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Design, synthesis and melatoninergic potency of new *N*-acyl 8,9-dihydro-4-methoxy-7*H*-2-benzo[*de*]quinolinalkanamines

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Abstract

A series of new *N*-acyl 8,9-dihydro-4-methoxy-7*H*-2-benzo[*de*]quinolinalkanamines 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. Compounds with a single methylene spacer in the side chain (**7**) have no agonist activity, but are weak antagonists in the *Xenopus* melanophore assay, irrespectively of the size or shape of the R substituent ($R = CH_3$ to c-C_4H_7). In contrast, compounds with two (**8**) or three (**9**) methylene spacers show partial agonist activity, though this does vary with the nature of the R substituent. Interestingly, the cyclopropane and cyclobutane R substituents, which are usually linked with antagonism, render the cyclopropanecarboxamido analog **9d** and its cyclobutanecarboxamido congener **9e** weak agonists. It seems, therefore, that in these compounds the R substituent constitutes a functional probe in the dynamic agonist–antagonist conformational equilibrium. One of the new molecules, antagonist **8c**, exhibits a noteworthy MT_2 subtype selectivity (13-fold), whereas the acetamido analog **9a** (with a three methylene units spacer) also acts as an antagonist and is the only analog exhibiting MT_1 selectivity (>10-fold). In contrast to the analogous *N*1–C7 annulated indole derivatives, recently reported, the new C1–C8 condensed isoquinolines are not all pure antagonists. Despite their modest receptor affinity at the binding site these

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compounds demonstrate that the nature of the response (agonist or antagonist activity) is dependent, in this case, on both the side chain spacer's length and the size and shape of the R group.

Keywords: C1–C8 annulated isoquinolines; Synthesis; Melatoninergic activity

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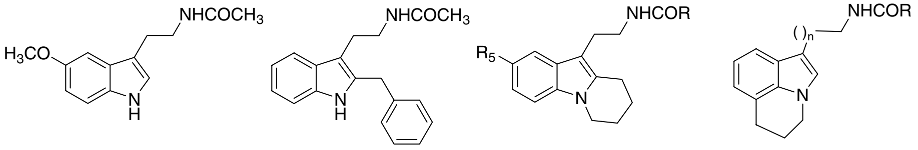
1. Introduction

Melatonin, (Fig. 1), first discovered in 1958 [1], is secreted by the pineal gland during the night in a wide range of species. This cycle of melatonin secretion is a robust hormonal marker of the 'biological clock' which can indicate time of day to tissues containing melatonin receptors [2]. In photoperiodic species such as sheep, in which reproductive cycles are dependent on the seasons, seasonal changes in night length are encoded as changes in melatonin secretion. This hormonal signal synchronizes the onset of the reproductive cycle during the appropriate season [3,4]. In humans, the circadian rhythm of melatonin secretion is thought to contribute to other functions of the circadian clock, such as consolidation of sleep [5] and regulation of the circadian rhythm of core body temperature [6]. Melatonin has also been implicated in Alzheimer's and other neurotic disorders [7], in certain cancers [8] and in Parkinson's disease [9]. Furthermore, due to its amphiphilic nature [10,11] melatonin can easily enter cells and effectively scavenge both water-soluble peroxyl ($ROO^{\bullet}$) [12] and lipoperoxy ($LOO^{\bullet}$) radicals [13,14].

The physiological actions of melatonin in regulating seasonal and circadian rhythms are mediated through its high affinity MT receptors. A low affinity binding site was recently shown to be the enzyme quinine reductase 2 [15]. The MT receptor is a member of the large family of receptors with seven membrane-spanning domains—a family that includes the serotonin and *β*-adrenergic receptors. MT receptors are of clear importance to the nervous system. Molecular cloning revealed that there are at least three subtypes of MT receptors: MT_1, MT_2, and Mel_{1c} [16]. To date, Mel_{1c} receptors have not been found in mammals. Of particular interest are the MT_1 and MT_2 receptors. These receptors are classic G-protein-linked receptors that inhibit adenylate cyclase. Additionally, MT_1 receptors activate protein kinase C-β, whereas MT_2 receptors inhibit the soluble guanylate cyclase pathway [17] while stimulating protein kinase C [18,19]. MT_1 receptors have the most widespread distribution in the rodent brain, accounting for the majority of melatonin-binding sites in most target tissues, and are widely thought to mediate many melatonin actions in the brain [20].



