Full Paper

N-Acetyl-5-arylalkoxytryptamine Analogs: Probing the Melatonin Receptors for MT_1-Selectivity

Christian Markl[1], William P. Clafshenkel[2], Mohamed I. Attia[1†], Shalini Sethi[2], Paula A. Witt-Enderby[2], and Darius P. Zlotos[3]

[1] Institute of Pharmacy and Food Chemistry, Pharmaceutical Chemistry, University of Würzburg, Würzburg, Germany
[2] Division of Pharmaceutical Sciences, School of Pharmacy, Duquesne University, Pittsburgh, PA, USA
[3] The German University in Cairo, Department of Pharmaceutical Chemistry, New Cairo City, Cairo, Egypt

A series of melatonin analogs obtained by the replacement of the ether methyl group with larger arylalkyl and aryloxyalkyl substituents was prepared in order to probe the melatonin receptors for MT_1-selectivity. The most MT_1-selective agents **11** and **15** were substituted with a $Ph(CH_2)_3$ or a $PhO(CH_2)_3$ group. Compounds **11** and **15** displayed 11.5-fold and 11-fold higher affinity for the MT_1 receptors than for the MT_2 subtype. Interestingly, in our binding assay **11** and **15** have shown considerably higher MT_1-affinity and selectivity than the reference ligand, the dimeric agomelatine **1a**.

Keywords: Melatonin analogs / Melatonin receptors / MT_1 receptor / MT_2 receptor

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Introduction

The neurohormone melatonin (*N*-acetyl-5-methoxytryptamine, MLT, Fig. 1) exerts its diverse physiological actions mostly through activation of the two high affinity G-protein-coupled MT_1 and MT_2 receptors [1].

An accurate characterization of melatonin receptor-mediated functions requires MT_1 and MT_2-selective ligands. While many series of MT_2-selective agents have been published in the last decade, pronounced MT_1-selectivity is still a challenge with only few examples of MT_1-selective agents reported up to date [2]. A common structural feature in the recently reported MT_2-selective ligands, most of them behaving as antagonists, is a lipophilic substituent located out of the plane of their core nucleus in a position corresponding to N1 or C2 of MLT [3]. The most MT_1-selective agents (Table 1) are dimers of the non-selective agonist agomelatine obtained by connecting two agomelatine units via their ether oxygen atoms by a polymethylene spacer. The optimal distance between the dimer head groups for MT_1-selectivity was determined to be three methylene groups [4]. The resulting compound **1a** behaved as MT_1 antagonist and displayed 224-times higher affinity for the MT_1 receptors ($K_i = 0.5$ nM) than for the MT_2 receptors ($K_i = 112$ nM) expressed in the HEK-cells. In the CHO-cells, the corresponding K_i MT_1/K_i MT_2 ratio was reduced to 38 [5]. Extension of the spacer to four methylene units to give compound **1b** resulted in a reduced MT_1-selectivity in HEK-cells (121-fold) and similar MT_1-selectivity in CHO-cells (54-fold) when compared to the C_3-analog **1a** [5]. Recently, a series of heterodimer analogs of **1b**, formally obtained by replacement of one of its agomelatine units with various aryl moieties showing MT_1-selectivity between 5- and 93-fold (CHO-cells) has been synthesized [6]. The most MT_1-selective ligand in this series is the biphenylcarboxylic acid analog **1c**. The findings indicate that a bivalent ligand is not necessary to achieve MT_1 selectivity. Another dimeric MT_1-selective agent was obtained by direct coupling of two agomelatine units *via* the aromatic carbon atoms to give the agonist **2** [4]. Diverse monomeric ligands displayed only moderate MT_1-selectivity with the 4-phenylbutyl substituted benzoxazole analog **3** [7] and the hexyloxy

Correspondence: Darius P. Zlotos, The German University in Cairo, Department of Pharmaceutical Chemistry, New Cairo City, 11835 Cairo, Egypt.
E-mail: darius.zlotos@guc.edu.eg
Fax: +20 27581041

†Current address: Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

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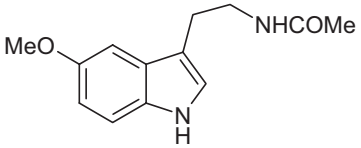


Figure 1. Structure of melatonin (MLT).

chromane analog **4** [4] being the most selective examples.

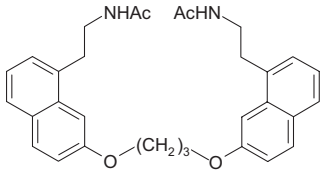
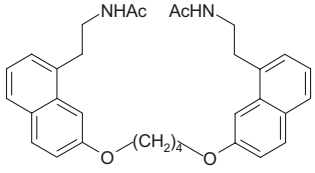
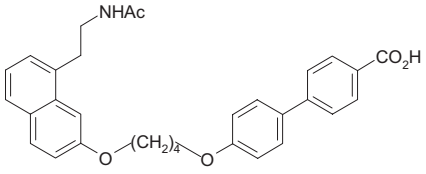
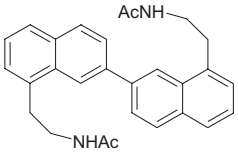
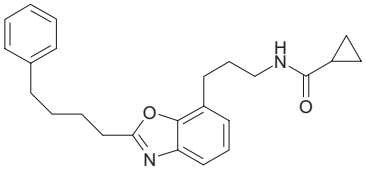
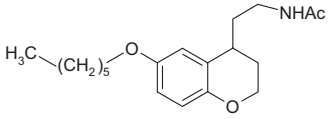
The only common structural feature of MT_1-selective ligands seems to be the the presence of a bulky hydrophobic substituent in a position topologically equivalent to the methoxy group of MLT. However, the optimal length of this substituent, as well as the nature of the terminal aromatic ring generating MT_1-selectivity has not been investigated until now. In this paper, we describe the synthesis and pharmacological evaluation of a novel extensive series of MLT analogs obtained by replacement of the ether methyl group with arylalkyl and aryloxyalkyl moieties of different chain lengths. The binding affinities of the novel compounds for the human MT_1 and MT_2 melatonin receptors expressed in CHO-cells were determined and compared to those of the most known MT_1-selective reference compound **1a** tested in the same binding assay.

