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European Journal of Pharmaceutical Sciences 18 (2003) 297–304
EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES
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Synthesis and biological evaluation of new β,β'-disubstituted 6,7,8,9-tetrahydropyrido[1,2-*a*]indol-10-yl ethylamido melatoninergic ligands

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Received 11 November 2002; accepted 17 January 2003

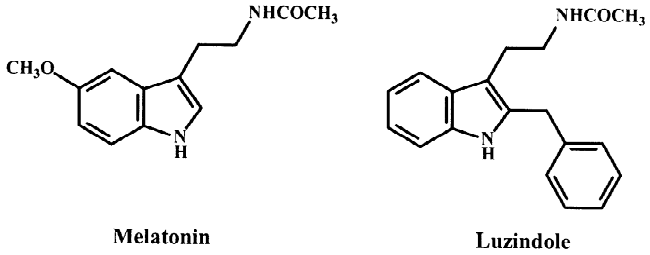
Abstract

Tricyclic analogs of melatonin with alkyl and cycloalkyl moieties in the β position of the ethylamido chain have been prepared and tested for their ability to activate pigment granule aggregation in *Xenopus laevis* melanophores. The introduction of two methyl groups in the β position of the side-chain of the methoxyl-substituted ligands induces a synergistic effect in agonist potency, which, importantly, is maintained after the methoxyl substituent is removed. The presence of more bulky β-substituents, regardless of the size of the R group, seems to lead to antagonism.

Keywords: N1–C2 annelated indoles; Synthesis; Melatoninergic activity

1. Introduction

Melatonin (*N*-acetyl 5-methoxytryptamine) (Scheme 1) is the principal hormone of the vertebrate pineal gland and is secreted mainly during darkness (Reiter, 1991). It is now well recognized that it regulates seasonal breeding in photoperiodic species and can entrain circadian rhythms in mammals, including man (Bartness et al., 1993; Reiter, 1993). Melatonin has also been reported to have anti-oxidant (Reiter et al., 1999; Iakovou et al., 2002) and antiproliferative effects (Molis et al., 1995).



Melatonin **Luzindole**

Scheme 1. Structures of melatonin and luzindole.

A number of these effects are mediated through a family of high-affinity G-protein-coupled cell-membrane receptors, MT_1, MT_2, and Mel_{1c} (Reppert et al., 1995), which are particularly abundant in tissues which are known to respond to melatonin (e.g. the body's biological clock in the suprachiasmatic nuclei of the hypothalamus and the retina). Melatonin receptors have been subjected to a number of modelling studies based on both the amino acid sequence (Garratt et al., 1996; Navajas et al., 1996; Sugden et al., 1995) and pharmacophore models (Jansen et al., 1996; Spadoni et al., 1997; Sicsic et al., 1997; Marot et al., 1998) and a number of active conformations have been proposed. These models have been compared and assessed in a recent review (Mor et al., 1999).

During the last decade we have sought to understand how melatonin interacts with its receptors. A number of structure–affinity relationships have been identified (Faust et al., 2000) and, recently, we and other researchers proposed molecular models of the melatonin binding site (Navajas et al., 1996; Sugden et al., 1995; Spadoni et al., 1997; Sicsic et al., 1997; Grol and Jansen, 1996; Mor et al., 1998).

In our ongoing effort to probe the stereoelectronic

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0928-0987/03/$ – see front matter
doi:10.1016/S0928-0987(03)00020-4

requirements for optimal activity we have previously reported the synthesis and biological activity of novel 2-phenyltryptamines annelated on the *a*-face of the pyrrole moiety by the introduction of one, two or three methylene groups, **1**: $n=1$–3, Scheme 2 (Faust et al., 2000). This work was recently extended by the synthesis of novel *N*-[2-(6,7,8,9-tetrahydropyrido[1,2-*a*]indol-10-yl)ethyl]alkanamides (**2**) and *N*-[2-(2-methoxy-6,7,8,9-tetrahydropyrido[1,2-*a*]indol-10-yl)ethyl]alkanamides (**3**) (Scheme 2) (Tsotinis et al., 2001). In the *Xenopus* melanophore pigment aggregation model, the melatoninergic ligands **1**: $n=1$, are agonists, while their congeners **1**: $n=3$, were found to be antagonists. Molecules **1**: $n=2$, are either antagonists ($R_5=H$) or agonists ($R_5=OCH_3$). Similarly, the non-methoxy-substituted compounds (**2**) are antagonists, while their 2-methoxy counterparts (**3**) are full agonists, the butyramido analog (**3**, $R=C_3H_7$) being almost as potent ($pEC_{50}=9.91$) as melatonin ($pEC_{50}=10.07$).

Compounds **1**–**3** probe the constraints at the receptor site with regard to the lower *N*1–C2 region of the indole moiety. In order to explore the possible synergistic influence on potency upon introducing α- and β-methyl substituents on the C-3 ethylamido side-chain, we also synthesized and evaluated the *N*1-phenethyl-substituted indole derivatives **4** and **5** (Scheme 2) (Tsotinis et al., 2002). Although the presence of methyl substituents in the side-chain of these molecules was found to increase their activity, the lack of annulation of a carbocyclic moiety on the *a*-face of the pyrrole ring seems to reduce their ability to dock onto the receptor.

Based on these findings we designed and synthesized the β,β'-disubstituted 6,7,8,9-tetrahydropyrido[1,2-*a*]indol-10-yl ethylamido melatoninergic ligands **16**, **17** (Scheme 3), **22** and **23** (Scheme 4). The results show that the new 5-methoxy (numbering with respect to the indole ring)-substituted analogs **17a**,**b**, like their analogous congeners **3** ($R=CH_3$ and n-C_3H_7), are full agonists. In contrast, the activity of the new non-methoxy-substituted molecules ranges from antagonists, in the case of compounds **22**–**25**, to partial agonists in the case of ligands **16a**,**b**. The activity of **16a**,**b** contrasts with that of their analogous counterparts **2**, which are all antagonists.