1: melatonin

2: luzindole

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=as above

5a: n=1, R=CH_3
5b: n=1, R=C_2H_5
5c: n=1, R=n-C_3H_7
5d: n=1, R=c-C_3H_5

5e: n=1, R=c-C_4H_7
6: n=2, R=as above

Fig. 1. Structures of melatonin, luzindole, *N*1–C2 annulated analogs **3** and **4** and *N*1–C7 annulated analogs **5** and **6**.

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 an effort to take advantage of the biological actions of melatonin for treatment of sleep and other circadian-linked disturbances in humans, we [21,22a,b] and others [22c–g] have developed melatonin analogs that act as agonists or antagonists at melatonin receptors. These melatoninergics include several nuclei, such as naphthalenic [22c], amidotetralin [22d], amido indane [22e], benzofuran [22f] and benzothiophene [22g], which serve as bioisosteres of the indole ring of melatonin.

In an attempt to probe the constraints at the receptor site with regard to the lower *N*1–C2 region of the indole moiety, we have reported the synthesis and biological activity of the *N*-[2-(6,7,8,9-tetrahydropyrido[1,2-*a*]indol-10-yl)ethyl]alkanamides, **3** and *N*-[2-(2-methoxy-6,7,8,9-tetrahydropyrido[1,2-*a*]indol-10-yl)ethyl]alkanamides, **4** (Fig. 1) [23]. In the *Xenopus* melanophore pigment aggregation model, the non-methoxy substituted compounds, **3**, are antagonists, while their 2-methoxy counterparts, **4**, are full agonists, the butyramido analog **4** ($R = C_3H_7$) being almost as potent ($pEC_{50} = 9.91$) as melatonin ($pEC_{50} = 10.07$). This work was recently extended by the synthesis of the *N*1–C7 annulated 2,3-dihydro-1*H*-pyrrolo[3,2,1-*ij*]quinolino-6-alkanamides **5** and **6** (Fig. 1) [22b]. Similarly to their *a*-face annelated congeners **3**, analogs **5** are antagonists in the *Xenopus* melanophore assay. The antagonistic character remains unaltered and in the case of the *N*-acylated propanamines **6**.

Based on these findings we report herein on the synthesis and biology of the novel *N*-acyl 8,9-dihydro-4-methoxy-7*H*-2-benzo[*de*]quinolinomethanamines **7a**–**e** and their congeners **8a**–**c** and **9a**–**e**. In all of these molecules, the pyrrole ring of the indole nucleus has been replaced by pyridine, producing thus the skeleton of isoquinoline, which is condensed between C1 and C8 with cyclohexane. The methoxyl group, which *albeit* not being an essential requirement for biological activity [24], has been retained in the new structures, since it is known to enhance potency by forming a hydrogen bond with His211 in the putative transmembrane domain 5 (TM5) [25,26]. Furthermore, analogs **7**–**9**, probe the influence of the relevant distance between the alkanamido side chain and the methoxyl group on meletoninergic activity (6 (**7a**–**e)** to 8 (**9a**–**e**) bonds distance).

Conversely to the *N*1–C7 annulated indole derivatives, recently reported [22b], the new C1–C8 condensed isoquinolines are not all pure antagonists, as the nature of their activity depends in this case on both the side chain spacer's size and the R group.

2. Materials and methods

2.1. General

Melting points were determined on a Büchi 530 apparatus and are uncorrected. ^1H 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 taken at 50 MHz on a Bruker AC 200 spectrometer. Tetramethylsilane was used as internal standard. Mass spectrometry was performed on a Finnigan AQA single-quadrupole MS (ThermoQuest, Manchester, UK) instrument, equipped with an ESI source. 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.

