chose the non-methoxy compound **4a** as our initial target in order to establish a viable synthetic route to the more biologically relevant molecule **4b**. In the course of our studies on allosteric ligands of muscarinic $M_2$ receptors, we recently reported the synthesis of 6*H*,13*H*-pyrazino[1,2-*a*;4,5-*a*′]diindole (6a*S*,13a*S*-**7a**).[5] The synthetic sequence

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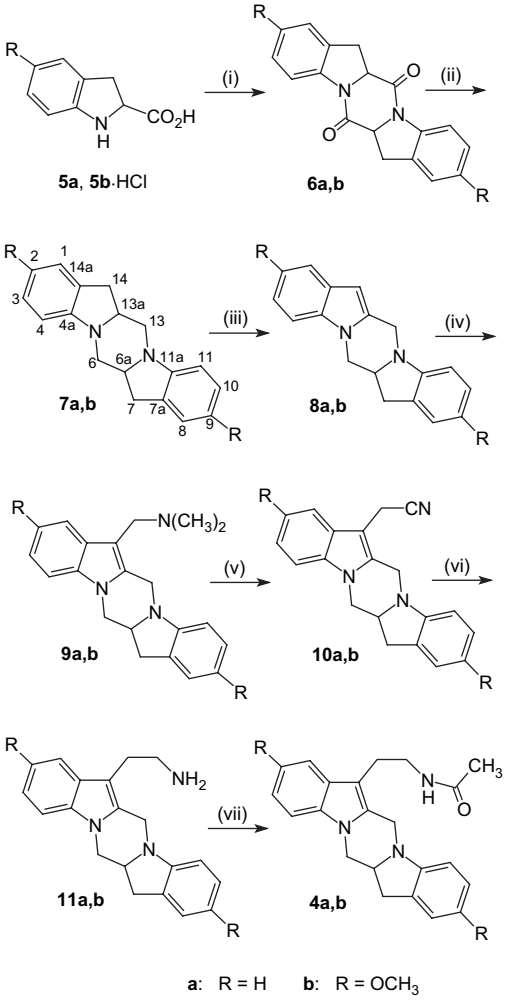
involved the self-condensation of (2*S*)-(−)-indoline-2-carboxylic using DCC,[6] followed by reduction of the resulting dilactam with borane. Starting from the commercially available racemic indoline-2-carboxylic acid (**5a**), we now applied the same strategy to afford 6*H*,13*H*-pyrazino[1,2-*a*;4,5-*a*′]diindole (**7a**) (Scheme 1). Thus, self-coupling of **5a** using DCC in THF afforded dilactam (**6a**) in 41% yield. Reduction of both carbonyl groups of **6a** could be achieved by heating at reflux in THF with borane to afford the tetrahydro-6*H*,13*H*-pyrazino[1,2-*a*;4-5-*a*′]diindole **7a** in excellent yield (98%). In the next critical step, one of the two indoline moieties of **7a** should be selectively dehydrogenated. Keeping in mind that by refluxing 6a*S*,13a*S*-**7a** in toluene with an excess of Pd/C 10%, introduction of both C6a–C7 and C13a–C14 double bonds occured,[5] more gentle reaction conditions should be applied. We were pleased to find that heating of **7a** in toluene at 80 °C with reduced amount of Pd/C 10% provided the monodehydrogenation product (**8a**) in 48% yield. Under these conditions, both starting material and the didehydrogenation product were present in the reaction mixture, as indicated by TLC. We were gratified to find that the desired compound **8a** could be separated from the aforementioned side products by means of silica gel chromatography.



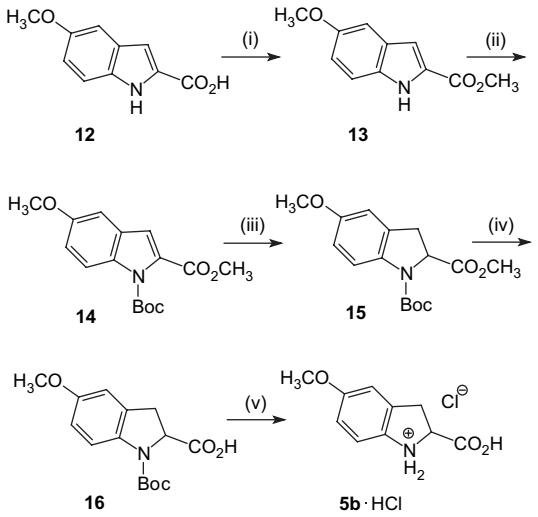

**Scheme 1**. Reagents: (i) **6a**: DCC, THF; **6b**: DCC, $Et_3N$, $CH_2Cl_2$; (ii) borane–THF complex, THF; (iii) **8a**: Pd/C 10%, toluene; **8b**: Pd/C 10%; (iv) $CH_2{=}NMe_2^+I^-$, $CH_2Cl_2$; (v) 1. MeI, $CH_2Cl_2$, 2. KCN, dicyclohexyl-[18]-crown-[6], MeCN; (vi) $LiALH_4$, $Et_2O$; and (vii) acetic anhydride, $Et_3N$, $CH_2Cl_2$.

With this key intermediate in hand, we were now prepared for introduction of the ethylamine side chain. In our first attempt we applied the glyoxyamide method involving reaction with $(COCl)_2$ followed by treatment with ammonia and subsequent reduction of both carbonyl groups. Thus, **8a** could be converted to the corresponding 14-glyoxyamide by treatment with $(COCl)_2$ in diethyl ether and subsequent introduction of ammonia gas in 91% yield. Unfortunately, all reduction attempts to transform the **8a**-glyoxyamide to the corresponding ethylamine analog using $LiAlH_4$ and borane failed. We were pleased to observe that another approach involving 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, proved to be successful. Thus, aminomethylation of **8a** using dimethylmethyleneammonium iodide (Eschenmoser's salt) in dichloromethane afforded the Mannich base (**9a**) in 77% yield. Treatment of **9a** with methyl iodide in dichloromethane and refluxing of the resulting trimethylammonium iodide with potassium cyanide and dicyclohexyl-[18]-crown-[6] afforded the nitrile (**10a**) in 69% overall yield. Reduction of **10a** using $LiAlH_4$ in diethyl ether and toluene provided the ethylamine (**11a**), which could be easily converted to the desired melatoninergic ligand **4a** by acetylation using acetic anhydride in dichloromethane in 88% yield.

