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Original article

Design, synthesis and melatoninergic activity of new unsubstituted and β,β′-difunctionalised 2,3-dihydro-1*H*-pyrrolo[3,2,1-*ij*]quinolin-6-alkanamides

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Abstract

A series of new 2,3-dihydro-1*H*-pyrrolo[3,2,1-*ij*]quinolin-6-alkanamides, with and without alkyl and cycloalkyl moieties in the β-position of the alkanamido side chain, have been prepared and tested for their ability to activate pigment granule aggregation in *Xenopus laevis* melanophores and bind to the recombinant human MT_1 and MT_2 melatonin receptor subtypes expressed in NIH 3T3 cells. An increase of the spacer's length in the side chain by a methylene unit (from **17d** to **21d**) leads to a six-fold decrease in antagonistic activity. On the other hand, the introduction of two methyl groups in the β-position of the side chain of **17a** induces agonist potency (compound **24**), implying thus that the two β-methyl groups are not only tolerated by the receptor, but constitute functional probes in its dynamic agonist–antagonist conformational equilibrium. The presence of more bulky β-substituents, regardless of the size of the R group, compounds **24a**,**b**, seems to lead to antagonism and to a noteworthy MT_2 subtype selectivity. Last, the new *N*1–C7 annulated derivatives presented herein are substantially more potent than their respective *N*1–C2 annulated counterparts, previously reported.

Keywords: *N*1–C7 annulated indoles; Synthesis; Melatoninergic activity

1. Introduction

Melatonin (Fig. 1) is a hormone synthesized by the pineal gland of mammals, including humans [1]. It plays a critical role in the regulation of reproduction in seasonally breeding mammals, where it has been commercially exploited [2]. It has a major role in the regulation of circadian rhythms in non-mammalian vertebrates and is a component of their regulation in mammals [3]. Melatonin has a hypnotic action in animals and humans [4] and ROZEREM™ (ramelteon) (Fig. 1), a potent melatonin MT_1 and MT_2 receptor agonist, has recently been granted approval for the treatment of insomnia associated with sleep onset in the USA [5]. Sleep problems are common in the elderly [6] and the lack of melatonin, which decreases with age, may be a major factor. It also has therapeutic potential in Seasonal Affective Disorders (SAD) [7] and as an agent in restoring circadian rhythms both in the blind and where these have been disturbed by shift-work or jet-lag [8]. Melatonin has also been implicated in Alzheimer's and other neurological disorders [9], in certain cancers [10] and in Parkinson's disease [11]. Furthermore, due to its hydrophobic and hydrophilic nature [1,12] melatonin can easily enter cells and partition itself between ''shallow subsurface'' and intracellular compartments, where it effectively scavenges both water-soluble peroxy ($ROO^{\bullet}$) [13] and lipoperoxy ($LOO^{\bullet}$) radicals [14,15].

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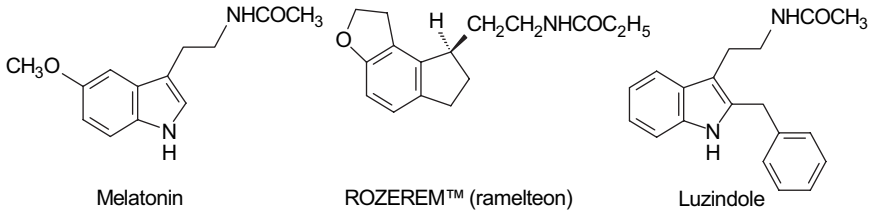


Fig. 1. Structures of melatonin, ROZEREM™ (ramelteon) and luzindole.

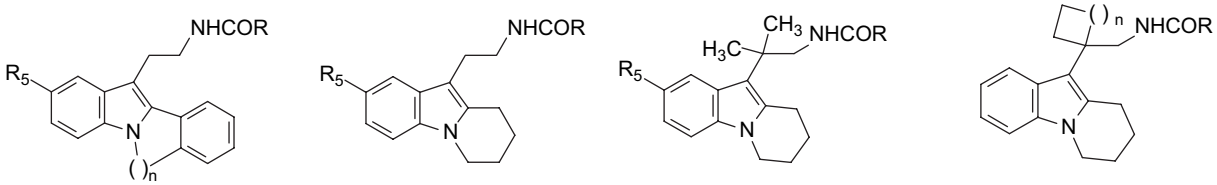
The physiological actions of melatonin in regulating seasonal and circadian rhythms are mediated through a family of specific, high affinity, G-protein-coupled cell membrane receptors [16]. Radioligand binding studies using 2-[^{125}I]-melatonin have revealed a widespread distribution of binding sites throughout the nervous system [17]. The distribution of binding sites shows that they are particularly abundant in tissues that respond to melatonin, including the retina, suprachiasmatic nucleus (SCN), the *pars tuberalis* of the pituitary, and cerebral and tail arteries [18]. Two receptor subtypes have been cloned in mammals, MT_1 (Mel_{1a}) and MT_2 (Mel_{1b}), and a third, Mel_{1c}, in chicken, the *Xenopus laevis* and zebrafish [19].

The way in which melatonin binds at these receptors and the possible therapeutic potential of melatonin in a wide variety of clinical conditions has led to a surge of interest in the synthesis of agonists and antagonists to its actions. The preparation of compounds that can discriminate between the receptor types is a major goal [20], since together with the genetic disruption of specific receptors [21] these could provide tools for targeting specific functions.

In our ongoing effort to probe the stereoelectronic requirements for optimal melatoninergic activity we have previously reported the synthesis and biological activity of novel 2-phenyltryptamines annulated on the *a*-face of the pyrrole moiety by the introduction of 1, 2 or 3 methylene groups, **2**: $n = 1, 2, 3$ (Fig. 2) [20]. This work was extended by the synthesis of the *N*-[2-(6,7,8,9-tetrahydropyrido[1,2-*a*]indol-10-yl)ethyl]alkanamides, **3a–e** and *N*-[2-(2-methoxy-6,7,8,9-tetrahydropyrido[1,2-*a*]indol-10-yl)ethyl]alkanamides, **4a–e** (Fig. 2) [22]. In the *Xenopus* melanophore pigment aggregation model, the melatoninergic ligands **2**: $n = 1$ are agonists while their congeners **2**: $n = 3$ were found to be antagonists. Molecules **2**: $n = 2$ are either antagonists ($R_5 = H$) or agonists ($R_5 = OCH_3$). Similarly, the non-methoxy substituted compounds, **3**, are antagonists while their 2-methoxy counterparts, **4**, are full agonists, the butyramido analog **4** ($R = n\text{-}C_3H_7$) being almost as potent ($pEC_{50} = 9.91$) as melatonin ($pEC_{50} = 10.07$).

Compounds, **2–4**, probe the constraints at the receptor site with regard to the lower *N*1–C2 region of the indole moiety. In order to explore a possible synergistic influence on potency upon introducing alkyl and cycloalkyl groups in the β-position of the alkanamido side chain, we recently reported the synthesis and biology of the β,β′–disubstituted 6,7,8,9-tetrahydropyrido[1,2-*a*]indol-10-yl ethylamido melatoninergic ligands **5a–d** and **6–9** (Fig. 2) [23]. The results we obtained suggested that the new 5-methoxy (numbering with respect to the indole ring) substituted analogs **5c**,**d**, like their analogous congeners **4** ($R = CH_3$ and $n\text{-}C_3H_7$) are full agonists. Conversely, the activity of the new non-methoxy substituted molecules ranges from antagonists, in the case of compounds **6–9**, to partial agonists in the case of ligands **5a**,**b**. The activity of **5a**,**b** contrasts with that of their analogous counterparts **3** which are all antagonists.