Results and discussion

Chemistry

The dimeric agomelatine **1a** was prepared from *O*-demethylagomelatine and 1,3-dibromopropane using K_2CO_3 in MeCN as described by Descamps-Francois et al. [3] in 66% yield. However, as we observed a considerably different melting point (mp 113–116°C) from the one reported (mp 101–103°C) [3], compound **1a** was fully characterized by NMR, MS, HPLC and combustion analysis to confirm its structure. Moreover, a by-product, *O*-allyl-*O*-demethylagomelatine, was observed as a result of HBr elimination from the monoalkylated intermediate. In order to obtain the corresponding dimer of MLT **5**, the same reaction conditions were applied to *N*-acetyl-5-hydroxytryptamine **6** yielding the allyl ether **7** as a major product. The desired dimeric MLT **5** could be obtained in 35% yield under liquid-liquid-liquid phase transfer catalysis conditions (TBAB, NaOH, NaCl, toluene, H_2O) [8] using 1-bromo-3-chloropropane as starting material (Scheme 1). All other target compounds were prepared by alkylation of **6** with different alkyl halides. For the commercially available **6**, we developed two new synthetic approaches starting from the lower-priced 5-benzyloxyindole-3-acetonitrile (Scheme 1). A two step reaction sequence involved reduction of the nitrile group using $NaBH_4$, $NiCl_2 \cdot 6\,H_2O$, Ac_2O followed by debenzylation of the resulting (benzyloxyindolyl)-ethylacetamide **8** by means of ammonium formate, 10% Pd/C. A more convenient one step reaction applied Raney Ni-Pd/C catalytic hydrogenation in the presence of Ac_2O. *O*-Alkylation of **6** with a variety of arylalkylhalides was carried out using the standard procedure (K_2CO_3, CH_3CN, 18 h reflux) to give the target

Table 1. MT_1-Selective ligands and their binding constants K_i [nM] at the MT_1 and MT_2 receptors.

		MT_1	MT_2
1a		0.5 [a] 3.9 [b]	112 [a] 149 [b]
1b		0.6 [a] 3.1 [b]	73 [a] 167 [b]
1c		0.55 [b]	51 [b]
2		3.2 [a] 5.2 [b]	253 [a] 246 [b]
3		0.63 [c]	22 [c]
4		1.2 [a] 3.4 [b]	29 [a] 21 [b]

[a] HEK-cells, [b] CHO-cells, [c] NIH3T3-cells

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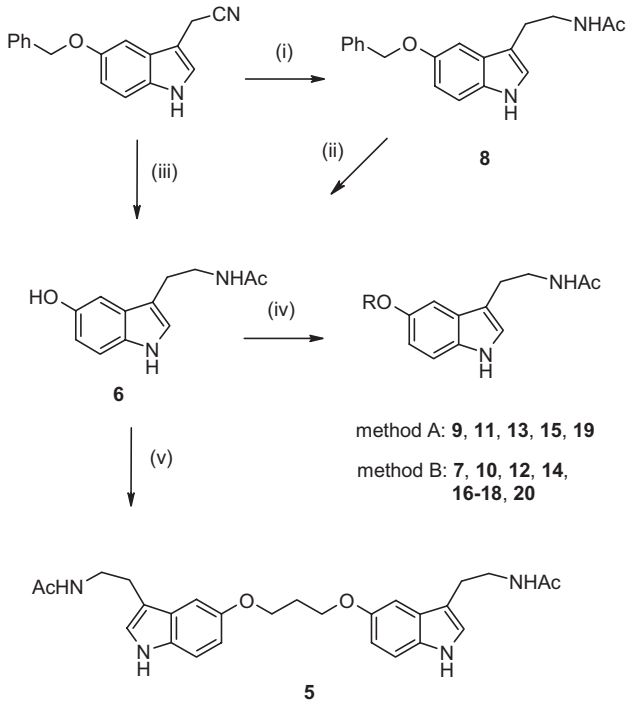

Scheme 1. Reagents and conditions: (i) $NaBH_4$, Ac_2O, $NiCl_2 \cdot 6\ H_2O$, MeOH, rt, 20 h; (ii) ammonium formate, 10% Pd/C, EtOH, reflux, 2 h; (iii) Raney Ni, 10% Pd/C, 400 kPa H_2, THF, Ac_2O, 50°C, 12 h; (iv) method A: K_2CO_3, CH_3CN, respective alkylation agent, reflux, 18 h, method B: Cs_2CO_3, CH_3CN, respective alkylation agent, 60°C, 4 h; (v) NaOH, NaCl, TBAB, H_2O, Br-$(CH_2)_3$-Cl, toluene, 50°C, 3 h.

ethers **9**, **11**, **13**, **15**, and **19** (see Table 2 for structures). The remaining target compounds **7**, **10**, **12**, **14**, **16–18**, and **20** were obtained according to a modified alkylating protocol using Cs_2CO_3 as base, lower temperature (60°C) and shorter reaction time (4 h).

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 was in the range of the reported literature. For the sake of comparison, the most MT_1-selective ligand reported to date, the dimeric agomelatine analog **1a**, was included in our study The results are compiled in Table 2. One of the most MT_1-selective ligands **15** was subjected to functional studies using cyclic AMP assay in CHO cells expressing human MT_1 or MT_2 receptors. The results are shown in Fig. 2.

Discussion

To our surprise, the reference MT_1-selective ligand, the dimeric agomelatine **1a**, displayed a much lower affinity for the MT_1 receptors (K_i = 112 nM) than the affinity reported by Descamps-Francois et al. [3] and Audinot et al. (K_i = 3.9 nM) [4]. Moreover, instead of the expected high MT_1-selectivity (K_i MT_2/K_i MT_1 = 38) [4] we observed lower selectivity (3.2-fold) for the MT_1 subtype. It should be mentioned that the same cell-lines (CHO-cells) were used in both laboratories. Moreover, MLT competition assays run in parallel as control experiments gave similar MT_1 (0.46 nM vs. 0.22 nM [5]) and MT_2 (0.95 nM vs. 0.35 nM [5]) binding constants. Though these differences cannot be explained, these findings underscore the importance of running multiple reference compounds when conducting pharmacological analyses of compounds that have ideally different binding profiles to minimize the variability of results between labs. This then allows for better more direct comparisons of the pharmacological data obtained from the different laboratories.

The structure of agomelatine has been derived from MLT by the bioisosteric replacement of the indole ring with a naphthalene scaffold. In order to examine which of the two ring systems generates higher MT_1-selectivity, we synthesized a dimeric MLT **5** obtained by connecting two MLT units via C_3 spacer. Compound **5** displayed a 4-fold lower affinity for the MT_1 receptors (K_i = 464 nM) and an 8-fold lower affinity for the MT_2 subtype (K_i = 2828 nM) than the dimeric agomelatine **1a** resulting in an increased MT_1-selectivity ratio of 6. The higher affinities of the agomelatine analog for both MLT receptor subtypes are in agreement with the binding profile of the monomers, as agomelatine displays higher binding for both receptors than MLT [3].

A common structural feature of MT_1-selective ligands is the the presence of a bulky hydrophobic substituent in a position topologically equivalent to the methoxy group of MLT. In order to examine the optimal length of this substituent, as well as the nature of the terminal aromatic ring generating MT_1-selectivity, we replaced the ether methyl group of MLT with arylalkyl and aryloxyalkyl moieties of different chain lengths. The allyl (**7**) and 2-naphthyl (**9**) analogs displayed low nanomolar affinities for both MT_1 and MT_2 receptors without pronounced subtype selectivity. The *O*-benzyl derivative (**8**) maintained the high affinity for the MT_2 receptors (K_i = 1.8 nM) when compared to MLT (K_i = 1 nM) while its binding for the MT_1-subtype was 30-times reduced (K_i = 14.6 nM) relative to the parent compound resulting in 8-fold selectivity toward the MT_2 receptors. Compound **8** has already been reported to be MT_2-selective displaying 18-fold higher affinity for MT_2-receptors (K_i = 6.6 nM) than for the MT_1-receptors (K_i = 118 nM) expressed in NIH3T3 cells [9]. The differences in binding affinities and selectivity ratio can most likely be explained by different cell lines used in both binding assays.

