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Synthesis and pharmacological evaluation of 1,2,3,4-tetrahydropyrazino [1,2-*a*]indole and 2-[(phenylmethylamino)methyl]-1*H*-indole analogues as novel melatoninergic ligands

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ABSTRACT

Two novel series of melatonin-derived compounds have been synthesized and pharmacologically evaluated at the MT_1 and MT_2 subtypes of melatonin receptors. Compounds **12b–c** are non-selective high-affinity MT_1 and MT_2 receptor ligands (K_i = 7–11 nM). Compound **12b** had little intrinsic activity at the MT_1 receptor and no intrinsic activity at the MT_2 receptor. Compound **20d** displayed the highest MT_2 binding affinity (K_i = 2 nM) and moderate selectivity toward the MT_2 subtype (K_i MT_1/MT_2 ratio = 8) behaving as MT_2 antagonist and MT_1 agonist (IC_{50} = 112 pM). The findings help define SARs around the positions 1 and 2 of melatonin with respect to binding affinity, MT_2 selectivity, and intrinsic activity.

1. Introduction

The neurohormone melatonin **1** exerts its diverse physiological actions mostly through activation of the two high affinity G-protein-coupled MT_1 and MT_2 receptors[1] (Fig. 1).

An accurate characterization of melatonin receptor-mediated functions requires MT_1 and MT_2 selective ligands. However, pronounced subtype selectivity is still a challenge, and only recently a limited number of selective compounds have been identified.[2–4] The majority of subtype selective ligands behave as MT_2 receptor antagonists. A common structural feature in most of the of MT_2-selective antagonists is the presence of a lipophilic substituent located out-of-the plane of their core nucleus in a position corresponding to positions 1 and 2 in melatonin.[5] The most recently published series include *N*-(3,3-diphenylpropenyl)alkanamides,[6] N-substituted (anilinoethyl)amides,[7] 3-phenylnaphthalenic derivatives,[8] and 2-[(2,3-dihydro-1*H*-indol-1-yl)methyl]melatonin analogues,[9] However, there are only limited studies exploring the steric and electronic properties of the hydrophobic binding pocket accommodating this lipophilic group.[8–13] In the course of our studies on novel ligands for melatonin receptors, we have recently synthesized rigid pentacyclic compounds **2a–c** (Fig. 2) possessing an indoline moiety attached to the positions 1 and 2 of melatonin.[14,15] While the non-methoxy derivative **2a** displayed only micromolar binding affinity for both receptor subtypes (MT_1: K_i = 1 μM; MT_2:

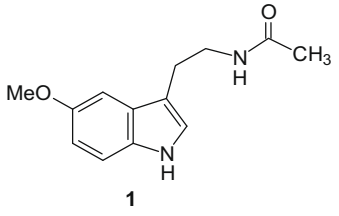

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Figure 1.

K_i = 1.7 μM), the dimethoxy analogue **2b** exhibited nanomolar affinity for MT_2 receptors (K_i = 410 nM) being 4.4-fold higher than for the MT_1 subtype (K_i = 1.8 μM). Removal of the methoxy group from the indoline moiety of **2b** led to a sixfold increase in binding affinity at both receptor subtypes (compound **2c** MT_1: K_i = 320 nM; MT_2: K_i = 65 nM).[15] The rather poor selectivity and moderate binding of **2b–c** can be most likely explained by the bulkiness and/or the unfavorable spatial orientation of the indoline moiety, which is, due to the nearly planar geometry of the dihydropyrazino-diindole ring system, not able to occupy the lipophilic binding pocket of the MT_2 receptors. In this paper, we report the synthesis of two novel series of melatoninergic ligands formally derived from compound **2c** by a removal of the indoline benzene ring and by a simultaneous cleavage of the central piperazine ring and the indoline moiety, respectively, as shown in Figure 2. Additionally, the piperazine ring of the tricyclic tetrahydro[1,2-*a*]indole scaffold was expanded to the seven-membered 1,4-diazepane skeleton and cleaved to give the corresponding ethyl(methyl)-aminomethyl

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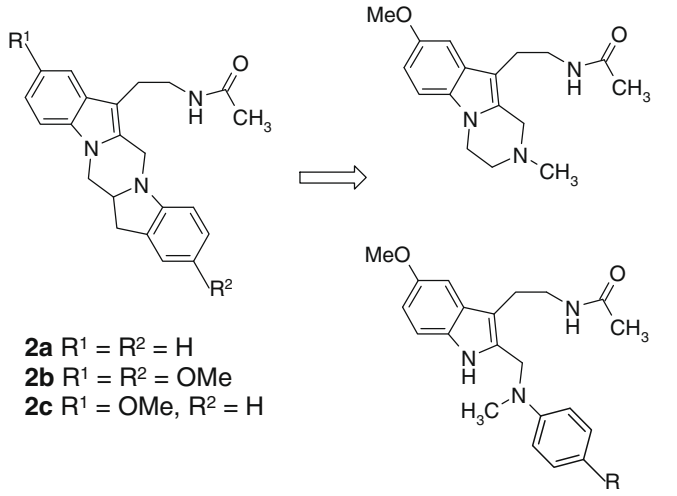


Figure 2.

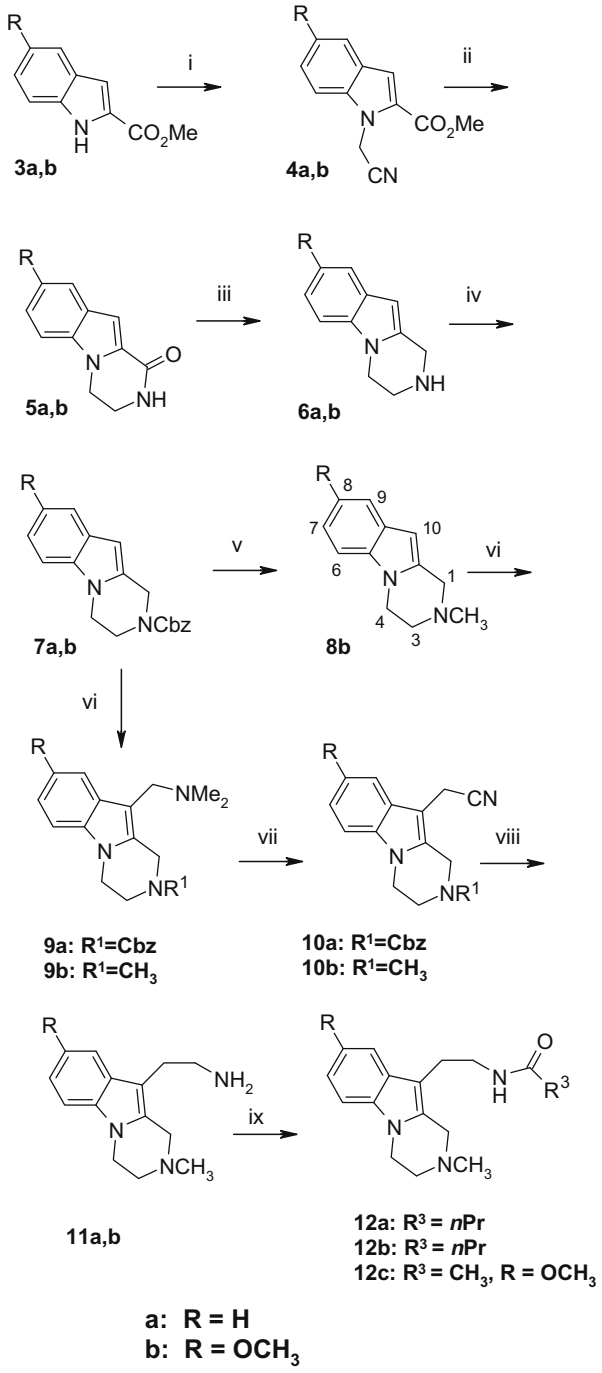
substituted analogue. The novel melatonin-derived compounds help define SARs around the positions 1 and 2 of melatonin with respect to binding affinity, MT_2 subtype selectivity, and intrinsic activity.

2. Results and discussion

2.1. Chemistry

We chose the non-methoxy tricyclic compound **12a** as our initial target in order to establish a viable route to the more biologically relevant melatonin-derived molecules **12b**,**c**. The synthetic route applied for the preparation of **12a** is shown in Scheme 1. The key intermediate is the unsubstituted tetrahydropyrazino[1,2-*a*]indole **6a**. Guandalini et al. obtained **6a** by *N*-cyanomethylation of indole-2-carboxylic acid ethyl ester and subsequent reductive cyclization using $LiAlH_4$ in only 24%.[16] The low yield was due to the formation of the corresponding aminoalcohol resulting from simultaneous reduction of both cyano and ester moieties. In order to avoid the formation of this undesired by-product, we applied catalytic hydrogenation for the crucial cyclization step. Thus, the cyanomethylated indole-2-carboxylic acid methyl ester **4a** was selectively reduced to the corresponding primary ammonium salt using H_2 (25 hPa) Pd/C in methanol/HCl_{concd}. The spontaneous ring closure proceeded by adding aqueous ammonia to yield lactam **5a** (76%) which was reduced to the key intermediate **6a** (95%) using $LiAlH_4$. For the introduction of the melatonin-like ethylamine side chain our standard procedure 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 was applied.[14,15] In order to avoid methylation of the secondary amino group of the piperazine ring of **6a**, the latter was protected by a Cbz group prior to the aminomethylation step. Mannich reaction of the Cbz-protected tricycle **7a** using dimethylmethyleniminium iodide, quaternization of the Mannich base **9a** with methyl iodide, and subsequent substitution of the trimethylammonium group with potassium cyanide provided nitrile **10a**. The simultaneous reduction of the cyano and the Cbz groups using $LiAlH_4$ yielded the crude amine **11a** which was subjected to N-acylation using butyric anhydride to give butyramide **12a**.

For the synthesis of the 8-methoxy-tetrahydropyrazino[1,2-*a*]indole analogs **12b**,**c**, 5-methoxy-indole-2-carboxylic acid methyl ester **3b** was used as starting material (see Scheme 1). N-



Scheme 1. Reagents and conditions: (i) (1) KO*t*Bu, DMF; (2) chloroacetonitrile 65 °C–rt; (ii) **5a**: (1) 25 hPa H_2, 10% Pd/C, MeOH, HCl_{concd}, rt; (2) 25% NH_3; **5b**: $NaBH_4$, $CoCl_2$, MeOH/THF, rt; (iii) $LiAlH_4$, Et_2O, THF, 0 °C–reflux; (iv) CbzCl, 2 M NaOH, dioxane, 2 M Na_2CO_3, 0 °C; (v) $LiAlH_4$, Et_2O, THF, reflux; (vi) $(CH_2{=}NMe_2)^+I^-$, CH_2Cl_2, reflux; (vii) (1) MeI, CH_2Cl_2, rt; (2) KCN, dicyclohexyl-[18]-crown-[6], MeCN, reflux; (viii) **11a**: $LiAlH_4$, Et_2O, THF, 0 °C–reflux; **11b**: $NaBH_4$, $CoCl_2$, MeOH/THF, rt; (ix) butyric anhydride or acetic anhydride, Et_3N, CH_2Cl_2, 0 °C–rt.

Cyanomethylation of the latter provided the nitrile **4b**. The reductive cyclization of **4b** applying similar reaction conditions as for the synthesis of **4a** (25 hPa H_2, 10% Pd/C) yielded the lactam **5b** in only 49%. A better yield of **5b** (60%) could be achieved by using cobalt(II)-chloride/sodium borohydride as reducing agent.[17] After $LiAlH_4$ reduction, the resulting amine **6b** was N-protected to give the carbamate **7b**. Unfortunately, after treatment of **7b** with Eschenmoser's salt, we were unable to isolate the desired pure Mannich

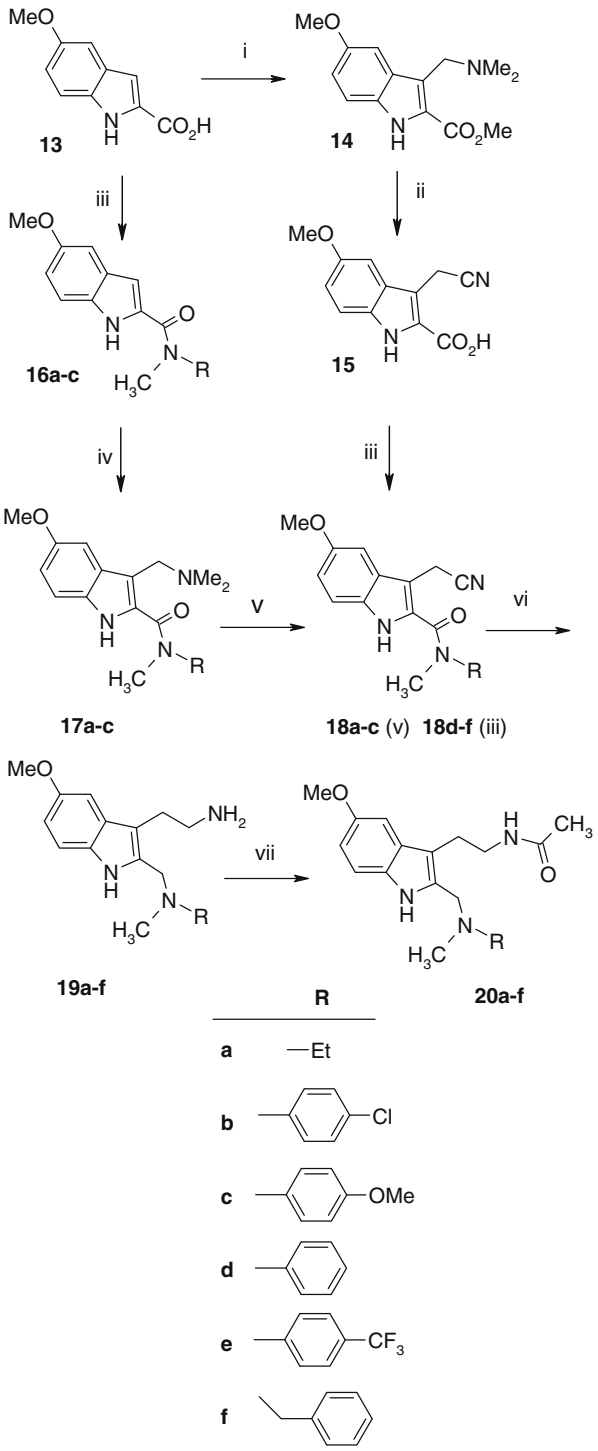
product. An alternative approach involving reduction of the Cbz moiety and subsequent Mannich reaction proved to be successful. Thus, treatment of **7b** with $LiAlH_4$ and aminomethylation of the resulting amine **8b** gave the Mannich base **9b**. The subsequent quaternization with methyl iodide proceeded regioselectively affecting exclusively the sterically less hindered *N*,*N*-dimethylmethylamine side chain. Nucleophilic substitution of the resulting trimethyl ammonium group by potassium cyanide provided the cyanomethyl analogue **10b** which was converted to the corresponding ethylamine **11b** by means of $NaBH_4/CoCl_2$ reduction. Finally, the crude **11b** was acylated using butyric and acetic anhydride to give the respective melatoninergic ligands **12b** and **12c**. Another approach toward the target compound **12c** starting from 2-[(*N*-benzyl-*N*-methyl)aminomethyl]melatonin **20f** is discussed later (see Scheme 3).