## 2.2. Synthesis of the methoxy compound 4b

With a viable synthetic route in hand, we were now prepared for the synthesis of the melatonin-derived agent **4b**. The synthesis of the commercially unavailable starting material, 5-methoxyindoline-2-carboxylic acid (**5b**) was reported in the patent literature.[7] Stanton et al. obtained **5b**·HCl in three steps involving N-acetylation of 5-methoxyindole-2-carboxylic acid (**12**) followed by catalytic hydrogenation using platinum oxide and a subsequent deacetylation using 2 M aqueous HCl. Unfortunately, by adopting this literature strategy we were unable to obtain **5b**·HCl, after the deacetylation step, in any detectable amount. However, we were fortunate to develop a practical and an efficient alternative route to **5b**·HCl (Scheme 2). Our approach commenced with the acid catalyzed esterification of the free carboxylic acid functionality of **12** to give the known methyl ester (**13**) in 96% yield. Subsequent protection of the indole nitrogen of **13** by introduction of *tert*-butoxycarbonyl (Boc) group in acetonitrile in the presence of DMAP furnished **14** in 97% yield. The Boc protected ester was reduced to the corresponding dihydroindole derivative (**15**) by catalytic hydrogenation at 20 bar over Pd/C 10% in methanol. Hydrolysis of the ester functionality of **15** using LiOH in THF/$H_2O$ followed by N-deprotection in HCl/ethyl acetate mixture afforded the desired compound **5b**·HCl mp 175–178 °C (lit.[7] 90–92 °C) in an overall yield of 91% based on **12**. Such a huge difference of melting points indicates that the compound described in the patent literature can impossibly be **5b**·HCl. It should be mentioned that attempts to hydrolyze the ester functionality of **15** to the corresponding carboxylic acid **5b** without protection of the indolic nitrogen gave decomposition products or non-separable reaction mixture.

The next two reaction steps proceeded similarly to those in the non-methoxy series. Thus, condensation of **5b** using




**Scheme 2**. Reagents: (i) $H_2SO_4$, $CH_3OH$; (ii) $(Boc)_2O$, DMAP, MeCN; (iii) $H_2$, Pd/C 10%; MeOH; (iv) LiOH, THF, $H_2O$; and (v) HCl/ethyl acetate.

DCC in dichloromethane afforded dilactam (**6b**) in 45% yield. Reduction of both carbonyl groups of **6b** could be achieved by heating at reflux in THF with borane to afford the dimethoxytetrahydro-6*H*,13*H*-pyrazino[1,2-*a*;4-5-*a*′]diindole (**7b**) in excellent yield (95%). Introduction of the double bond in one of the indoline moieties of **7b** proved to be extremely difficult. While in the non-methoxy series, refluxing of **7a** in toluene with Pd/C 10% led to dehydrogenation of both indoline moieties,[5] attempts to introduce at least one double bond in **7b** with Pd/C 10% in refluxing toluene or xylene were unsuccessful. Furthermore, γ-$MnO_2$,[8] TCCA,[9] DDQ,[10] palladium dichloride,[11] sulfur,[12] and Swern oxidation[13] also failed to give the desired product, although each of these reagents had been reported to oxidize indoline to indole. Ultimately, we were pleased to find that dehydrogenation of **7b** to give **8b** could be achieved by heating **7b** at 150 °C without solvent in the presence of Pd/C 10% in 50% yield. For the introduction of the ethylamine side chain, we applied the same procedure as in the non-methoxy series. Thus, aminomethylation of **8b** using dimethylmethyleneammonium iodide (Eschenmoser's salt) in dichloromethane afforded the Mannich base (**9b**) in 98% yield. Quaternization of **9b** with methyl iodide in dichloromethane and refluxing of the resulting trimethylammonium iodide with potassium cyanide and dicyclohexyl-[18]-crown-[6] afforded the nitrile (**10b**) in 65% overall yield. Reduction of **10b** using $LiAlH_4$ in diethyl ether and THF provided the ethylamine (**11b**) (95%), which could be converted to the desired melatonin-derived agent **4b** by acetylation using acetic anhydride in dichloromethane in 60% yield.

### 2.3. Pharmacological studies

The affinity of compounds **4a** and **4b** for the human $MT_1$ or $MT_2$ melatonin receptors was measured by competition binding analysis using the radioligand 2-[$^{125}$I]-iodomelatonin. Melatonin competition assays were run in parallel and the affinity of melatonin for the $MT_1$ or $MT_2$ melatonin receptors is in the range of the reported literature. As shown in Table 1, compound **4a** displays micromolar affinity for both melatonin receptors and has 1700–2000 times less affinity for the receptors when compared to melatonin. However, compound **4b** displays nanomolar affinity for $MT_2$ receptors and, in fact, has 4.4-fold higher affinity for $MT_2$ receptors than $MT_1$ suggesting that **4b** may be more selective for $MT_2$ than $MT_1$ receptors. Also, compound **4b** has a better affinity profile for the $MT_2$ receptors when compared against melatonin with it having 68 times less affinity for the $MT_2$ receptors compared to 4000 times less affinity for the $MT_1$ receptors.

**Table 1**. Competition of **4a**, **4b**, and melatonin for 2-[$^{125}$I]-iodomelatonin binding to human $MT_1$ and $MT_2$ melatonin receptors expressed in CHO cells

| | $K_i$ (range of SEM) | | |
|---|---|---|---|
| Compound | $MT_1$ | $MT_2$ | *N* |
| **4a** | 1.0 μM (1.0–1.1 μM) | 1.7 μM (1.5–2.0 μM) | 5 |
| Melatonin | 0.45 nM (0.39–0.53 nM) | 1.0 nM (0.88–1.2 nM) | 5 |
| **4b** | 1.8 μM (1.1–2.9 μM) | 0.41 μM (0.22–0.77 μM) | 7 |
| Melatonin | 0.44 nM (0.12–1.1 nM) | 6.0 nM (1.9–19 nM) | 6 |

All competition binding experiments were performed on CHO whole cell lysates using 90–125 pM 2-[$^{125}$I]-iodomelatonin at 25 °C. The affinity ($K_D$) of 2-[$^{125}$I]-iodomelatonin for $MT_1$ and $MT_2$ receptors expressed in CHO cells is 80 pM and 150 pM, respectively; *N*: number of experiments.