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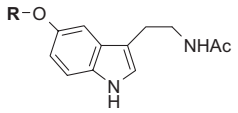
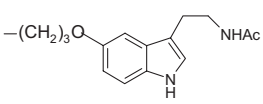
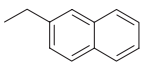
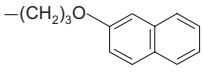
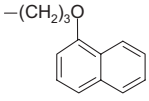
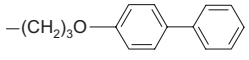
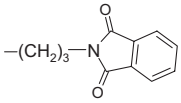
Table 2. Binding affinity[a] of compounds **1a** and **5–20** for the human MT_1 and MT_2 receptors expressed in CHO cells obtained in competition radioligand binding assays using 2-[^{125}I]-iodomelatonin.



	R	MT_1 pK_i ± SEM (K_i, nM)	MT_2 pK_i ± SEM (K_i, nM)	K_i MT_2/K_i MT_1
1a	–	6.95 ± 0.03 (112)	6.45 ± 0.03 (355)	3.2
MLT	$-CH_3$	9.34 ± 0.10 (0.46)	9.02 ± 0.09 (0.95)	2.1
5		6.33 ± 0.02 (464)	5.55 ± 0.04 (2828)	6.1
7	$-CH_2-CH=CH_2$	8.20 ± 0.03 (6.3)	8.76 ± 0.01 (1.7)	0.27
9		8.09 ± 0.14 (8.1)	7.74 ± 0.02 (18.3)	2.3
8	$-CH_2$-Ph	7.84 ± 0.02 (14.6)	8.74 ± 0.12 (1.8)	0.12
10	$-(CH_2)_2$-Ph	9.83 ± 0.12 (0.15)	9.72 ± 0.04 (0.19)	1.3
11	$-(CH_2)_3$-Ph	8.40 ± 0.12 (3.9)	7.35 ± 0.41 (45.0)	11.5
12	$-(CH_2)_4$-Ph	8.33 ± 0.08 (4.7)	7.99 ± 0.02 (10.2)	2.2
13	$-(CH_2)_5$-Ph	7.13 ± 0.07 (74)	8.09 ± 0.07 (8.2)	0.11
14	$-(CH_2)_2$-OPh	8.97 ± 0.09 (1.1)	8.57 ± 0.05 (2.7)	2.5
15	$-(CH_2)_3$-OPh	8.10 ± 0.08 (7.9)	7.06 ± 0.15 (87)	11
16		7.31 ± 0.06 (49.2)	7.78 ± 0.03 (16.7)	0.34
17		8.38 ± 0.04 (4.1)	8.13 ± 0.02 (7.4)	1.8
18		7.63 ± 0.03 (23.6)	7.74 ± 0.22 (18.1)	0.77
19		7.15 ± 0.04 (71.6)	6.84 ± 0.02 (145)	2.0
20	$-(CH_2)_5-CH_3$	8.31 ± 0.08 (4.9)	8.18 ± 0.12 (6.6)	1.3

[a] pK_i values were calculated from IC_{50} values obtained from competitive curves according to the method of Cheng and Prusoff [12] and are the mean of three determinations.

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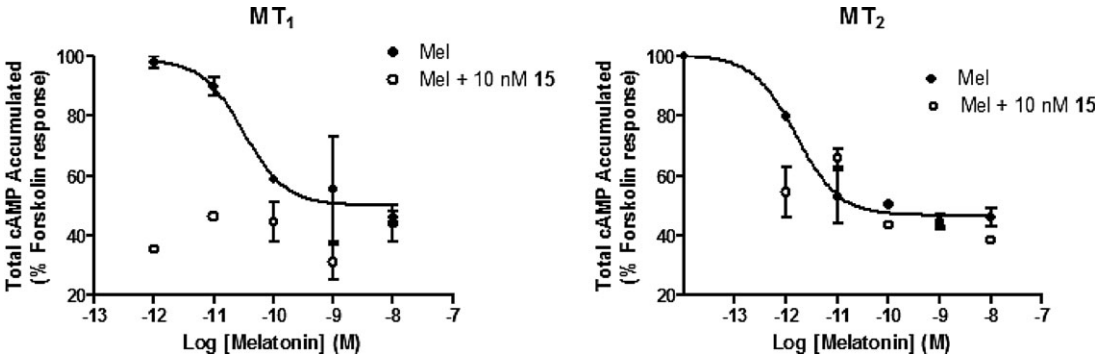

Figure 2. Functional analysis of melatonin (1 pM–10 nM; closed circles) in the absence or presence of **15** (10 nM; open circles) to inhibit forskolin-induced cAMP accumulation in CHO cells expressing the human MT_1 melatonin receptor (MT_1-CHO) or human MT_2 melatonin receptor (MT_2-CHO). Each data point represents the average ± SEM of two experiments. Where appropriate, curves were fit by non-linear regression analysis by 1-site fit to obtain potency (IC_{50}) and maximal inhibitory values using GraphPad Prism software.

Elongation of the benzyl side chain in **8** by one methylene group led to a complete loss of MT_2-selectivity. The resulting phenylethyl analog **10** exhibited the highest affinities for melatonin receptors of the whole series (K_i = 0.15 and 0.19 nM for MT_1 and MT_2). A further increase in chain length reversed the subtype selectivity generating the most MT_1-selective agent **11**. Compound **11** with a C_3 spacer between MLT and the phenyl group displayed a 11.5-fold higher binding for the MT_1 (K_i = 3.9 nM) than for the MT_2 receptors (K_i = 45 nM). Its MT_1-selectivity is 3.6-times higher than that of the reference agent **1a**. A further elongation of the polymethylene spacer abolished MT_1-selectivity. In the C_4-homolog **12**, the additional CH_2-group caused a 4-fold increase of binding for MT_2 receptors (K_i = 10.2 nM) while the MT_1-affinity was unchanged (K_i = 4.7 nM) when compared to the C_3 analog **11** resulting in considerable reduction of MT_1-selectivity. The C_5-homolog **13** displayed a 16-fold decrease of binding to MT_1 receptors (K_i = 74 nM) and unchanged MT_2-affinity relative to the C_4-analog **12** generating a 9-fold selectivity for the MT_2 receptors. The data in the homologous C_1-C_5 series **8**, **10**–**13** revealed the optimal chain length for MT_1 -selectivity to be C_3 which is in agreement with the results in the dimeric agomelatine series [3]. Introduction of a second ether oxygen to the most MT_1-selective ligand **11** maintained its superior pharmacological profile. The resulting phenyloxy analog **15** displayed an 11-fold higher affinity for the MT_1 (K_i = 7.9 nM) than for the MT_2 receptors (K_i = 87 nM). Reduction of the spacer length from C_3 to C_2 resulted in loss of MT_1-selectivity giving a high-affinity non-selective ligand **14** (K_i = 1.1 and 2.7 nM for MT_1 and MT_2).