For the preparation of 2-[(phenylmethylamino)-methyl]-indole analogues **20a–f** we applied two different synthetic routes displayed in Scheme 2. Starting from the commercially available 5-methoxyindole-2-carboxylic acid **13**, the target compounds **20a**–**c** were prepared in five steps commencing with the amide formation using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDCI·HCl) as a coupling reagent and the appropriate N-substituted methylamine to give *N*-ethyl (**16a**), *N*-*p*-chlorophenyl (**16b**), and *N*-*p*-methoxyphenyl (**16c**) amides. For the subsequent Mannich–Eschenmoser aminomethylation, more vigorous reaction conditions were required due to the electron-withdrawing effect of the amide function in position 2 of the indole ring. Satisfactory yields were achieved using Eschenmoser's salt in refluxing chloroform. The Mannich bases **17a–c** were converted to the cyanomethyl analogues **18a–c** using our standard procedure (1. MeI; 2. KCN, CH_3CN, dicylohexyl-[18]-crown[6]). For the synthesis of the cyanomethyl derivatives **18d–f**, we applied another reaction sequence using our key intermediate **15** which was prepared via the aminomethylated 5-methoxyindole-2-carboxylic acid methyl ester **14** as previously reported.[9] Thus, condensation of **15** with *N*-methylaniline, *p*-CF_3-*N*-methylaniline, and *N*-methylbenzylamine provided the amides **18d–f**. Simultaneous nitrile and amide reduction using $LiAlH_4$ afforded the ethylamines **19a–f** which were converted to the desired melatoninergic ligands **20a–f** by N-acylations using acetic anhydride.

Starting from the benzylamine analogue **20f**, we developed another approach toward the 1,2,3,4-tetrahydropyrazino[1,2-*a*]indole melatonin derivative **12c** based on reductive ring closure of the *N*-cyanomethyl analogue **21** outlined in Scheme 3.[18]

Thus, compound **21**, obtained by cyanomethylation of **20f**, was subjected to catalytic hydrogenation to give **12c** via partial hydrogenation of a nitrile function to an iminium ion followed by an intramolecular nucleophilic substitution with the debenzylated methylamine acting as a nucleophile and ammonia as a leaving group.

The same strategy was applied to build the seven-membered ring of the 2,3,4,5-tetrahydro-1*H*-[1,4]-diazepino[1,2-*a*]indole analogue **23**. Thus, N-cyanoethylation of compound **20f** using acrylonitrile provided the nitrile **22**. Following the reductive cyclization procedure, **22** was converted to the desired target compound **23** by catalytic hydrogenation.



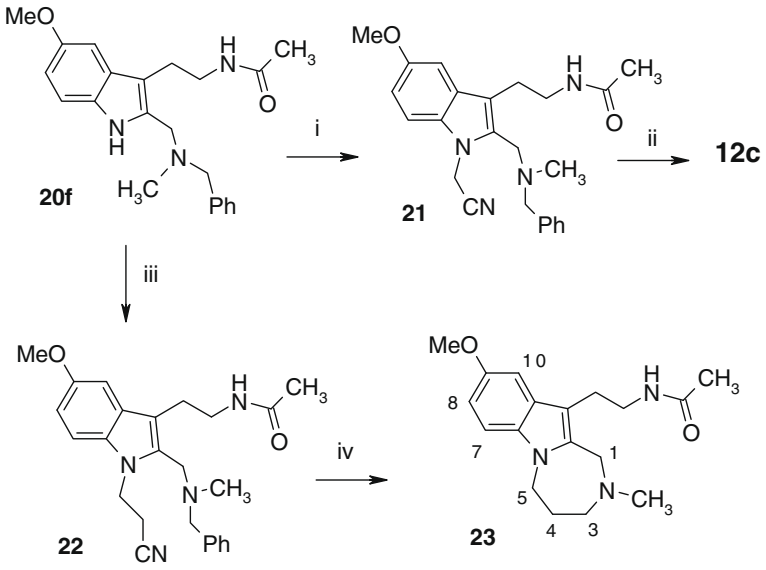
Scheme 2. Reagents and conditions: (i) (1) MeOH, H_2SO_4, reflux; (2) $(CH_2{=}NMe_2)^+I^-$, $CHCl_3$, reflux; (ii) (1) MeI, CH_2Cl_2, rt; (2) KCN, dicyclohexyl-[18]-crown-[6], MeCN, reflux; (3) LiOH, THF, rt; (4) 2 M HCl_{aq}; (iii) $HN(CH_3)R$, EDCI·HCl, CH_2Cl_2, rt; (iv) $(CH_2{=}NMe_2)^+I^-$, $CHCl_3$, reflux; (v) (1) MeI, CH_2Cl_2, rt; (2) KCN, dicyclohexyl-[18]-crown-[6], MeCN, reflux; (vi) $LiAlH_4$, Et_2O, THF, 0 °C–rt; (vii) Ac_2O, Et_3N, CH_2Cl_2, 0 °C–rt.

2.2. Pharmacology

The affinity of the target compounds for human MT_1 or MT_2 melatonin receptors expressed in CHO cells 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. The results are compiled in Table 1. Compounds having the highest binding affinity and/or selectivity (**12b** and **20d**) were subjected to functional studies using cyclic AMP assay in CHO cells expressing human MT_1 or MT_2 receptors. The results are shown in Figure 3.

2.3. Discussion

The tricyclic 1,2,3,4-tetrahydropyrazino[1,2-*a*]indole analogues **12a–c** derived from the pentacyclic ligands **2a–c** by removal of



Scheme 3. Reagents and conditions: (i) (1) KO*t*Bu, DMF; (2) bromoacetonitrile 65 °C–rt; (ii) H_2 (7 hPa), 10% Pd/C, AcOH; (iii) $H_2C{=}CH{-}CN$, Triton-B®, THF, dioxane, 0 °C–rt; (iv) H_2 (7 hPa), 10% Pd/C, AcOH.

Table 1
Binding affinity[a] of the target compounds for the human MT_1 and MT_2 receptors expressed in CHO cells obtained in competition radioligand binding assays using 2-[^{125}-I]-iodomelatonin

	pK_i MT_1 ± SEM	pK_i MT_2 ± SEM
Melatonin	9.34 ± 0.10	9.02 ± 0.09
2a[10]	6.00 ± 0.01	5.77 ± 0.06
2b[10]	5.74 ± 0.20	6.39 ± 0.27
2c[11]	6.50 ± 0.03	7.19 ± 0.03
12a	6.59 ± 0.03	7.07 ± 0.11
12b	8.18 ± 0.03	8.16 ± 0.07
12c	7.93 ± 0.04	8.11 ± 0.05
20a	6.00 ± 0.20	6.65 ± 0.08
20b	6.59 ± 0.12	7.37 ± 0.05
20c	8.09 ± 0.06	8.03 ± 0.12
20d	7.81 ± 0.19	8.64 ± 0.12
20e	6.96 ± 0.02	7.20 ± 0.01
20f	5.89 ± 0.04	6.18 ± 0.06
23	5.25 ± 08	6.05 ± 0.02

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

the indoline benzene ring exhibited considerably higher binding affinities for both MT_1 and MT_2 receptor subtypes than the parent compounds. Within the series, the non-methoxy butyramide **12a** displayed much lower affinities for melatonin receptors (MT_1: K_i = 256 nM; MT_2: K_i = 86 nM) than the methoxy analogues **12b**,**c** which is in agreement with findings observed for other melatoninergic ligands and confirms the importance of the methoxy group for binding at both receptor subtypes.[2] The methoxy butyramide **12b** (MT_1: K_i = 6.6 nM; MT_2: K_i = 6.9 nM) and acetamide **12c** (MT_1: K_i = 11.7 nM; MT_2: K_i = 7.8 nM) are nearly equipotent high-affinity ligands. However, compared to the slightly MT_2-selective pentacyclic ligand **2c**, **12b**, and **12c** are not able to differentiate between MT_1 and MT_2 receptors indicating that the *N*-methylpiperidine ring attached to positions 1 and 2 of melatonin is not able to reach the lipophilic binding pocket of MT_2 receptors in the region corresponding to N1–C2 of melatonin. Even though affinity values of **12b** for binding to each of the receptors is similar, functional analysis reveals that **12b** has partial agonist activity at MT_1 receptors with a potency (IC_{50}) value of 3.14 nM and about 12% maximum inhibition at 10 nM. By contrast, **12b** displayed no intrinsic activity at MT_2 receptors as no concentration-dependent inhibition of forskolin-induced cAMP accumulation occurred over a wide range of concentrations (Fig. 3). Interestingly, the tetrahydropyrido[1,2-*a*]indole analogue of **12b**, formally obtained by replacement of the NCH_3 moiety with a CH_2 group, was reported to be a full melatoninergic agonist in the *Xenopus laevis* melanophore assay.[19]

Expansion of the six-membered piperazine ring of the acetamide **12c** to the seven-membered 1,4-diazepane ring produced a dramatical decrease of binding affinity at both receptor subtypes. The resulting compound **23** (MT_1: K_i = 5.62 μM; MT_2: K_i = 883 nM) showed 480-fold and 110-fold higher binding constants at MT_1 and MT_2 receptors, respectively, than the parent ligand **12c**. In contrast to the non-selective agent **12c**, compound **23** displayed slight selectivity (sixfold) for the MT_2 receptors.

In order to examine whether the lacking MT_2 selectivity of the tricyclic compounds **12b** and **12c** is caused by the non-flexibility of the piperazine ring, we synthesized a ring-opened analogue of **12c** by formally breaking one of the C–N bonds of the piperazine ring. The resulting compound **20a** showed dramatically reduced binding affinities for both receptor subtypes (85-fold for MT_2 and 30-fold for MT_1) when compared to the parent ligand **12c**. Replacement of the *N*-ethyl group of **20a** by a phenyl ring generated a compound having the best pharmacological profile of the whole series. The resulting ligand **20d** exhibited an excellent MT_2 binding affinity (K_i = 2.3 nM) being 7-times more selective for the MT_2 than for the MT_1 receptors (MT_1: K_i = 15.6 nM). The findings are in agreement with the structures of the most MT_2-selective ligands, all of them possessing an aromatic ring in a position topologically equivalent to N1–C2 region of melatonin.

Functional analysis of **20d** revealed that it displayed strong agonist activity at MT_1 receptors with a potency value IC_{50} = 112 pM and maximal (60%) inhibition at 1 μM. By contrast, **20d** did not possess intrinsic activity at MT_2 receptors as no concentration-dependent inhibition of forskolin-induced cAMP accumulation occurred over wide range of concentrations (Fig. 3).

In the further optimization attempt, we substituted the benzene ring of **20d** with groups that are present in some of the most MT_2-selective agents such as chloro[10] and methoxy[12] groups. Unfortunately, the binding affinity of the *p*-chloro-substituted analogue

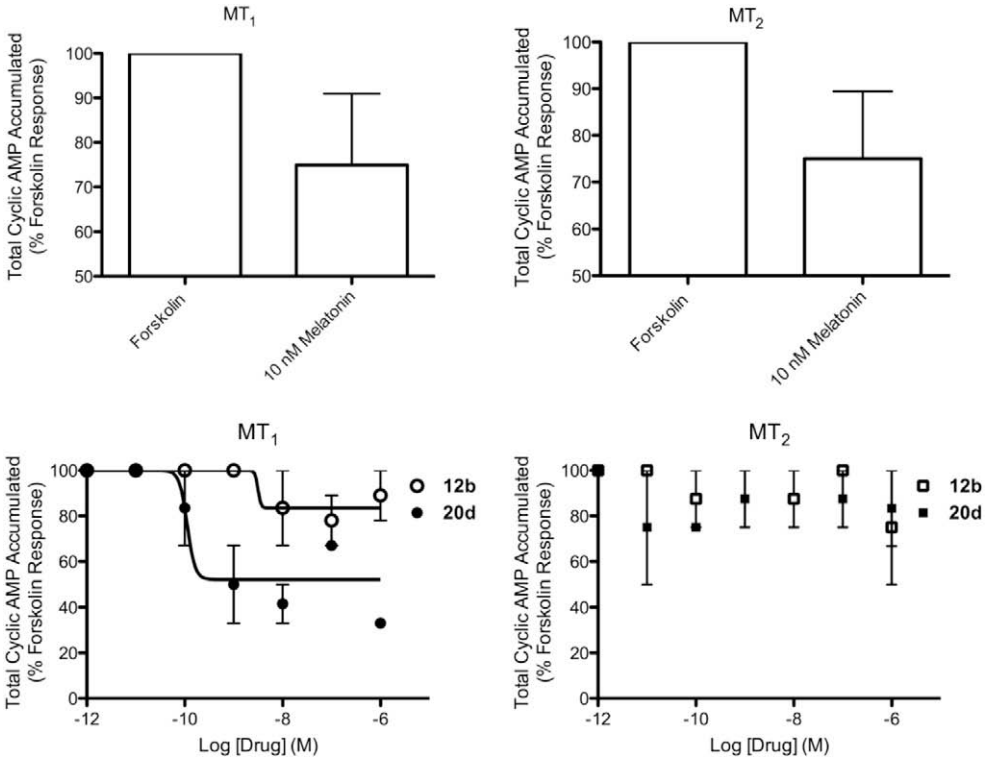


Figure 3. Functional analysis of 10 nM melatonin (top graphs) to inhibit forskolin-induced cAMP accumulation in MT_1-CHO cells (left graph, top) or MT_2-CHO cells (right graph, top). Bottom graphs show the results from the functional analysis for compounds **12b** or **20d** at MT_1 receptors (left graph, bottom) or MT_2 receptors (right graph, bottom) expressed in CHO cells. Each data point represents the mean ± standard error of two to three independent experiments. Where appropriate, curves were fit by non-linear regression analysis by 1-site fit to obtain potency (IC_{50}) values using GraphPad Prism software.