Based on these findings we designed and synthesized the *N*1–C7 annulated 2,3-dihydro-1*H*-pyrrolo[3,2,1-*ij*]quinolino-6-alkanamides **17a–e** and **21a–e** (Scheme 1), and their β,β′-difunctionalised counterparts **24** and **27a**,**b** (Scheme 2). Similarly to their *a*-face annelated congeners **3**, the new β-unsubstituted analogs **17** are antagonists in the *Xenopus* melanophore assay. The antagonistic character remains unaltered



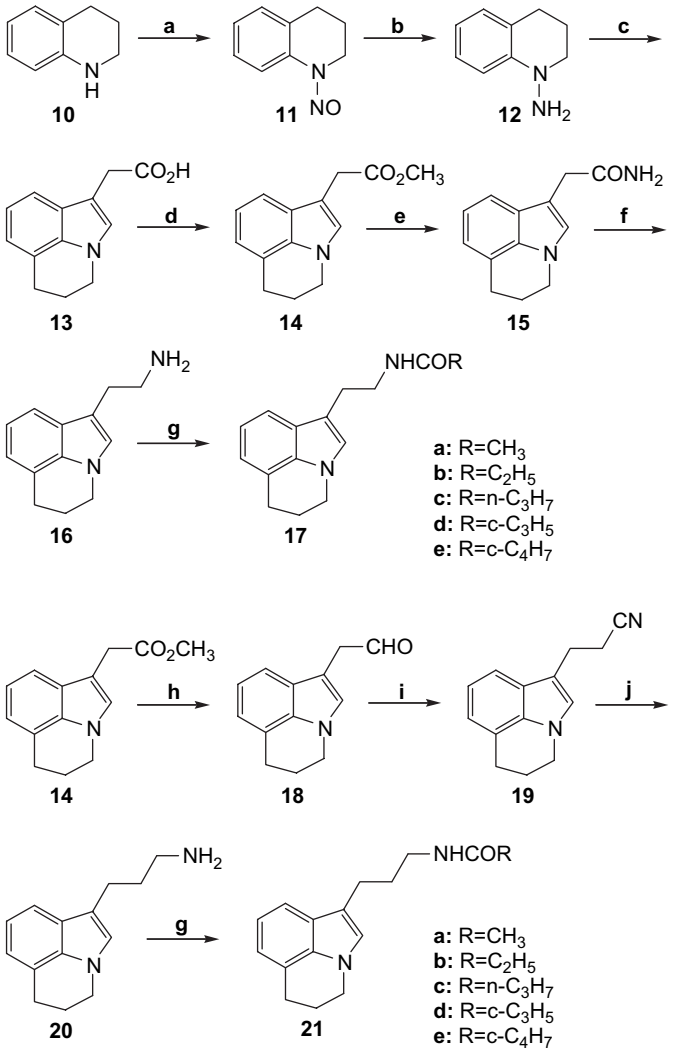
2: n = 1, 2, 3

3a: R_5 = H, R = CH_3
3b: R_5 = H, R = C_2H_5
3c: R_5 = H, R = n-C_3H_7
3d: R_5 = H, R = c-C_3H_5
3e: R_5 = H, R = c-C_4H_7
4: R_5 = OCH_3, R = CH_3, C_2H_5, n-C_3H_7, c-C_3H_7, c-C_4H_7

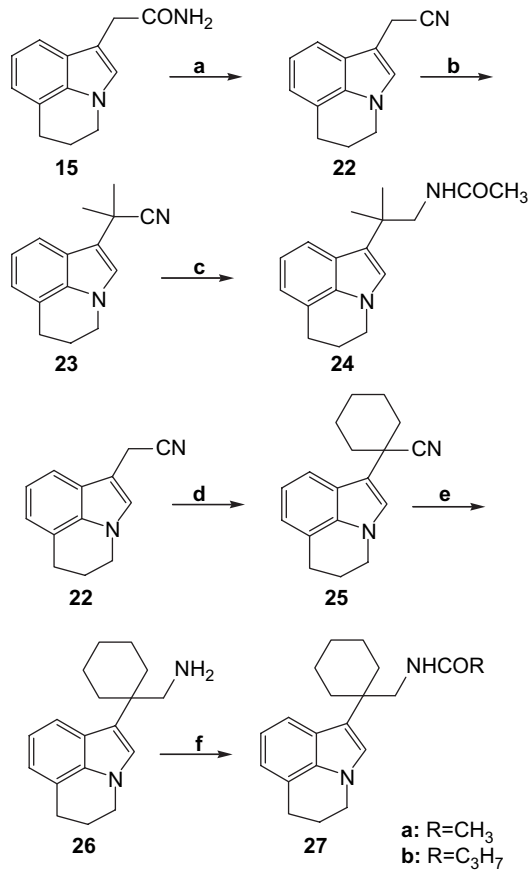
5a: R_5 = H, R = CH_3
5b: R_5 = H, R = n-C_3H_7
5c: R_5 = OCH_3, R = CH_3
5d: R_5 = OCH_3, R = n-C_3H_7

6: n = 1, R = CH_3
7: n = 1, R = n-C_3H_7
8: n = 3, R = CH_3
9: n = 3, R = n-C_3H_7

Fig. 2. Structures of melatoninergic ligands **2–9**.



Scheme 1. (a) $NaNO_2$, conc. HCl, H_2O; (b) $LiAlH_4$, THF; (c) $HO_2C(CH_2)_2$-$COCO_2H$, conc. HCl, AcOH, 80 °C; (d) CH_3I, K_2CO_3, DMF; (e) NH_3 (gas), CH_3OH; (f) $LiAlH_4$, THF, reflux; (g) $(RCO)_2O$ or RCOCl, Et_3N, CH_2Cl_2; (h) DIBAL-H, toluene, −78 °C; (i) TosMIC, *t*-BuOK, DME, −30 °C to 60 °C; (j) $LiAlH_4$, C_6H_6/Et_2O, reflux.



Scheme 2. (a) $(CF_3CO)_2O$, Et_3N, CH_2C_{l2}, 0 °C; (b) CH_3I, NaH, DMF; (c) H_2/Raney-Ni, Ac_2O, AcONa; (d) 1,5-dibromopentane, NaH, DMF/Et_2O; (e) $LiAlH_4$, C_6H_6/Et_2O, reflux; (f) $(RCO)_2O$, Et_3N, CH_2Cl_2.

and in the case of the *N*-acylated propanamines **21**. Within the new β,β′-difunctionalised series, the antagonistic potency drops on moving from R = *n*-C_3H_7 (**27b**) to R = CH_3 (**27a**). An interesting shift towards partial agonism is observed when R and β,β′ are all methyl (**24**). Compounds **27a**,**b** have some MT_2 subtype selectivity.

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 on a Bruker DRX 400 (400 MHz) spectrometer, and the spectra are reported in δ. ^{13}C NMR spectra were taken 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. 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. Flash chromatography was performed using Sorbsil c60-A silica as the stationary phase. Spinning plate chromatography (SPC) was performed in a Chromatotron apparatus (Model 7924), using plates of 4 mm thickness coated with Merck Kieselgel GF_{254} silica gel.

2.2. Synthesis of 1-nitroso-1,2,3,4-tetrahydroquinoline (*11*)

The title compound was prepared upon nitrosation of commercially available 1,2,3,4-tetrahydroquinoline (**10**), following the method of Paris et al. [24].

2.3. Synthesis of 1-amino-1,2,3,4-tetrahydroquinoline (*12*)

The desired compound was obtained in 80% yield upon reduction of 1-nitroso-1,2,3,4-tetrahydroquinoline (**11**) with

lithium aluminum hydride in THF. The ^{1}H NMR spectral data are in full agreement with those reported [24].

2.4. Synthesis of 2,3-dihydro-1H-pyrrolo-[3,2,1-ij]quinolin-6-acetic acid (**13**)

The title compound was prepared by reacting hydrazine **12** with α-ketoglutaric acid in the presence of a mixture of conc. HCl and acetic acid. Yield 48%; Mp 86–89 °C. The ^{1}H NMR spectral data are in full agreement with those reported [24].