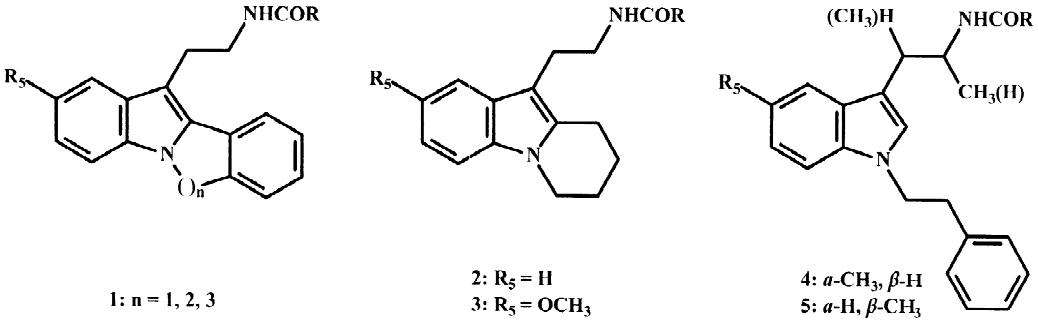
2. Experimental

2.1. Instrumentation and chemicals

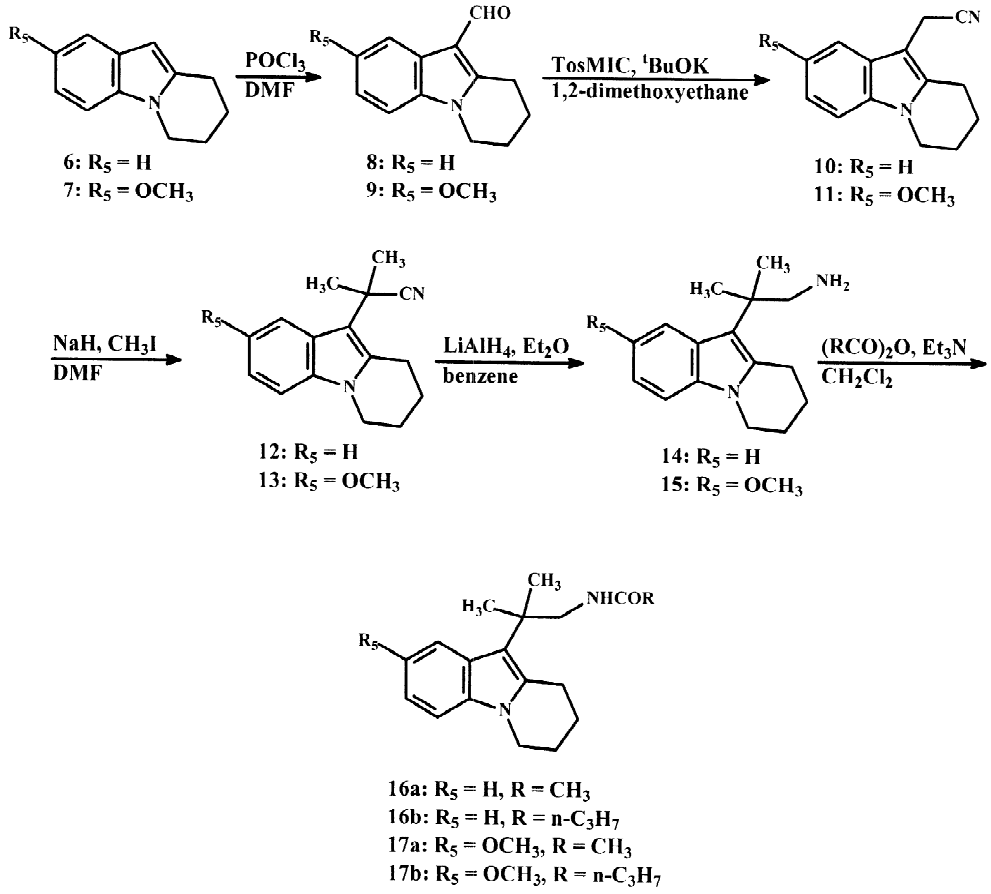
Melting points were determined on a Büchi 530 apparatus and are uncorrected. ^{1}H NMR spectra were taken in $CDCl_3$ and recorded either on a Bruker AC 200 (200 MHz) or a Bruker DRX 400 (400 MHz) spectrometer, and the spectra are reported in δ. ^{13}C NMR spectra were recorded at 50 MHz on a Bruker AC 200 spectrometer. Tetramethylsilane was used as internal standard. All the experiments were carried out under an atmosphere of argon. The solvents used were dried as follows: benzene using sodium wire, triethylamine over sodium hydroxide, dimethylformamide and dichloromethane over molecular sieves (4 Å), diethyl ether and tetrahydrofuran over calcium hydride and acetone over molecular sieves (4 Å). Elemental analyses (C, H, N) were carried out by the Microanalytical Section of the Institute of Organic and Pharmaceutical Chemistry, NHRF. DC-Alufolien plates (Kieselgel 60 F_{254}, Schichtdicke 0.2 mm, Merck) were used for analytical TLC and were visualized with ultraviolet light or developed with iodine or phosphomolybdic acid. The following abbreviations are used: DMF, *N*,*N*′-dimethylformamide; TosMIC, tosylmethyl isocyanide; DMSO, dimethyl sulfoxide.