20b for both receptor subtypes were reduced by a factor of approx. 17 (MT_1: K_i = 257 nM; MT_2: K_i = 42 nM) when compared to the unsubstituted ligand **20d** while the moderate selectivity ratio was maintained (K_i MT_1/MT_2 = 6). Introduction of the more lipophilic *p*-CF_3 substituent further worsened the pharmacological profile yielding a non-selective ligand **20e** (MT_1: K_i = 111 nM; MT_2: K_i = 63 nM). Interestingly, the *p*-OMe analogue **20c** is a high-affinity non-selective agent (MT_1: K_i = 8 nM; MT_2: K_i = 9 nM). This is in agreement with our previous findings in the 2-[(2,3-dihydro-1*H*-indol-1-yl)methyl]-melatonin series with the *p*-OMe-indoline analogue showing also no subtype specificity.[9] The non-selective binding behavior of **20c** is probably caused by the competition of both methoxy groups for binding at the MT_2 receptor region accommodating the methoxy group of melatonin inducing the unfavorable ligand orientation. In order to probe the sterical requirements of the hydrophobic binding pocket, we modified the structure of our most MT_2-selective agent **20d** by replacing the phenyl group with a longer benzyl substituent. The resulting ligand **20f** showed dramatically reduced binding affinities for both receptor subtypes (80-fold for MT_1, 285-fold for MT_2) indicating a sterically restricted MT_2-binding pocket.

3. Conclusions

In summary, we report the synthesis and pharmacological evaluation of two novel series of melatonin-derived ligands at the MT_1 and MT_2 subtypes of melatonin receptors. The 1,2,3,4-tetrahydropyrazino[1,2-*a*]indole analogues **12b**,**c** are high-affinity MT_1 and MT_2 receptor ligands displaying no subtype selectivity (K_i = 7–11 nM). Functional analysis of **12b** showed that it was a partial agonist at MT_1 receptors and possessed no intrinsic activity at MT_2 receptors. From the 2-[(phenyl-methylamino)methyl]-melatonin derivatives **20b–f**, the unsubstituted phenyl analogue **20d** had the best pharmacological profile showing high binding affinity (K_i = 2 nM) and moderate selectivity (K_i MT_1/MT_2 ratio = 8) toward the MT_2 subtype. Interestingly, **20d** showed strong agonist properties at the MT_1 receptor with no intrinsic activity at the MT_2 receptor. The novel compounds help defining SARs around the positions 1 and 2 of melatonin with respect to binding affinity, MT_2 subtype selectivity, and intrinsic activity.

4. Experimental

4.1. Chemistry

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. Bruker AV-400 spectrometer was used to obtain ^1H NMR and ^{13}C NMR spectra. Proton chemical shifts are referred to $CHCl_3$ (7.24 ppm). Carbon chemical shifts are referred to $CDCl_3$ (77.00 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, Finnigan MAT 90, 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. General procedure for the synthesis of 4a,b

To a solution of the respective methyl ester **3a**,**b** (6.34 mmol) in anhydrous DMF (15 mL) potassium *tert*-butoxide (1.06 g, 9.45 mmol) was added at room temperature. After 40 min,

chloroacetonitrile (0.79 mL, 12.6 mmol) was added dropwise and the solution was heated at 65 °C for 30 min and left stirring at room temperature for 20 h. The reaction mixture was poured onto ice (20 g) and the mixture was extracted with ethyl acetate (5 × 30 mL). The combined organic layers were washed with saturated $NaHCO_3$ solution (2 × 20 mL), saturated sodium chloride solution (2 × 20 mL) and water (20 mL). The extracts were dried (Na_2SO_4) and evaporated under vacuum. Recrystallization from methanol gave **4a**,**b**.

4.1.1.1. Methyl 1-(cyanomethyl)-1*H*-indole-2-carboxylate (4a).

Compound **4a** (1.15 g, 85%) was obtained from **3a** (1.11 g) as pale yellow needles; mp: 117 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.92 (s, 3H, OC*H*$_3$), 5.56 (s, 2H, C*H*$_2$CN), 7.22 (ddd, *J* = 1.4, 6.6, 7.9 Hz, 1H, H-6), 7.35 (d, *J* = 1.0 Hz, 1H, H-3), 7.38–7.40 (m, 1H, H-7), 7.41–7.45 (m, 1H, H-5), 7.69 ppm (d, *J* = 7.3, 1H, H-4); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 32.28 (*C*H$_2$CN), 52.07 (O*C*H$_3$), 109.59 (C-3), 112.79 (C-7), 114.85 (*C*N), 122.05 (C-4), 123.23 (C-6), 126.26 (C-2), 126.35 (C-3a), 126.47 (C-5), 138.72 (C-7a), 162.26 ppm (C=O); IR: ν = 2950, 2836, 1703, 1520, 1437 cm^{-1}; MS (EI, 70 eV) *m/z* (%): 214 (83) [M$^+$], 199 (100), 183 (23), 182 (35), 154 (39), 143 (26), 128 (20), 127 (16), 115 (21). Anal. Calcd for $C_{12}H_{10}N_2O_2$: C, 67.28; H, 4.71; N, 13.08. Found: C, 67.62; H, 4.71; N, 13.03.

4.1.1.2. Methyl 1-(cyanomethyl)-5-methoxy-1*H*-indole-2-carboxylate (4b).

Compound **4b** (1.29 g, 83%) was obtained from **3b** (1.11 g) as pale brown needles; mp: 131–132 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.81 (s, 3H, C-5-OC*H*$_3$), 3.90 (s, 3H, CO_2CH_3), 5.51 (s, 2H, C*H*$_2$CN), 7.06–7.07 (m, 1H, H-3), 7.06–7.07 (m, 1H, H-4), 7.23 (d, *J* = 8.8 Hz, 1H, H-7), 7.27 ppm (d, *J* = 8.8 Hz, 1H, H-6); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 32.38 (*C*H$_2$CN), 51.99 (CO_2CH_3), 55.69 (O*C*H$_3$), 109.59 (C-3), 103.36 (C-4), 110.35 (C-7), 112.21 (C-6), 114.89 (*C*N), 126.53 (C-2), 126.80 (C-3a), 134.06 (C-7a), 155.55 (C-5), 162.19 ppm (C=O); IR: ν = 2999, 2924, 2824, 2853, 1626, 1526, 1439 cm^{-1}; MS (EI, 70 eV) *m/z* (%): 244 (98) [M$^+$], 229 (100), 201 (14), 184 (20), 173 (12), 158 (14), 142 (11), 141 (15). Anal. Calcd for $C_{13}H_{12}N_2O_3$: C, 62.93; H, 4.95; N, 11.47. Found: C, 62.81; H, 4.96; N, 11.40.

4.1.2. 3,4-Dihydropyrazino[1,2-*a*]indol-1(2*H*)-one (5a)

To a solution of **4a** (1.34 g, 6.26 mmol) in MeOH (140 mL) and concentrated hydrochloric acid (3.1 mL) were added 10% Pd/C (310 mg) in one portion. The mixture was hydrogenated (25 hPa) at room temperature for 24 h. The catalyst was removed by filtration and washed with methanol. The resulting green solution was evaporated and the resulting green solid was dissolved in water (168 mL). The solution was extracted with CH_2Cl_2 (3 × 50 mL) and the aqueous layer was made basic with aqueous ammonia (pH >10). The mixture was stirred for 80 min to accomplish ring closure, extracted with $CHCl_3$ (5 × 50 mL) and the extract was dried over Na_2SO_4. Evaporation of the solvent afforded **5a** (882 mg, 76%) as colorless crystals. The spectral data of **5a** were identical to those previously reported.[20]

4.1.3. 8-Methoxy-3,4-dihydropyrazino[1,2-*a*]indol-1(2*H*)-one (5b)

To a solution of **4b** (3.32 g, 13.6 mmol) in absolute methanol (160 mL) and absolute THF (80 mL) was added anhydrous cobalt chloride (3.60 g, 28.0 mmol) followed by sodium borohydride (2.56 g, 68.0 mmol) in small portions. Evolution of hydrogen gas was observed and black precipitate appeared. When the addition was complete, stirring was continued for 1 h at room temperature. Hydrochloric acid (10%, 80 mL) was added to dissolve the black precipitate and methanol was removed under reduced pressure. 25% Aqueous ammonia (3.4 mL) was added and the mixture was extracted with ethyl acetate (6 × 40 mL). The combined extracts were washed with brine (2 × 20 mL) and dried (Na_2SO_4). Removal of solvent at reduced pressure afforded a yellow solid that was purified by silica gel chromatography (methanol–ethyl acetate–*n*-pentane 1:2:2) to afford **5b** (1.76 g, 60%). The spectral data of **5b** were identical to those previously reported.[21]

4.1.4. General procedure for the synthesis of 6a,b

A solution of the respective lactam **5a**,**b** (8.16 mmol) in absolute THF (50 mL) was added to a stirred suspension of $LiAlH_4$ (625 mg, 16.5 mmol) in absolute diethyl ether (50 mL) at 0–5 °C. The reaction mixture was refluxed for 4 h. The reaction was quenched by a slow addition of water at 0–5 °C. The formed precipitate was filtered off through a pad of Celite®545 and washed with ethyl acetate (300 mL). The combined organic layers were extracted with 2 M hydrochloric acid (3 × 100 mL). The aqueous layer was separated and basified with 25% ammonia. The resulting suspension was extracted with ethyl acetate (4 × 50 mL). Drying of the organic layer over Na_2SO_4 and evaporation of the solvent afforded the crude amines **6a**,**b** which were used for the next step without further purification.

4.1.4.1. 1,2,3,4-Tetrahydropyrazino[1,2-*a*]indole (6a).

Compound **6a** (1.35 g, 95%) was obtained from **5a** (1.52 g) as a colorless viscous oil that crystallized after 5 min. The spectral data of **6a** were identical to those previously reported.[22]

4.1.4.2. 8-Methoxy-1,2,3,4-tetrahydropyrazino[1,2-*a*]indole (6b).

Compound **6b** (1.55 g, 94%) was obtained from **5b** (1.77 g) as a pale brown solid. The spectral data of **6b** were identical to those previously reported.[22]

4.1.5. General procedure for the synthesis of 7a,b

The respective amine **6a**,**b** (16.4 mmol) was dissolved in a 4:1 mixture of 2 M Na_2CO_3/1,4-dioxane (83 mL) and the solutions of benzyl chloroformate (5.63 mL, 40.1 mmol) in 1,4-dioxane (34 mL) and 2 M aqueous NaOH (20.7 mL, 41.4 mmol) were added dropwise and simultaneously under ice-cooling. After 3.5 h of stirring, the organic solvent was removed under reduced pressure and the mixture was extracted with CH_2Cl_2 (4 × 100 mL). Drying of the combined organic layers (Na_2SO_4) and removal of the solvent gave **7a**,**b**. Analytical samples were obtained by recrystallization from methanol.

4.1.5.1. Benzyl 3,4-dihydropyrazino[1,2-*a*]indole-2(1*H*)-carboxylate (7a).

Compound **7a** (4.16 g, 83%) was obtained from **6a** (2.82 g) as a pale yellow viscous oil, that crystallized after 5 h. The spectral data of **7a** were identical to those previously reported.[16]

4.1.5.2. Benzyl 8-methoxy-3,4-dihydropyrazino[1,2-*a*]indole-2(1*H*)-carboxylate (7b).

Compound **7b** (4.41 g, 80%) was obtained from **6b** (3.32 g) as colorless crystals: mp 122 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.84 (s, 3H, OC*H*$_3$), 3.96–4.11 (m, 4H, H-3, H-4), 4.87 (s, 2H, H-1), 5.20 (s, 2H, C*H*$_2$Ph), 6.21 (s, 1H, H-10), 6.84 (dd, *J* = 8.6, 2.4 Hz, 1H, H-7), 7.04 (d, *J* = 2.4 Hz, 1H, H-9), 7.14 (d, *J* = 8.6 Hz, 1H, H-6), 7.32–7.34 ppm (m, 5H, Ph-*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 41.44 (C-1, C-4), 42.30 (C-3), 55.78 (O*C*H$_3$), 67.58 (*C*H$_2$Ph), 96.60 (C-9), 102.20 (C-10), 109.19 (C-6), 110.98 (C-7), 128.02, 128.29, 128.51 (*C*-Ph), 131.25 (C-5a), 132.21 (C-9a), 132.40 (C-10a), 136.25 (C-1′), 154.49 (C=O), 155.13 (C-8) ppm; IR: ν = 3015, 2959, 2937, 2893, 2838, 1689, 1484, 1417 cm^{-1}; MS (EI, 70 eV) *m/z* (%): 336 (14) [M$^+$], 245 (100), 201 (31), 158 (10), 91 (27), 65 (7). Anal. Calcd for $C_{20}H_{20}N_2O_3$: C, 71.41; H, 5.99; N, 8.33. Found: C, 71.32; H, 6.02; N, 8.27.