### 2.4. Discussion

According to the existing pharmacophore model, potent $MT_2$ selective ligands include a methoxy group and a bulky lipophilic substituent in positions corresponding to N1 or C2 of melatonin that is located out of the plane of the indole ring.[3] Both ligands **4a** and **4b** are derived from desmethoxymelatonin and melatonin, respectively, by attaching an indoline moiety to the positions 1 and 2 of desmethoxymelatonin and melatonin, respectively, via methylene groups. Compound **4a** exhibited rather poor affinity for both $MT_1$ and $MT_2$ receptors ($K_i$=1.0 and 1.7 μM, respectively) when compared to melatonin and to other potent melatoninergic ligands.[2] Interestingly, while the introduction of the methoxy group in the indole ring of **4a** to give the melatonin-derived compound **4b** did not affect the $MT_1$ binding ($K_i$=1.8 μM), the affinity for the $MT_2$ receptors was increased 4.4-times confirming the importance of the methoxy group for binding at the $MT_2$ receptors. However, the $MT_2$ binding constant of **4b** ($K_i$=0.41 μM) indicates only a moderate affinity for $MT_2$ receptors when compared to other melatoninergic ligands.[2] The most likely explanation for the poor selectivity and moderate binding affinity of **4b** is the unfavorable spatial orientation of the indoline moiety, which is, due to its rigidity and bulkiness, not able to occupy the lipophilic binding pocket of the $MT_2$ receptors. Therefore, synthetic work on more flexible analogs of **4b** is continued in our laboratory.

## 3. Conclusions

In summary, in search for subtype selective ligands of melatonin receptors, we prepared two derivatives of the novel 6a,7-dihydro-6*H*,13*H*-pyrazino[1,2-*a*;4,5-*a*′]diindole ring system. The non-methoxy and methoxy analogs, **4a** and **4b**, respectively, were prepared in seven steps starting from the corresponding indoline-2-carboxylic acids **5a** and

**5b**, respectively. For the commercially unavailable 5-methoxy-indoline-2-carboxylic acid **5b**, a novel and an efficient synthesis was developed. While **4a** exhibited similar micromolar affinities for both melatonin receptors, the methoxy analog **4b** displayed moderate affinity for $MT_2$ receptors ($K_i$=0.41 μM) being 4.4-fold higher than for the $MT_1$ subtype.

## 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. Proton chemical shifts are referred to $CHCl_3$ (7.24 ppm) and DMSO-$d_6$ (2.55 ppm). Carbon chemical shifts are referred 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 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. 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-1,2-dicarboxylic acid 1-*tert*-butyl ester 2-methyl ester (14).** A solution of **13** (4.70 g, 22.9 mmol), di-*tert*-butyl dicarbonate (5.50 g, 25.2 mmol), and a catalytical amount of 4-(dimethylamino)-pyridine (0.55 g) in acetonitrile (60 ml) was stirred at ambient temperature for 72 h. The volatiles were removed under reduced pressure and the residue was dissolved in diethyl ether (100 ml). The resulted solution was washed with 1 M HCl (2×20 ml), water (2×20 ml), dried ($Na_2SO_4$), and evaporated under vacuum to give 6.76 g (97%) of **14** as a pale yellow solid, which was pure enough to be used in the next step without further purification. Analytical sample was obtained by silica gel chromatography (*n*-pentane/diethyl ether, 1:1) to afford **14** as a colorless crystalline solid mp 65–66 °C. FTIR (ATR) $\nu$=1740, 1715, 1321, 1067, 666 cm$^{-1}$. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 1.59 (s, 9H, –C($CH_3$)$_3$), 3.81 (s, 3H, $OCH_3$), 3.89 (s, 3H, $CO_2CH_3$), 6.99–7.02 (m, 3H, H-3, H-4, H-6), 7.95 (d, 1H, *J*=9.6 Hz, H-7). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 27.7 (C($CH_3$)$_3$), 52.2 ($CO_2CH_3$), 55.6 ($OCH_3$), 84.3 ($C$($CH_3$)$_3$), 103.6 (C-3), 114.5 (C-4), 115.7 (C-6), 116.3 (C-7), 128.1 (C-2), 130.8 (C-3a), 132.6 (C-7a), 149.2 (urethane), 156.2 (C-5), 162.2 (ester). MS (EI): *m*/*z* (%)=305 ($M^+$, 8), 205 (46), 173 (100). Anal. Calcd for $C_{16}H_{19}NO_5$: C, 62.93; H, 6.28; N, 4.59. Found: C, 63.05; H, 6.31; N, 4.57.

**4.1.2. 5-Methoxy-2,3-dihydro-1*H*-indole-1,2-dicarboxylic acid 1-*tert*-butyl ester 2-methyl ester (15).** Pd/C 10% (0.60 g) was added to a stirred solution of **14** (4.41 g, 14.4 mmol) in methanol (160 ml). The reaction mixture was hydrogenated under 20 bar of $H_2$ for 18 h at room temperature. The catalyst was removed by filtration and the solvent was evaporated in vacuo to give **15** as a white solid, which was recrystallized from methanol to yield 3.82 g (86%) of pure **15** as colorless crystals mp 88–90 °C. FTIR (ATR) $\nu$=1737, 1704, 1491, 1021, 629 cm$^{-1}$. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 1.46 (s, 9H, –C($CH_3$)$_3$), 3.05 (dd, 1H, *J*=16.7, 4.3 Hz, H$^a$-3), 3.45 (dd, 1H, *J*=16.7, 12.1 Hz, H$^b$-3), 3.70 (s, 3H, $CO_2CH_3$), 3.73 (s, 3H, $OCH_3$), 4.81–4.89 (m, 1H, H-2), 6.67–6.71 (m, 2H, $H_{arom.}$), 6.75–7.78 (m, 1H, $H_{arom.}$). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 28.3 (C($CH_3$)$_3$), 32.8 (C-3), 52.3 ($CO_2CH_3$), 55.7 ($OCH_3$), 60.6 (C-2), 81.1 ($C$($CH_3$)$_3$), 110.9, 112.5, 115.1 ($C_{arom.}$), 129.2 (C-3a), 136.2 (C-7a), 151.6 (urethane), 155.7 (C-5), 172.5 (ester). MS (EI): *m*/*z* (%)=307 ($M^+$, 5), 207 (28), 148 (100). Anal. Calcd for $C_{16}H_{21}NO_5$: C, 62.51; H, 6.89; N, 4.56. Found: C, 62.50; H, 6.71; N, 4.49.