2.5. Synthesis of the methyl ester of 2,3-dihydro-1H-pyrrolo[3,2,1-ij]quinolin-6-acetic acid (**14**)

Potassium carbonate (2.35 g, 17.03 mmol) is added to a solution of the acid **13** (3.39 g, 15.77 mmol) in DMF (31 mL) at ambient temperature. The mixture is sonicated for 10 min and methyl iodide (1.2 mL, 19.55 mmol) is then added dropwise. The resulting suspension is stirred for 60 min prior to the addition of H_2O (20 mL) and then extracted with AcOEt (3 × 50 mL). The organic phases are washed with H_2O (2 × 50 mL), saturated aqueous NaCl (2 × 50 mL) and dried (Na_2SO_4). The solvent is evaporated *in vacuo* and the residue obtained is triturated with AcOEt (5 mL) to give the title compound as an off-white powder. Yield 67%; Mp 103–105 °C (recryst. EtOH); ^{1}H NMR: $\delta = 2.00$–2.35 (m, 2H), 2.85–3.10 (m, 2H), 3.67 (s, 3H), 3.70 (s, 2H), 4.00 (t, 2H, $J = 6.0$ Hz), 6.90 (s, 1H), 7.35–7.65 (m, 3H); ^{13}C NMR: $\delta = 32.8$, 33.3, 34.4, 50.4, 58.5, 112.4, 117.8, 119.4, 121.3, 121.8, 126.4, 132.0, 139.9, 171.0.

2.6. Synthesis of 2,3-dihydro-1H-pyrrolo-[3,2,1-ij]quinolin-6-acetamide (**15**)

A fine stream of dry ammonia gas is bubbled for 15 min through a solution of the ester **14** (0.64 g, 2.79 mmol) in methanol (10.5 mL) at 0 °C. The reaction mixture is then left in the fridge (4 °C) for 16 h, chilled to 0 °C and ammonia gas is bubbled for 15 min. After a period of 16 more hours in the fridge the desired compound precipitates as a white solid and filtered. The cake is washed with methanol and dried *in vacuo* (0.1 mm/Hg), until a constant weight is obtained. Yield 97%; Mp 119–123 °C; ^{1}H NMR: $\delta = 2.00$–2.33 (m, 2H), 2.81–3.10 (m, 2H), 3.63 (s, 2H), 3.98 (t, 2H, $J = 6.0$ Hz), 5.80 (br s, 1H), 6.10 (br s, 1H), 6.88 (s, 1H), 7.35–7.63 (m, 3H); ^{13}C NMR: $\delta = 32.8$, 33.3, 36.0, 58.5, 112.4, 117.8, 119.4, 121.3, 121.8, 126.4, 132.0, 139.9, 172.7.

2.7. Synthesis of 2,3-dihydro-1H-pyrrolo-[3,2,1-ij]quinolin-6-ethanamine (**16**)

A solution of the amide **15** (1.00 g, 4.67 mmol) in dry THF (8 mL) is added dropwise to a suspension of lithium aluminum hydride (0.36 g, 9.35 mmol) in dry THF (5 mL) at 0 °C. The reaction mixture is then left stirring at ambient temperature for 16 h, chilled once again to 0 °C and treated with H_2O (1 mL) in order to destroy the excess of lithium aluminum hydride. The resulting suspension is stirred for 30 more minutes and filtered through Celite. The filtrate is diluted with AcOEt (20 mL), washed with H_2O (2 × 20 mL) and saturated aqueous NaCl (2 × 25 mL) and dried (Na_2SO_4). The solvent is removed under reduced pressure and the desired amine **16** is obtained as a pale yellow viscous liquid, which is used as such for further reactions.

2.8. General procedure for the preparation of amides **17a–e**

Triethylamine (1.5 ml) and the appropriate acid anhydride (0.88 mmol) (compounds **17a–c**) or acid chloride (0.88 mmol) (compounds **17d,e**) are added dropwise to a chilled (0 °C) solution of amine **16** (0.15 g, 0.76 mmol) in dichloromethane (2.5 mL). The resulting mixture is stirred at room temperature for 30–60 min prior to being transferred to a beaker containing H_2O (5 mL). The biphasic mixture is separated and the organic phase is diluted with AcOEt (40 mL), washed with H_2O (2 × 20 mL) and saturated aqueous NaCl (2 × 25 mL) and dried (Na_2SO_4). The solvents are removed under reduced pressure and the dark residue obtained is triturated with AcOEt (1 mL). The dark brown solids produced are then recrystallised from ethanol to give the desired amides **17a–e** as beige powders.

2.8.1. Synthesis of N-(2,3-dihydro-1H-pyrrolo[3,2,1-ij]-quinolin-6-ethyl)acetamide (**17a**)

Acetamide **17a** is prepared according to the general method given in Section 2.8. Yield 55%; Mp 149–152 °C; ^{1}H NMR: $\delta = 1.86$ (s, 3H), 2.00–2.33 (m, 2H), 2.81–3.10 (m, 2H), 3.15 (m, 2H), 3.01 (t, 2H, $J = 6.4$ Hz), 3.66 (m, 2H), 5.54 (br s, 1H), 6.88 (s, 1H), 7.35–7.63 (m, 3H); ^{13}C NMR: $\delta = 18.0$, 30.9, 33.3, 36.0, 46.0, 58.5, 112.4, 117.8, 119.4, 121.3, 121.8, 126.4, 132.0, 139.9, 170.9. Anal. Calcd for $C_{15}H_{18}N_2O$ (242.32): C, 74.35; H, 7.49; N, 11.56. Found: C, 74.08; H, 7.69; N, 11.28.

2.8.2. Synthesis of N-(2,3-dihydro-1H-pyrrolo-[3,2,1-ij]quinolin-6-ethyl)propionamide (**17b**)

The title compound **17b** is prepared according to the general method given in Section 2.8. Yield 46%; Mp 167–169 °C; ^{1}H NMR: $\delta = 1.13$ (t, 3H, $J = 7.5$ Hz), 2.16 (q, 2H, $J = 7.5$ Hz), 2.20–2.33 (m, 2H), 2.81–3.10 (m, 2H), 3.15 (m, 2H), 3.01 (t, 2H, $J = 6.4$ Hz), 3.66 (m, 2H), 5.51 (br s, 1H), 6.88 (s, 1H), 7.36–7.64 (m, 3H); ^{13}C NMR: $\delta = 9.9$, 29.9, 30.9, 33.3, 36.0, 46.0, 58.5, 112.4, 117.8, 119.4, 121.3, 121.8, 126.4, 132.0, 139.9, 173.5. Anal. Calcd for $C_{16}H_{20}N_2O$ (256.35): C, 74.97; H, 7.86; N, 10.93. Found: C, 75.13; H, 7.98; N, 10.75.

2.8.3. Synthesis of N-(2,3-dihydro-1H-pyrrolo-[3,2,1-ij]quinolin-6-ethyl)butyramide (**17c**)

Amide **17c** is prepared according to the general procedure given in Section 2.8. Yield 61%; Mp 158–161 °C; ^{1}H NMR: $\delta = 0.90$ (t, 3H, $J = 7.4$ Hz), 1.57–1.65 (m, 2H), 2.09 (t, 2H, $J = 7.1$ Hz), 2.20–2.33 (m, 2H), 2.81–3.10 (m, 2H),

3.01 (t, 2H, $J = 6.4$ Hz), 3.05–3.15 (m, 2H), 3.66 (m, 2H), 5.51 (br s, 1H), 6.86 (s, 1H), 7.36–7.64 (m, 3H); ^{13}C NMR: $\delta = 13.6$, 19.2, 30.9, 33.3, 36.0, 38.6, 46.0, 58.5, 112.4, 117.8, 119.4, 121.3, 121.8, 126.4, 132.0, 139.9, 172.8. Anal. Calcd for $C_{17}H_{22}N_2O$ (270.37): C, 75.52; H, 8.20; N, 10.36. Found: C, 75.43; H, 8.09; N, 10.11.

2.8.4. Synthesis of N-cyclopropanecarboxamido (2,3-dihydro-1H-pyrrolo[3,2,1-ij]quinolin-6-yl)ethanamine (**17d**)

The title compound is obtained according to the general method given in Section 2.8. Yield 41%; Mp 198–202 °C; ^{1}H NMR: $\delta = 0.70$–0.79 (m, 2H), 0.93–1.03 (m, 2H), 1.23 (m, 1H), 2.20–2.33 (m, 2H), 2.69 (t, 2H, $J = 6.4$ Hz), 2.85–3.15 (m, 2H), 3.47 (q, 2H, $J = 6.3$ Hz), 4.00 (t, 2H, $J = 6.0$ Hz), 5.81 (br s, 1H), 6.86 (s, 1H), 7.36–7.64 (m, 3H); ^{13}C NMR: $\delta = 7.2$, 14.9, 30.9, 33.3, 36.0, 46.0, 58.5, 112.4, 117.8, 119.4, 121.3, 121.8, 126.4, 132.0, 139.9, 172.8. Anal. Calcd for $C_{17}H_{20}N_2O$ (268.36): C, 76.09; H, 7.51; N, 10.44. Found: C, 75.92; H, 7.65; N, 10.22.