4.1.6. 8-Methoxy-2-methyl-1,2,3,4-tetrahydropyrazino[1,2-*a*]indole (8b)

A solution **7b** (415 mg, 1.23 mmol) in absolute diethyl ether (6.5 mL) and absolute THF (2 mL) was added dropwise to a stirred

suspension of $LiAlH_4$ (94 mg, 2.48 mmol) in absolute diethyl ether (8 mL) at 0–5 °C. The reaction mixture was refluxed for 4 h. The reaction was quenched by a slow addition of water at 0–5 °C. The formed precipitate was filtered off through a pad of Celite®545 and washed with ethyl acetate (120 mL). The organic layer was separated and the aqueous layer extracted with ethyl acetate (3 × 30 mL). Drying of the combined organic layers over Na_2SO_4 and evaporation of the solvent afforded **8b** (255 mg, 96%) as a white solid. The spectral data of **8b** were identical to those previously reported.[23]

4.1.7. Benzyl 10-[(dimethylamino)methyl]-3,4-dihydro pyrazino[1,2-*a*]indole-2(1*H*)-carboxylate (9a)

Dimethylmethyleniminium iodide (2.09 g, 13.6 mmol) was added to a solution of compound **7a** (2.00 g, 6.53 mmol) in absolute CH_2Cl_2 (105 mL). After heating for 2.5 h at reflux, the reaction mixture was made basic with 25% ammonia. The organic layer was separated, washed with water and dried over Na_2SO_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** (2.29 g, 96%) as a yellow viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.26 (s, 6H, N(CH_3)$_2$), 3.56 (s, 2H, CH_2N(CH_3)$_2$), 3.90–4.14 (m, 4H, H-3, H-4), 4.91 (s, 2H, H-1), 5.21 (s, 2H, CH_2Ph), 7.11–7.23 (m, 2H, H-7, H-8), 7.25 (m, 5H, Ph-*H*), 7.32–7.42 (m, 1H, H-6), 7.65 (d, *J* = 7.8 Hz, 1H, H-9) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 30.83 (C-1), 41.44 (C-4), 41.66 (C-3), 45.24 (N(CH_3)$_2$), 53.05 (CH_2N(CH_3)$_2$), 67.57 (CH_2Ph), 106.85 (C-10), 108.42 (C-6), 118.88 (C-9), 119.97 (C-8), 121.09 (C-7), 128.16, 128.37, 128.51 (C_{ar}), 130.04 (C-9a), 136.03 (C-10a), 136.30 (C-5a, C-1′), 155.14 (C=O) ppm; IR: ν = 3032, 2940, 2855, 2810, 1699, 1455, 1418 cm^{-1}; HRMS-ESI *m/z* [M+H]$^+$ calcd for $C_{22}H_{26}N_3O_2$: 364.2025, found: 364.2012.

4.1.8. 1-(8-Methoxy-2-methyl-1,2,3,4-tetrahydro-pyrazino[1,2-*a*]indol-10-yl)-*N*,*N*-dimethylmethanamine (9b)

Dimethylmethyleniminium iodide (185 mg, 1.00 mmol) was added to a solution of **8b** (108 mg, 0.499 mmol) in absolute CH_2Cl_2 (12 mL). After heating for 2.5 h at reflux the resulting white precipitate was filtered off, dissolved in water (150 mL) and the solution was made basic with 25% ammonia. The resulting suspension was extracted with CH_2Cl_2 (5 × 25 mL). The filtrate of the original reaction mixture was basified with 25% ammonia, the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layers were washed with water, dried over Na_2SO_4 and evaporated in vacuo to give **9b** (103 mg, 76%) as a yellow viscous oil. The crude product was used for the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.24 (s, 6H, N(CH_3)$_2$), 2.50 (s, 3H, N(CH_3)), 2.88 (t, *J* = 5.6 Hz, 2H, H-3) 3.47 (s, 2H, CH_2N(CH_3)$_2$), 3.73 (s, 2H, H-1), 3.85 (s, 3H, OCH_3) 4.03 (t, *J* = 5.6 Hz, 2H, H-4), 6.79 (dd, *J* = 8.6, 2.3 Hz, 1H, H-7), 7.08 (d, *J* = 2.3 Hz, 1H, H-9), 7.11 (d, *J* = 8.6 Hz, 1H, H-6) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 41.87 (C-4), 45.33 (N(CH_3)$_2$), 45.96 (NCH_3), 52.22 (C-1), 52.38 (C-3), 53.22 (CH_2N(CH_3)$_2$), 55.98 (OCH_3), 101.20 (C-9), 105.65 (C-10), 109.03 (C-6), 110.31 (C-7), 129.18 (C-9a), 131.17 (C-5a), 133.40 (C-10a), 154.27 (C-8) ppm; IR: ν = 2930, 2858, 1583, 1483, 1452 cm^{-1}; HRMS-ESI *m/z* [M−NH(CH_3)$_2$]$^+$ calcd for $C_{14}H_{17}N_2O$: 229.1335, found: 229.1332.

4.1.9. Benzyl 10-(cyanomethyl)-3,4-dihydropyrazino[1,2-*a*]indole-2(1*H*)-carboxylate (10a)

Methyl iodide (3.82 mL, 60.3 mmol) was added to a solution of **9a** (2.29 g, 6.30 mmol) in absolute CH_2Cl_2 (20 mL). The reaction mixture was stirred at room temperature for 15 h. The volatiles were removed in vacuo to afford **9a** methoiodide. This crude ammonium salt was suspended in absolute acetonitrile (420 mL), dicyclohexyl-[18]-crown-[6] (2.70 g, 7.25 mmol) and potassium cyanide (5.29 g, 8.12 mmol) were added. The resulting reaction mixture was heated at reflux for 2.5 h. The solvent was evaporated under reduced pressure and the residue was extracted with ethyl acetate (3 × 100 mL). The organic layer was washed with water (2 × 100 mL), dried (Na_2SO_4), and evaporated in vacuo. The residue was purified by silica gel chromatography ($CHCl_3$/methanol/25% ammonia, 100:10:1) to afford **10a** (2.09 g, 96%) as a red-brown viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.73 (s, 2H, CH_2CN), 3.96–4.01 (m, 2H, H-3), 4.04–4.13 (m, 2H, H-4), 4.88 (s, 2H, H-1), 5.20 (s, 2H, CH_2Ph), 7.16–7.38 (m, 3H, H-6, H-7, H-8), 7.24 (m, 5H, Ph-*H*), 7.57 (d, *J* = 7.6 Hz, 1H, H-9) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.58 (CH_2CN), 29.67 (C-1), 40.93 (C-4), 41.47 (C-3), 67.87 (CH_2Ph), 108.90 (C-6), 117.43 (CN), 117.73 (C-9), 120.63 (C-7), 121.92 (C-8), 126.49 (C-9a), 128.15, 128.33, 128.60 (*C*-Ph), 135.66 (C-10a), 136.06 (C-5a, C-1′), 155.05 (C=O) ppm; IR: ν = 2922, 2853, 2361, 1734, 1704, 1458 cm^{-1}; HRMS-ESI *m/z* [M+Na]$^+$ calcd for $C_{21}H_{19}N_3O_2Na$: 368.1354, found: 368.1369.

4.1.10. (8-Methoxy-2-methyl-1,2,3,4-tetrahydropyrazino[1,2-*a*]indol-10-yl)acetonitrile (10b)

Methyl iodide (0.09 mL, 1.46 mmol) was added to a solution of **9b** (200 mg, 0.732 mmol) in absolute CH_2Cl_2 (6 mL). The reaction mixture was stirred at room temperature for 2 h. The volatiles were removed in vacuo to afford **9b** methoiodide. This crude ammonium salt was suspended in absolute acetonitrile (49 mL), dicyclohexyl-[18]-crown-[6] (314 mg, 0.843 mmol) and potassium cyanide (615 mg, 9.44 mmol) were added. The resulting reaction mixture was heated at reflux for 2.5 h. The solvent was evaporated under reduced pressure and the residue was dissolved in ethyl acetate (50 mL). The organic layer was washed with water (2 × 15 mL), dried (Na_2SO_4), and evaporated in vacuo. The residue was purified by silica gel chromatography ($CHCl_3$/methanol/25% ammonia, 100:10:1) to afford **10b** (155 mg, 83%) as a yellow viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.50 (s, 3H, NCH_3), 2.89 (t, *J* = 5.6 Hz, 2H, H-3), 3.67 (s, 2H, CH_2CN), 3.73 (s, 2H, H-1), 3.85 (s, 3H, OCH_3), 3.94 (d, *J* = 5.6 Hz, 2H, H-4), 6.83 (dd, *J* = 8.8, 2.4 Hz, 1H, H-7), 6.97 (d, *J* = 2.4 Hz, 1H, H-9), 7.14 (d, *J* = 8.8 Hz, 1H, H-6) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.51 (CH_2CN), 41.85 (C-4), 45.83 (NCH_3), 51.57 (C-1), 51.97 (C-3), 55.85 (OCH_3), 96.15 (C-10), 99.59 (C-9), 109.62 (C-6), 111.33 (C-7), 117.72 (*C*N), 127.07 (C-9a), 130.83 (C-5a), 132.41 (C-10a), 154.62 (C-8) ppm; IR: ν = 2930, 2858, 1583, 1483, 1452 cm^{-1}; HRMS-ESI *m/z* [M+H]$^+$ calcd for $C_{15}H_{18}N_3O$: 256.1444, found: 256.1448.

4.1.11. 2-(2-Methyl-1,2,3,4-tetrahydropyrazino[1,2-*a*]indol-10-yl)ethylamine (11a)

A solution of **10a** (2.09 g, 6.05 mmol) in absolute THF (174 mL) was added to a stirred suspension of $LiAlH_4$ (2.94 g, 78.7 mmol) in absolute diethyl ether (192 mL) at 0–5 °C. The reaction mixture was refluxed for 3 h and quenched by a slow addition of water at 0–5 °C. The formed precipitate was filtered off through a pad of Celite®545 and washed with diethyl ether (500 mL). The organic layer was separated, washed with water (2 × 60 mL) and dried over Na_2SO_4. The volatiles were removed under vacuum to afford **11a** as a yellow viscous oil that was used in the next step without further purification.

4.1.12. 2-(8-Methoxy-2-methyl-1,2,3,4-tetrahydropyrazino[1,2-*a*]indol-10-yl)ethylamine (11b)

To a solution of **10b** (295 mg, 1.16 mmol) in absolute methanol (13.5 mL) was added anhydrous cobalt chloride (306 mg, 2.38 mmol) followed by sodium borohydride (217 mg, 5.78 mmol) in small portions. Evolution of hydrogen gas was observed and black precipitate appeared. When the addition was completed, stirring was continued for 1 h at room temperature. Hydrochloric

acid (10%, 7 mL) was added to dissolve the black precipitate and methanol was removed in vacuo. 25% Ammonia was added and the aqueous mixture was extracted with ethyl acetate (7 × 20 mL). The combined extracts were dried (Na_2SO_4) and the volatiles were removed under vacuum to afford **11b** as a yellow viscous oil that was used in the next step without further purification.

4.1.13. General procedure for the synthesis of 12a–c

A stirred solution of the respective amine **11a**,**b** (1 equiv) in absolute CH_2Cl_2 (90 mL) was treated with triethylamine (3.5 equiv) and the respective acylation agent (2.5 equiv) at 0–5 °C. The reaction mixture was stirred at ambient temperature for 7 h. The solvent was evaporated under reduced pressure and the residue was purified by silica gel chromatography ($CHCl_3$–MeOH–25% NH_3 100:10:1).

4.1.13.1. *N*-[2-(2-Methyl-1,2,3,4-tetrahydropyrazino[1,2-a]indol-10-yl)ethyl]butanamide (12a). Compound **12a** (107 mg, 10%) was obtained from **11a** (810 mg) and butyric anhydride (1.72 mL) as yellow crystals. Mp 177–178 °C (diethyl ether); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.89 (t, J = 7.6 Hz, 3H, $CH_2CH_2CH_3$), 1.50–1.67 (m, 2H, $CH_2CH_2CH_3$), 2.02 (t, J = 7.1 Hz, 2H, $CH_2CH_2CH_3$), 2.48 (s, 3H, NCH_3), 2.86 (t, J = 6.5 Hz, 2H, CH_2CH_2N), 2.87 (t, J = 5.3 Hz, 2H, H-3), 3.47 (q, J = 6.5 Hz, 2H, CH_2CH_2N), 3.68 (s, 2H, H-1), 4.05 (t, J = 5.3 Hz, 2H, H-4), 5.59 (s br, 1H, C(O)N*H*), 7.06–7.18 (m, 2H, H-7, H-8), 7.22–7.26 (m, 1H, H-6), 7.15 (d, J = 7.6 Hz, 1H, H-9) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.73 ($CH_2CH_2CH_3$), 18.97 ($CH_2CH_2CH_3$), 23.91 (CH_2CH_2N), 38.66 (CH_2CH_2N), 39.86 ($CH_2CH_2CH_3$), 41.86 (C-4), 46.00 (NCH_3), 52.11 (C-1), 52.46 (C-3), 105.55 (C-10), 108.64 (C-6), 118.03 (C-9), 119.48 (C-8), 120.80 (C-7), 128.03 (C-9a), 131.36 (C-10a), 135.91 (C-5a), 172.89 (C=O) ppm; IR: ν = 3308, 3053, 2961, 2930, 2873, 2340, 1634, 1460 cm^{-1}; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{18}H_{26}N_3O$ 300.2070, found: 300.2070.