*2.2. 7-Methoxy-8-bromo-1-tetralone (**11**)*

The title compound was obtained as a pale red solid (92%) following the method reported by Bohlmann and Fritz [31]. Mp 98–100 °C (diethyl ether) (99 °C [31]).

*2.3. 8-Bromo-7-methoxy-3,4-dihydronaphthalen-1(2H)-one ethylene acetal (**12**)*

This compound was prepared in 82% yield following the method of Hickey et al. [32]. Mp 88–89 °C (*n*-hexane) (90–91 °C [32]).

*2.4. 8-Formyl-7-methoxy-3,4- dihydronaphthalen-1(2H)-one ethylene acetal (**13**)*

The title compound was obtained as a transparent oil (70%) following the method reported by Hickey et al. [32].

*2.5. Methyl a-azido-3′,4′-dihydro-β-[2′H-spiro[1,3-dioxolan-2,1′(2′H)naphthalen]] propenoate (**14**)*

This compound was prepared in 45% yield following the methods of Allen and coworkers [33] and Hickey et al. [32].

*2.6. Methyl2-azido-3-(7-methoxy-1-oxo-1,2,3,4-tetrahydronaphthalen-8-yl)propenoate (**15**)*

The title compound was obtained as an off-white solid (75%) following the method reported by Hickey et al. [32]. Mp 98 °C (cyclohexane) (100 °C [32]).

*2.7. Methyl 8,9-dihydro-4-methoxy-7H-benzo[de]quinoline-2-carboxylate (**16**)*

This compound was obtained as an off-white flaky solid (95%) following the method of Hickey et al. [32]. Mp 119–121 °C (*n*-hexane) (122–123 °C [32]).

*2.8. 8,9-Dihydro-4-methoxy-7H-2-benzo[de]quinolinocarboxamide (**17**)*

A fine stream of dry ammonia gas was bubbled for 15 min through a solution of the ester **16** (0.29 g, 1.13 mmol) in methanol (4.5 mL) at 0 °C. The saturation of the reaction mixture with dry ammonia was repeated several times until all of the starting material had been consumed (TLC control, AcOEt). The solvent was removed *in vacuo* and the residue was taken up with EtOAc (50 mL). The solution was washed with brine and concentrated *in vacuo* to give a residue, which was purified by flash column chromatography (AcOEt/cyclohexane 93:7). The title compound was obtained as a transparent viscous oil (yield 95%). ^{1}H NMR ($CDCl_3$): $\delta = 2.14$–2.22 (quintet, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2$), 3.02–3.08 (t, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2$), 3.27–3.33 (t, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2$), 3.98 (s, 3H, OCH_3), 5.56 (bs, 1H, N*H* amide), 6.95 (dd, $J = 8.1$ Hz, 1H, H_{arom}), 7.30 (dd,

$J = 8.1$ Hz, 1H, H_{arom}), 8.06 (bs, 1H, N*H* amide), 8.82 (s, 1H, H_{arom}). $C_{14}H_{14}N_2O_2$ (242.3): Calcd: C, 69.41; H, 5.82; N, 11.56. Found: C, 69.37; H: 5.79; N: 11.49.

*2.9. 8,9-Dihydro-4-methoxy-7H-2-benzo[de]quinolinomethanamine (**18**)*

A solution of the amide **17** (105 mg, 0.44 mmol) in dry THF (5 mL) was added dropwise to a suspension of lithium aluminum hydride (0.10 g, 2.60 mmol) in dry THF (2.5 mL) at 0 °C. The reaction mixture was then refluxed for 2 h, chilled once again to 0 °C and treated with H_2O (3 mL) in order to destroy the excess of lithium aluminum hydride. The resulting suspension was stirred for 30 more min and filtered through celite. The filtrate was diluted with AcOEt (45 mL), washed with H_2O (2 × 20 mL) and saturated aqueous NaCl (2 × 25 mL) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the desired amine **18** was obtained as a pale yellow viscous liquid, which was used as such for further reactions.