2.8.5. Synthesis of N-cyclobutanecarboxamido-(2,3-dihydro-1H-pyrrolo[3,2,1-ij]quinolin-6-yl)-ethanamine (**17e**)

Amide **17e** was made following the general procedure given in Section 2.8. Yield 46%; Mp 177–179 °C; ^{1}H NMR: $\delta = 1.80$–1.93 (m, 2H), 2.04–2.12 (m, 1H), 2.15–2.33 (m, 5H), 2.69 (t, 2H, $J = 6.4$ Hz), 2.85–3.15 (m, 3H), 3.47 (q, 2H, $J = 6.3$ Hz), 4.00 (t, 2H, $J = 6.0$ Hz), 5.45 (br s, 1H), 6.86 (s, 1H), 7.36–7.64 (m, 3H); ^{13}C NMR: $\delta = 18.2$, 25.4, 30.9, 33.3, 36.0, 40.1, 46.0, 58.5, 112.4, 117.8, 119.4, 121.3, 121.8, 126.4, 132.0, 139.9, 175.1. Anal. Calcd for $C_{18}H_{22}N_2O$ (282.38): C, 76.56; H, 7.85; N, 9.92. Found: C, 76.38; H, 7.74; N, 10.05.

2.9. Synthesis of 2,3-dihydro-1H-pyrrolo-[3,2,1-ij]quinolin-6-acetaldehyde (**18**)

DIBAL-H (2.2 mL, 12.17 mmol) is added dropwise to a stirred solution of the methyl ester **14** (0.20 g, 0.87 mmol) in dry toluene (7.5 mL) at −78 °C. The mixture is stirred at this temperature for 25 min and then allowed to reach 0 °C. HCl (1 N, 2.5 mL) is added and the resulting suspension is filtered through Celite. The filtrate is extracted with AcOEt (3 × 50 mL) and the combined organic phases are washed with saturated aqueous NaCl. After drying over Na_2SO_4, the solvents are evaporated *in vacuo* to give the title compound as a clear oil, pure enough to be used in the following reaction. ^{1}H NMR: $\delta = 2.19$–2.31 (quintet, 2H, $CH_2CH_2CH_2$, $J = 5.7$ Hz), 2.98–3.11 (t, 2H, $CH_2CH_2CH_2$, $J = 5.7$ Hz), 3.79–3.80 (d, 2H, CH_2CHO, $J = 2.3$ Hz), 4.12–4.21 (t, 2H, NCH_2, $J = 5.7$ Hz), 6.96–7.11 (m, 3H, H_{arom}), 7.36–7.40 (d, 1H, H_{arom}, $J = 7.7$ Hz), 9.75–9.77 (t, 1H, CHO, $J = 2.3$ Hz).

2.10. Synthesis of 3-(2,3-dihydro-1H-pyrrolo-[3,2,1-ij]quinolin-6-yl)propionitrile (**19**)

A solution of *p*-tolouenesulfonylmethyl isocyanide (TosMIC) (0.22 g, 1.13 mmol) in dimethoxyethane (DME) (1.5 mL) is added dropwise to a suspension of potassium *tert*-butoxide (0.26 g, 2.32 mmol) in DME (1.5 mL) at −30 °C. After the addition, the mixture is cooled to −60 °C and a solution of the aldehyde **18** (210 mg, 1.05 mmol) in DME (3.5 mL) is cautiously added. The reaction is stirred at this temperature for 1 h and methanol (2.5 mL) is added. The mixture is allowed to thaw and then refluxed for 15 min. Upon completion of the reaction, the solvent is removed *in vacuo* and H_2O (5 mL) followed by addition of acetic acid (0.1 mL). The resulting suspension is extracted with dichloromethane (3 × 50 mL), the combined organics washed with saturated aqueous $NaHCO_3$ and NaCl and dried (Na_2SO_4). The solvent is evaporated under reduced pressure and the residue is purified by column chromatography (cyclohexane/ethyl acetate, 60:40) to give the desired compound as an off-white solid. Total yield of the above two reactions 41%; Mp 54–57 °C (recryst. EtOH); ^{1}H NMR: $\delta = 2.17$–2.29 (quintet, 2H, $CH_2CH_2CH_2$, $J = 5.9$ Hz), 2.64–2.71 (t, 2H, CH_2CH_2CN, $J = 7.3$ Hz), 2.95–3.01 (t, 2H, $CH_2CH_2CH_2$, $J = 5.9$ Hz), 3.08–3.15 (t, 2H, CH_2CH_2CN, $J = 7.3$ Hz), 4.09–4.15 (t, 2H, $CH_2CH_2CH_2N$, $J = 5.9$ Hz), 6.91–7.11 (m, 3H, H_{arom}), 7.33–7.38 (d, 1H, H_{arom}, $J = 7.7$ Hz); ^{13}C NMR: $\delta = 19.0$, 22.0, 22.8, 24.6, 44.0, 111.1, 115.8, 118.8, 119.6, 119.9, 120.2, 122.0, 124.2, 124.5.

2.11. Synthesis of 3-(2,3-dihydro-1H-pyrrolo-[3,2,1-ij]quinolin-6-yl)propylamine (**20**)

A solution of the nitrile **19** (75 mg, 0.35 mmol) in dry benzene (1 mL) is added dropwise to a suspension of lithium aluminum hydride (0.06 g, 1.58 mmol) in dry ether (2 mL) at 0 °C. The mixture is then refluxed for 1.5 h, cooled to 0 °C and treated carefully with H_2O (2 mL). The resulting suspension is filtered through Celite and the filtrate is extracted with AcOEt (3 × 50 mL). The combined organics are washed with a saturated aqueous NaCl solution and dried (Na_2SO_4). The solvent is removed *in vacuo* to give the desired amine as a yellowish oil, pure enough to be used as such in the following reactions.

2.12. General procedure for the preparation of amides **21a–e**

These amides were prepared by the general method, given in Section 2.8, followed for the synthesis of their counterparts **17a–e**. Their purification was effected by flash column chromatography eluting with AcOEt.

2.12.1. Synthesis of N-[3-(2,3-dihydro-1H-pyrrolo-[3,2,1-ij]quinolin-6-propyl]acetamide (**21a**)

Following the general procedure given in Section 2.12, amide **21a** is obtained as a viscous oil. Yield 42%; ^{1}H

NMR: $\delta = 1.82-1.97$ (m, 5H, $CH_2CH_2CH_2NH$, CH_3CO), 2.14–2.25 (quintet, 2H, $CH_2CH_2CH_2$, $J = 5.9$ Hz), 2.74–2.81 (t, 2H, $IndCH_2CH_2CH_2NH$, $J = 7.3$ Hz), 2.93–2.99 (t, 2H, $CH_2CH_2CH_2$, $J = 5.9$ Hz), 3.24–3.34 (t, 2H, CH_2NHCO, $J = 6.6$ Hz), 4.05–4.10 (t, 2H, NCH_2, $J = 5.9$ Hz), 5.60 (br s, 1H, NHCO), 6.86–7.02 (m, 3H, H_{arom}), 7.34–7.38 (d, 1H, H_{arom}, $J = 7.7$ Hz) ppm; ^{13}C NMR: $\delta = 18.0$, 26.9, 33.3, 34.4, 34.9, 42.4, 58.5, 114.1, 116.3, 118.4, 119.1, 121.7, 123.6, 125.0, 134.6, 174.8 ppm. Anal. Calcd for $C_{16}H_{20}N_2O$ (256.35): C, 74.97; H, 7.86; N, 10.93. Found: C, 74.84; H, 7.78; N, 10.81.