4.1.13.2. *N*-[2-(8-Methoxy-2-methyl-1,2,3,4-tetrahydropyrazino[1,2-*a*]indol-10-yl)ethyl]butanamide (12b). Compound **12b** (446 mg, 38%) was obtained from **11b** (923 mg) and butyric anhydride (1.54 mL) as yellow viscous oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.81 (t, J = 7.3 Hz, 3H, $CH_2CH_2CH_3$), 1.51–1.63 (m, 2H, $CH_2CH_2CH_3$), 2.03 (t, J = 7.6 Hz, 2H, $CH_2CH_2CH_3$), 2.46 (s, 3H, NCH_3), 2.75–2.87 (m, 4H, CH_2CH_2N, H-3), 3.46 (q, J = 6.9 Hz, 2H, CH_2CH_2N), 3.63 (s, 2H, H-1), 3.81 (s, 3H, OCH_3), 3.98 (t, J = 5.5 Hz, 2H, H-4), 5.63 (br s, 1H, N*H*), 6.79 (dd, J = 8.8, 2.3 Hz, 1H, H-7), 6.95 (d, J = 2.2 Hz, 1H, H-9), 7.10 (d, J = 8.8 Hz, 1H, H-6) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 3.69 ($CH_2CH_2CH_3$), 18.97 ($CH_2CH_2CH_3$), 23.89 (CH_2CH_2N), 38.63 (CH_2CH_2N), 39.53 ($CH_2CH_2CH_3$), 41.87 (C-4), 45.92 (NCH_3), 52.04 (C-1), 52.38 (C-3), 55.91 (OCH_3), 100.38 (C-9), 105.23 (C-10), 109.28 (C-6), 110.49 (C-7), 128.38 (C-9a), 131.26 (C-5a), 132.08 (C-10a), 154.19 (C-8), 172.81 (C=O) ppm; IR: 3283, 2931, 2853, 2791, 1643, 1549, 1483 cm^{-1}; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{19}H_{28}N_3O_2$: 330.2176, found: 330.2175.

4.1.13.3. *N*-[2-(8-Methoxy-2-methyl-1,2,3,4-tetrahydropyrazino[1,2-*a*]indol-10-yl)ethyl]acetamide (12c). Compound **12c** (397 mg, 37%) was obtained from **11b** (923 mg) and acetic anhydride (0.89 mL) as a yellow viscous oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.87 (s, 3H, C(O)CH_3), 2.47 (s, 3H, NCH_3), 2.81 (t, J = 6.5 Hz, 2H, CH_2CH_2N), 2.84 (t, J = 5.6 Hz, 2H, H-3), 3.42 (q, J = 6.5 Hz, 2H, CH_2CH_2N), 3.64 (s, 2H, H-1), 3.82 (s, 3H, OCH_3), 3.99 (t, J = 5.6 Hz, 2H, H-4), 5.67 (br s, 1H, N*H*), 6.79 (dd, J = 8.6, 2.3 Hz, 1H, H-7), 6.96 (d, J = 2.3 Hz, 1H, H-9), 7.11 (d, J = 8.6 Hz, 1H, H-6) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 23.29 (C(O)CH_3), 23.77 (CH_2CH_2N), 39.90 ($CH_2CH_2N_2$), 41.89 (C-4), 45.94 (NCH_3), 52.05 (C-1), 52.40 (C-3), 55.98 (OCH_3), 100.34 (C-9), 105.24 (C-10), 109.37 (C-6), 110.56 (C-7), 128.41 (C-9a), 131.28 (C-5a), 132.06 (C-10a), 154.23 (C-8), 170.02 (C=O) ppm; IR: ν = 3286, 2933, 2849, 2730, 2363, 1641, 1483, 1452, 1432, 1229, 1164, 1040, 795 cm^{-1}; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{17}H_{24}N_3O_2$: 302.1863, found: 302.1866.

4.1.14. General procedure for the synthesis of 16a–c

The appropriate *N*-methylamine (1 equiv) was added to a solution of the 5-methoxy-1*H*-indole-2-carboxylic acid (**13**) (1 equiv) and EDCI·HCl (1.5 equiv) in absolute CH_2Cl_2 (25 mL). The mixture was stirred for 20 h and the participated solid was collected by filtration. The filtrate was concentrated under vacuum yielding some more precipitate that was again collected by filtration. The combined precipitates were washed with 2 M hydrochloric acid, saturated $NaHCO_3$ solution, and water and dried in vacuo to give the crude amides **16a**–**c** which were used in the next step without further purification. Analytical samples of **16a**–**c** were obtained by recrystallization from isopropanol.

4.1.14.1. *N*-Ethyl-5-methoxy-*N*-methyl-1*H*-indole-2-carboxamide (16a). Compound **16a** (0.900 g, 74%) was obtained from **13** (1.0 g) as a pale yellow solid; mp 128–130 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.30 (t, J = 7.1 Hz, 3H, CH_3–CH_2), 3.31 (s, 3H, N–CH_3), 3.72 (q, J = 7.1 Hz, 2H, CH_3–CH_2), 3.83 (s, 3H, OCH_3), 6.74 (s, 1H, H-3), 6.93 (dd, J = 2.3, 8.8 Hz, 1H, H-6), 7.05 (d, J = 2.3 Hz, 1H, H-4), 7.33 (d, J = 8.8 Hz, 1H, H-7), 9.88 (br, 1H, NH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.87 (CH_3–CH_2), 35.38 (N–CH_3), 44.57 (CH_3–CH_2), 55.60 (OCH_3), 102.13 (C-4), 104.77 (C-3), 112.71 (C-7), 115.44 (C-6), 128.05, 130.26, 130.99 (C-2, C-3a, C-7a), 154.39 (C-5), 162.72 ppm (C=O); IR: ν = 3254, 2963, 1596, 1401 cm^{-1}; MS (EI, 70 eV) m/z (%): 232 [M]$^+$, 173 (100), 158 (21), 119 (17). Anal. Calcd for $C_{13}H_{16}N_2O_2$: C, 67.15; H, 7.02; N, 11.89. Found: C, 67.22; H, 6.94; N, 12.06.

4.1.14.2. *N*-(4-Chlorophenyl)-5-methoxy-*N*-methyl-1*H*-indole-2-carboxamide (16b). Compound **16b** (1.00 g, 61.0%) was obtained from **13** (1.00 g) as a pale yellow solid; mp 245–246 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.41 (s, 3H, N–CH_3), 3.71 (s, 3H, OCH_3), 5.43 (d, J = 1.5 Hz, 1H, H-3), 6.84 (dd, J = 2.5, 8.8 Hz, 1H, H-6), 6.89 (d, J = 2.5 Hz, 1H, H-4), 7.34 (d, J = 8.8 Hz, 1H, H-7), 7.45 (m, 2H, H_{ar}), 7.57 (m, 2H, H_{ar}), 11.52 (br, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 38.34 (N–CH_3), 55.11 (OCH_3), 101.69 (C-4), 105.45 (C-3), 112.98 (C-7), 115.09 (C-6), 129.53, 129.63 (C_{ar}), 126.95, 130.10, 130.94 (C-2, C-3a, C-7a), 132.06, 143.31 (C_{ar}), 153.67 (C-5), 161.43 (C=O) ppm; IR: ν = 3330, 2950, 1610, 1586, 1390 cm^{-1}; MS (EI, 70 eV) m/z (%):314 (66 [M]$^+$, 174 (100), 173 (95), 158 (21), 141 (45). Anal. Calcd for $C_{17}H_{15}ClN_2O_2$: C, 64.52; H, 4.89; N, 8.90. Found: C, 64.87; H, 4.80; N, 8.89.

4.1.14.3. 5-Methoxy-*N*-(4-methoxyphenyl)-*N*-methyl-1*H*-indole-2-carboxamide (16c). Compound **16c** (2.92 g, 94%) was obtained from **13** (1.91 g) as a pale yellow solid; mp: 211–212 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.45 (s, 3H, NCH_3), 3.74 (s, 3H, C-4′-OCH_3), 3.88 (s, 3H, C-5-OCH_3), 6.78 (d, J = 2.3 Hz, 1H, H-4), 6.82 (dd, J = 8.8, 2.3 Hz, 1H, H-6), 6.95–7.01 (m, 2H, H-3′, H-5′), 7.16–7.25 (m, 2H, H-2′, H-6′), 7.24 (s, 1H, H-3), 7.26 (d, J = 8.8 Hz, 1H, H-7), 9.49 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 39.13 (NCH_3), 56.57 (C-5-OCH_3), 56.60 (C-4′-OCH_3), 102.30 (C-4), 106.61 (C-6), 112.45 (C-7), 115.03 (C-3′), 115.96 (C-3), 127.26 (C-1′), 129.06 (C-2′), 130.09 (C-3a), 130.65 (C-7a), 136.92 (C-2), 154.28 (C-5), 159.38 (C-4′), 162.10 ppm (C=O); IR: ν = 3273, 1618, 1597, 1509, 1390 cm^{-1}; MS (EI, 70 eV) m/z (%): 310 (26) [M]$^+$, 174 (11), 137 (100), 122 (21), 119 (11). Anal. Calcd for $C_{20}H_{19}N_3O_2$: C, 69.66; H, 5.85; N, 9.03. Found: C, 69.47; H, 5.81; N, 8.97.

4.1.15. General procedure for the synthesis of 17a–c

N,*N*-Dimethylmethyleniminium iodide (2.5 equiv) was added to a solution of the respective amide **16a**–**c** (1 equiv) in absolute

$CHCl_3$ (50 mL). After heating for 16 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 to give the crude products **17a–c** which were pure enough to be used in the next step without further purification as indicated by ^{1}H NMR.

4.1.15.1. 3-[(Dimethylamino)methyl]-*N*-ethyl-5-methoxy-*N*-methyl-1*H*-indole-2-carboxamide (17a). Compound **17a** (0.60 g, 75%) was obtained from **16a** (0.64 g) as a pale yellow viscous oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.15 (t, J = 6.7 Hz, 3H, C*H*$_3$–CH$_2$), 2.19 (s, 6H, NMe_2), 3.00 (s, 3H, N–CH_3), 3.48 (q, J = 6.7 Hz, 2H, CH_3–C*H*$_2$), 3.59 (s, 2H, C*H*$_2$–NMe_2), 3.81 (s, 3H, OCH_3), 6.83 (dd, J = 2.3, 8.8 Hz, 1H, H-6), 7.17–7.19 (m, 2H, H-4, H-7), 9.29 ppm (br, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.66 (*C*H$_3$–CH$_2$), 35.17 (N–CH_3), 44.36 (CH_3–*C*H$_2$), 45.22 (NMe_2), 54.23 (*C*H$_2$–NMe_2), 55.77 (OCH_3), 101.80 (C-4), 111.84 (C-3), 112.19 (C-7), 113.78 (C-6), 127.97, 130.14, 130.94 (C-2, C-3a, C-7a), 154.12 (C-5), 165.39 ppm (C=O).

4.1.15.2. *N*-(4-Chlorophenyl)-3-[(dimethylamino)methyl]-5-methoxy-*N*-methyl-1*H*-indole-2-carboxamide (17b). Compound **17b** (0.42 g, 89.0%) was obtained from **16b** (0.40 g) as a light brown viscous oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.14 (s, 6H, NMe_2), 3.27 (s, 2H, C*H*$_2$–NMe_2), 3.43 (s, 3H, N–CH_3), 3.76 (s, 3H, OCH_3), 6.79 (dd, J = 2.5, 8.8 Hz, 1H, H-6), 7.02 (ddd, J = 2.0, 3.0, 8.6 Hz, 2H, H_{ar}), 7.06 (m, 2H, H-4, H-7), 7.15 (ddd, J = 2.0, 3.0, 8.6 Hz, 2H, H_{ar}), 8.62 ppm (br, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 38.03 (N–CH_3), 45.36 (NMe_2), 53.79 (*C*H$_2$–NMe_2), 55.71 (OCH_3), 101.81 (C-4), 112.19 (C-7), 114.82 (C-3), 114.92 (C-6), 126.87 (ArCH), 127.97, 129.11, 130.96, (C-2, C-3a, C-7a), 129.23 (C_{ar}), 131.94, 142.53 (C_{ar}), 154.19 (C-5), 164.91 ppm (C=O).

4.1.15.3. 3-[(Dimethylamino)methyl]-5-methoxy-*N*-(4-methoxyphenyl)-*N*-methyl-1*H*-indole-2-carboxamide (17c). Compound **17c** (572 mg, 78%) was obtained from **16c** (300 mg) as a colorless viscous oil; δ_H ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.52 (s, 6H, N(C*H*$_3$)$_2$), 3.94 (s, 3H, NC*H*$_3$), 3.76 (s, 3H, C-4′-OCH_3), 3.85 (s, 3H, C-5-OCH_3), 3.97 (s, 2H, CH_2N), 6.81–6.87 (m, 3H, H-6, H-3′, H-5′), 7.00 (d, J = 9.1 Hz, 1H, H-7), 7.10–7.17 (m, 2H, H-2′, H-6′), 7.30 (d, J = 2.0 Hz, 1H, H-4), 7.83 ppm (br s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 38.92 (N*C*H$_3$), 44.16 (N(*C*H$_3$)$_2$), 53.07 (*C*H$_2$N), 55.07 (C-5-O*C*H$_3$), 56.23 (C-4′-O*C*H$_3$), 101.37 (C-4), 112.33 (C-6), 113.27 (C-3), 115.02 (C-3′), 116.05 (C-7), 127.57 (C-1′, C-2′), 129.39 (C-2), 130.43 (C-3a), 136.16 (C-7a), 154.83 (C-5), 158.66 (C-4′), 163.49 ppm(C=O); IR: ν = 3230, 2934, 2832, 3770, 1619, 1510, 1457, 1435 cm^{-1}; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{21}H_{26}N_3O_3$: 368.1969, found 368.1969.