The structure of the MT_1-selective ligand **15** was further modified by replacing the terminal phenyl group with bulkier aromatic ring systems, such as 2-naphthyl (**16**), 1-naphthyl (**17**) and biphenyl (**18**) resulting in loss of MT_1-selectivity. It is worth noting that the 2-naphthyl analog **16** represents a structural modification of the reference ligand **1a** obtained by the removal of the ethylacetamide side chain from one naphthyl moiety and replacement of the other naphthyl scaffold by the indole ring. These structural changes led to a 2.3-fold decrease of MT_1 (49 nM) and 21-fold decrease of MT_2-affinity (16.7 nM) generating a reversed 3-fold preference toward the MT_2 subtype.

Another replacement of the phenyl group by the phthalimide moiety in the MT_1-selective agent **11** to give compound **19** also led to reduced both MT_1-affinity (71.6 nM) and selectivity (MT_1-selectivity ratio 2). Finally, compound **20** bearing a hexyl group which is also present in the MT_1-selective ligand **4** exhibited nearly the same binding constants to both MLT receptor subtypes (K_i = 4.9 and 6.6 nM for MT_1 and MT_2).

Functional analysis of one of the two most MT_1-selective ligands, **15**, showed it to have agonist activity at both MT_1 (MT1-CHO) and MT_2 (MT2-CHO) receptors expressed in CHO cells. As shown in Fig. 2, melatonin (1 pM–10 nM) inhibited forskolin-induced cAMP accumulation in MT1-CHO cells in a concentration-dependent manner with a potency (IC_{50}) value of 32 pM and a maximum inhibition (49%) occurring at 10 nM melatonin. The addition of **15** (10 nM) with melatonin (1 pM–10 nM) did not competitively antagonize melatonin's actions at MT_1 receptors but rather acted as an agonist producing a maximal inhibitory (~63%) effect on forskolin-induced cAMP accumulation when combined with a concentration of melatonin (i.e., 1 pM) that produced no inhibition alone. Similarly, melatonin (1 pM–10 nM) inhibited forskolin-induced cAMP accumulation in MT_2-CHO cells with a potency value of 1.6 pM and maximum inhibition (~53%) occurring at 10 nM melatonin. **15** (10 nM), added in combination with melatonin (1 pM–10 nM) did not competitively antagonize melatonin's actions at MT_2 receptors but acted as an agonist producing a 25% further inhibition of forskolin-induced cAMP accumulation to that

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produced by 1 pM melatonin alone. For both MT_1-CHO and MT_2-CHO cell lines, **15**, added in combination with melatonin, produced maximum inhibition of forskolin-induced cAMP accumulation at all concentrations of melatonin tested.

In summary, a novel series of MLT analogs obtained by the systematic replacement of the ether methyl group with larger arylalkyl and aryloxyalkyl substituents was prepared in order to probe the melatonin receptors for MT_1-selectivity. The most MT_1-selective agents **11** and **15** were substituted with a $Ph(CH_2)_3$ or a $PhO(CH_2)_3$ group confirming the optimal chain length for MT_1-selectivity to be C_3. Interestingly, in our binding assay, **11** and **15** have shown considerably higher MT_1-affinity and selectivity than the standard highly MT_1-selective ligand, the dimeric agomelatine **1a**. Our findings are useful for the future design of MT_1-selective ligands.

Experimental

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. A Bruker AV-400 spectrometer was used to obtain ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra respectively. Proton chemical shifts are referred to $CHCl_3$ (7.24 ppm) and DMSO-d_6 (2.55 ppm). Coupling constants (*J* values) are given in hertz (Hz). 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 ESI-microTOF spectrometers. Elemental analysis was performed by the microanalytical section of the Instiute of Inorganic Chemistry, University of Würzburg. All reactions were carried out under an argon atmosphere. All chemicals were purchased from commercial suppliers and used directly without any further purification. Agomelatine was synthesized as previously reported [10]. HPLC analysis was performed on an Agilent 1100 HPLC system with a Synergi MAX RP C-12 column (4 μm, 150 × 4.60 mm). 99.9% methanol/0.1% formic acid (mobile phase B) and 99.9% water/0.1% formic acid (mobile phase A) were used at a flow rate 1.0 mL/min in linear gradient elution of 10 to 100% B over 18 min holding 4 min at 100% B; compound purities obtained by integration of chromatograms at λ = 254.8 nm were ≥95% throughout.

*N-[2-(7-{3-[8-(2-Acetylaminoethyl)naphthalen-2-yloxy]-propoxy}-naphthalen-1-yl)ethyl]acetamide (**1a**)*

1a was synthesized from *O*-demethylagomelatine and 1,3-dibromopropane according to a previously described procedure [3] applying an additional purification step by column chromatography (silica, gel, ethyl acetate to THF gradient elution). Contrary to the reported melting point (mp 101–103°C, toluene), a different melting point (mp 113–116°C, toluene) was observed. The ^{1}H-NMR data were identical to those previously reported [3]. ^{13}C-NMR ($CDCl_3$): δ 20.7, 26.8, 31.0, 37.6, 62.4, 101.5, 116.2, 121.2, 124.5, 124.9, 126.9, 128.1, 130.9, 132.3, 154.7, 167.4. MS (EI): *m/z* (%) = 499 (M^+, 7), 498 (19), 440 (31), 439 (96), 381 (21), 380 (71), 368 (21), 212 (21), 211 (100), 210 (30), 199 (33), 197 (16), 190 (25), 183 (23), 171 (18), 170 (26), 169 (30), 158 (15). 157 (48), 153 (21), 141 (23), 140 (14), 128 (20). Anal. calcd. for $C_{27}H_{28}N_2O_3$: C, 74.67; H, 6.87; N, 5.62. Found: C, 74.95; H, 7.04; N, 5.38; RP-HPLC t_R = 18.3 min.