**4.1.3. 5-Methoxy-2,3-dihydro-1*H*-indole-2-carboxylic acid hydrochloride (5b·HCl).** Lithium hydroxide solution 2 M (30 ml) was added to a stirred solution of **15** (4.40 g, 14.3 mmol) in THF (80 ml). The resulting reaction mixture was stirred at room temperature for 18 h and the solvent was evaporated under reduced pressure. The residue was diluted with water (20 ml) and extracted with diethyl ether (2×25 ml). The aqueous layer was acidified using 0.5 M potassium hydrogen sulfate solution and extracted with diethyl ether (3×30 ml). The organic extracts were combined, dried ($Na_2SO_4$), and evaporated under vacuum to give 4.19 g (100%) of 5-methoxy-2,3-dihydro-1*H*-indole-1,2-dicarboxylic acid 1-*tert*-butyl ester **16** as a pale yellow powder mp 104–105 °C. The crude **16** was dissolved in approximately 2.5 M dry hydrogen chloride/ethyl acetate solution (33 ml) and the reaction mixture was stirred at ambient temperature for 1 h. The precipitated solid was filtered off, washed with diethyl ether (20 ml), and dried to give 2.81 g (85%) of **5b**·HCl as a white solid mp 175–178 °C (lit.[7] 90–92 °C). FTIR (ATR) $\nu$=2967–2523, 1741, 1499, 1026, 663 cm$^{-1}$. $^1$H NMR (400 MHz, $D_2O$): $\delta$ 3.39 (dd, 1H, *J*=16.7, 6.6 Hz, H$^a$-3), 3.60 (dd, 1H, *J*=16.7, 9.6 Hz, H$^b$-3), 3.73 (s, 3H, $OCH_3$), 4.95 (dd, 1H, *J*=9.6, 6.6 Hz, H-2), 6.89 (dd, 1H, *J*=8.6, 1.76 Hz, H-6), 6.93 (s, 1H, H-4), 7.32 (d, 1H, *J*=8.6 Hz, H-7). $^{13}$C NMR (100 MHz, $D_2O$): $\delta$ 33.0 (C-3), 55.8 ($OCH_3$), 60.6 (C-2), 110.9 (C-4), 114.8 (C-6), 120.0 (C-7), 127.3 (C-3a), 135.4 (C-7a), 160.6 (C-5), 171.4 (ester). MS (EI): *m*/*z* (%)=193 ($M^+$, 31), 148 (100), 133 (53). Anal. Calcd for $C_{10}H_{12}ClNO_3$: C, 52.39; H, 5.28; N, 6.11. Found: C, 52.02; H, 5.22; N, 5.99.

**4.1.4. 6a,7,13a,14-Tetrahydropyrazino[1,2-*a*;4,5-*a*′]diindole-6,13-dione (6a).** DCC (6.0 g, 29.1 mmol) was added to the solution of the racemic 2,3-dihydro-1*H*-indole-2-carboxylic acid **5a** (2.5 g, 15.3 mmol) in anhydrous THF and the reaction mixture was stirred at room temperature for 2 h. Precipitates were filtered off and the filtrate was evaporated under reduced pressure. The oily residue was purified by silica gel chromatography ($CH_2Cl_2$) to give **6a** (0.92 g, 42%) as a colorless solid mp 264 °C. FTIR (ATR) $\nu$=2918, 2851, 1674, 1599, 1483, 1458, 1413 cm$^{-1}$. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 3.43 (dd, 2H, *J*=16.7, 10.4 Hz, H$^a$-7, H$^a$-14), 3.79 (dd, 2H, *J*=16.7, 8.8 Hz, H$^b$-7, H$^b$-14), 4.96 (dd, 2H, *J*=10.4, 8.8 Hz, H-6a, H-13a), 7.09 (m, 2H, H-2, H-9), 7.24 (m, 4H, H-1, H-8, H-3, H-10), 8.09 (d, 2H, *J*=7.8 Hz, H-4, H-11). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 30.1 (C-7, C-14), 61.7 (C-6a, C-13a), 115.8

(C-4, C-11), 125.0 (C-3, C-10, C-2, C-9), 127.9 (C-1, C-8), 129.7 (C-7a, C-14a), 140.5 (C-4a, C-11a), 164.2 (2×C=O). MS (EI): *m/z* (%)=290.1 ($M^+$, 100%), 143.1 (10), 117.1 (84). Anal. Calcd for $C_{18}H_{14}N_2O_2$: C, 74.47; H, 4.86; N, 9.65. Found: C, 74.52; H, 4.91; N, 9.44.

**4.1.5. 2,9-Dimethoxy-6a,7,13a,14-tetrahydropyrazino[1,2-*a*;4,5-*a′*]diindole-6,13-dione (6b).** Triethylamine (2.5 ml, 17.8 mmol) and DCC (8.00 g, 38.8 mmol) were added to a stirred suspension of **5b**·HCl (4.00 g, 17.4 mmol) in dry $CH_2Cl_2$ (80 ml) at ambient temperature. The resulting reaction mixture was stirred at room temperature for 18 h. The precipitated solids were filtered off and washed with $CH_2Cl_2$ (20 ml). The combined filtrate and washing were washed with 1 N HCl (2×15 ml), water (2×20 ml), and cooled at −30 °C for 18 h. The precipitates were filtered off and the organic solvent was removed under vacuum. The residue was recrystallized from methanol to afford 1.37 g (45%) of **6b** as a pale yellow solid mp 254–255 °C. FTIR (ATR) $\nu$=1660, 1600, 1488, 1400, 822 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 3.37 (dd, 2H, *J*=16.9, 10.4 Hz, $H^a$-7, $H^a$-14), 3.72 (dd, 2H, *J*=16.9, 8.9 Hz, $H^b$-7, $H^b$-14), 3.77 (s, 6H, 2×$OCH_3$), 4.94 (t, 2H, *J*=9.4 Hz, H-6a, H-13a), 6.75 (dd, 2H, *J*=8.6, 2.5 Hz, H-3, H-10), 6.79–6.82 (m, 2H, H-1, H-8), 7.98 (d, 2H, *J*=8.6 Hz, H-4, H-11). $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 30.5 (C-7, C-14), 55.6 (2×$OCH_3$), 61.8 (C-6a, C-13a), 111.0 (C-1, C-8), 112.6 (C-3, C-10), 116.4 (C-4, C11), 131.4, 134.2 (C-4a, C-11a, C-7a, C-14a), 157.0 (C-2, C-9), 163.4 (2×lactam). MS (EI): *m/z* (%)=350 ($M^+$, 41), 147 (100), 132 (56). Anal. Calcd for $C_{20}H_{18}N_2O_4$: C, 68.55; H, 5.18; N, 8.00. Found: C, 68.39; H, 5.27; N, 7.90.