4.1.16. General procedure for the synthesis of 18a–c

Methyl iodide (1.59 mL, 25.5 mmol) was added to a solution of the appropriate *Mannich* base **17a–c** (4.25 mmol) in absolute CH_2Cl_2 (150 mL). The reaction mixture was stirred at room temperature for 3 h. The volatiles were removed in vacuo to afford **17a–c** methoiodides. This crude ammonium salt was suspended in absolute acetonitrile (170 mL). Dicyclohexyl-[18]-crown-[6] (2.03 g, 5.45 mmol) and potassium cyanide (3.98 g, 61.1 mmol) were added and the resulting reaction mixture was heated at reflux for 3 h. The solvent was evaporated under reduced pressure and the residue was extracted with ethyl acetate (2 × 50 mL). The organic layer was washed with water (2 × 85 mL), dried (Na_2SO_4) and evaporated in vacuo.

4.1.16.1. 3-(Cyanomethyl)-*N*-ethyl-5-methoxy-*N*-methyl-1*H*-indole-2-carboxamide (18a). Compound **18a** (0.27 g, 60%) was obtained from **17a** (0.48 g) after purification by silica gel chromatography ($CHCl_3$/ethyl acetate, 1:1) as a light brown solid, mp: 133–135 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.09 (t, J = 7.1 Hz, 3H, C*H*$_3$–CH$_2$), 2.94 (s, 3H, N–CH_3), 3.39 (q, J = 7.1 Hz, 2H, CH_3–C*H*$_2$), 3.76 (s, 2H, CH_2–CN), 3.77 (s, 3H, OCH_3), 6.82 (dd, J = 2.3, 8.8 Hz, 1H, H-6), 6.94 (d, J = 2.3 Hz, 1H, H-4), 7.12 (d, J = 8.8 Hz, 1H, H-7), 9.39 ppm (br, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.88 (*C*H$_3$–CH$_2$), 13.78 (*C*H$_2$–CN), 34.79 (N–CH_3), 44.15 (CH_3–*C*H$_2$), 55.92 (OCH_3), 100.20 (C-4), 104.07 (CN), 113.15 (C-7), 115.39 (C-6), 117.49 (C-3), 126.58, 129.44, 130.86 (C-2, C-3a, C-7a), 154.99 (C-5), 164.30 ppm (C=O); MS (EI, 70 eV) m/z (%): 271 (61) [M]$^+$, 212 (100), 184 (31); IR: ν = 3263, 2965, 2221, 1611, 1548 cm^{-1}. Anal. Calcd for $C_{15}H_{17}N_3O_2$: C, 66.40; H, 6.32; N, 15.49. Found: C, 66.01; H, 5.95; N, 15.19.

4.1.16.2. *N*-(4-Chlorophenyl)-3-(cyanomethyl)-5-methoxy-*N*-methyl-1H-indole-2-carboxamide (18b). Compound 18b (0.16 g, 56%) was obtained from **17b** (0.30 g) after purification by silica gel chromatography (ethyl acetate) as a light red viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.48 (s, 3H, N–CH_3), 3.78 (s, 2H, CH_2–CN), 3.81 (s, 3H, OCH_3), 6.89 (dd, J = 2.3, 8.8 Hz, 1H, H-6), 6.94 (d, J = 2.3 Hz, 1H, H-4), 7.06 (m, 1H, H-7), 7.09 (m, 2H, H_{ar}), 7.28 (m, 2H, H_{ar}), 8.17 ppm (br, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 3.79 (C*H*$_2$–CN), 38.37 (N–CH_3), 55.69 (OCH_3), 99.64 (C-4), 107.88 (CN), 112.86 (C-7), 116.58 (C-6), 117.62 (C-3), 126.25, 128.0, 130.51 (C-2, C-3a, C-7a), 127.15, 129.23, 133.04, 142.02 (C_{ar}), 154.95 (C-5), 162.92 (C=O) ppm; m/z (EI): 353 (60, M$^+$), 212 (63), 141 (100); IR: ν = 3283, 3059, 2939, 2198, 1625, 1458 cm^{-1}; MS (EI, 70 eV) m/z (%): 353 (60) [M]$^+$, 212 (63), 141 (100). Anal. Calcd for $C_{19}H_{16}ClN_3O_2$: C, 64.50; H, 4.56; N, 11.88. Found: C, 63.89; H, 4.14; N, 11.78.

4.1.16.3. 3-(Cyanomethyl)-5-methoxy-*N*-(4-methoxyphenyl)-*N*-methyl-1*H*-indole-2-carboxamide (18c). Compound **18c** (1.34 g, 90%) was obtained from **17c** (1.56 g) after purification by silica gel chromatography (CH_2Cl_2–MeOH–25% NH_3 160:10:1) as colorless crystals. Analytical sample was obtained by recrystallization from *t*-butyl methyl ether; mp: 126 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.44 (s, 3H, NC*H*$_3$), 3.77 (s, 3H, C-4′-O*C*H$_3$), 3.81 (s, 3H, C-5-O*C*H$_3$), 3.94 (s, 2H, C*H*$_2$CN), 6.82–6.90 (m, 2H, H-3′, H-5′), 6.95–7.02 (m, 3H, H-4, H-6, H-7), 7.08–7.15 (m, 2H, H-2′, H-6′), 7.72 ppm (br s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.86 (*C*H$_2$CN), 38.73 (N*C*H$_3$), 55.53 (C-4′-O*C*H$_3$), 55.70 (C-5-O*C*H$_3$), 99.65 (C-4), 108.73 (*C*N), 112.70 (C-7), 115.23 (C-3′), 116.34 (C-6), 117.95 (C-2), 126.23 (C-3), 127.54 (C-2′), 128.14 (C-3a), 130.30 (C-7a), 136.15 (C-1′), 154.79 (C-5), 156.63 (C-4′), 158.73 ppm (C=O); IR: ν = 3287 2359, 1654, 1591, 1580, 1510, 1456, 1436 cm^{-1}; MS (EI, 70 eV) m/z (%): 349 (19) [M]$^+$, 137 (100), 122 (27). Anal. Calcd for $C_{20}H_{19}N_3O_3$: C, 68.75; H, 5.48; N, 12.03. Found: C, 68.64; H, 5.31; N, 11.93.

4.1.17. General procedure for the synthesis of 18d–f

The appropriate *N*-methyl amine (1.00 mmol) was added to a solution of **15** (230 mg, 1.00 mmol) and EDCI·HCl (288 mg, 1.50 mmol) in absolute CH_2Cl_2 (1.5 mL). The mixture was stirred for 14 h and poured into 2 M hydrochloric acid (8 mL). The phases were separated und the aqueous layer was extracted with CH_2Cl_2 (3 × 8 mL). The combined organic layers were washed with $NaHCO_3$ solution and water. After drying (Na_2SO_4) the solvent was evaporated under reduced pressure and the residue was subjected to silica gel chromatography (ethyl acetate for **18d**, ethyl acetate/*n*-pentane 3:2 for **18e**. **18f** was used without further purification). Analytical samples of **18d–f** were obtained by recrystallization from *t*-butyl methyl ether.

4.1.17.1. 3-(Cyanomethyl)-5-methoxy-*N*-methyl-*N*-phenyl-1*H*-indole-2-carboxamide (18d). Compound **18d** (192 mg, 60%) was obtained from **15** (230 mg) and methylphenylamine (107 mg) as pale red crystals; mp: 126–127 °C; ^{1}H NMR (400 MHz, $CDCl_3$):

δ = 3.50 (s, 3H, NCH_3), 3.80 (s, 3H, OCH_3), 3.88 (s, 2H, CH_2CN), 6.86 (dd, J = 2.5, 8.8 Hz, 1H, H-6), 6.95–7.00 (m, 2H, H-4, H-7), 7.15–7.38 (m, 5H, H_{ar}), 7.75 ppm (br s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.82 (*C*H_2CN), 38.46 (N*C*H_3), 55.71 (O*C*H_3), 99.69 (C-4), 112.67 (C-7), 116.48 (C-6), 117.83 (*C*N), 126.24 (C-3), 126.26 (C-2′, C-6′), 127.60 (C-4′), 128.09 (C-2), 130.06 (C-3′, C-5′), 130.35 (C-3a), 132.54 (C-7a), 143.51 (C-1′), 154.85 (C-5), 162.57 ppm (C=O); IR: ν = 3269, 2360, 2330, 2623, 2604, 1623, 1604, 1582, 1538, 1493, 1456, 1434 cm^{-1}; MS (EI, 70 eV) *m/z* (%): 319 (49) [M]$^+$, 213 (28), 212 (48), 108 (19), 107 (100), 106 (19), 73 (15), 44 (16). Anal. Calcd for $C_{19}H_{17}N_3O_2$: C, 71.46; H, 5.37; N, 13.16. Found: C, 71.19; H, 5.41; N, 12.95.

4.1.17.2. 3-(Cyanomethyl)-5-methoxy-*N*-methyl-*N*-[4-(trifluoromethyl)phenyl]-1*H*-indole-2-carboxamide (18e). Compound **18e** (217 mg, 56%) was obtained from **15** (230 mg) and methyl-[4-(trifluoromethyl)phenyl)] amine (175 mg) as colorless crystals; mp: 166–167 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.53 (s, 3H, NCH_3), 3.70 (s, 2H, CH_2CN), 3.80 (s, 3H, OCH_3), 6.86–6.95 (m, 2H, H-4, H-6), 7.08 (d, J = 8.6 Hz, 1H, H-7), 7.25 (d, J = 8.3 Hz, 2H, H-3′, H-5′), 7.56 (d, J = 8.3 Hz, 2H, H-2′, H-6′), 8.35 ppm (br s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.75 (*C*H_2CN), 38.15 (N*C*H_3), 55.69 (O*C*H_3), 99.64 (C-4), 112.93 (C-7), 116.79 (C-6), 117.43 (*C*N), 122.15 (*C*F_3) 124.85 (C-3), 125.81 (C-2′, C-6′), 126.30 (C-2), 126.92 (C-3′, C-5′), 127.93 (C-4′), 129.05 (C-3a), 130.86 (C-7a), 146.68 (C-1′), 155.03 (C-5), 163.20 ppm (C=O); IR: ν = 3364, 2359, 2342, 1630, 1611 cm^{-1}; MS (EI, 70 eV) *m/z* (%): 388 (10), 387 (40) [M]$^+$, 213 (52), 212 (100), 184 (10), 170 (12). Anal. Calcd for $C_{20}H_{16}F_3N_3O_2$: C, 62.01; H, 4.16; N, 10.8. Found: C, 61.97; H, 4.42; N, 10.57.

4.1.17.3. *N*-Benzyl-3-(cyanomethyl)-5-methoxy-*N*-methyl-1*H*-indole-2-carboxamide (18f). Compound **18f** (293 mg, 88%) was obtained from **15** (230 mg) and benzylmethylamine (121 mg) as colorless crystals; mp: 177–178 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.04 (3H, s, NCH_3), 3.85 (s, 3H, OCH_3), 3.92 (s, 2H, CH_2CN), 4.68 (s, 2H, CH_2Ph), 6.92 (dd, J = 2.3, 8.8 Hz, 1H, H-6), 7.06 (d, J = 2.3 Hz, 1H, H-4), 7.16 (d, J = 8.8, 1H, H-7), 7.22–7.40 (m, 5H, H_{ar}), 8.63 ppm (br s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.74 (*C*H_2CN), 35.34 (N*C*H_3), 54.66 (*C*H_2Ph), 55.81 (O*C*H_3), 99.90 (C-4), 104.09 (*C*N), 112.86 (C-7), 115.89 (C-6), 117.37 (C-3), 126.68 (C-2), 127.54 (C-4′), 128.04 (C-2′, C-6′), 128.74 (C-3a), 128.83 (C-3′, C-5′), 130.50 (C-1′), 136.18 (C-7a), 155.07 (C-5), 164.36 ppm (C=O); IR: ν = 3256, 3244, 1602, 1549, 1485, 1457, 1412 cm^{-1}; MS (EI, 70 eV) *m/z* (%): 333 (26) [M]$^+$, 242 (100), 212 (23), 211 (47), 186 (15), 120 (25), 91 (100). Anal. Calcd for $C_{20}H_{19}N_3O_2$: C, 72.05; H, 5.74; N, 12.60. Found: C, 72.11; H, 5.35; N, 12.72.

4.1.18. General procedure for the synthesis of 19a–f

A solution of the appropriate amide **18a**–**f** (1 equiv) in absolute THF (25 mL) was added to a stirred suspension of $LiAlH_4$ (11 equiv) in absolute diethyl ether (30 mL) at 0–5 °C. The reaction mixture was refluxed for 3 h. The reaction was quenched by a slow addition of water at 0–5 °C. The formed precipitate was filtered off through a pad of Celite®545 and washed with ethyl acetate (150 mL). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 75 mL). The combined organic layers were washed with water (2 × 75 mL) and dried over Na_2SO_4. The volatiles were removed under vacuum to afford the respective amines **19a**–**f** as viscous oils that were used in the next step without further purification.

4.1.19. General procedure for the synthesis of 20a–f

A stirred solution of the respective amine **19a**–**f** (1 equiv) in absolute CH_2Cl_2 (25 mL) was treated with triethylamine (3.5 equiv) and acetic anhydride (2.5 equiv) 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.