*N-[2-(5-{3-[3-(2-Acetylamino-ethyl)-1H-indol-5-yloxy]-propoxy}-1H-indol-3-yl)ethyl]acetamide (**5**)*

A mixture of **6** (215 mg, 0.985 mmol), sodium hydroxide (50 mg, 1.23 mmol), sodium chloride (488 mg, 8.37 mmol), TBAB (253 mg, 0.787 mmol) and water (1.5 mL) was stirred in a sealed tube until completely dissolved. 1-Bromo-3-chloropropane (77 mg, 0.491 mmol) und toluene (1.5 mL) were added and the three-layer-emulsion was stirred rapidly (1000 rpm) at 50°C for 3 h. Ethyl acetate (40 mL) and water (40 mL) were added, the organic phase was separated, and the aqueous phase was extracted with ethyl acetate (2 × 30 mL). The combined organic layers were dried (Na_2SO_4) and evaporated under reduced pressure. The crude product was purified by a two-step silica gel chromatography (1. chloroform/methanol/ammonia, 10:1:0.1; 2. THF/ammonia, 10:0.1). **5** was isolated as a pale yellow solid (83 mg, 35%), mp 220°C (decomp.). ^{1}H-NMR (d_6-DMSO): δ 1.85 (s, 6H), 2.21–2.29 (m, 2H), 2.81 (t, 4H, *J* = 7.3 Hz), 3.34 (qua, 4H, *J* = 6.7 Hz), 4.21 (t, 4H, *J* = 6.2 Hz), 6.68 (dd, 2H, *J* = 2.2, 8.7 Hz), 7.11 (d, 2H, *J* = 2.2 Hz), 7.14 (s, 2H), 7.27 (d, 2H, *J* = 8.7 Hz), 7.68 (br, 2H), 10.69 (br, 2H). ^{13}C-NMR (d_6-DMSO): δ 22.7, 25.2, 29.2, 38.9, 64.9, 101.3, 111.5, 111.7, 111.9, 123.3, 127.6, 131.5, 152.1, 169.1. HRMS-ESI *m/z* $[M + Na]^+$ calcd. for $C_{27}H_{32}N_4O_4Na$: 499.2315, found: 499.2315; RP-HPLC t_R = 13.7 min.

*N-{2-[5-(benzyloxy)-1H-indol-3-yl]ethyl}acetamide (**8**)*

$NaBH_4$ (0.790 g, 21.0 mmol) was cautiously added to a stirred solution of 5-benzyloxyindole-3-acetonitrile (0.770 g, 3.00 mmol), acetic anhydride (0.60 mL, 6 mmol) and $NiCl_2 \cdot 6\ H_2O$ (0.71 g, 3.00 mmol) in methanol (30 mL) at 0°C. A black precipitate was formed following a vigorous reaction and once the reaction had subsided, stirring was continued at room temperature for 20 h. Methanol was removed under vacuum and the residue was dissolved in ethyl acetate (40 mL) and $NaHCO_3$ (20 mL), filtered, and the precipitate was washed with ethyl acetate (3 × 10 mL). The combined organic layer was dried (Na_2SO_4) and evaporated under reduced pressure to give 0.860 g (93%) of crude **8** as light brown viscous oil which was used as such in the next step. RP-HPLC t_R = 15.2 min.

*N-[2-(5-hydroxy-1H-indol-3-yl)ethyl]acetamide (**6**)*

(Method A) A solution of 5-benzyloxyindole-3-acetonitrile (525 mg, 2.00 mmol) in THF (12 mL) and acetic anhydride (2.65 mL) was hydrogenated over Raney nickel and 10% Pd/C (145 mg) at 400 kPa H_2 for 12 h at 50°C. The catalysts were filtered off on Celite® and washed with THF. The filtrate was concentrated in vacuo and the residue was purified by column chromatography ($CHCl_3$/MeOH, 4:1) to give 311 mg (71%) of **6** as a colorless foam. (Method B) A mixture of **8** (0.310 g, 1.0 mmol), ammonium formate (0.300 g, 4.76 mmol) and 10% Pd/C (0.600 g) in ethanol (30 mL) was heated at reflux for 2 h. The reaction mixture was cooled to room temperature and filtered. The filtrate was evaporated under reduced pressure to afford 0.190 g (85%) of pure **6** as a pale yellow viscous oil. The spectral data of **6** were identical with those previously documented [11].

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*General procedure (method A) for the synthesis of N-{2-[5-(substituted alkoxy)-1H-indol-3-yl]ethyl}acetamides (**9**, **11**, **13**, **15**, and **19**)*

A mixture of **6** (1 equiv.), anhydrous K_2CO_3, (2 equiv.), the respective alkylating agent (1.5 equiv.), and a catalytical amount of KI (10 mg) in dry acetonitrile (20 mL) was heated at reflux for 18 h. The reaction mixture was cooled to room temperature, filtered and the filtrate was evaporated under vacuum to afford the crude products which were purified by column chromatography (chloroform/methanol/ammonia, 10:1:0.1).

*N-{2-[5-(Naphthalen-2-ylmethoxy)-1H-indol-3-yl]ethyl}-acetamide (**9**)*

Compound **9** (0.140 g, 47%) was obtained from **6** (0.180 g, 0.83 mmol) and 2-(bromomethyl)naphthalene (0.280 g, 1.25 mmol) in the presence of K_2CO_3 (0.230 g, 1.66 mmol) as pale yellow solid mp 56–58°C. ^{1}H-NMR ($CDCl_3$): δ 1.77 (s, 3H), 2.79 (t, 2H, J = 6.8 Hz), 3.41–3.46 (m, 2H), 5.14 (s, 2H), 5.52 (br., 1H), 6.83 (d, 1H, J = 2.3 Hz), 6.87 (dd, 1H, J = 2.5, 8.8 Hz), 7.07 (d, 1H, J = 2.8 Hz), 7.12–7.15 (m, 1H), 7.36–7.38 (m, 2H), 7.47 (dd, 1H, J = 1.8, 8.8 Hz), 7.72–7.81 (m, 4H), 8.21 (br., 1H). ^{13}C-NMR ($CDCl_3$): δ 23.3, 25.3, 39.8, 71.2, 102.4, 112.1, 112.6, 113.0, 123.0, 125.6, 125.9, 126.2, 126.4, 127.8, 127.9, 128.0, 128.3, 131.9, 133.1, 133.4, 135.1, 153.2, 170.3. MS (EI): m/z (%) = 358 (M^+, 14), 299 (19), 286 (10), 141 (100). Anal. calcd. for $C_{23}H_{22}N_2O_2$: C, 77.07; H, 6.19; N, 7.82. Found: C, 76.75; H, 7.04; N, 7.00; RP-HPLC t_R = 16.8 min.

*N-{2-[5-(3-phenylpropoxy)-1H-indol-3-yl]ethyl}acetamide (**11**)*

Compound **11** (0.100 g, 50%) was obtained from **6** (0.130 g, 0.60 mmol) and (3-bromopropyl)benzene (0.170 g, 0.90 mmol) in the presence of K_2CO_3 (0.17 g, 1.2 mmol) as a light brown viscous oil. ^{1}H-NMR ($CDCl_3$): δ 1.81 (s, 3H), 2.02–2.08 (m, 2H), 2.76 (t, 2H, J = 8.2 Hz), 2.83 (t, 2H, J = 7.07 Hz), 3.45–3.50 (m, 2H), 3.93 (t, 2H, J = 6.7 Hz), 5.54 (br., 1H), 6.79 (dd, 1H, J = 2.5, 8.6 Hz), 6.89–6.94 (m, 2H), 7.10–7.20 (m, 6H), 8.16 (br., 1H). ^{13}C-NMR ($CDCl_3$): δ 23.4, 25.3, 31.1, 32.3, 39.8, 67.9, 101.8, 112.0, 112.9, 122.9, 125.9, 126.2, 127.8, 128.4, 128.5, 128.6, 141.8, 153.4, 170.2. MS (EI): m/z (%) = 336 (M^+, 29), 277 (100), 264 (73), 159 (46). Anal. calcd. for $C_{21}H_{24}N_2O_2$: C, 74.97; H, 7.19; N, 8.33. Found: C, 74.75; H, 7.21; N, 8.39; RP-HPLC t_R = 16.6 min.