**4.1.6. 6a,7,13a,14-Tetrahydro-6*H*,13*H*-pyrazino[1,2-*a*;4,5-*a′*]diindole (7a).** Borane–THF complex 1 M (15 ml) was added dropwise to a stirred solution of **6a** (0.90 g, 3.1 mmol) in dry THF (100 ml) at room temperature. The reaction mixture was refluxed for 17 h, allowed to cool and 2 M HCl (8 ml) was added dropwise under ice-cooling. After heating for ½ h at reflux, the reaction mixture was basified with 25% ammonia under ice-cooling and extracted with $CH_2Cl_2$ (3×100 ml). The combined organic layers were washed with water (3×15 ml), dried ($MgSO_4$), and evaporated in vacuo to yield 0.80 g (98%) of **7a** as a white solid, which was pure enough to be used in the next step without further purification. Analytical sample was obtained by recrystallization from ethyl acatate to give **7a** as colorless crystals mp 140 °C. The IR, NMR, and MS data of **7a** are identical with the corresponding data of the (6a*S*,13a*S*)-**7a** stereomer.[5]

**4.1.7. 2,9-Dimethoxy-6a,7,13a,14-tetrahydro-6*H*,13*H*-pyrazino[1,2-*a*;4,5-*a′*]diindole (7b).** Borane–THF complex 1 M (8.5 ml) was added dropwise to a stirred suspension of **6b** (0.63 g, 1.8 mmol) in dry THF (40 ml) at room temperature. The reaction mixture was refluxed for 17 h, allowed to cool and 2 M HCl (8.5 ml) was added dropwise under ice-cooling. The resulting reaction mixture was refluxed for 1 h, allowed to cool, basified with 25% ammonium hydroxide solution and extracted with ethyl acetate (3×20 ml). The combined organic layers were washed with water (2×15 ml), dried ($Na_2SO_4$), and evaporated in vacuo to yield 0.55 g (95%) of **7b** as a white solid, which was pure enough to be used in the next step without further purification. Analytical sample was obtained by recrystallization from ethyl acetate to give **7b** as colorless crystals mp 129–131 °C. FTIR (ATR) $\nu$=1490, 1215, 1136, 636 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 2.69 (dd, 2H, *J*=16.2, 2.6 Hz, $H^a$-7, $H^a$-14), 2.88 (dd, 2H, *J*=12.1, 10.4 Hz, $H^a$-6, $H^a$-13), 3.25 (dd, 2H, *J*=16.2, 9.6 Hz, $H^b$-7, $H^b$-14), 3.39 (dd, 2H, *J*=10.1, 3.0 Hz, $H^b$-6, $H^b$-13), 3.72 (s, 6H, 2×$OCH_3$), 4.35–4.42 (m, 2H, H-6a, H-13a), 6.34 (d, 2H, *J*=8.3 Hz, H-4, H-11), 6.62 (dd, 2H, *J*=8.3, 2.5 Hz, H-3, H-10), 6.70–6.72 (m, 2H, H-1, H-8). $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 33.2 (C-7, C-14), 51.8 (C-6, C-13), 55.7 (2×$OCH_3$), 57.0 (C-6a, C-13a), 108.3 (C-4, C-11), 111.8 (C-1, C-8), 112.1 (C-3, C-10), 129.7 (C-7a, C-14a), 146.1 (C-4a, C-11a), 153.4 (C-2, C-9). HRMS (ESI, pos.) $C_{20}H_{20}N_2O_2H^+$: *m/z* calcd 323.1759, *m/z* found 323.1755.

**4.1.8. 6a,7-Dihydro-6*H*,13*H*-pyrazino[1,2-*a*;5-*a′*]diindole (8a).** Pd/C 10% (0.25 g) was added to a solution of **7a** (0.65 g, 2.5 mmol) in toluene (100 mL). The reaction mixture was heated for 3 h at 80 °C. Pd/C was filtered off through a pad of Celite®545 and washed with toluene. The solvent was removed in vacuo and the residue purified by silica gel chromatography ($CH_2Cl_2$/hexane, 1:1) to give **8a** (0.31 g, 48%) as a yellow crystalline solid mp 170 °C. FTIR (ATR) $\nu$=2833, 2787, 1609, 1476, 1450, 1371, 1336, 1240, 1173, 734 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 2.89 (dd, 1H, *J*=14.9, 10.9 Hz, $H^a$-7), 3.24 (dd, 1H, *J*=14.9, 7.6 Hz, $H^b$-7), 3.83 (dddd, 1H, *J*=10.9, 10.9, 7.6, 3.8 Hz, H-6a), 3.97 (t, 1H, *J*=10.9 Hz, $H^a$-6), 4.19 (dd, 1H, *J*=14.9, 1.2 Hz, $H^a$-13), 4.52 (dd, 1H, *J*=10.9, 3.8 Hz, $H^b$-6), 4.90 (d, 1H, *J*=14.9 Hz, $H^b$-13), 6.35 (s, 1H, H-14), 6.63 (d, 1H, *J*=7.6 Hz, H-11), 6.79 (m, 1H, H-9), 7.09–7.20 (m, 4H, H-2, H-10, H-3, H-8), 7.31 (d, 1H, *J*=8.1 Hz, H-4), 7.57 (d, 1H, *J*=7.8 Hz, H-1). $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 33.2 (C-7), 44.6 (C-13), 47.2 (C-6), 61.6 (C-6a), 97.2 (C-14), 107.4 (C-11), 108.6 (C-4), 119.4 (C-9), 120.0, 120.2, 120.9 (C-2, C-1, C-8), 124.7 (C-3), 127.8 (C-10), 128.9 (C-14a), 132.6 (C-7a), 135.9 (C-4a), 150.9 (C-11a). MS (EI): *m/z* (%)=260.1 ($M^+$, 35), 143.1 (100), 115.1 (28). Anal. Calcd for $C_{18}H_{16}N_2$: C, 83.05; H, 6.19; N, 10.76. Found: C, 82.82; H, 6.52; N, 10.42.

**4.1.9. 2,9-Dimethoxy-6a,7-dihydro-6*H*,13*H*-pyrazino[1,2-*a*;5-*a′*]diindole (8b).** A mixture of **7b** (0.6 g, 1.86 mmol) and Pd/C 10% (0.10 g) was heated at 150 °C for 1 h. The reaction mixture was allowed to cool, $CH_2Cl_2$ (2×25 ml) was added and the catalyst was removed by filtration. The organic extracts were combined, dried ($Na_2SO_4$), and evaporated under reduced pressure. The residue was recrystallized from chloroform to afford 0.3 g (50%) of **8b** as a light brown solid mp 256–257 °C. FTIR (ATR) $\nu$=2831, 2360, 1485, 1238, 1026, 844, 735 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 2.85 (dd, 1H, *J*=14.6, 7.3 Hz, $H^a$-7), 3.17 (dd, 1H, *J*=14.6, 7.3 Hz, $H^b$-7), 3.69–3.73 (m, 1H, H-6a), 3.75 (s, 3H, $OCH_3$), 3.84 (s, 3H, $OCH_3$), 3.91–3.97 (m, 1H, $H^a$-6), 4.05 (d, 1H, *J*=14.4 Hz, $H^a$-13), 4.44 (dd, 1H, *J*=10.6, 3.8 Hz, $H^b$-6), 4.81 (d, 1H, *J*=14.4 Hz, $H^b$-13), 6.26 (s, 1H, H-14), 6.54 (d, 1H, *J*=8.3 Hz, H-11), 6.71 (dd, 1H, *J*=8.3, 2.5 Hz, H-10), 6.82–6.84 (m, 2H, H-3, H-8), 7.04 (d, 1H, *J*=2.3 Hz, H-1), 7.18 (d, 1H, *J*=8.6 Hz, H-4). $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 33.5 (C-7), 45.6 (C-13), 47.2 (C-6), 55.9, 56.0 (2×$OCH_3$), 62.4 (C-6a), 96.9 (C-1), 102.3 (C-14), 107.8 (C-11), 109.2 (C-4), 110.8 (C-3), 111.9 (C-10),

112.4 (C-8), 128.9, 130.6, 131.3, 133.5, 145.1, (C-4a, C-7a, C-11a, C-13a, C-14a), 153.9, 154.5 (C-2, C-9). HRMS (ESI, pos.) $C_{20}H_{20}N_2O_2^{\bullet}H^+$-2: *m/z* calcd 319.1447, *m/z* found 319.1441.