4.1.19.1. *N*-[2-(2-{[Ethyl(methyl)amino]methyl}-5-methoxy-1*H*-indol-3-yl)ethyl]acetamide (20a). Compound **20a** (0.10 g, 41%, elution with $CHCl_3$–MeOH–25% NH_3, 100:10:1) was obtained from **19a** (0.21 g) as a light brown viscous oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.13 (t, J = 7.1 Hz, 3H, CH_3–CH$_2$), 1.84 (s, 3H, C(O)CH_3), 2.20 (s, 3H, N–CH_3), 2.53 (q, J = 7.1 Hz, 2H, CH$_3$–CH_2), 2.89 (t, J = 6.3 Hz, 2H, CH_2–CH$_2$–N), 3.44–3.48 (m, 2H, CH$_2$–CH_2–N), 3.56 (s, 2H, CH_2–N–CH$_3$), 3.81 (s, 3H, OCH_3), 6.79 (dd, J = 2.3, 8.8 Hz, 1H, H-6), 6.93 (br, 1H, NH), 6.95 (d, J = 2.3 Hz, 1H, H-4), 7.19 (d, J = 8.8 Hz, 1H, H-7), 8.63 ppm (br, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.99 (*C*H$_3$–CH$_2$), 22.99 (C(O)CH$_3$), 23.41 (*C*H$_2$–CH$_2$–N), 40.31 (*C*H$_2$–N–CH$_3$), 41.54 (N–CH$_3$), 51.62 (CH$_3$–*C*H$_2$), 52.26 (CH$_2$–*C*H$_2$–N), 55.90 (OCH$_3$), 100.25 (C-4), 110.50 (C-3), 111.64 (C-7), 111.96 (C-6), 128.44, 130.51, 133.33 (C-2, C-3a, C-7a), 153.99 (C-5), 170.27 ppm (C=O); IR: ν = 3841, 2934, 1645 cm^{-1}; HRMS-ESI *m/z* [M+Na]$^+$ calcd for $C_{17}H_{25}N_3O_2Na$: 326.1846, found: 326.1842.

4.1.19.2. *N*-[2-(2-{[(4-Chlorophenyl)(methyl)amino]methyl}-5-methoxy-1*H*-indol-3-yl)ethyl]acetamide (20b). Compound **20b** (520 mg, 48%, elution with ethyl acetate) was obtained from **19b** (0.10 g) as a yellow powder; mp: 61–63 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.88 (s, 3H, C(O)CH$_3$), 2.87 (s, 3H, N–CH$_3$), 2.93–2.95 (m, 2H, CH_2–CH$_2$–N), 3.45–3.50 (m, 2H, CH$_2$–CH_2–N), 3.83 (s, 3H, OCH$_3$), 4.49 (s, 2H, CH_2–N–CH$_3$), 5.69 (br, 1H, NH), 6.76 (m, 2H, H_{ar}), 6.79 (dd, J = 2.3, 8.8 Hz, 1H, H-6), 6.99 (d, J = 2.3 Hz, 1H, H-4), 7.14 (m, 1H, H-7), 7.17 (m, 2H, H_{ar}), 8.05 ppm (br, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 23.31 (C(O)CH$_3$), 24.13 (*C*H$_2$–CH$_2$–N), 38.74 (N–CH$_3$), 40.21 (*C*H$_2$–N–CH$_3$), 49.61 (CH$_2$–*C*H$_2$–N), 55.95 (OCH$_3$), 100.35 (C-4), 109.31 (C-3), 111.67 (C-6), 116.89 (C-7), 115.0, 123.19, 128.94, 130.44 (C-2, C-3a, C-7a), 129.19, 133.25, 148.76 (C_{ar}), 154.20 (C-5), 170.10 (C=O) ppm; IR: ν = 3269, 2926, 1645 cm^{-1}; HRMS-ESI *m/z* [M+Na]$^+$ calcd for $C_{21}H_{24}ClN_3O_2Na$: 408.1455, found: 408.1449.

4.1.19.3. *N*-[2-(5-Methoxy-2-{[(4-methoxyphenyl)(methyl)amino]methyl}-1*H*-indol-3-yl)-ethyl]acetamide (20c). Compound **20c** (46 mg, 12%, elution with ethyl acetate) was obtained from **19c** (349 mg) as colorless foam; mp: 45–49 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.84 (s, 3H, C(O)CH_3), 2.78 (s, 3H, NCH_3), 2.93 (t, J = 6.5 Hz, 2H, CH_2CH$_2$N), 3.48 (q, J = 6.5 Hz, 2H, CH$_2$CH_2N), 3.75 (s, 3H, C-4′-OCH_3), 3.83 (s, 3H, C-5-OCH_3), 4.37 (s, 2H, CH_2N), 5.95 (br s, 1H, C(O)NH), 6.78–6.94 (m, 5H, H-6, H-2′, H-3′, H-5′, H-6′), 6.99 (d, J = 2.3 Hz, 1H, H-4), 7.16 (d, J = 8.8 Hz, 1H, H-7), 8.25 ppm (br, 1H, N-1-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 23.24 (C(O)CH$_3$), 23.96 (*C*H$_2$CH$_2$N), 39.92 (NCH$_3$), 40.23 (CH$_2$CH$_2$N), 51.56 (*C*H$_2$N), 55.62 (C-4′-OCH$_3$), 55.93 (C-5-OCH$_3$), 100.31 (C-4), 109.62 (C-3), 111.68 (C-7), 111.90 (C-6), 114.58 (C-3′, C-5′), 117.38 (C-2′, C-6′), 128.10 (C-3a), 128.81 (C-7a), 130.44 (C-2), 133.07 (C-1′), 153.76 (C-4′), 154.12 (C-5), 170.11 ppm (C=O); IR: ν = 3277, 3063, 2995, 2934, 2833, 1639, 1510, 1485, 1452, 1437 cm^{-1}; HRMS-ESI *m/z* [M+H]$^+$ calcd for $C_{22}H_{28}N_3O_3$: 382.2125, found 382.2133. Anal. Calcd for $C_{22}H_{27}N_3O_3$: C, 69.27; H, 7.13; N, 11.02. Found: C, 68.90; H, 7.10; N, 10.92.

4.1.19.4. *N*-[2-(5-Methoxy-2-{[(methyl(phenyl)amino]methyl}-1*H*-indol-3-yl)ethyl]acetamide (20d). Compound **20d** (49 mg, 14%, elution with $CHCl_3$–MeOH–25% NH_3 100:10:1) was obtained from **19d** (319 mg) as a yellow viscous oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.84 (s, 3H, C(O)CH_3), 2.90 (t, J = 6.5 Hz, 2H, CH_2CH$_2$N), 2.92 (s, 3H, NCH_3), 3.45 (q, J = 6.5 Hz, 2H, CH$_2$CH_2N), 3.83 (s, 3H, OCH_3), 4.49 (s, 2H, CH_2N), 5.84 (br s, 1H, C(O)NH), 6.75–6.84 (m,

3H, H-3′, H-4′, H-5′), 6.86 (d, J = 8.8 Hz, 1H, H-6), 6.98 (d, J = 2.2 Hz, 1H, H-4), 7.12 (d, J = 8.8 Hz, 1H, H-7); 7.20–7.28 (m, 2H, H-2′,H-6′), 8.27 ppm (br, 1H, N-1-*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 23.19 (C(O)*C*H$_3$), 24.03 (*C*H$_2$CH$_2$N), 38.56 (N*C*H$_3$), 40.16 (CH$_2$*C*H$_2$N), 49.67 (*C*H$_2$N), 55.88 (O*C*H$_3$), 100.28 (C-4), 109.13 (C-3), 111.64 (C-6), 111.69 (C-7), 114.02 (C-2′, C-6′), 118.57 (C-4′), 128.24 (C-3a), 128.90 (C-7a), 129.39 (C-3′, C-5′), 130.43 (C-2), 150.02 (C-1′), 154.06 (C-5), 170.14 ppm (C=O); IR: 3270, 3063, 2934, 2831, 1633, 1595, 1487, 1450, 1435 cm^{-1}; HRMS-ESI *m/z* [M+Na]$^+$ calcd for $C_{21}H_{25}N_3O_2Na$: 374.1839, found: 374.1836.

4.1.19.5. *N*-{2-(5-Methoxy-2-({[methyl[4-(trifluoromethyl)phenyl]amino}]-methyl)-1*H*-indol-3-yl)]ethyl}acetamide (20e).

Compound **20e** (78 mg, 21%, elution with $CHCl_3$–MeOH–25% NH_3 100:10:1) was obtained from **19e** (350 mg) as colorless crystals; mp:165–167 °C. Analytical sample was obtained by recrystallization from $CHCl_3$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.90 (s, 3H, C(O)C*H*$_3$), 2.95 (t, J = 6.5 Hz, 2H, C*H*$_2$CH$_2$N), 2.98 (s, 3H, NC*H*$_3$), 3.48 (q, J = 6.5 Hz, 2H, CH$_2$C*H*$_2$N), 3.83 (s, 3H, OC*H*$_3$), 4.62 (s, 2H, C*H*$_2$N), 5.65 (br s, 1H, C(O)N*H*), 6.77–6.87 (m, 3H, H-6, H-2′, H-6′), 7.00 (d, J = 2.3 Hz, 1H, H-4), 7.15 (d, J = 8.8 Hz, 1H, H-7); 7.46 (d, J = 8.6 Hz, 2H, H-3′,H-5′), 8.01 ppm (br, 1H, N-1-*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 23.34 (C(O)*C*H$_3$), 24.22 (*C*H$_2$CH$_2$N), 38.30 (N*C*H$_3$), 40.16 (CH$_2$*C*H$_2$N), 48.24 (*C*H$_2$N), 55.95 (O*C*H$_3$), 100.39 (C-4), 109.52 (C-3), 111.72 (C-6), 112.01 (C-2′, C-6′), 112.13 (C-7), 123.47 (C-4′), 126.15 (*C*F$_3$), 126.71 (C-3′, C-5′), 128.94 (C-3a), 130.52 (C-7a), 132.68 (C-2), 150.02 (C-1′), 151.96 (C-5), 170.11 ppm (C=O); IR: ν = 3206, 2937, 2846, 1659, 1613 cm^{-1}; HRMS-ESI *m/z* [M+Na]$^+$ calcd for: $C_{22}H_{24}F_3N_3O_2Na$: 442.1713, found: 442.1710. Anal. Calcd for $C_{22}H_{24}F_3N_3O_2$: C, 63.00; H, 5.77; N, 10.02. Found: C, 62.65; H, 5.81; N, 9.82.

4.1.19.6. *N*-[2-(2-{[(Benzyl(methyl)amino]methyl}-5-methoxy-1*H*-indol-3-yl)ethyl]acetamide (20f).

Compound **20f** (350 mg, 96% , elution with $CHCl_3$–MeOH–25 NH_3 100:10:1) was obtained from **19f** (333 mg) as a light brown viscous oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.76 (s, 3H, C(O)C*H*$_3$), 2.19 (s, 3H, NC*H*$_3$), 2.90 (t, J = 6.3 Hz, 2H, C*H*$_2$CH$_2$N), 3.48 (q, J = 5.8 Hz, 2H, CH$_2$C*H*$_2$N), 3.56 (s, 2H, C*H*$_2$N), 3.82 (s, 3H, OC*H*$_3$), 6.56 (br s, 1H, C(O)N*H*), 6.81 (d, J = 8.8, 1.8 Hz, 1H, H-6), 6.96 (d, J = 1.8 Hz, 1H, H-4), 7.20 (d, J = 8.6 Hz, 1H, H-7), 7.24–7.34 (m, 5H, H-2′, H-3′, H-4′, H-5′, H-6′), 8.38 (br, 1H, N-1-*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 23.05 (C(O)*C*H$_3$), 23.38 (*C*H$_2$CH$_2$N), 40.22 (CH$_2$*C*H$_2$N), 42.33 (N*C*H$_3$), 52.54 (*C*H$_2$N), 55.91 (O*C*H$_3$), 62.70 (*C*H$_2$Ph), 100.28 (C-4), 110.23 (C-3), 111.52 (C-6), 111.87 (C-7), 127.45 (C-4′), 128.41 (C-3′, C-5′), 128.62 (C-3a), 129.25 (C-2′, C-6′), 130.43 (C-7a), 133.82 (C-2), 137.90 (C-1′), 154.00 (C-5), 170.14 ppm (C=O); IR: ν = 3242, 3061, 3029, 2935, 2876, 2832, 2795, 2358, 2340, 1649, 1556, 1485, 1453 cm^{-1}; HRMS-ESI *m/z* [M+H]$^+$ calcd for $C_{22}H_{28}N_3O_2$: 366.2176, found: 366.2173.

4.1.19.7. *N*-[2-(2-{[(Benzyl(methyl)amino]methyl}-1-(cyano)-methyl-5-methoxy-1*H*-indol-3-yl)ethyl]acetamide (21).

To a solution of the acetamide **20f** (365 mg, 1.00 mmol) in absolute DMF (2.5 mL) potassium *tert*-butoxide (134 mg, 1.20 mmol) was added at room temperature. After 40 min, bromoacetonitrile (0.07 mL, 1.03 mmol) was added dropwise and the solution was heated at 65 °C for 30 min and left stirring at room temperature for 5 h. Another portion of bromoacetonitrile (0.05 mL) was added and the reaction was stirred for a further hour. The reaction mixture was poured onto ice (20 g) and the mixture was extracted with $CHCl_3$ (5 × 15 mL). The extracts were dried (Na_2SO_4), evaporated under vacuum and the residue was purified by silica gel chromatography (CH_2Cl_2/THF 1:1) to afford **21** as yellow viscous oil (183 mg, 45%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.90 (s, 3H, C(O)C*H*$_3$), 2.48 (s, 3H, NC*H*$_3$), 2.91 (t, J = 6.4 Hz, 2H, C*H*$_2$CH$_2$N), 3.43 (q, J = 6.4 Hz, 2H, CH$_2$C*H*$_2$N), 3.53 (s, 4H, C*H*$_2$N, C*H*$_2$Ph), 3.83 (s, 3H, OC*H*$_3$), 5.16 (s, 2H, C*H*$_2$CN), 5.78 (br, 1H, C(O)N*H*), 6.90 (dd, J = 8.8, 2.2 Hz, 1H, H-6), 7.01 (d, J = 2.2 Hz, 1H, H-4), 7.12–7.40 ppm (m, 6H, H-7, H-2′, H-3′, H-4′, H-5′, H-6′); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.59 (C(O)*C*H$_3$), 23.28 (*C*H$_2$CN), 23.38 (*C*H$_2$CH$_2$N), 40.14 (CH$_2$*C*H$_2$N), 42.01 (N*C*H$_3$), 44.01 (*C*H$_2$N), 55.95 (O*C*H$_3$), 62.39 (*C*H$_2$Ph), 101.34 (C-4), 109.29 (C-6), 112.61 (C-7), 113.09 (C-3), 115.18 (*C*N), 127.53 (C-4′), 128.46 (C-3a), 128.52 (C-3′, C-5′), 129.12 (C-2′, C-6′), 131.40 (C-7a), 133.01 (C-2), 138.11 (C-1′), 154.87 (C-5), 170.16 ppm (C=O); IR: ν = 3299, 3028, 2929, 2834, 2794, 2342, 1632, 1484, 1454, 1432, 1364 cm^{-1}; HRMS-ESI *m/z* [M+Na]$^+$ calcd for $C_{24}H_{28}N_4O_2Na$: 427.2104, found: 427.2113.