*N-{2-[5-(5-phenylpentyl)oxy)-1H-indol-3-yl]ethyl}-acetamide (**13**)*

Compound **13** (0.130 g, 35%) was obtained from **6** (0.230 g, 1.05 mmol) and (5-bromopentyl) benzene (0.360 g, 1.58 mmol) in the presence of K_2CO_3 (0.290 g, 2.10 mmol) as dark yellow viscous oil. ^{1}H-NMR ($CDCl_3$): δ 1.4–1.48 (m, 2H), 1.57–1.65 (m, 2H), 1.70–1.77 (m, 2H), 1.81 (s, 3H), 2.55 (t, 2H, J = 7.7 Hz), 2.82 (t, 2H, J = 6.7 Hz), 3.44–3.49 (m, 2H), 3.89 (t, 2H, J = 6.6 Hz), 5.60 (br., 1H), 6.76 (dd, 1H, J = 2.5, 8.8 Hz), 6.84–6.94 (m, 2H), 7.06–7.20 (m, 6H). ^{13}C-NMR ($CDCl_3$): δ 23.4, 25.3, 25.9, 29.4, 31.3, 35.9, 39.8, 68.9, 101.7, 112.0, 112.5, 112.9, 122.9, 125.7, 127.8, 128.3, 128.5, 131.7, 142.6, 153.5, 170.3. MS (EI): m/z (%) = 364 (M^+, 32), 305 (100), 292 (38), 159 (56). Anal. calcd. for $C_{23}H_{28}N_2O_2$: C, 75.79; H, 7.74; N, 7.69. Found: C, 75.35; H, 7.94; N, 7.75; RP-HPLC t_R = 17.8 min.

*N-{2-[5-(3-phenoxypropoxy)-1H-indol-3-yl]ethyl}-acetamide (**15**)*

Compound **15** (0.200 g, 56%) was obtained from **6** (0.220 g, 1.008 mmol) and 3-phenoxypropyl bromide (0.370 g, 1.72 mmol) in the presence of K_2CO_3 (0.28 g, 2.02 mmol) as light brown solid mp 112–114°C. ^{1}H-NMR ($CDCl_3$): δ 1.79 (s, 3H), 2.12–2.19 (m 2H), 2.76–2.82 (m, 2H), 3.40–3.47 (m, 2H), 4.04–4.11 (m, 4H), 5.66 (br., 1H), 6.74–7.19 (m, 9H), 8.43 (br., 1H). ^{13}C-NMR ($CDCl_3$): δ 23.3, 25.3, 29.6, 39.9, 64.6, 65.5, 101.8, 112.1, 122.4, 112.8, 114.6, 120.7, 123.1, 127.8, 129.5, 131.8, 153.2, 158.9, 170.4. MS (EI): m/z (%) = 352 (M^+, 32), 293 (100), 280 (13), 159 (37). Anal. calcd. for $C_{21}H_{24}N_2O_3$: C, 71.57; H, 6.86; N, 7.95. Found: C, 71.20; H, 6.90; N, 8.03; RP-HPLC t_R = 16.2 min.

*N-(2-{5-[3-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)-propoxy]-1H-indol-3-yl}ethyl)-acetamide (**19**)*

Compound **19** (0.120 g, 29%) was obtained from **6** (0.220 g, 1.008 mmol) and 2-(3-chloropropyl)-1*H*-isoindole-1,3-(2*H*)-dione (0.340 g, 1.51 mmol) in the presence of K_2CO_3 (0.280 g, 2.02 mmol) as light brown solid, mp 60–62°C. ^{1}H-NMR ($CDCl_3$): δ 1.81 (s, 3H), 2.05–2.11 (m, 2H), 2.79 (t, 2H, J = 6.7 Hz), 3.42–3.47 (m, 2H), 3.82 (t, 2H, J = 6.9 Hz), 3.95 (t, 2H, J = 5.9 Hz), 5.81 (br., 1H), 6.62 (dd, 1H, J = 2.5, 8.8 Hz), 6.84–6.87 (m, 2H), 7.08 (d, 1H, J = 8.8 Hz), 7.58–7.60 (m, 2H), 7.69–7.72 (m, 2H), 8.51 (br., 1H). ^{13}C-NMR ($CDCl_3$): δ 23.3, 25.2, 28.4, 35.6, 39.8, 66.6, 101.9, 111.9, 112.4, 112.7, 122.9, 123.2, 127.7, 131.8, 132.1, 133.9, 152.9, 168.4, 170.3. MS (EI): m/z (%) = 405 (M^+, 13), 346 (33), 333 (23) 188 (100), 160 (31). Anal. calcd. for $C_{23}H_{23}N_3O_4$: C, 68.13; H, 5.72; N, 10.36. Found: C, 67.84; H, 5.79; N, 10.45; RP-HPLC t_R = 14.4 min.

*General procedure (method B) for the synthesis of N-{2-[5-(substituted alkoxy)-1H-indol-3-yl]ethyl}acetamides (**7**, **10**, **12**, **14**, **16–18** and **20**)*

A mixture of **6** (1.00 mmol), cesium carbonate (1.710 g, 5.25 mmol) and the respective alkylating agent (1.03 mmol) in abs. acetonitrile (10 mL) was stirred at 60°C for 4 h. The reaction mixture was concentrated *in vacuo*, the residue was diluted with THF and filtered through a Celite® pad. The filtrate was collected and concentrated *in vacuo*. The crude product was purified by silica gel chromatography ($CHCl_3$/methanol, 10:1). Further purification was carried out for **14**, **16–18** and **20** by recrystallization from THF/MTBE.

*N-[2-(5-Allyloxy-1H-indol-3-yl)-ethyl]acetamide (**7**)*

Compound **7** (137 mg, 53%) was obtained from **6** (218 mg) and 3-bromopropene (125 mg, 1.03 mmol) as colorless viscous oil. ^{1}H-NMR ($CDCl_3$): δ 1.90 (s, 3H), 2.82–2.97 (m, 2H), 3.48–3.62 (m, 2H), 4.55 (d, 2H, J = 4.5 Hz), 5.26 (dd, 1H, J = 1.7, 10.4 Hz), 5.42 (dd, 1H, J = 1.7, 17.2 Hz), 5.67 (br, 1H), 6.01–6.16 (m, 1H), 6.80–7.09 (m, 3H), 7.23 (d, 1H, J = 8.1 Hz), 8.30 (br, 1H). ^{13}C-NMR ($CDCl_3$): δ 23.4, 25.2, 39.7, 69.8, 102.1, 112.0, 112.5, 112.9, 117.4, 122.9, 127.7, 131.7, 133.8, 152.9, 170.2. MS (EI): m/z (%) = 258 (M^+, 8), 237 (30), 236 (16), 235 (100), 224 (24), 222 (68), 199 (16), 186 (16), 159 (21), 158 (20), 146 (25), 145 (20). Anal. calcd. for $C_{15}H_{18}N_2O_2$: C, 69.74; H, 7.02; N, 10.84. Found: C, 69.29; H, 7.20; N, 10.64; RP-HPLC t_R = 13.1 min.