**4.1.10. (13a,14-Dihydro-6*H*,13*H*-pyrazino[1,2-*a*;4,5-*a*′]-diindol-7-yl)-dimethylamine (9a).** Dimethylmethylenimminium iodide (0.40 g, 2.2 mmol) was added to a solution of compound **8a** (0.34 g, 1.3 mmol) in dry $CH_2Cl_2$ (100 mL). After heating for 1 h at reflux, the reaction mixture was made basic with 25% ammonia. The organic layer was separated, washed with water and dried over $MgSO_4$. The solvent was removed in vacuo and the residue purified by silica gel chromatography ($CHCl_3$/methanol/25% ammonia, 100:10:1) to give **9a** (0.32 g, 77%) as a colorless crystalline solid mp 132 °C. FTIR (ATR) $\nu$=2957, 2932, 2814, 1605, 1457, 1334, 1244, 757, 740 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 2.27 (s, 6H, $NMe_2$), 2.89 (dd, 1H, *J*=14.6, 10.9 Hz, $H^a$-7), 3.24 (dd, 1H, *J*=14.6, 7.6 Hz, $H^b$-7), 3.55 (d, 1H, *J*=13.1 Hz, –*H*CH–$NMe_2$), 3.61 (d, 1H, *J*=13.1 Hz, –HC*H*–$NMe_2$), 3.82 (dddd, 1H, *J*=10.9, 10.9, 7.6, 3.8 Hz, H-6a), 3.96 (t, 1H, *J*=10.9 Hz, $H^a$-6), 4.12 (d, 1H, *J*=15.4 Hz, $H^a$-13), 4.51 (dd, 1H, *J*=10.9, 3.8 Hz, $H^b$-6), 4.95 (d, 1H, *J*=15.1 Hz, $H^b$-13), 6.68 (d, 1H, *J*=7.6 Hz, H-11), 6.79 (m, 1H, H-9), 7.11–7.21 (m, 4H, H-2, H-10, H-3, H-8), 7.29 (d, 1H, *J*=8.1 Hz, H-4), 7.67 (d, 1H, *J*=7.8 Hz, H-1). $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 33.3 (C-7), 43.1 (C-13), 45.8 (–$NMe_2$), 47.2 (C-6), 53.5 (–*C*$H_2$–$NMe_2$), 61.5 (C-6a), 107.0 (C-14), 107.5 (C-11), 108.6 (C-4), 118.9 (C-1), 119.4 (C-9), 119.8 (C-2), 120.1 (C-3), 124.8 (C-10), 127.8 (C-8), 128.8, 128.9 (C-7a, C-14a), 130.9 (C-13a), 135.7 (C-4a), 151.0 (C-11a). HRMS (ESI, pos.) $C_{21}H_{23}N_3^{\bullet}H^+$: *m/z* calcd 318.1970, *m/z* found 318.1965.

**4.1.11. 2,9-Dimethoxy-14-[(dimethylamino)methyl]-6a,7-dihydro-6*H*,13*H*-pyrazino[1,2-*a*;4,5-*a*′]diindole (9b).** Dimethylmethylenimminium iodide (0.27 g, 1.46 mmol) was added to a solution of **8b** (0.38 g, 1.19 mmol), in dry $CH_2Cl_2$ (150 ml). The reaction mixture was refluxed for 1 h, 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 give 0.44 g (98%) of **9b** as a dark red solid, which was used in the next step without further purification. Analytical sample was obtained by recrystallization from methanol to give **9b** as a light red solid mp 180–182 °C. FTIR (ATR) $\nu$=2941, 2360, 1487, 1236, 1030, 794, 609 $cm^{-1}$. $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.27 (s, 6H, $NMe_2$), 2.83 (dd, 1H, *J*=14.6, 11.4 Hz, $H^a$-7), 3.15 (dd, 1H, *J*=14.6, 7.3 Hz, $H^b$-7), 3.63–3.70 (m, 1H, H-6a), 3.76 (s, 3H, $OCH_3$), 3.87 (s, 3H, $OCH_3$), 3.89 (s, 2H, C*H*$_2$–$NMe_2$), 3.91–3.94 (m, 1H, $H^a$-6), 3.97 (d, 1H, *J*=14.9 Hz, $H^b$-6), 4.41 (dd, 1H, *J*=10.6, 3.8 Hz, $H^b$-6), 4.87 (d, 1H, *J*=14.9 Hz, $H^b$-13), 6.60 (d, 1H, *J*=8.6 Hz, H-11), 6.72 (dd, 1H, *J*=8.6, 2.5 Hz, H-10), 6.82–6.85 (m, 2H, H-3, H-8), 7.12 (d, 1H, *J*=2.3 Hz, H-1), 7.16 (d, 1H, *J*=8.6 Hz, H-4). $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ 33.5 (C-7), 44.5 (C-13), 45.5 ($NMe_2$), 47.2 (C-6), 53.5 ($CH_2$–$NMe_2$), 56.01, 56.03 (2×$OCH_3$), 62.3 (C-6a), 101.3 (C-1), 107.9 (C-11), 109.1 (C-4), 110.7 (C-3), 111.9 (C-10), 112.4 (C-8), 128.2, 130.6, 130.8, 132.7, 145.0 (C-4a, C-7a, C-11a, C-13a, C-14a), 153.4, 153.9 (C-2, C-9). HRMS (ESI, pos.) $C_{23}H_{27}N_3O_2^{\bullet}H^+$-2: *m/z* calcd 376.2025, *m/z* found 376.2008.