*2.1.1. General procedure for the synthesis of acetonitriles **10** and **11***

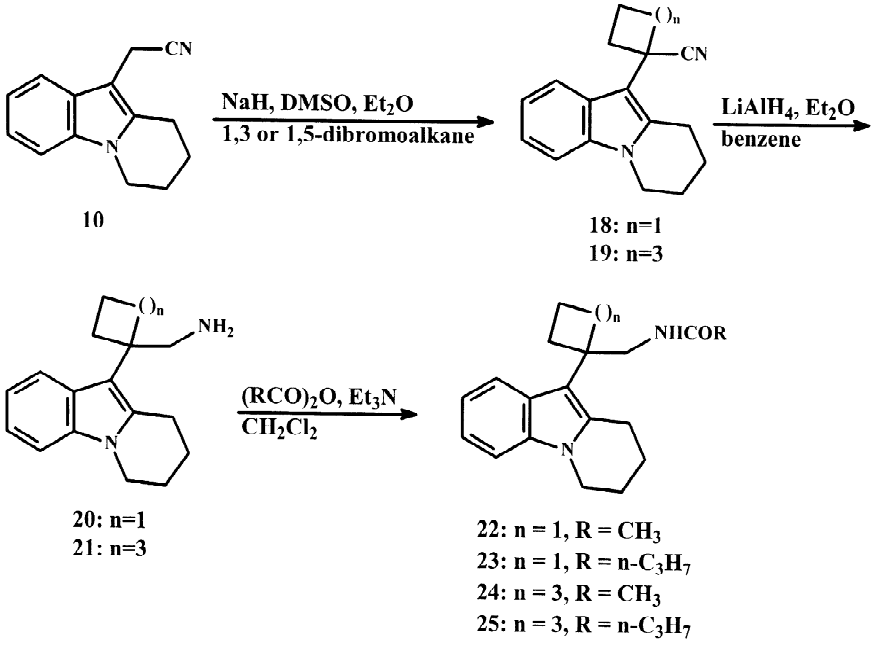
A solution of TosMIC (1.8 g, 9.2 mmol) in 1,2-dimethoxyethane (10 ml) was added dropwise to a stirred suspension of potassium *tert*-butoxide (2.1 g, 18.7 mmol) in 1,2-dimethoxyethane (10 ml) at −30 °C. A solution of the corresponding aldehyde **8** or **9** (8.7 mmol) in 1,2-dimethoxyethane (10 ml) was then added dropwise to the mixture at −60 °C, which was left under stirring at this temperature for 60 min. The reaction was quenched by the addition of MeOH (25 ml), brought to room temperature and then heated to reflux for 15 min. After removal of the solvent in vacuo the residue was taken up in a mixture of H_2O (25 ml) and AcOH (0.1 ml) and extracted with CH_2Cl_2. The extracts were washed with a saturated solution of $NaHCO_3$, dried over Na_2SO_4 and concentrated



Scheme 2. Structures of melatoninergic ligands **1**–**5**.



Scheme 3. Synthesis of the melatoninergic analogs **16a**,**b** and **17a**,**b**.



Scheme 4. Synthesis of the melatoninergic ligands **22**–**25**.

under reduced pressure. The crude product was purified by flash column chromatography (cyclohexane/AcOEt, 9:1, v/v) and triturated with AcOEt to give acetonitriles **10** and **11** as off-white solids.

*2.1.1.1. (6,7,8,9-Tetrahydropyrido[1,2-a]indol-10-yl)-acetonitrile (**10**)*

Yield, 58%; m.p., 119–120 °C; ^{1}H NMR, $\delta = 1.87–2.01$ (m, 2H, 8-H), 2.04–2.14 (m, 2H, 7-H), 2.95 (t, 2H, 9-H, $J = 6.2$ Hz), 3.75 (s, 2H, CH_2CN), 4.06 (t, 2H, 6-H, $J = 6.2$ Hz), 7.16–7.22 (m, 2H H_{arom}), 7.25–7.28 (m, 1H, H_{arom}), 7.54–7.59 (m, 1H, H_{arom}) ppm; ^{13}C NMR, $\delta = 12.6$, 21.3, 23.4, 24.3, 42.1, 98.5, 108.6, 117.5, 119.4, 120.3, 121.2, 128.1, 135.8, 136.1 ppm. Anal. Calc. for $C_{14}H_{14}N_2$ (210.28): C, 79.97; H, 6.71; N, 13.32. Found: C, 79.85; H, 6.68; N, 13.40.

*2.1.1.2. (2-Methoxy-6,7,8,9-tetrahydropyrido[1,2-a]indol-10-yl)acetonitrile (**11**)*

Yield, 96%; m.p., 102–103 °C; ^{1}H NMR, $\delta = 1.88–1.94$ (m, 2H, 8-H), 2.03–2.08 (m, 2H, 7-H), 2.90 (t, 2H, 9-H, $J = 6.2$ Hz), 3.70 (s, 2H, CH_2CN), 3.86 (s, 3H, OCH_3), 3.99 (t, 2H, 6-H, $J = 6.2$ Hz), 6.81 (dd, 1H, 3-H, $J = 8.8$, 2.2 Hz), 6.97 (d, 1H, 1-H, $J = 2.2$ Hz), 7.15 (d, 1H, 4-H, $J = 8.8$ Hz) ppm; ^{13}C NMR, $\delta = 12.7$, 21.4, 23.3, 24.2, 42.4, 55.9, 98.2, 108.5, 117.3, 119.6, 121.4, 127.9, 135.5, 136.9, 153.9 ppm. Anal. Calc. for $C_{15}H_{16}N_2O$ (240.30): C, 74.97; H, 6.71; N, 11.66. Found: C, 74.85; H, 6.68; N, 11.58.

*2.1.2. General procedure for the synthesis of the methylpropionitriles **12** and **13***

A solution of acetonitrile **10** or **11** (0.73 mmol) and methyl iodide (0.10 ml, 1.82 mmol) in DMF (1.5 ml) was added dropwise at 0 °C to a stirred suspension of sodium hydride (60% dispersion in mineral oil, 0.04 g, 1.82 mmol) in DMF (1.5 ml). The reaction mixture was then allowed to warm to room temperature and after stirring for 5 h was treated with saturated NH_4Cl (5 ml) and extracted with AcOEt. The extract was washed with H_2O and brine, dried over Na_2SO_4 and the solvent removed in vacuo. The crude product formed was flash chromatographed (cyclohexane/AcOEt, 9:1, v/v) to give the desired compounds as off-white solids.