2.12.2. Synthesis of N-[3-(2,3-dihydro-1H-pyrrolo-[3,2,1-ij]quinolin-6-propyl]propionamide (21b)

Amide **21b** is obtained as an off-white solid, following the general method given in Section 2.12. Yield 48%; Mp 58–61 °C (recryst. EtOH); ^1H NMR: $\delta = 1.06-1.10$ (t, 3H, CH_3CH_2CO, $J = 7.3$ Hz), 1.86–1.94 (quintet, 2H, $CH_2CH_2CH_2NH$, $J = 7.3$ Hz), 2.09–2.14 (q, 2H, $COCH_2CH_3$, $J = 7.3$ Hz), 2.17–2.23 (quintet, 2H, $CH_2CH_2CH_2$, $J = 5.9$ Hz), 2.76–2.80 (t, 2H, $CH_2CH_2CH_2NH$, $J = 7.3$ Hz), 2.94–2.97 (t, 2H, $CH_2CH_2CH_2$, $J = 5.9$ Hz), 3.28–3.33 (t, 2H, CH_2NHCO, $J = 6.6$ Hz), 4.07–4.09 (t, 2H, NCH_2, $J = 5.9$ Hz), 5.50 (br s, 1H, NHCO), 6.87–7.03 (m, 3H, H_{arom}), 7.35–7.37 (d, 1H, H_{arom}, $J = 8.1$ Hz) ppm; ^{13}C NMR: $\delta = 9.7$, 26.9, 27.1, 33.3, 34.4, 34.9, 42.7, 58.5, 114.1, 116.3, 118.4, 121.7, 123.6, 126.4, 132.0, 139.9, 175.4. Anal. Calcd for $C_{17}H_{22}N_2O$ (270.37): C, 75.52; H, 8.20; N, 10.36. Found: C, 75.43; H, 8.09; N, 10.11.

2.12.3. Synthesis of N-[3-(2,3-dihydro-1H-pyrrolo-[3,2,1-ij]quinolin-6-propyl]butyramide (21c)

Compound **21c** is obtained as a viscous yellowish oil, following the general procedure given in Section 2.12. Yield 38%; ^1H NMR: $\delta = 0.87-0.94$ (t, 3H, $CH_3CH_2CH_2CO$, $J = 7.3$ Hz), 1.49–1.67 (sextet, 2H, $CH_3CH_2CH_2CO$, $J = 7.3$ Hz), 1.82–1.97 (quintet, 2H, $CH_2CH_2CH_2NH$, $J = 7.3$ Hz), 2.02–2.10 (t, 2H, $COCH_2CH_2CH_3$, $J = 7.3$ Hz), 2.14–2.26 (quintet, 2H, $CH_2CH_2CH_2$, $J = 5.9$ Hz), 2.74–2.81 (t, 2H, $CH_2CH_2CH_2NH$, $J = 7.3$ Hz), 2.93–2.99 (t, 2H, $CH_2CH_2CH_2$, $J = 5.9$ Hz), 3.26–3.36 (t, 2H, CH_2NHCO, $J = 6.6$ Hz), 4.05–4.11 (t, 2H, NCH_2, $J = 5.9$ Hz), 5.55 (br s, 1H, NHCO), 6.87–7.03 (m, 3H, H_{arom}), 7.35–7.38 (d, 1H, H_{arom}, $J = 7.7$ Hz); ^{13}C NMR: $\delta = 13.0$, 18.8, 26.9, 33.3, 34.4, 34.9, 36.5, 42.7, 58.5, 114.1, 116.3, 118.4, 119.1, 121.7, 123.6, 125.0, 139.9, 174.7. Anal. Calcd for $C_{18}H_{24}N_2O$ (284.40): C, 76.02; H, 8.51; N, 9.85. Found: C, 75.63; H, 8.19; N, 10.09.

2.12.4. Synthesis of N-cyclopropanecarboxamido-3-(2,3-dihydro-1H-pyrrolo[3,2,1-ij]quinolin-6-yl)-propylamine (21d)

The title compound **21d** was obtained as a beige solid, following the general procedure given in Section 2.12. Yield 47%; Mp 92–94 °C (recryst. EtOH); ^1H NMR: $\delta = 0.63-0.74$ (m, 2H, CH_2 cyclopropyl), 0.83–0.98 (m, 2H, CH_2 cyclopropyl), 1.18–1.28 (m, 1H, CHCO), 1.83–1.98 (quintet, 2H, $CH_2CH_2CH_2NH$, $J = 7.3$ Hz), 2.14–2.26 (quintet, 2H, $CH_2CH_2CH_2$, $J = 5.9$ Hz), 2.74–2.82 (t, 2H, $CH_2CH_2CH_2NH$, $J = 7.3$ Hz), 2.92–2.98 (t, 2H, $CH_2CH_2CH_2$, $J = 5.9$ Hz), 3.28–3.38 (t, 2H, CH_2NHCO, $J = 6.6$ Hz), 4.05–4.11 (t, 2H, NCH_2, $J = 5.9$ Hz), 5.57 (br s, 1H, NHCO), 6.87–7.02 (m, 3H, H_{arom}), 7.35–7.38 (d, 1H, H_{arom}, $J = 7.7$ Hz); ^{13}C NMR: $\delta = 9.1$, 13.5, 26.9, 33.3, 34.4, 34.9, 43.0, 58.5, 114.1, 116.3, 118.4, 119.1, 121.7, 123.6, 132.0, 139.9, 180.7. Anal. Calcd for $C_{18}H_{22}N_2O$ (282.38): C, 76.56; H, 7.85; N, 9.92. Found: C, 76.41; H, 7.76; N, 9.81.

2.12.5. Synthesis of N-cyclobutanecarboxamido-3-(2,3-dihydro-1H-pyrrolo[3,2,1-ij]quinolin-6-yl)-propylamine (21e)

Amide **21e** is obtained as a white solid, following the general procedure given in Section 2.12. Yield 45%; Mp 68–71 °C (recryst. EtOH); ^1H NMR: $\delta = 1.66-2.31$ (m, 10H, $CH_2CH_2CH_2NH$, $CH_2CH_2CH_2$, CH_2 cyclobutyl), 2.74–2.81 (t, 2H, $CH_2CH_2CH_2NH$, $J = 7.3$ Hz), 2.84–2.99 (m, 3H, $CH_2CH_2CH_2$, CHCO), 3.26–3.35 (t, 2H, CH_2NHCO, $J = 6.6$ Hz), 4.05–4.11 (t, 2H, NCH_2, $J = 5.9$ Hz), 5.34 (br s, 1H, NHCO), 6.87–7.04 (m, 3H, H_{arom}), 7.34–7.38 (d, 1H, H_{arom}, $J = 7.7$ Hz); ^{13}C NMR: $\delta = 18.7$, 22.8, 22.9, 24.7, 25.3, 29.7, 30.1, 39.3, 39.9, 43.8, 114.1, 116.3, 118.4, 119.1, 121.7, 123.6, 125.0, 134.6, 179.9. Anal. Calcd for $C_{19}H_{24}N_2O$ (296.41): C, 76.99; H, 8.16; N, 9.45. Found: C, 76.85; H, 8.02; N, 9.37.

2.13. Synthesis of 2,3-dihydro-1H-pyrrolo-[3,2,1-ij]quinolin-6-acetonitrile (22)

Trifluoroacetic acid anhydride (0.5 mL, 3.6 mmol) is added in one portion to a suspension of 2,3-dihydro-1*H*-pyrrolo[3,2,1-*ij*]quinolin-6-acetamide (**15**) (0.60 g, 2.78 mmol) in triethylamine (1.5 mL, 8.33 mmol) and dichloromethane (17 mL) at 0 °C. The mixture is stirred at this temperature for 2 min and then transferred to a beaker containing ice-water. The resulting suspension is stirred for 10 more minutes and then extracted with dichloromethane (3×25 mL). The combined extracts are washed with H_2O (2×30 mL) and dried (Na_2SO_4). The solvent is evaporated *in vacuo* to leave a dark brown solid, which is purified by spinning plate chromatography (SPC) (cyclohexane/dichloromethane 80:20) to give the desired acetonitrile as a pale yellow solid. Yield 93%; Mp 112–114 °C (recryst. AcOEt); ^1H NMR: $\delta = 2.05-2.15$ (m, 2H, $N-CH_2CH_2CH_2$), 2.55 (t, 2H, $N-CH_2CH_2CH_2$, $J = 5.9$ Hz), 3.55 (s, 2H, CH_2CN), 3.85 (t, 2H, $CH_2CH_2CH_2-N$, $J = 5.8$ Hz), 6.35 (s, 1H, H_{arom}), 6.63–6.75 (m, 1H, H_{arom}), 7.10–7.15 (m, 1H, H_{arom}), 7.28–7.33 (m, 1H, H_{arom}); ^{13}C NMR: $\delta = 17.8$, 33.3, 34.4, 58.5, 112.4, 114.9, 117.8, 119.4, 121.3, 121.8, 126.4, 132.0, 139.9.