**4.1.12. (6a,7-Dihydro-6*H*,13*H*-pyrazino[1,2-*a*;4,5-*a*′]-diindol-14-yl)-acetonitrile (10a).** Methyl iodide (0.5 ml) was added to a solution of **9a** (0.17 g, 0.54 mmol) in dry $CH_2Cl_2$ (50 ml). The reaction mixture was stirred at room temperature for 1 h. The volatiles were removed under vacuum and the residual ammonium salt was dissolved in dry acetonitrile (150 ml). Dicyclohexyl-[18]-crown-[6] (0.30 g) and potassium cyanide (0.50 g) were added and resulting reaction mixture was heated at reflux for 2 h. The solvent was evaporated under reduced pressure and the residue was subjected to silica gel chromatography ($CHCl_3$) to afford **10a** (0.11 g, 69%) as a brown solid mp 154 °C. FTIR (ATR) $\nu$=2881, 2830, 1607, 1474, 1454, 1340, 1250, 754, 736 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 2.90 (dd, 1H, *J*=14.9, 10.9 Hz, $H^a$-7), 3.25 (dd, 1H, *J*=14.9, 7.6 Hz, $H^b$-7), 3.75–3.86 (m, 3H, H-6a, –C*H*$_2$CN), 3.96 (d, 1H, *J*=10.9 Hz, $H^a$-6), 4.15 (d, 1H, *J*=14.9 Hz, $H^a$-13), 4.51 (dd, 1H, *J*=10.9, 3.8 Hz, $H^b$-6), 4.93 (d, 1H, *J*=15.1 Hz, $H^b$-13), 6.68 (d, 1H, *J*=7.8 Hz, H-11), 6.81 (m, 1H, H-9), 7.15–7.27 (m, 4H, H-2, H-10, H-3, H-8), 7.32 (d, 1H, *J*=7.8 Hz, H-4), 7.59 (d, 1H, *J*=7.8 Hz, H-1). $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 12.9 (–*C*$H_2$CN), 33.3 (C-7), 43.3 (C-13), 47.3 (C-6), 61.3 (C-6a), 97.5 (–CN), 107.6 (C-11), 109.0 (C-4), 117.6 (C-14), 117.7 (C-1), 119.8 (C-9), 120.6 (C-2), 121.9 (C-3), 124.8 (C-10), 127.9 (C-8), 126.9 (C-14a), 128.8 (C-7a), 130.4 (C-13a), 135.7 (C-4a), 150.6 (C-11a). MS (EI): *m/z* (%)=299.1 ($M^+$, 63), 272.1 (14), 259.1 (14), 182.1 (100). Anal. Calcd for $C_{20}H_{17}N_3$: C, 80.24; H, 5.72; N, 14.04. Found: C, 79.81; H, 5.75; N, 13.64.

**4.1.13. (2,9-Dimethoxy-6a,7-dihydro-6*H*,13*H*-pyrazino[1,2-*a*;4,5-*a*′]diindol-14-yl)-acetonitrile (10b).** Methyl iodide (0.1 ml) was added to a solution of **9b** (0.21 g, 0.56 mmol) in dry $CH_2Cl_2$ (20 ml). The reaction mixture was stirred at room temperature for 1 h. The volatiles were removed under vacuum to afford **9b** methoiodide mp 134–136 °C. This crude ammonium salt was suspended in dry acetonitrile (20 ml), dicyclohexyl-[18]-crown-[6] (0.24 g) and potassium cyanide (0.47 g) were added. The resulting reaction mixture was heated at reflux for 3 h. The solvent was evaporated under reduced pressure and the residue was dissolved in ethyl acetate (40 ml). The organic layer was washed with water (3×15 ml), dried ($Na_2SO_4$), and evaporated in vacuo. The residue was purified by silica gel chromatography (ethyl acetate/chloroform, 8:2) to afford **10b** (0.13 g, 65%) as a dark red viscous oil that solidified on cooling in the freezer. FTIR (ATR) $\nu$=2918, 2850, 2361, 1485, 1230, 799, 735 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 2.82 (dd, 1H, *J*=14.9, 11.6 Hz, $H^a$-7), 3.15 (dd, 1H, *J*=14.9, 7.3 Hz, $H^b$-7), 3.69–3.71 (m, 1H, H-6a), 3.73 (s, 2H, C*H*$_2$–CN), 3.75 (s, 3H, $OCH_3$), 3.85 (s, 3H, $OCH_3$), 3.90 (d, 1H, *J*=10.9 Hz, $H^a$-6), 3.98 (d, 1H, *J*=14.9 Hz, $H^a$-13), 4.40 (dd, 1H, *J*=10.9, 3.8 Hz, $H^b$-6), 4.82 (d, 1H, *J*=14.9 Hz, $H^b$-13), 6.57 (d, 1H, *J*=8.3 Hz, H-11), 6.71 (dd, 1H, *J*=8.3, 2.5 Hz, H-10), 6.82 (d, 1H, *J*=2.2 Hz, H-8), 6.87 (dd, 1H, *J*=8.8, 2.2 Hz, H-3), 6.99 (d, 1H, *J*=2.3 Hz, H-1), 7.17 (d, 1H, *J*=8.8 Hz, H-4). $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 12.8 (*C*$H_2$CN), 33.4 (C-7), 44.1 (C-13), 47.2 (C-6), 55.9, 56.0 (2×$OCH_3$), 62.1 (C-6a), 97.1 (CN), 99.7 (C-1), 107.9 (C-11), 109.7 (C-4), 111.8 (C-3), 111.9 (C-10), 112.5 (C-8), 106.1, 117.7, 127.2, 128.8, 130.5, 144.7 (C-4a, C-7a, C-11a, C-13a, C-14, C-14a), 154.2, 154.8 (C-2,

C-9). HRMS (ESI, pos.) $C_{23}H_{27}N_3O$;$2H^+$-2: *m*/*z* calcd 358.1555, *m*/*z* found 358.1550.