*2.1.2.1. 2-(6,7,8,9-Tetrahydropyrido[1,2-a]indol-10-yl)-2-methylpropionitrile (**12**)*

Yield, 31%; m.p., 117–118 °C; ^{1}H NMR, $\delta = 1.88–1.98$ (m, 8H, 8-H + $C(CH_3)_2$), 1.99–2.11 (m, 2H, 7-H), 3.18 (t, 2H, 9-H, $J = 6.2$ Hz), 4.04 (t, 2H, 6-H, $J = 6.2$ Hz), 7.06–7.19 (m, 2H, H_{arom}), 7.22–7.28 (m, 1H, H_{arom}), 7.34–7.78 (m, 1H, H_{arom}) ppm; ^{13}C NMR, $\delta = 21.4$, 23.2, 24.4, 27.8, 29.7, 42.4, 98.9, 108.6, 117.1, 119.5, 120.1, 121.4, 128.3, 136.5, 137.2 ppm. Anal. Calc. for $C_{16}H_{18}N_2$ (238.33): C, 80.63; H, 7.61; N, 11.75. Found: C, 80.58; H, 7.55; N, 11.60.

*2.1.2.2. 2-(2-Methoxy-6,7,8,9-tetrahydropyrido[1,2-a]indol-10-yl)-2-methyl propionitrile (**13**)*

Yield, 34%; m.p., 109–111 °C; ^{1}H NMR, $\delta = 1.82–1.96$ (m, 8H, 8-H + $C(CH_3)_2$), 1.97–2.10 (m, 2H, 7-H), 3.14 (t, 2H, 9-H, $J = 6.2$ Hz), 3.84 (s, 3H, OCH_3), 4.00 (t, 2H, 6-H, $J = 6.2$ Hz), 6.82 (dd, 1H, 3-H, $J = 8.8$, 2.2 Hz), 7.14 (d, 1H, 4-H, $J = 8.8$ Hz), 7.23 (d, 1H, 1-H, $J = 2.2$ Hz) ppm; ^{13}C NMR, $\delta = 21.2$, 23.3, 24.2, 27.6, 29.5, 42.3, 56.0, 99.2, 108.6, 117.4, 119.3, 121.3, 128.1, 134.1, 136.4, 153.1 ppm. Anal. Calc. for $C_{17}H_{20}N_2O$ (268.36): C, 76.09; H, 7.51; N, 10.44. Found: C, 76.00; H, 7.45; N, 10.35.

*2.1.3. General procedure for the synthesis of the amines **14** and **15***

A solution of the methylpropionitrile **12** or **13** (1.81 mmol) in benzene (2 ml) was added dropwise to a suspension of lithium aluminum hydride (0.21 g, 5.46 mmol) in dry diethyl ether (10 ml) at 0 °C. The mixture was stirred at room temperature for 30 min and upon cooling to 0 °C was carefully treated with water (5 ml). The resulting suspension was filtered through Celite and the filtrate diluted with AcOEt. The organic layer was washed with H_2O and brine, dried over Na_2SO_4 and concentrated under reduced pressure to give the crude amines **14** or **15** as yellow oils, which were used without further purification in the acylation reactions.

*2.1.3.1. 2-(6,7,8,9-Tetrahydropyrido[1,2-a]indol-10-yl)-2-methylpropionamine (**14**)*

Yield, 93%.

*2.1.3.2. 2-(2-Methoxy-6,7,8,9-tetrahydropyrido[1,2-a]indol-10-yl)-2-methyl propionamine (**15**)*

Yield, 71%.

*2.1.4. General procedure for the synthesis of the amides **16a,b** and **17a,b***

Triethylamine (0.07 ml, 0.48 mmol) was added to a solution of the amine **14** or **15** (0.36 mmol) in CH_2Cl_2 (1 ml) at 0 °C. The mixture was stirred at this temperature for 10 min and the appropriate acid anhydride (0.42 mmol) was then added. After the addition the mixture was left under stirring at room temperature for 60 min before H_2O was added. The resulting mixture was extracted with CH_2Cl_2, washed with H_2O and brine and dried over Na_2SO_4. The solvent was removed in vacuo to give the crude amides as viscous oils. Unless otherwise indicated the amides were triturated with AcOEt (1 ml) to afford the title compounds as amorphous solids.

*2.1.4.1. N-[2-(6,7,8,9-Tetrahydropyrido[1,2-a]indol-10-yl)-2-methylpropyl]acetamide (**16a**)*

Yield, 37%; m.p., 152–154 °C; ^{1}H NMR, $\delta = 1.52$ (s, 6H, $C(CH_3)_2$), 1.84 (s, 3H, $COCH_3$), 1.85–1.92 (m, 2H, 8-H), 1.94–2.06 (m, 2H, 7-H), 3.10 (t, 2H, 9-H, $J = 6.2$

Hz), 3.64 (d, 2H, CH_2NH, $J = 5.8$ Hz), 4.04 (t, 2H, 6-H, $J = 6.2$ Hz), 5.25 (br s, 1H, NH), 6.99–7.09 (m, 1H, H_{arom}), 7.10–7.19 (m, 1H, H_{arom}), 7.25–7.29 (m, 1H, H_{arom}), 7.72–7.80 (m, 1H, H_{arom}) ppm; ^{13}C NMR, $\delta =$ 21.2, 23.4, 23.6, 24.0, 26.5, 35.7, 42.3, 48.9, 99.2, 108.7, 117.6, 119.3, 120.4, 127.9, 134.1, 136.0, 172.8 ppm. Anal. Calc. for $C_{18}H_{24}N_2O$ (284.40): C, 76.02; H, 8.50; N, 9.85. Found: C, 75.95; H, 8.45; N, 9.80.