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*N-[2-(5-Phenethyloxy-1H-indol-3-yl)-ethyl]acetamide (**10**)*

Compound **10** (210 mg, 65%) was obtained from **6** (218 mg) and (2-bromoethyl)benzene (191 mg, 1.03 mmol) as colorless viscous oil. ^{1}H-NMR ($CDCl_3$): δ 1.98 (s, 3H), 2.83 (t, 2H, J = 6.0 Hz), 3.05 (t, 2H, J = 7.1 Hz), 3.48 (qua, 2H, J = 6.0 Hz), 4.15 (t, 2H, J = 7.1 Hz), 5.56 (br, 1H), 6.79 (dd, 1H, J = 1.8, 8.6 Hz), 6.91 (s, 1H), 6.95 (d, 1H, J = 1.8 Hz), 7.13–7.28 (m, 6H), 8.17 (br, 1H). ^{13}C-NMR ($CDCl_3$): δ 20.9, 25.2, 35.0, 39.7, 69.7, 101.7, 112.0, 112.5, 112.9, 122.9, 126.4, 127.7, 128.4, 129.0, 131.7, 138.5, 153.1, 170.1. HRMS-ESI m/z $[M + Na]^+$ calcd. for $C_{20}H_{22}N_2O_2Na$: 345.1578, found: 345.1573; RP-HPLC t_R = 15.9 min.

*N-{2-[5-(4-Phenylbutoxy)-1H-indol-3-yl]-ethyl}acetamide (**12**)*

Compound **12** (60 mg, 17%) was obtained from **6** (218 mg) and (4-chlorobutyl)benzene (173 mg, 1.03 mmol) as colorless viscous oil. ^{1}H-NMR ($CDCl_3$): δ 1.54 (qui, 2H, J = 6.8 Hz), 1.64 (qui, 2H, J = 6.8 Hz), 1.96 (s, 3H), 2.61 (t, 2H, J = 6.8 Hz), 2.83 (t, 2H, J = 6.3 Hz), 3.48 (qua, 2H, J = 6.3 Hz), 3.93 (t, 2H, J = 6.8 Hz), 5.58 (br, 1H), 6.77 (dd, 1H, J = 2.3, 8.7 Hz), 6.89 (s, 1H), 6.94 (d, 1H, J = 2.3 Hz), 7.07–7.24 (m, 6H), 8.19 (br, 1H). ^{13}C-NMR ($CDCl_3$): δ 20.9, 25.0, 25.2, 29.1, 35.6, 39.7, 68.7, 101.7, 111.9, 112.5, 112.8, 122.8, 125.7, 127.7, 128.2, 128.4, 131.6, 142.3, 153.4, 170.2. HRMS-ESI m/z $[M + Na]^+$ calcd. for $C_{22}H_{26}N_2O_2Na$: 373.1891, found: 373.1886; RP-HPLC t_R = 17.2 min.

*N-{2-[5-(2-Phenoxyethoxy)-1H-indol-3-yl]ethyl}acetamide (**14**)*

Compound **14** (186 mg, 55%) was obtained from **6** (218 mg) and (2-bromoethoxy)benzene (207 mg, 1.03 mmol) as pale yellow crystals, mp 150–151°C. ^{1}H-NMR ($CDCl_3$): δ 1.90 (s, 3H), 2.91 (t, 2H, J = 6.0 Hz), 3.55 (qua, 2H, J = 6.0 Hz), 4.25–4.40 (m, 4H), 5.33 (br, 1H), 6.87–7.02 (m, 5H), 7.08 (s, 1H), 7.22–7.31 (m, 3H), 8.07 (br, 1H). ^{13}C-NMR ($CDCl_3$): δ 23.4, 25.3, 39.8, 66.7, 67.6, 102.3, 112.0, 112.7, 113.1, 114.7, 121.0, 122.9, 127.7, 129.5, 131.9, 153.1, 158.7, 170.2. MS (EI): m/z (%) = 338 (M^+, 28), 280 (21), 279 (100), 266 (71), 159 (41), 146 (25), 145 (22). Anal. calcd. for $C_{20}H_{22}N_2O_3$: C, 70.99; H, 6.55; N, 8.28. Found: C, 70.60; H, 6.71; N, 8.37; RP-HPLC t_R = 15.1 min.

*N-(2-{5-[3-(Naphthalen-2-yloxy)propoxy]-1H-indol-3-yl}-ethyl)acetamide (**16**)*

Compound **16** (173 mg, 43%) was obtained from **6** (218 mg) and 2-(3-bromopropoxy)naphthalene (273 mg, 1.03 mmol) as white solid mp 140°C. ^{1}H-NMR (d_6-DMSO): δ 1.84 (s, 3H), 2.31 (qui, 2H, J = 5.9 Hz), 2.81 (t, 2H, J = 7.1 Hz), 3.30–3.42 (m, 2H), 4.23 (t, 2H, J = 5.6 Hz), 4.34 (t, 2H, J = 6.1 Hz), 6.80 (dd, 1H, J = 2.3, 8.8 Hz), 7.09–7.17 (m, 2H), 7.20–7.30 (m, 2H), 7.34–7.44 (m, 2H), 7.45–7.54 (m, 1H), 7.81–7.91 (m, 3H), 7.96 (br, 1H), 10.64 (br, 1H). ^{13}C-NMR (d_6-DMSO): δ 22.7, 25.1, 28.9, 39.4, 64.5, 64.7, 101.4, 106.7, 111.4, 111.9, 112.3, 118.7, 123.3, 123.5, 126.3, 126.7, 127.4, 127.6, 128.4, 129.2, 131.5, 134.3, 152.1, 156.4, 169.0. MS (EI): m/z (%) = 403 (16), 402 (M^+, 60), 344 (24), 343 (100), 330 (56), 200 (32), 185 (27), 172 (17), 159 (35), 146 (24), 127 (15). Anal. calcd. for $C_{25}H_{26}N_2O_3$: C, 74.60; H, 6.51; N, 6.96. Found: C, 74.34; H, 6.77; N, 6.95; RP-HPLC t_R = 17.5 min.