**4.1.14. *N*-[2-(6a,7-Dihydro-6*H*,13*H*-pyrazino[1,2-*a*;4,5-*a*′]diindol-14-yl)-ethyl]-acetamide (4a).** A solution of **10a** (0.100 g, 0.334 mmol) in dry toluene (30 ml) was added to a stirred suspension of $LiAlH_4$ (0.30 g) in dry diethyl ether (30 ml) at 0–5 °C. The reaction mixture was heated at 40 °C for 2 h. Water (3 ml) was added dropwise under ice-cooling and the reaction mixture was stirred for 1 h at room temperature. The precipitate was filtered off and washed with diethyl ether. The combined filtrate and washings were dried ($Na_2SO_4$), filtered, and evaporated under vacuum to afford 0.100 g (99%) of 2-(6a,7-dihydro-6*H*,13*H*-pyrazino[1,2-*a*;4,5-*a*′]diindol-14-yl)-ethylamine **11a** as a pale yellow viscous oil. A stirred solution of **11a** (0.100 g, 0.329 mmol) in dry $CH_2Cl_2$ (10 ml) was treated with triethylamine (0.30 ml) and acetic anhydride (0.20 ml, 2.11 mmol) at 0–5 °C. The reaction mixture was stirred at ambient temperature for 3 h. The solvent was evaporated and the residue was purified by silica gel chromatography (chloroform/ methanol/25% ammonia 100:10:1) to afford **4a** (0.110 g, 88%) as a yellow solid mp 88 °C. FTIR (ATR) $\nu$=2925, 1648, 1608, 1551, 1477, 1454, 1373, 1339, 1298, 1247, 741 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 1.90 (s, 3H, $CH_3$), 2.87 (dd, 1H, *J*=14.8, 10.9 Hz, $H^a$-7), 2.91–3.03 (m, 2H, –C$H_2$–$CH_2$–NH–), 3.24 (dd, 1H, *J*=14.9, 7.6 Hz, $H^b$-7), 3.46–3.62 (m, 2H, –$CH_2$–C$H_2$–NH–), 3.79 (dddd, 1H, *J*=11.0, 11.0, 7.6, 3.6 Hz, H-6a), 3.94 (d, 1H, *J*=10.9 Hz, $H^a$-6), 4.05 (d, 1H, *J*=14.9 Hz, $H^a$-13), 4.51 (dd, 1H, *J*=10.9, 3.6 Hz, $H^b$-6), 4.86 (d, 1H, *J*=14.9 Hz, $H^b$-13), 5.52 (br, 1H, NH), 6.66 (d, 1H, *J*=7.8 Hz, H-11), 6.80 (m, 1H, H-9), 7.11–7.23 (m, 4H, H-2, H-10, H-3, H-8), 7.31 (d, 1H, *J*=8.0 Hz, H-4), 7.55 (d, 1H, *J*=7.8 Hz, H-1). $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 23.4 ($CH_3$), 24.1 (–C$H_2$–$CH_2$–NH–), 33.3 (C-7), 39.9 (–$CH_2$–C$H_2$–NH–), 43.5 (C-13), 47.2 (C-6), 61.7 (C-6a), 106.5 (C-14), 107.6 (C-11), 108.8 (C-4), 118.2 (C-1), 119.6 (C-9), 119.8 (C-2), 121.2 (C-3), 124.7 (C-10), 127.8 (C-8), 128.1 (C-14a), 128.9 (C-7a), 129.8 (C-13a), 136.0 (C-4a), 150.8 (C-11a), 170.1 (C═O). MS (EI): *m*/*z* (%)=345.2 ($M^+$, 24), 259.1 (100), 259.1 (14), 156.1 (42). Anal. Calcd for $C_{22}H_{23}N_3O$: C, 76.49; H, 6.71; N, 12.16. Found: C, 76.10; H, 6.70; N, 11.80.

**4.1.15. *N*-[2-(2,9-Dimethoxy-6a,7-dihydro-6*H*,13*H*-pyrazino[1,2-*a*;4,5-*a*′]diindol-14-yl)-ethyl]-acetamide (4b).** A solution of **10b** (0.300 g, 0.83 mmol) in dry THF (30 ml) was added to a stirred suspension of $LiAlH_4$ (0.650 g, 17.00 mmol) in dry diethyl ether (20 ml) at 0–5 °C. The reaction mixture was refluxed for 3 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 filtrates and washings were dried ($Na_2SO_4$), filtered and evaporated under vacuum to afford 0.290 g (96%) of 2-(2,9-dimethoxy-6a,7-dihydro-6*H*,13*H*-pyrazino[1,2-*a*;4,5-*a*′]diindol-14-yl)-ethylamine **11b** as a pale yellow viscous oil. A stirred solution of **11b** (0.290 g, 0.80 mmol) in dry $CH_2Cl_2$ (20 ml) was treated with triethylamine (0.39 ml, 2.80 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 and the residue was purified by silica gel chromatography (ethyl acetate/chloroform, 8:2) to yield **4b** (0.190 g, 60%) as an orange solid mp 101–104 °C. FTIR (ATR) $\nu$=3480–3360, 2361, 1639, 1488, 1228, 742 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 1.90 (s, 3H, $CH_3$), 2.82–2.96 (m, 3H, $H^a$-7, C$H_2$–$CH_2$–N), 3.17 (dd, 1H, *J*=14.9 Hz, $H^b$-7), 3.45–3.58 (m, 2H, $CH_2$–C$H_2$–N), 3.62–3.70 (m, 1H, H-6a), 3.75 (s, 3H, $OCH_3$), 3.85 (s, 3H, $OCH_3$), 3.88–3.94 (m, 2H, $H^a$-6, $H^a$-13), 4.43 (dd, 1H, *J*=10.9, 3.8 Hz, $H^b$-6), 4.77 (d, 1H, *J*=14.7 Hz, $H^b$-13), 5.57 (br, 1H, NH), 6.58 (d, 1H, *J*=8.3 Hz, H-11), 6.71 (dd, 1H, *J*=8.3, 1.9 Hz, H-10), 6.82 (s, 1H, H-8), 6.85 (dd, 1H, *J*=8.8, 2.0 Hz, H-3), 7.00 (d, 1H, *J*=2.0 Hz, H-1), 7.18 (d, 1H, *J*=8.8 Hz, H-4). $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 23.5 ($CH_3$), 24.1 (C$H_2$–$CH_2$–N), 33.5 (C-7), 39.8 ($CH_2$–C$H_2$–N), 44.4 (C-13), 47.2 (C-6), 55.99, 56.03 (2×$OCH_3$), 62.5 (C-6a), 100.4 (C-1), 107.9 (C-11), 109.4 (C-4), 111.1 (C-3), 111.5 (C-10), 112.5 (C-8), 106.1, 128.5, 130.6, 130.7, 131.2, 145.0 (C-4a, C-7a, C-11a, C-13a, C-14, C-14a), 154.1, 154.4 (C-2, C-9), 170.1 (C═O). HRMS (ESI, pos.) $C_{24}H_{27}N_3O$;$3H^+$-2: *m*/*z* calcd 404.1974, *m*/*z* found 404.1970.

### 4.2. Binding assays

To assess the affinity, competition binding assays were performed on compounds **4a** and **4b**. Chinese hamster ovary (CHO) cells stably transfected with the human $MT_1$ or $MT_2$ melatonin receptors were grown to confluence on 10-cm diameter culture dishes and then resuspended in 50 mM ice-cold Tris–HCl (pH 7.4 containing protease inhibitors). CHO whole cell lysates were then added to tubes containing Tris–HCl (50 mM containing protease inhibitors) and 2-[$^{125}$I]-iodomelatonin (90–125 pM) in the absence or presence of compound **4a** or **4b** (1 pM–100 μM). All reactions were incubated for 1 h at 25 °C, rapidly filtered and counted in a gamma counter.

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