*2.1.4.2. N-[2-(6,7,8,9-Tetrahydropyrido[1,2-a]indol-10-yl)-2-methylpropyl]butyramide (**16b**)*

Yield, 33%; m.p., 129–131 °C; ^1H NMR, $\delta = 0.96$ (t, 3H, CH_2CH_3, $J = 7.3$ Hz), 1.51 (s, 6H, $C(CH_3)_2$), 1.58–1.76 (sextet, 2H, CH_2CH_3, $J = 7.3$ Hz), 1.78–1.92 (m, 2H, 8-H), 1.94–2.06 (m, 2H, 7-H), 2.41 (t, 2H, $COCH_2$, $J = 7.3$ Hz), 3.09 (t, 2H, 9-H, $J = 6.2$ Hz), 3.64 (d, 2H, CH_2NH, $J = 6.2$ Hz), 4.03 (t, 2H, 6-H, $J = 6.2$ Hz), 5.25 (br s, 1H, NH), 6.99–7.08 (m, 1H, H_{arom}), 7.09–7.17 (m, 1H, H_{arom}), 7.20–7.27 (m, 1H, H_{arom}), 7.72–7.79 (m, 1H, H_{arom}) ppm; ^{13}C NMR, $\delta = 13.7$, 19.2, 21.5, 23.5, 24.3, 26.5, 35.7, 38.8, 42.3, 48.6, 97.5, 108.5, 117.4, 119.6, 120.1, 128.2, 136.3, 137.1, 173.0 ppm. Anal. Calc. for $C_{20}H_{28}N_2O$ (312.45): C, 76.88; H, 9.03; N, 8.96. Found: C, 76.80; H, 9.00; N, 8.90.

*2.1.4.3. N-[2-(2-Methoxy-6,7,8,9-tetrahydropyrido[1,2-a]-indol-10-yl)-2-methylpropyl]acetamide (**17a**)*

Yield, 35%; m.p., 136–137 °C; ^1H NMR, $\delta = 0.84$ (t, 3H, CH_2CH_3, $J = 7.3$ Hz), 1.50 (s, 6H, $C(CH_3)_2$), 1.77–1.92 (m, 5H, 8-H + $COCH_3$), 1.93–2.08 (m, 2H, 7-H), 3.07 (t, 2H, 9-H, $J = 6.2$ Hz), 3.63 (d, 2H, CH_2NH, $J = 5.8$ Hz), 3.83 (s, 3H, OCH_3), 4.00 (t, 2H, 6-H, $J = 6.2$ Hz), 5.26 (br s, 1H, NH), 6.81 (dd, 1H, 3-H, $J = 8.8$, 2.2 Hz), 7.14 (d, 1H, 4-H, $J = 8.8$ Hz), 7.23 (d, 1H, 1-H, $J = 2.2$ Hz) ppm; ^{13}C NMR, $\delta = 21.3$, 23.5, 23.7, 24.1, 26.7, 35.8, 42.4, 49.0, 55.8, 98.8, 108.6, 117.5, 119.4, 128.0, 134.3, 136.1, 153.3, 172.5 ppm. Anal. Calc. for $C_{19}H_{26}N_2O_2$ (314.43): C, 72.58; H, 8.33; N, 8.91. Found: C, 72.48; H, 8.30; N, 8.90.

*2.1.4.4. N-[2-(2-Methoxy-6,7,8,9-tetrahydropyrido[1,2-a]-indol-10-yl)-2-methylpropyl]butyramide (**17b**)*

Yield, 24%; m.p., 123–124 °C; ^1H NMR, $\delta = 1.50$ (s, 6H, $C(CH_3)_2$), 1.53–1.62 (m, 2H, CH_2CH_3), 1.81–1.89 (m, 2H, 8-H), 1.96–2.05 (m, 4H, 7-H + $COCH_2$), 3.07 (t, 2H, 9-H, $J = 6.2$ Hz), 3.63 (d, 2H, CH_2NH, $J = 5.8$ Hz), 3.83 (s, 3H, OCH_3), 4.00 (t, 2H, 6-H, $J = 6.2$ Hz), 5.25 (br s, 1H, NH), 6.81 (dd, 1H, 3-H, $J = 8.8$, 2.2 Hz), 7.14 (d, 1H, 4-H, $J = 8.8$ Hz), 7.23 (d, 1H, 1-H, $J = 2.2$ Hz) ppm; ^{13}C NMR, $\delta = 13.5$, 19.1, 21.4, 23.6, 24.4, 26.6, 35.8, 38.7, 42.2, 48.5, 55.5, 97.8, 108.4, 117.5, 119.5, 128.1, 136.4, 137.2, 154.0, 172.9 ppm. Anal. Calc. for $C_{21}H_{30}N_2O_2$ (342.48): C, 73.65; H, 8.83; N, 8.18. Found: C, 73.55; H, 8.80; N, 8.10.

*2.1.5. 1-(6,7,8,9-Tetrahydropyrido[1,2-a]indol-10-yl)-cyclobutanecarbonitrile (**18**)*