*N-(2-{5-[3-(Naphthalen-1-yloxy)propoxy]-1H-indol-3-yl}-ethyl)acetamide (**17**)*

Compound **17** (229 mg, 57%) was obtained from **6** (218 mg) and 1-(3-bromopropoxy)naphthalene (273 mg, 1.03 mmol) as white solid, mp 147–148°C. ^{1}H-NMR (d_6-DMSO): δ 1.83 (s, 3H), 2.38 (qui, 2H, J = 6.1 Hz), 2.79 (t, 2H, J = 7.3 Hz), 3.32 (qua, 2H, J = 6.8 Hz), 4.30 (t, 2H, J = 6.2 Hz), 4.40 (t, 2H, J = 6.1 Hz), 6.80 (dd, 1*H*, J = 2.3, 8.7 Hz), 7.03–7.16 (m, 3H), 7.26 (d, 1H, J = 8.7 Hz), 7.43–7.58 (m, 4H), 7.90 (d, 1H, J = 8.1 Hz), 7.94 (t, 1H, J = 5.4 Hz), 8.25 (d, 1H, J = 7.6), 10.69 (br, 1H). ^{13}C-NMR (d_6-DMSO): δ 23.7, 25.2, 30.0, 39.4, 64.7, 64.9, 101.4, 105.2, 111.4, 111.9, 112.3, 119.9, 121.4, 123.3, 125.2, 126.2, 126.4, 127.4, 127.5, 127.6, 131.5, 140.0, 152.1, 154.0, 168.9. MS (EI): m/z (%) = 403 (17), 402 (M^+, 60), 344 (22), 343 (88), 330 (22), 200 (100), 186 (23), 159 (22), 158 (21), 157 (20), 146 (26), 115 (25). Anal. calcd. for $C_{25}H_{26}N_2O_3$: C, 74.60; H, 6.51; N, 6.96. Found: C, 74.25; H, 6.69; N, 7.02; RP-HPLC t_R = 17.7 min.

*N-(2-{5-[3-(Biphenyl-4-yloxy)-propoxy]-1H-indol-3-yl}-ethyl)acetamide (**18**)*

18 (236 mg, 55%) was obtained from **6** (218 mg) and 4-(3-bromopropoxy)biphenyl (300 mg, 1.03 mmol) as white solid, mp 141°C. ^{1}H-NMR (d_6-DMSO): δ 1.84 (s, 3H), 2.31 (qui, 2H, J = 5.9 Hz), 2.81 (t, 2H, J = 7.1 Hz), 3.30–3.40 (m, 2H), 4.23 (t, 2H, J = 5.6 Hz), 4.34 (t, 2H, J = 6.1 Hz), 6.80 (dd, 1H, J = 2.3, 8.7 Hz), 7.09–7.18 (m, 4H), 7.20–7.54 (m, 4H), 7.82–7.92 (m, 4H), 7.96 (br, 1H), 10.68 (br, 1H). ^{13}C-NMR (d_6-DMSO): δ 22.7, 25.2, 28.9, 39.5, 64.5, 64.7, 101.4, 111.5, 112.0, 112.3, 114.9, 123.3, 126.1, 126.7, 127.6, 127.7, 128.8. 131.5, 132.5, 139.8, 152.1, 158.2, 169.0. MS (EI): m/z (%) = 429 (M^+, 12), 428 (36), 370 (26), 369 (100), 357 (16), 355 (52), 200 (27), 185 (15), 159 (25), 146 (19). Anal. calcd. for $C_{27}H_{28}N_2O_3$: C, 75.68; H, 6.59; N, 6.54. Found: C, 75.30; H, 6.64; N, 6.81; RP-HPLC t_R = 18.0 min.

*N-[2-(5-Hexyloxy-1H-indol-3-yl)-ethyl]acetamide (**20**)*

Compound **20** (206 mg, 68%) was obtained from **6** (218 mg) and 1-bromohexane (170 mg, 1.03 mmol) as white solid, mp 175°C. ^{1}H-NMR ($CDCl_3$): δ 0.89 (t, 3H, J = 6.9 Hz), 1.27–1.38 (m, 4H), 1.45 (qui, 2H, J = 7.2 Hz), 1.78 (qui, 2H, J = 6.8 Hz), 1.89 (s, 3H), 2.89 (t, 2H, J = 6.2 Hz), 3.54 (qua, 2H, J = 6.2 Hz), 3.97 (t, 2H, J = 6.6 Hz), 5.75 (br, 1H), 6.84 (dd, 1H, J = 1.8, 8.8 Hz), 6.93 (s, 1H), 7.01 (d, 1H, J = 1.8 Hz), 7.23 (d, 1H, J = 8.8 Hz), 8.48 (br, 1H). ^{13}C-NMR ($CDCl_3$): δ 14.0, 22.5, 23.2, 25.2, 25.7, 29.4, 31.3, 39.7, 68.9, 101.6, 111.9, 112.3, 112.7, 122.8, 125.6, 127.6, 131.5, 170.0. MS (EI): m/z (%) = 302 (M^+, 37), 244 (18), 243 (100), 231 (15), 230 (69), 159 (63), 146 (44). Anal. calcd. for $C_{18}H_{26}N_2O_2$: C, 71.49; H, 8.67; N, 9.26. Found: C, 71.39; H, 8.77; N, 9.36; RP-HPLC t_R = 17.2 min.

Pharmacology

Competition binding analysis

All synthesized compounds were tested for their binding affinity and selectivity for each of the melatonin receptor subtypes, MT_1 and MT_2 using competition binding analysis. Briefly, cells expressing the human MT_1 or MT_2 melatonin receptor (MT_1-CHO, MT_2-CHO) were grown to confluence on 10 cm cell culture plates until they reached approximately 80% confluence. Next, cells were washed, lifted, and added to tubes containing 80–100 pM.

2-[^{125}I]-iodomelatonin in the absence (total binding) or presence of melatonin (1 fM to 1 μM) or the test compounds

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(1 pM to 1 μM). The reactions were incubated for 1 h at 25°C, and then terminated following the addition of cold Tris-HCl solution (50 mM, pH 7.4) and filtered through glass fiber filters (Schleicher and Schuell, Keene, NH) saturated in polyethylenimine 0.5% solution (v/v). Radioactive counts were counted using a gamma counter. Data points were fit by 1- or 2-site nonlinear regression analysis based upon the lowest residual sum of squares (GraphPad Prism) and affinity constants (K_i) values were calculated.

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 min at 37°C: (a) 30 μM rolipram alone (basal), (b) 30 μM rolipram and 100 μM forskolin (maximal accumulation), (c) 30 μM rolipram, 100 μM forskolin and melatonin (in concentrations ranging from 10^{-12} to 10^{-7} M) or (d) 30 μM rolipram, 100 μM forskolin and melatonin (in concentrations ranging from 10^{-12} to 10^{-7} M) plus **15** (at a concentration of 10^{-8} M). Cyclic AMP accumulation was expressed as a percentage of forskolin response within each group. Curves were fit using non-linear regression analysis (one-site or two-site) and potency (IC_{50}) values were calculated using the commercially available software (GraphPad PRISM®, GraphPad Prism, Inc., San Diego, CA).

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