2.14. Synthesis of 2-methyl-2-(2,3-dihydro-1H-pyrrolo-[3,2,1-ij]quinolin-6-yl)propionitrile (23)

To a stirred suspension of sodium hydride (60%) (0.08 g, 1.9 mmol) in DMF (2.0 mL) is added dropwise a mixture of

the acetonitrile **22** (0.15 g, 0.77 mmol) and methyl iodide (0.1 mL, 1.91 mmol) in DMF (2 mL). The resulting suspension is stirred at room temperature for 18 h and then treated with a saturated aqueous solution of ammonium chloride (15 mL). The mixture is taken up with AcOEt (3 × 25 mL), washed with H_2O (2 × 20 mL) and a saturated aqueous solution of NaCl (2 × 20 mL). The extracts are dried (Na_2SO_4) and the solvent is removed under reduced pressure to leave a yellowish oil, which is purified by spinning plate chromatography (SPC) (cyclohexane/AcOEt 95:5) to give the title compound as a white solid. Yield 29%; Mp 134–136 °C (recryst. AcOEt); ^{1}H NMR: $\delta = 1.90$ (s, 6H, $C(CH_3)_2$), 2.05–2.111 (m, 2H, N–$CH_2CH_2CH_2$), 2.53 (t, 2H, N–$CH_2CH_2CH_2$, $J = 5.8$ Hz), 3.85 (t, 2H, $CH_2CH_2CH_2$–N, $J = 5.6$ Hz), 6.35 (s, 1H, H_{arom}), 6.63–6.75 (m, 1H, H_{arom}), 7.12–7.15 (m, 1H, H_{arom}), 7.28–7.30 (m, 1H, H_{arom}); ^{13}C NMR: $\delta = 25.6$, 33.6, 34.5, 58.9, 112.4, 117.8, 119.2, 119.4, 121.6, 121.8, 126.1, 132.1, 138.9.

2.15. Synthesis of N-[2-methyl-2-(2,3-dihydro-1H-pyrrolo[3,2,1-ij]quinolin-6-yl)propyl]acetamide (24)

A mixture consisting of the nitrile **23** (0.025 g, 0.11 mmol), acetic anhydride (1.5 mL), sodium acetate (0.014 g, 0.17 mmol) and Raney-Ni (5 mg) is hydrogenated in a Parr apparatus for 16 h at 50 °C. The catalyst is then filtered off through Celite and the mixture concentrated *in vacuo*. The residue obtained is diluted with dichloromethane (10 mL), washed with H_2O (3 × 15 mL) and a saturated aqueous solution of NaCl (2 × 15 mL) and dried (Na_2SO_4). The solvent is removed under reduced pressure to give the desired amide **24** as a white amorphous solid. Yield 30%; Mp 132–135 °C (recryst. EtOH); ^{1}H NMR: $\delta = 1.50$ (s, 6H, $C(CH_3)_2$), 1.88 (s, 3H, $COCH_3$), 2.05–2.11 (m, 2H, N–$CH_2CH_2CH_2$), 2.53 (t, 2H, N–$CH_2CH_2CH_2$, $J = 5.7$ Hz), 3.63 (d, 2H, CH_2NH, $J = 5.8$ Hz), 3.85 (t, 2H, $CH_2CH_2CH_2$–N, $J = 5.6$ Hz), 6.35 (s, 1H, H_{arom}), 6.65–6.76 (m, 1H, H_{arom}), 7.13–7.16 (m, 1H, H_{arom}), 7.26–7.28 (m, 1H, H_{arom}); ^{13}C NMR: $\delta = 12.4$, 18.0, 27.5, 33.3, 34.4, 38.4, 58.5, 58.8, 112.4, 117.8, 119.2, 119.4, 121.6, 121.8, 126.1, 132.1, 139.9, 170.9. Anal. Calcd for $C_{17}H_{22}N_2O$ (270.37): C, 75.52; H, 8.20; N, 10.36. Found: C, 75.36; H, 8.03; N, 10.18.

2.16. Synthesis of 1-(2,3-dihydro-1H-pyrrolo-[3,2,1-ij]quinolin-6-yl)cyclohexanecarbonitrile (25)

To a stirred suspension of sodium hydride (60%) (60 mg, 1.53 mmol) in DMSO (1.5 mL) is added dropwise a solution 2,3-dihydro-1*H*-pyrrolo[3,2,1-*ij*]quinolin-6-acetonitrile (**22**) (100 mg, 0.51 mmol) and 1,5-dibromopentane (0.1 mL, 0.62 mmol) in DMSO (1.0 mL) and diethyl ether (1.0 mL). The mixture is stirred at ambient temperature for 2 h and then quenched with H_2O (2 mL). Extraction with AcOEt (3 × 50 mL), followed by washing with a saturated aqueous solution of NaCl (2 × 15 mL), drying (Na_2SO_4) and solvent evaporation under reduced pressure, results in the formation of a dark liquid, which is purified by spinning plate chromatography (SPC) (cyclohexane/AcOEt 95:5) to give the title compound as a clear viscous oil. Yield 78%; ^{1}H NMR: $\delta = 1.79$–1.96 (m, 8H, $4CH_2$ cyclohexano), 2.16–2.27 (quintet, 2H, $CH_2CH_2CH_2$, $J = 5.6$ Hz), 2.32–2.45 (m, 2H, CH_2 cyclohexano), 2.95–3.01 (t, 2H, $CH_2CH_2CH_2N$, $J = 5.9$ Hz), 4.08–4.13 (t, 2H, NCH_2, $J = 5.7$ Hz), 6.93–7.09 (m, 3H, H_{arom}), 7.60–7.64 (d, 1H, H_{arom}, $J = 7.7$ Hz).

2.17. Synthesis of [1-(2,3-dihydro-1H-pyrrolo-[3,2,1-ij]quinolin-6-yl)cyclohexane]methylamine (26)

The synthesis of the title compound was accomplished by following the method reported for the preparation of its congener **20**. Amine **26** was used without any chromatographic purification in the next reaction.

2.18. Synthesis of N-[1-(2,3-dihydro-1H-pyrrolo-[3,2,1-ij]quinolin-6-yl)cyclohexanemethyl]acetamide-(27a) and N-[1-(2,3-dihydro-1H-pyrrolo-[3,2,1-ij]quinolin-6-yl)cyclohexanemethyl]-butyramide (27b)

Acetamide **27a** was prepared by the general method given in Section 2.12, reported for its counterpart **21a**. Yield 52%; Mp 109–111 °C (recryst. EtOH); ^{1}H NMR: $\delta = 1.32$–1.85 (m, 11H, $4CH_2$ cyclohexano and CH_3CO), 1.98–2.12 (m, 2H, CH_2 cyclohexano), 2.20–2.28 (m, 2H, $CH_2CH_2CH_2N$), 2.96–3.02 (t, 2H, $CH_2CH_2CH_2N$, $J = 5.9$ Hz), 3.59–3.61 (d, 2H, CH_2NH, $J = 5.9$ Hz), 4.11–4.16 (t, 2H, NCH_2, $J = 6.6$ Hz), 5.13 (br s, 1H, *NH*CO), 6.83–7.04 (m, 3H, H_{arom}), 7.51–7.54 (d, 1H, H_{arom}, $J = 7.3$ Hz); ^{13}C NMR: $\delta = 13.7$, 19.1, 22.5, 22.6, 24.8, 26.4, 34.5, 38.8, 39.9, 44.1, 48.0, 60.4, 118.5, 118.6, 119.2, 122.0, 123.7, 124.6, 135.2, 172.9. Anal. Calcd for $C_{20}H_{26}N_2O$ (310.44): C, 77.38; H, 8.44; N, 9.02. Found: C, 77.27; H, 8.35; N, 8.91.