A solution of the acetonitrile **10** (0.15 g, 0.71 mmol) and 1,3-dibromopropane (143.4 mg, 0.71 mmol) in DMSO (2 ml) was added dropwise at 0 °C to a stirred suspension of sodium hydride (60% dispersion in mineral oil, 0.08 g, 2.13 mmol) in DMSO (2.5 ml). The reaction mixture was then allowed to warm to room temperature. After stirring for 18 h, the resulting suspension was treated with saturated NH_4Cl (5 ml) and extracted with AcOEt. The extract was washed with H_2O and brine, dried over Na_2SO_4 and the solvent removed in vacuo. The crude product formed was triturated with AcOEt to give the desired compounds as a white solid: Yield, 27%; m.p., 116-119 °C; ^1H NMR, $\delta = 1.81$–1.97 (m, 2H, 8-H), 1.98–2.12 (m, 3H, 7-H + 1H cyclobut.), 2.43–2.70 (m, 1H, cyclobut.), 2.73–2.83 (m, 1H, cyclobut.), 2.84–2.96 (m, 5H, 9-H + 3H cyclobut.), 4.03 (t, 2H, 6-H, $J = 6.2$ Hz), 7.03–7.20 (m, 2H, H_{arom}), 7.21–7.29 (m, 1H, H_{arom}), 7.44–7.52 (m, 1H, H_{arom}) ppm; ^{13}C NMR, $\delta = 17.8$, 21.3, 23.8, 33.8, 34.0, 42.5, 98.7, 108.8, 117.5, 119.7, 120.2, 128.0, 135.8, 136.6, 137.0 ppm. Anal. Calc. for $C_{17}H_{18}N_2$ (250.34): C, 81.56; H, 7.25; N, 11.19. Found: C, 81.46; H, 7.20; N, 11.15.

*2.1.6. 1-(6,7,8,9-Tetrahydropyrido[1,2-a]indol-10-yl)-cyclohexanecarbonitrile (**19**)*

A solution of the acetonitrile **10** (0.24 g, 1.14 mmol) and 1,5-dibromopropane (315 mg, 1.37 mmol) in a mixture of Et_2O (1 ml) and DMSO (1 ml) was added dropwise at 0 °C to a stirred suspension of sodium hydride (60% dispersion in mineral oil, 0.14 g, 3.42 mmol) in DMSO (3 ml). The reaction mixture was then allowed to warm to room temperature. After stirring for 18 h, the suspension was treated with saturated NH_4Cl (5 ml) and extracted with AcOEt. The extract was washed with H_2O and brine, dried over Na_2SO_4 and the solvent removed in vacuo. The crude product formed was triturated with AcOEt to give the desired compound as a white solid. Yield, 45%; m.p., 118–119 °C; ^1H NMR, $\delta = 1.28$–1.39 (m, 2H, cyclohex.), 1.56–1.63 (m, 1H, cyclohex.), 1.79–1.95 (m, 9H, 8-H + 7H cyclohex.), 2.00–2.08 (m, 2H, 7-H), 3.20 (t, 2H, 9-H, $J = 6.2$ Hz), 4.04 (t, 2H, 6-H, $J = 6.2$ Hz), 7.05–7.11 (m, 1H, H_{arom}), 7.12–7.17 (m, 1H, H_{arom}), 7.23–7.26 (m, 1H, H_{arom}), 7.78–7.82 (m, 1H, H_{arom}) ppm; ^{13}C NMR, $\delta =$ 21.6, 23.5, 23.7, 24.4, 25.3, 37.4, 42.3, 44.3, 99.1, 108.5, 117.3, 119.4, 121.7, 128.2, 136.4, 137.2 ppm. Anal. Calc. for $C_{19}H_{22}N_2$ (278.40): C, 81.97; H, 7.96; N, 10.06. Found: C, 81.87; H, 7.86; N, 10.00.

*2.1.7. 1-(6,7,8,9-Tetrahydropyrido[1,2-a]indol-10-yl)-cyclobutylmethylamine (**20**)*

This amine was prepared by the general method described for amines **14** and **15**. Yellow oil, yield 78%.

*2.1.8. 1-(6,7,8,9-Tetrahydropyrido[1,2-a]indol-10-yl)-cyclohexylmethylamine (**21**)*

This amine was prepared by the general method described for amines **14** and **15**. Yellow oil, yield 73%.

*2.1.9. N-[1-(6,7,8,9-Tetrahydropyrido[1,2-a]indol-10-yl)-cyclobutylmethyl]acetamide (**22**)*

This amide was prepared by the general method described for amides **16a**,**b** and **17a**,**b**. Yield, 36%; m.p., 163–165 °C; ^{1}H NMR, $\delta = 1.82$ (s, 3H, $COCH_3$), 1.83–2.00 (m, 2H, 8-H), 2.01–2.10 (m, 2H, 7-H), 2.24–2.34 (m, 2H, cyclobut.), 2.50–2.59 (m, 2H, cyclobut.), 2.79–2.85 (m, 2H, cyclobut.), 3.06 (t, 2H, 9-H, $J = 6.2$ Hz), 3.71 (d, 2H, CH_2NH, $J = 5.8$ Hz), 4.02 (t, 2H, 6-H, $J = 6.2$ Hz), 5.34 (br s, 1H, NH), 7.01–7.05 (m, 1H, H_{arom}), 7.10–7.12 (m, 1H, H_{arom}), 7.23–7.25 (m, 1H, H_{arom}), 7.38–7.40 (m, 1H, H_{arom}) ppm; ^{13}C NMR, $\delta = 15.7$, 21.3, 23.2, 23.7, 24.1, 30.5, 42.4, 46.6, 46.8, 98.8, 108.6, 117.5, 119.1, 120.8, 127.6, 134.0, 136.3, 173.0 ppm. Anal. Calc. for $C_{19}H_{24}N_2O$ (312.41): C, 73.05; H, 7.74; N, 8.97. Found: C, 72.95; H, 7.70; N, 8.90.