4.1.19.8. *N*-[2-(2-{[(Benzyl(methyl)amino]methyl}-1-[2-(cyano)ethyl]-5-methoxy-1*H*-indol-3-yl)ethyl]acetamide (22).

To a stirred solution of acetamide **20f** (410 mg, 1.12 mmol) and acrylonitrile (0.16 mL, 2.24 mmol) in dioxane–THF (6.7 mL, 1:1) at 0 °C was added dropwise a catalytical amount of Triton-B® (40% benzyltrimethylammonium hydroxide solution in MeOH, 0.06 mL). The cooling bath was removed and reaction mixture was stirred for 2 h. The mixture was acidified with 2 M hydrochloric acid (4 mL) and the volatiles were evaporated in vacuo. The residue was purified by column chromatography on silica gel ($CHCl_3$–MeOH–25% NH_3 100:10:1) to yield **22** (288 mg, 61%) as a yellow viscous oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.90 (s, 3H, C(O)C*H*$_3$), 2.14 (s, 3H, NC*H*$_3$), 2.72 (t, J = 7.2 Hz, 2H, CH$_2$C*H*$_2$CN), 2.88–2.94 (m, 2H, C*H*$_2$CH$_2$N), 3.44 (q, J = 6.4 Hz, 2H, CH$_2$C*H*$_2$N), 3.55 (s, 2H, C*H*$_2$Ph), 3.58 (s, 2H, C*H*$_2$N), 3.83 (s, 3H, OC*H*$_3$), 4.37 (t, J = 7.2 Hz, 2H, C*H*$_2$CH$_2$CN), 5.88 (br, 1H, C(O)N*H*), 6.86 (dd, J = 8.8, 2.3 Hz, 1H, H-6), 7.01 (d, J = 2.3 Hz, 1H, H-4), 7.12 (d, J = 8.8 Hz, 1H, H-7), 7.23–7.39 ppm (m, 5H, H-2′, H-3′, H-4′, H-5′, H-6′); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 23.16 (C(O)*C*H$_3$), 18.19 (CH$_2$*C*H$_2$CN), 24.15 (*C*H$_2$CH$_2$N), 39.17 (*C*H$_2$CH$_2$CN), 40.02 (CH$_2$*C*H$_2$N), 41.91 (N*C*H$_3$), 50.22 (*C*H$_2$N), 55.90 (O*C*H$_3$), 62.81 (*C*H$_2$Ph), 101.04 (C-4), 109.27 (C-6), 112.06 (C-3), 112.19 (C-7), 117.56 (*C*N), 127.50 (C-4′), 128.08 (C-3a), 128.52 (C-3′, C-5′), 129.29 (C-2′, C-6′), 130.93 (C-7a), 133.61 (C-2), 138.21 (C-1′), 154.36 (C-5), 170.14 ppm (C=O); IR: ν = 3287, 2927, 2832, 2361, 2340, 1649, 1542, 1482, 1454, 1423 cm^{-1}; HRMS-ESI *m/z* [M+Na]$^+$ calcd for $C_{25}H_{30}N_4O_2Na$: 441.2266, found: 441.2261.

4.1.20. General procedure for the synthesis of 12c, 23

To a solution of the respective acetamide **21**, **22** (0.717 mmol) in glacial acetic acid (1.45 mL) was added 10% Pd/C (37 mg) in one portion. The mixture was hydrogenated (7 hPa) at room temperature for 48 h. The catalyst was removed by filtration through a pad of Celite®545 and washed with $CHCl_3$ (50 mL). The resulting solution was made basic with 25% aqueous NH_3 and extracted with $CHCl_3$. The combined $CHCl_3$ solutions were dried over Na_2SO_4 and the solvent was evaporated under vacuum. The residue was purified by silica gel chromatography ($CHCl_3$–MeOH–25% NH_3 100:10:1).

4.1.20.1. *N*-[2-(8-Methoxy-2-methyl-1,2,3,4-tetrahydropyrazino[1,2-*a*]indol-10-yl)ethyl]acetamide (12c).

Compound **12c** (108 mg, 50%) was obtained from **21** (290 mg) as yellow viscous oil.

4.1.20.2. *N*-[2-(9-Methoxy-2-methyl-2,3,4,5-tetrahydro-1*H*-[1,4]diazepino[1,2-a]indol-11-yl)ethyl]acetamide (23).

Compound **23** (92 mg, 41%) was obtained from **22** (300 mg) as colorless foam; mp: 45–49 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.77–1.84 (m, 2H, H-4), 1.86 (s, 3H, C(O)C*H*$_3$), 2.36 (s, 3H, NC*H*$_3$), 2.85–2.94 (m, 4H, C*H*$_2$CH$_2$N, H-3), 3.44 (q, J = 6.1 Hz, 2H, CH$_2$C*H*$_2$N), 3.73 (s, 2H, H-1), 3.83 (s, 3H, OCH$_3$), 4.16 (t, J = 4.3 Hz, 2H, H-5), 5.99 (br 1H, C(O)N*H*), 6.83 (dd, J = 8.8, 2.5 Hz, 1H, H-8), 6.95 (d, J = 2.5 Hz, 1H,

H-10), 7.13 ppm (d, J = 8.8 Hz, 1H, H-7); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 23.05 (C(O)CH_3), 23.74 (CH_2CH_2N), 40.02 ($CH_2CH_2N_2$), 28.32 (C-4), 44.84 (NCH_3), 44.18 (C-1), 51.55 (C-5), 60.49 (C-3), 55.95 (OCH_3), 100.49 (C-10), 109.60 (C-11), 111.68 (C-7), 109.53 (C-8), 127.20 (C-10a), 131.53 (C-6a), 136.60 (C-11a), 153.75 (C-9), 170.16 (C=O) ppm; HRMS-ESI m/z $[M+H]^+$ calcd for: $C_{18}H_{26}N_3O_2$: 316.2019, found: 316.2023.

4.2. Pharmacology

4.2.1. Competition binding analysis

All compounds were tested for their binding affinity and selectivity for each of the melatonin receptor subtypes, MT_1 and MT_2 using competition binding analysis. Briefly, cells expressing the human MT_1 or MT_2 melatonin receptor (MT_1-CHO, MT_2-CHO) were grown to confluence on 10 cm cell culture plates until they reach approximately 80% confluence. Next, cells were washed, lifted, and added to tubes containing 80–100 pM 2-[^{125}I]-iodomelatonin in the absence (total binding) or presence of melatonin (1 pM to 1 μM) or the test compounds (1 pM to 1 μM). The reactions were incubated for 1 h at 25 °C, terminated following the addition of cold Tris-HCl solution (50 mM, pH 7.4) and then filtered through glass fiber filters (Schleicher and Schuell, Keene, NH) saturated in polyethylenimine 0.5% solution (v/v). Radioactivity was determined using a gamma counter. Data points were fit by site non-linear regression analysis based upon the lowest residual sum of squares (GraphPad Prism) and affinity constants (K_i) values calculated.

4.2.2. Cyclic AMP assays

The cAMP accumulation assays were carried out by Enzyme Immuno Antibody (EIA) kit according to manufacturer's directions. Stable CHO cell lines expressing human MT_1 or human MT_2 receptors were cultured on 10 cm plates in F-12 media containing 10% FBS and 1% pen/strep until they were 70–80% confluent, after which the cells were lifted and plated in 24- well plates. The following day, the cells were incubated in serum-free media containing one of the following treatment groups for 20 minutes at 37 °C—(a) 30 μM rolipram alone (basal), (b) 30 μM rolipram and 100 μM forskolin (maximal accumulation), (c) 30 μM rolipram, 100 μM forskolin, and 10 nM melatonin, or (d) 30 μM rolipram, 100 μM forskolin, and **12b** or **20d** (in concentrations ranging from 10^{-11} M to 10^{-6} M). Cyclic AMP accumulation was expressed as a percentage of forskolin response within each group. Where appropriate, curves were fit using non-linear regression analysis (one-site) and potency (IC_{50}) values were calculated using the commercially available software (GraphPad PRISM®).

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References and notes

1. Reppert, S. M.; Weaver, D. R.; Godson, C. *Trends Pharmacol. Sci.* **1996**, *17*, 100.
2. Zlotos, D. P. *Arch. Pharm. Chem. Life Sci.* **2005**, *338*, 229.
3. Spadoni, G.; Bedini, A. In *From Molecules to Therapy*; Pandi-Perumal, S. R., Cardinali, D. P., Eds.; Nova Science, 2007; pp 33–45.
4. Garratt, P. J.; Tsotinis, A. *Mini-Rev. Med. Chem.* **2007**, *10*, 1075.
5. Rivara, S.; Mor, M.; Lorenzi, S.; Lodola, A.; Plazzi, P. V.; Spadoni, G.; Bedini, A.; Tarzia, G. *Arkivoc* **2006**, *VIII*, 8.
6. Bedini, A.; Spadoni, G.; Gatti, G.; Lucarini, S.; Tarzia, G.; Rivara, S.; Lorenzi, S.; Lodola, A.; Mor, M.; Lucini, V.; Pannacci, M.; Scaglione, F. *J. Med. Chem.* **2006**, *49*, 7393.
7. Rivara, S.; Lodola, A.; Mor, M.; Bedini, A.; Spadoni, G.; Lucini, V.; Pannacci, M.; Fraschini, F.; Scaglione, F.; Sanchez, R. O.; Gobbi, G.; Tarzia, G. *J. Med. Chem.* **2007**, *50*, 6618.
8. Poissonnier-Durieux, S.; Ettaoussi, M.; Pérès, B.; Boutin, JA.; Audinot, V.; Bennejean, C.; Delagrange, P.; Caignard, DH.; Renard, P.; Berthelot, P.; Lesieur, D.; Yous, S. *Bioorg. Med. Chem.* **2008**, *16*, 8339.
9. Zlotos, D. P.; Attia, M. I.; Julius, J.; Shalini, S.; Witt-Enderby, P. A. *J. Med. Chem.* **2009**, *52*, 826.
10. Rivara, S.; Lorenzi, S.; Mor, M.; Plazzi, P. V.; Spadoni, G.; Bedini, A.; Tarzia, G. *J. Med. Chem.* **2005**, *48*, 4049.
11. Faust, R.; Garratt, P. J.; Jones, R.; Yeh, L.-K.; Tsotinis, A.; Panoussopoulou, M.; Calogeropoulou, T.; Teh, M.-T.; Sugden, D. *J. Med. Chem.* **2000**, *43*, 1050.
12. Wallez, V.; Durieux-Poissonier, S.; Chavatte, P.; Boutin, J. A.; Audinot, V.; Nicolas, J.-P.; Bennejean, C.; Delagrange, P.; Renard, P.; Lesieur, D. *J. Med. Chem.* **2002**, *45*, 2788.
13. Karageorge, G. N.; Bertenshaw, S.; Iben, L.; Xu, C.; Sarbin, N.; Gentile, A.; Dubowchik, G. M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5881.
14. Attia, M. I.; Julius, J.; Witt-Enderby, P. A.; Zlotos, D. P. *Tetrahedron* **2007**, *63*, 754.
15. Attia, M. I.; Witt-Enderby, P. A.; Julius, J. *Bioorg. Med. Chem.* **2008**, *16*, 7654.
16. Guandalini, L.; Martini, E.; Gualtieri, F.; Romanelli, M. N.; Varani, K. *Arkivoc* **2004**(V), 286.
17. Brimble, M. A.; Brimble, M. T.; Hodges, R.; Lane, G. *Aust. J. Chem.* **1988**, *41*, 1583.
18. Diker, K.; El Biach, K.; Döé de Maindreville, M.; Lévy, J. *J. Nat. Prod.* **1997**, *60*, 791.
19. Tsotinis, A.; Panoussopoulou, M.; Sivananthan, S.; Sugden, D. *Il Farmaco* **2001**, *56*, 725.
20. Campiani, G.; Butini, S.; Trotta, F.; Fattorusso, C.; Catalanotti, B.; Aiello, F.; Gemma, S.; Nacci, V.; Novellino, E.; Stark, J. A.; Cagnotto, A.; Fumagalli, E.; Carnovali, F.; Cervo, L.; Mennini, T. *J. Med. Chem.* **2003**, *46*, 3822.
21. Zaitsev, S. A.; Glushkov, R. G.; Mashkovskii, M. D.; Andreeva, N. I. *Khim.-Farm. Zh.* **1989**, *23*, 1201.
22. Rajur, S. B.; Merwade, A.-Y.; Hendi, S. B.; Basanagoudar, L. D. *Indian J. Chem., Sect. B* **1989**, *28B*, 1065.
23. Yamada, Y.; Takai, H.; Hatano, K.; Sakakibara, M.; Matsui, M. *Agric. Biol. Chem.* **1972**, *36*, 106.