Butyramide **27b** was prepared by the general method given in Section 2.12, reported for its counterpart **21c**. Yield 41%; Mp 106–108 °C (recryst. EtOH); ^{1}H NMR: $\delta = 0.81$–0.85 (t, 3H, $CH_3CH_2CH_2CO$, $J = 7.3$ Hz), 1.40–1.78 (m, 10H, $4CH_2$ cyclohexano and $CH_3CH_2CH_2CO$), 1.92–2.08 (m, 4H, $CH_3CH_2CH_2CO$ and CH_2 cyclohexano), 2.18–2.27 (m, 2H, $ArCH_2CH_2CH_2$), 2.97–3.00 (t, 2H, $CH_2CH_2CH_2NAr$, $J = 5.9$ Hz), 3.59–3.60 (d, 2H, CH_2NH, $J = 5.9$ Hz), 4.09–4.13 (t, 2H, $ArNCH_2$, $J = 6.6$ Hz), 5.11 (br s, 1H, *NH*CO), 6.87–6.98 (m, 3H, H_{arom}), 7.51–7.53 (d, 1H, H_{arom}, $J = 8.1$ Hz). Anal. Calcd for $C_{22}H_{30}N_2O$ (338.49): C, 78.06; H, 8.93; N, 8.28. Found: C, 78.28; H, 8.88; N, 8.19.

2.19. Pharmacological protocols

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

Melanophore cells were grown in 96-well tissue culture plates and growth medium was replaced with 0.7 × L-15 culture medium 18 h before analogs were tested [20, 25–28]. 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, U.K.), then cells were treated with the varying concentrations of the analogs. The maximal concentration used was 10^{-4} M. All experiments used triplicate wells at six concentrations of 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.

2.19.2. Binding affinity assay

The binding affinity of the analogs was determined in competition with radioligand binding assays using 2-[^{125}I]-iodomelatonin (specific activity 2200 Ci/mol, Perkin Elmer, U.K.), as described previously [27] on the recombinant human MT_1 and MT_2 subtypes expressed in NIH 3T3 cells.

3. Results and discussion

3.1. Chemistry

The synthesis of **17a–e** and **21a–e** is shown in Scheme 1. Commercially available 1,2,3,4-tetrahydroquinoline (**10**) was nitrosated with sodium nitrite and conc. HCl to the *N*-nitroso analog **11**, which was then converted to the hydrazine **12** on reduction with lithium aluminum hydride. Condensation of **12** with *a*-ketoglutaric acid in the presence of conc. HCl and glacial acetic acid afforded the tricyclic acetic acid **13** [24] in 40% overall yield. Basic esterification of the latter with methyl iodide in *N*,*N*-dimethylformamide gave ester **14**, which on treatment with gaseous ammonia in methanol was converted to the primary amide **15**. This was then reduced to the amine **16** with lithium aluminum hydride in tetrahydrofuran, which was not purified but immediately acylated with the appropriate reagent to give the target compounds **17a–e**.

The synthesis of their congeners, **21a–e**, involved the conversion of ester **14** to the aldehyde **18**, effected on treating the former with DIBAL-H at −78 °C in the presence of toluene, followed by the reaction of **18** with tosylmethyl isocyanide in the presence of potassium *tert*-butoxide and 1,2-dimethoxyethane, reduction of the resulting nitrile **19** with lithium aluminum hydride in the presence of benzene and diethyl ether (1:5) to the amine **20**, and immediate acylation with the appropriate reagent.

The synthesis of the side chain restrained analogs, **24** and **27a**,**b**, is depicted in Scheme 2. Amide **15** was dehydrated to the acetonitrile **22**, on treatment with trifluoroacetic anhydride in dichloromethane in the presence of triethylamine. Nitrile **22** was then reacted with excess methyl iodide in the presence of sodium hydride in *N*,*N*-dimethylformamide to give the β,β′-dimethyl analog **23**. Treatment of nitrile **22** with 1,5-dibromopentane in the same way gave the β-carbocyclic analog **25**. Compound **24** was obtained by a simultaneous Raney-Nickel hydrogenation/acetylation process, whilst its β,β′-disubstituted counterparts **27a**,**b** on reduction of **25**, with lithium aluminum hydride, in the presence of benzene and diethyl ether (1:5), to the amine **26** and immediate acylation 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 *X. laevis* melanophores. In these cells, granules containing melanin pigment are normally distributed evenly throughout the cell but move towards the cell centre when melatonin receptors are activated. The response is readily quantified by measuring the change in light (630 nm) absorbance of the cells as the pigment concentrates near the cell centre.

Table 1
Melatoninergic activity of compounds **17a–e** and **21a–e** in the *Xenopus laevis* melanophore assay and on human MT_1 and MT_2 receptors expressed in NIH 3T3 cells

Compound	R	Agonist pEC_{50}	Antagonist pIC_{50}	Human MT_1 (pK_i, nm)	Human MT_2 (pK_i, nm)	Selectivity (MT_2/MT_1)
Melatonin		10.07	NA	9.41 ± 0.09	9.45 ± 0.04	1.1
Luzindole		NA[a]	5.61 ± 0.08	6.10 ± 0.08	7.33 ± 0.06	17
17a	CH_3	NA	5.21 ± 0.03	NT	NT	–
17b	C_2H_5	NA	5.66 ± 0.01	NT	NT	–
17c	C_3H_7	NA	5.77 ± 0.05	NT	NT	–
17d	c-C_3H_5	NA	6.55 ± 0.01	NT	NT	–
17e	c-C_4H_7	NT[b]	NT	NT	NT	–
21a	CH_3	NA	5.29 ± 0.02	6.72 ± 0.05	7.02 ± 0.04	2.0
21b	C_2H_5	NA	5.33 ± 0.04	7.12 ± 0.01	7.76 ± 0.08	4.4
21c	C_3H_7	NA	5.85 ± 0.04	7.38 ± 0.02	7.93 ± 0.05	3.5
21d	c-C_3H_5	NA	5.82 ± 0.02	6.94 ± 0.05	7.15 ± 0.06	1.6
21e	c-C_4H_7	NA	5.80 ± 0.03	6.43 ± 0.04	6.73 ± 0.07	2.0

[a] NA = no agonist or antagonist effect detected at 100 μM. Agonist and antagonist data are the mean of triplicate experiments.
[b] NT = not tested.

Table 2
Melatoninergic activity of the side chain conformationally restrained analogs **24** and **27a**,**b** in the *Xenopus laevis* melanophore assay and on human MT_1 and MT_2 receptors expressed in NIH 3T3 cells

Compound	R	Agonist pEC_{50}	Antagonist pIC_{50}	Human MT_1 (pK_i, nm)	Human MT_2 (pK_i, nm)	Selectivity (MT_2/MT_1)
Melatonin		10.07	NA	9.41 ± 0.09	9.45 ± 0.04	1.1
Luzindole		NA[a]	5.61 ± 0.08	6.10 ± 0.08	7.33 ± 0.06	17
24	CH_3	5.72 ± 0.03	~4.00	NT[b]	NT	–
27a	CH_3	NA	5.93 ± 0.03	5.58 ± 0.23	6.63 ± 0.06	11.2
27b	C_3H_7	NA	5.69 ± 0.04	5.23 ± 0.02	6.39 ± 0.01	14.4

[a] NA = no agonist or antagonist effect detected at 100 μM.
[b] NT = not tested. Agonist and antagonist data are the mean of triplicate experiments.

It is apparent from the data presented in Table 1 that irrespective of the number of methylene units in the side chain spacer, analogs **17a**–**d** and **21a**–**e** are all antagonists in the melanophore assay. With the exception of the acetamides **17a** and **21a** and the propionamide **21b**, the potencies of the other new compounds are comparable to that of luzindole (Fig. 1), a commonly used melatonin receptor antagonist ($pIC_{50} = 5.61$). Moreover, there is a general trend of reduced potency upon spacer's elongation. Compound **17d** is of particular interest in that by increasing the spacer's length in the side chain by a methylene unit (from **17d** to **21d**) a six-fold decrease in potency occurs. This may indicate that in this series (**21a**–**e**) the long side chain is not well accommodated in its binding site and so cannot readily induce the receptor conformation needed for antagonist action.