*2.10. General procedure for the synthesis of N-acyl 8,9-dihydro-4-methoxy-7H-2-benzo[de] quinolinomethanamines (7**a**–**e**)*

Triethylamine (0.2 mL) and the appropriate acid anhydride (0.24 mmol) (compounds **7a**–**c**) or acid chloride (0.24 mmol) (compounds **7d**,**e**) were added dropwise to a chilled (0 °C) solution of amine **16** (0.21 mmol) in dichlorometane (2 mL). The resulting mixture was stirred at room temperature for 30–60 min prior to being transferred to a small beaker containing H_2O (2 mL). The biphasic mixture was extracted with CH_2Cl_2 (3 × 10 mL), washed with H_2O (2 × 10 mL) and saturated aqueous NaCl (2 × 15 mL) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the dark residue obtained was purified by flash chromatography to give the desired amides **7a**–**e** as clear viscous oils.

*2.11. N-Acetyl 8,9-dihydro-4-methoxy-7H-2-benzo[de]quinolinomethanamine (7**a**)*

Acetamide **7a** was prepared by following general method 2.10. Yield 35%. Flash column chromatography eluent system: AcOEt/MeOH 93:7. ^1H NMR ($CDCl_3$): $\delta = 2.03$ (s, 3H, CH_3CO), 2.08–2.17 (m, 2H, $CH_2CH_2CH_2$), 2.96–3.02 (t, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2$) 3.12–3.18 (t, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2$), 3.91 (s, 3H, OCH_3), 4.57–4.60 (d, $J = 5.1$ Hz, 2H, CH_2NH), 6.53 (bs, 1H, NHCO), 6.82–6.86 (d, $J = 8.0$ Hz, 1H, H_{arom}), 7.13–7.17 (d, $J = 8.0$ Hz, 1H, H_{arom}), 7.74 (s, 1H, H_{arom}). ^{13}C NMR ($CDCl_3$): $\delta = 23.4$, 23.5, 29.7, 34.2, 45.0, 55.6, 107.8, 111.3, 124.4, 124.7, 128.8, 129.7, 148.0, 152.7, 159.8, 170.1. $C_{16}H_{18}N_2O_2$ (270.3): Calcd: C, 71.09; H, 6.71; N, 10.36. Found: C, 70.95; H, 6.69; N, 10.25.

*2.12. N-Propanoyl 8,9-dihydro-4-methoxy-7H-2-benzo[de]quinolinomethanamine (7**b**)*

The title compound **7b** was prepared according to the general method 2.10. Yield 30%. Flash column chromatography eluent system: AcOEt/MeOH 95:5. ^1H NMR ($CDCl_3$): $\delta = 1.14$–1.18 (t, $J = 7.7$ Hz, 3H, CH_3CH_2CO), 2.06–2.30 (m, 4H, $CH_2CH_2CH_2$, CH_2CO), 2.94–3.00 (t, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2$), 3.10–3.17 (t, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2$), 3.90 (s, 3H, OCH_3), 4.57–4.59 (d, $J = 5.1$ Hz, 2H, CH_2NH), 6.62 (bs, 1H, NHCO), 6.80–6.84 (d, $J = 7.7$ Hz, 1H, H_{arom}), 7.11–7.15 (d, $J = 7.7$ Hz, 1H, H_{arom}), 7.73 (s, 1H, H_{arom}).

^{13}C NMR ($CDCl_3$): $\delta = 9.8$, 23.4, 29.7, 34.2, 44.9, 55.5, 107.7, 111.1, 124.3, 124.7, 128.9, 129.7, 148.2, 152.7, 159.8, 173.6. $C_{17}H_{20}N_2O_2$ (284.4): Calcd: C, 71.81; H, 7.09; N, 9.85. Found: C, 71.72; H, 7.15; N, 9.78.