*2.1.10. N-[1-(6,7,8,9-Tetrahydropyrido[1,2-a]indol-10-yl)-cyclobutylmethyl]butyramide (**23**)*

This amide was prepared by the general method described for amides **16a**,**b** and **17a**,**b**. Yield, 45%; m.p., 155–158 °C; ^{1}H NMR, $\delta = 0.89$ (t, 3H, CH_2CH_3, $J = 7.3$ Hz), 1.53–1.62 (m, 2H, CH_2CH_3, $J = 7.3$ Hz), 1.86–1.88 (m, 2H, 8-H), 1.97–2.02 (m, 4H, 7-H + 2H cyclobut.), 2.05–2.11 (m, 2H, cyclobut.), 2.31–2.34 (m, 2H, cyclobut.), 2.56 (t, 2H, $COCH_2$, $J = 7.3$ Hz), 2.85 (t, 2H, 9-H, $J = 6.2$ Hz), 3.75 (d, 2H, CH_2NH, $J = 5.8$ Hz), 4.05 (t, 2H, 6-H, $J = 6.2$ Hz), 5.36 (br s, 1H, NH), 7.04–7.07 (m, 1H, H_{arom}), 7.13–7.17 (m, 1H, H_{arom}), 7.20–7.25 (m, 1H, H_{arom}), 7.37–7.40 (m, 1H, H_{arom}) ppm; ^{13}C NMR, $\delta = 13.7$, 15.7, 19.2, 21.4, 23.4, 24.3, 30.5, 38.9, 42.2, 48.3, 98.9, 108.4, 117.1, 119.5, 120.2, 128.1, 136.2, 137.2, 173.0 ppm. Anal. Calc. for $C_{21}H_{28}N_2O$ (324.46): C, 77.74; H, 8.70; N, 8.63. Found: C, 77.70; H, 8.60; N, 8.55.

*2.1.11. N-[1-(6,7,8,9-Tetrahydropyrido[1,2-a]indol-10-yl)cyclohexylmethyl]acetamide (**24**)*

This amide was prepared by the general method described for amides **16a**,**b** and **17a**,**b**. Yield, 43%; m.p., 172–175 °C; ^{1}H NMR, $\delta = 1.35$–1.45 (m, 8H, cyclohex.), 1.60–1.71 (m, 2H, cyclohex.), 1.78 (s, 3H, $COCH_3$), 1.83–1.91 (m, 2H, 8-H), 1.99–2.07 (m, 2H, 7-H), 3.05 (t, 2H, 9-H, $J = 6.2$ Hz), 3.53 (d, 2H, CH_2NH, $J = 5.8$ Hz), 4.06 (t, 2H, 6-H, $J = 6.2$ Hz), 5.20 (br s, 1H, NH), 7.00–7.06 (m, 1H, H_{arom}), 7.10–7.16 (m, 1H, H_{arom}), 7.22–7.27 (m, 1H, H_{arom}), 7.74–7.79 (m, 1H, H_{arom}) ppm; ^{13}C NMR, $\delta = 21.3$, 22.9, 23.4, 24.1, 24.4, 25.4, 36.8, 42.3, 46.4, 47.1, 99.6, 108.7, 117.4, 119.4, 120.0, 128.1, 136.5, 136.8, 173.1. Anal. Calc. for $C_{21}H_{28}N_2O$ (324.46): C, 77.74; H, 8.70; N, 8.63. Found: C, 77.68; H, 8.65; N, 8.60.

*2.1.12. N-[1-(6,7,8,9-Tetrahydropyrido[1,2-a]indol-10-yl)cyclohexylmethyl]butyramide (**25**)*

This amide was prepared by the general method described for amides **16a**,**b** and **17a**,**b**. Yield, 46%; m.p., 181–182 °C; ^{1}H NMR, $\delta = 0.81$ (t, 3H, CH_2CH_3, $J = 7.3$ Hz), 1.29–1.76 (m, 12H, CH_2CH_3 + 10H cyclohex.), 1.78–1.97 (m, 2H, 8-H), 1.98–2.10 (m, 2H, 7-H), 2.34–2.49 (m, 2H, $COCH_2$), 3.04 (t, 2H, 9-H, $J = 6.2$ Hz), 3.54 (d, 2H, CH_2NH, $J = 5.8$ Hz), 4.06 (t, 2H, 6-H, $J = 6.2$ Hz), 5.20 (br s, 1H, NH), 6.98–7.07 (m, 1H, H_{arom}), 7.08–7.18 (m, 1H, H_{arom}), 7.22–7.28 (m, 1H, H_{arom}), 7.73–7.81 (m, 1H, H_{arom}) ppm; ^{13}C NMR, $\delta = 12.4$, 19.0, 21.4, 23.4, 24.0, 24.3, 25.4, 36.8, 38.5, 42.4, 46.5, 47.0, 99.0, 108.6, 117.5, 119.3, 120.1, 128.0, 136.5, 136.9, 173.3. Anal. Calc. for $C_{23}H_{32}N_2O$ (352.52): C, 78.36; H, 9.15; N, 7.95. Found: C, 78.30; H, 9.05; N, 7.90.

2.2. Xenopus melanophore model for the evaluation of agonist and antagonist activity

Melanophore cells were grown in 96-well tissue culture plates, and growth medium (Sugden, 1991, 1992) was replaced with 0.7× L-15 culture medium 18 h before analogs were tested. Initial absorbance (A_i, 630 nm) of cells (~8000 cells/well) was measured in each well using a Bio-Tek microtiter plate reader (model EL3115, Anachem, UK), then cells were treated with the varying concentrations of the analogs indicated. The maximal concentration used was 10^{-4} M. All experiments used triplicate wells at six concentrations of the analog. The final absorbance (A_f) was measured after 60 min, and the fractional change in absorbance ($1 - A_f/A_i$) was calculated. Vehicle did not alter pigment granule distribution itself or inhibit responses to melatonin. The concentration of analog producing 50% of the maximum agonist response (EC_{50}) was determined from concentration–response curves. For evaluation of antagonist potency, cells were treated with vehicle (1% DMSO or methanol) or varying concentrations (10^{-4}–10^{-9} M) of the analogs for 60 min before melatonin (10^{-9} M) was added. The concentration of analog reducing melatonin-induced pigment aggregation by 50% (IC_{50}) was determined.