Comparison of the new *N*1–C7 annulated derivatives **17a**–**d** with their *N*1–C2 annulated congeners **3a**–**e** (Fig. 2) shows that the former annulation enhances the antagonist potency in the *Xenopus* melanophore as much as 691-fold in the case of the cyclopropanamido analogs **3d** ($pIC_{50} = 3.71$) and **17d** ($pIC_{50} = 6.55$). This remarkable difference nicely illustrates that steric factors in the *N*1–C7 area increase the preference for the active conformation [29] but, as these effects are exerted closer to the pharmacophoric side chain the population of the preferred conformation declines.

All five analogs belonging to the **21** series exhibit a greater affinity at MT_2 than at the MT_1 receptor, indicating that the MT_2 receptor cavity is larger and/or more flexible than that of the MT_1 [29].

The MT_2 selectivity is also retained in the case of the side chain restrained analogs **27a**,**b**. There is a subtle difference though in the degree of selectivity, as these compounds are 11- and 14-fold MT_2 selective, respectively.

Conversely to the unsubstituted analog **17a** and the cyclohexane side chain substituted derivatives **27a**,**b**, the new *gem*-dimethyl compound **24** is a partial agonist ($pEC_{50} = 5.72$; Table 2) with ~80% of full agonist response. Thus, it seems that the introduction of two β-methyl groups is not only tolerated by the receptor but it constitutes a functional probe in its dynamic agonist–antagonist conformational equilibrium. Similar behaviour has been noticed in the case of the analogous *gem*-dimethyl *N*1–C2 annulated compound **5a** ($pEC_{50} = 5.86$; [23]).

Interestingly, the incorporation of a six-membered (**27a**,**b**) ring has either almost no effect in the degree of antagonism on *Xenopus* melanophores ($pIC_{50} = 5.66$ for **17b** vs. $pIC_{50} = 5.69$ for **27b**) or it increases antagonist potency by almost five-fold ($pIC_{50} = 5.21$ for **17a** vs. $pIC_{50} = 5.93$ for **27a**) (Table 2). However, the new *N*1–C7 annulated cyclohexane side chain substituted derivatives **27a**,**b**, are substantially more potent than their respective *N*1–C2 annulated counterparts **8** and **9** [23] ($pIC_{50} = 5.93$ for **27a** vs. $pIC_{50} = 5.10$ for **8** and $pIC_{50} = 5.69$ for **27b** vs. $pIC_{50} = 5.02$ for **9**). This is another example of a topographically induced enhancement of antagonism due to steric factors.

4. Conclusion

The biological data presented herein for the three series of new analogs suggest that the influence of steric factors in binding is significant, when it arises from the presence of bulky moieties in the β-position of the alkanamidoethyl side chain and/or elongated spacers, coupled with a suitable *N*1–C7 annulation. This was demonstrated by the partial agonist activity of **24** compared to the antagonist activity exhibited by its non-substituted congeners **17a**–**d** and **21a**–**e**. The presence of more bulky β-substituents, regardless of the size of the R group, compounds **27a**,**b**, seems to lead to reduced antagonism. Moreover, steric effects in the *N*1–C7 area increase the preference for the active conformation, but when these effects are exerted closer to the pharmacophoric side chain (*N*1–C2 annulation) the population of the preferred conformation declines. However, in order to validate these hypotheses, a recurrence of similar effects on a large number of melatonin analogs, acting either as agonists or antagonists, is necessary. The results from such comparative studies may disclose information on important synergism between steric effects and potency, which could be exploited in the design of high affinity, specific ligands.

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References

[1] R.J. Reiter, Endocr. Rev. 12 (1991) 151–180.
[2] G. Lincoln, Nature 302 (1983) 755.
[3] R.J. Reiter, Experientia 49 (1993) 654–664.
[4] D. Garfinkel, M. Laudon, D. Nof, N. Zisapel, Lancet 346 (1995) 541–544.
[5] R. Wurtman, Expert Rev. Neurother. 6 (2006) 957–964.
[6] W.C. Dement, L. Miles, M.A. Carskadon, J. Am. Geriatrics Soc. 30 (1982) 25–50.
[7] N.E. Rosenthal, D.A. Sack, F.M. Jacobsen, S.P. James, J. Arendt, L. Tamarkin, T.A. Wehr, J. Neural Transm. 21 (1986) 257–267.
[8] K. Petrie, J.V. Conaglen, L. Thompson, K. Chamberlain, Br. Med. J. 298 (1989) 705–707.
[9] B. Poeggeler, L. Miravalle, M.G. Zagorski, T. Wisniewski, Y.-J. Chyan, Y. Zhang, H. Shao, T. Bryant-Thomas, R. Vidal, B. Frangione, J. Giso, M.A. Pappolla, Biochemistry 40 (2001) 14995–15001.
[10] T.M. Molis, L.L. Spriggs, Y. Jupiter, S.M. Hill, J. Pineal Res. 18 (1995) 93–103.
[11] R. Sandyk, Int. J. Neurosci. 50 (1990) 37–53.
[12] C.S. Shida, A.M.L. Castrucci, M.T. Lamy-Freund, J. Pineal Res. 16 (1994) 198–201.
[13] C. Pieri, M. Marra, F. Moroni, R. Recchioni, F. Marcheselli, Life Sci. 55 (1994) PL271–PL276.
[14] M.A. Livrea, L. Tesoriere, D. D'Arpa, M. Morreale, Free Radic. Biol. Med. 23 (1997) 706–711.
[15] K. Iakovou, A. Varvaresou, A.P. Kourounakis, K. Stead, D. Sugden, A. Tsotinis, J. Pharm. Pharmacol. 54 (2002) 147–156.
[16] S.M. Reppert, D.R. Weaver, V.M. Cassone, C. Godson, L.F. Kolakowski, Neuron 15 (1995) 1003–1015.
[17] E.L. Bittman, D.R. Weaver, Biol. Reprod. 43 (1990) 986–993.
[18] P.J. Morgan, P. Barratt, H.E. Howell, R. Helliwell, Neurochem. Int. 24 (1994) 101–146.
[19] S.M. Reppert, C. Godson, C.D. Mahle, D.R. Weaver, S.A. Slaugenhaupt, J.F. Gusella, Proc. Natl. Acad. Sci. USA 92 (1995) 8734–8738.
[20] R. Faust, P.J. Garratt, R. Jones, L.-K. Yeh, A. Tsotinis, M. Panoussopoulou, T. Calogeropoulou, M.-T. Teh, D. Sugden, J. Med. Chem. 43 (2000) 1050–1061 and references cited therein.
[21] X. Jin, C. von Gall, R.L. Pieschl, V.K. Gribkoff, J.H. Stehle, S.M. Reppert, D.R. Weaver, Mol. Cell. Biol. 23 (2003) 1054–1060.
[22] A. Tsotinis, M. Panoussopoulou, S. Sivananthan, D. Sugden, Il Farmaco 56 (2001) 725–729.
[23] A. Tsotinis, M. Panoussopoulou, K. Hough, D. Sugden, Eur. J. Pharm. Sci. 18 (2003) 297–304.
[24] D. Paris, M. Cottin, P. Demonchaux, G. Augert, P. Dupassieux, P. Lenoir, M.J. Peck, D. Jasserand, J. Med. Chem. 38 (1995) 669–685.
[25] D. Sugden, Br. J. Pharmacol. 104 (1991) 922–927.
[26] D. Sugden, Eur. J. Pharmacol. 213 (1992) 405–408.
[27] M.-T. Teh, D. Sugden, Br. J. Pharmacol. 126 (1999) 1237–1245.
[28] D.J. Davies, P.J. Garratt, D.A. Tocher, S. Vonhoff, J. Davies, M.-T. Teh, D. Sugden, J. Med. Chem. 41 (1998) 451–467.
[29] A. Tsotinis, M. Vlachou, D.P. Papahatjis, T. Calogeropoulou, S.P. Nikas, P.J. Garratt, V. Piccio, S. Vonhoff, K. Davidson, M.-T. Teh, D. Sugden, J. Med. Chem. 49 (2006) 3509–3519.