3. Results and discussion

3.1. Chemistry

The synthesis for **16** and **17** is shown in Scheme 3. Aldehydes **8** and **9** were obtained by applying the Vilsmeier–Haack reaction to compounds **6** and **7**, which were prepared as previously reported (Tsotinis et al., 2001). The derived aldehydes were then converted to the nitriles **10** and **11**, respectively, using tosylmethyl isocyanide in the presence of potassium *tert*-butoxide and 1,2-dimethoxy-

ethane (VanLeusen and Oomkes, 1980). Acetonitriles **10** and **11** were treated with excess methyl iodide in the presence of sodium hydride and *N,N*-dimethylformamide to give the β,β'-dimethyl analogs **12** and **13**.

Treatment of nitrile **10** with either 1,3-dibromobutane or 1,5-dibromopentane gave the β-carbocyclic analogs **18** and **19**. The final products **16**, **17** and **22**–**25** (Scheme 4) were obtained by reducing the respective β,β'-disubstituted nitriles **12**, **13**, **18** and **19** with lithium aluminium hydride in the presence of benzene and diethyl ether (1:5) to the amines **14**, **15**, **20** and **21**, which were not purified but were immediately acylated with the appropriate reagent.

3.2. Pharmacological studies

The agonist and antagonist potency of the new analogs was assessed in a well-established, specific model of melatonin action, the pigment aggregation response of *Xenopus laevis* melanophores (Faust et al., 2000; Garratt et al., 1994; Davies et al., 1998). Many thousands of black pigment granules are distributed evenly throughout these cells. This response can be quantified by measuring the changes in light absorbance of the cells as the pigment concentrates near the cell center.

Like their non-5-OCH_3-substituted congeners **2**, all but two of the new non-methoxyl compounds, analogs **16a** and **16b**, are antagonists in the above assay with potencies comparable to that of luzindole (Scheme 1), a commonly used melatonin receptor antagonist ($pIC_{50} = 5.61$). In contrast to their respective counterparts in series **2**, the acetamido and butyramido analogs, **16a**,**b**, are partial agonists (Table 1), a property that could be ascribed in this case to the more favourable disposition of the side-chains into the receptor as a result of the steric effect exerted by the *gem*-dimethyl moiety. This effect, however, is not repeated in the cases of molecules **22**–**25**. In these compounds it appears likely that the cyclobutyl and cyclohexyl rings are more readily accommodated on the phenyl ring side of the molecule (Scheme 4).

The situation is quite different when a 2-methoxyl group is introduced in the skeleton of the new analogs (**17**). Unlike their non-methoxyl-substituted *gem*-dimethyl counterparts (**16**), they exhibit full agonistic activity (Table 1).

Comparison of the potency of **16a** with **17a** shows a 140-fold increase, an order of magnitude important enough to reinforce the earlier hypothesis that the 5-methoxyl group of melatonin (Mathe-Allainmat et al., 1999) and other melatoninergic ligands (Tsotinis et al., 2001) is a major contributor to their binding to the receptor. However, albeit being substantial, the change in potency between **16** and **17** is less than that observed between analogs **2** and **3**, as in the latter case there is a shift from agonism to antagonism (Tsotinis et al., 2001). Part of this difference, as we and others have previously postulated (Daniolos et al., 1990; Tsotinis et al., 2002), may be ascribed to the conformational constraint imposed on the alkanamidoethyl side-chain by the introduction of bulky substituents in either the α or β position. Depending on the size and orientation in space of the bulky moiety, the different conformationally restricted melatoninergics exhibit activities that range from agonistic or partial agonistic to antagonistic (Tsotinis et al., 2002; Davies et al., 1998; Potenza and Lerner, 1992).

4. Conclusion

The biological data presented here for the two series of analogs suggest that, although the 5-methoxyl oxygen (numbering with respect to the indole ring) is a major contributor to the stability of the ligand–receptor complex, most probably acting as an electron donor forming a hydrogen bond with histidine 211 in putative transmembrane domain 5 (TM5) (Garratt et al., 1996), the influence of steric factors, arising from the presence of a *gem*-dimethyl moiety in the β-position of the alkanamidoethyl side-chain, in binding is also significant. This was demonstrated by the partial agonist activity of **16a** and **16b**

Table 1
Agonistic and antagonistic activity of compounds **16a**,**b**, **17a**,**b** and **22**–**25** in the *Xenopus laevis* melanophore assay

Compound	R	R_5	β-Substituent	Agonist pEC_{50}	Antagonist pIC_{50}
Melatonin				10.07	–
Luzindole				NA[a]	5.61
16a	CH_3	H	*gem*-Dimethyl	5.86±0.01 (32%)	(28% at 10^{-4} M)
16b	n-C_3H_7	H	*gem*-Dimethyl	6.90±0.02 (64%)	4.48±0.01
17a	CH_3	OCH_3	*gem*-Dimethyl	7.99±0.002	NA
17b	n-C_3H_7	OCH_3	*gem*-Dimethyl	8.38±0.13	NA
22	CH_3	H	Cyclobutane	NA	4.55±0.03
23	CH_3	H	Cyclohexane	NA	5.10±0.06
24	n-C_3H_7	H	Cyclobutane	NA	5.72±0.09
25	n-C_3H_7	H	Cyclohexane	NA	5.02±0.01

Number in parentheses indicates the magnitude of the partial agonist response as a percentage of the maximum. Agonist and antagonist data on melanophores are the mean of triplicate experiments.

[a] NA, no agonist or antagonist effect detected at 100 μM.

compared to the full antagonism exhibited by their non-substituted congeners **2**. The presence of more β-substituents, regardless of the size of the R group (**22**–**25**) seems to lead to antagonism. However, in order to validate these hypotheses, the recurrence of similar effects on a large number of melatoninergics, acting either as agonists or antagonists, is necessary. The results from such comparative studies may disclose information on important synergism between steric and electronic interactions, which could be exploited to design high-affinity ligands.

Acknowledgements

Ms Panoussopoulou and Dr. Andrew Tsotinis wish to thank the General Secretariat for Research and Technology of the Greek Ministry for Development for financial support in the context of a Greek–Hungarian bilateral research program.

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