2.13. *N-Butanoyl 8,9-dihydro-4-methoxy-7H-2-benzo[de]quinolinomethanamine (**7c**)*

Butyramide **7c** was prepared by following general method 2.10. Yield 30%. Flash column chromatography eluent system: AcOEt/MeOH 97:3. ^1H NMR ($CDCl_3$): $\delta = 0.94$–1.02 (t, $J = 7.7$ Hz, 3H, $CH_3CH_2CH_2CO$), 1.68–1.78 (quintet, $J = 7.7$ Hz, 2H, $CH_3CH_2CH_2CO$) 2.14–2.30 (m, 4H, $CH_2CH_2CH_2$, $COCH_2CH_2$), 3.02–3.08 (t, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2$), 3.18–3.25 (t, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2$), 3.96 (s, 3H, OCH_3), 4.65–4.67 (d, $J = 5.1$ Hz, 2H, CH_2NH), 6.59 (bs, 1H, NHCO), 6.88–6.92 (d, $J = 8.0$ Hz, 1H, H_{arom}), 7.19–7.26 (d, $J = 8.0$ Hz, 1H, H_{arom}), 7.81 (s, 1H, H_{arom}). ^{13}C NMR ($CDCl_3$): $\delta = 13.8$, 19.1, 23.5, 29.7, 34.3, 38.7, 44.9, 55.5, 107.7, 111.1, 124.4, 124.7, 128.8, 129.7, 148.3, 152.7, 159.8, 172.9. $C_{18}H_{22}N_2O_2$ (298.4): Calcd: C, 72.46; H, 7.43; N, 9.39. Found: C, 72.38; H, 7.50; N, 9.30.

2.14. *N-Cyclopropanecarbonyl 8,9-dihydro-4-methoxy-7H-2-benzo[de]quinolinomethanamine (**7d**)*

The title compound **7d** was prepared according to the general method 2.10. Yield 25%. Flash column chromatography eluent system: AcOEt/MeOH 98:2. ^1H NMR ($CDCl_3$): $\delta = 0.72$–0.76 (m, 2H, CH_2 cycloprop.), 0.97–1.01 (m, 2H, CH_2 cycloprop.), 1.38–1.51 (m, 1H, COCH), 2.11–2.19 (quintet, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2$), 3.00–3.04 (t, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2$), 3.17–3.23 (t, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2$), 3.94 (s, 3H, OCH_3), 4.63–4.66 (d, $J = 5.1$ Hz, 2H, CH_2NH), 6.73 (bs, 1H, NHCO), 6.85–6.89 (d, $J = 8.0$ Hz, 1H, H_{arom}), 7.16–7.22 (d, $J = 8.0$ Hz, 1H, H_{arom}), 7.78 (s, 1H, H_{arom}). ^{13}C NMR ($CDCl_3$): $\delta = 7.1$, 14.9, 23.5, 29.8, 34.3, 45.2, 55.6, 107.9, 111.5, 124.4, 124.7, 129.0, 129.8, 148.2, 152.7, 159.8, 173.5. $C_{18}H_{20}N_2O_2$ (296.4): Calcd: C, 72.95; H, 6.80; N: 9.45. Found: C, 72.87; H, 6.75; N, 9.40.

2.15. *N-Cyclobutanecarbonyl 8,9-dihydro-4-methoxy-7H-2-benzo[de]quinolinomethanamine (**7e**)*

Amide **7e** was prepared by following general method 2.10. Yield 25%. Flash column chromatography eluent: AcOEt. ^1H NMR ($CDCl_3$): $\delta = 1.88$–2.32 (m, 9H, $CH_2CH_2CH_2$, CHCO, CH_2 cyclobut.), 2.99–3.05 (t, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2$), 3.15–3.21 (t, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2$), 3.94 (s, 3H, OCH_3), 4.61–4.63 (d, $J = 5.1$ Hz, 2H, CH_2NH), 6.45 (bs, 1H, NHCO), 6.85–6.89 (d, $J = 7.7$ Hz, 1H, H_{arom}), 7.16–7.19 (d, $J = 7.7$ Hz, 1H, H_{arom}), 7.77 (s, 1H, H_{arom}). ^{13}C NMR ($CDCl_3$): $\delta = 18.2$, 23.5, 25.4, 29.7, 34.3, 40.0, 44.8, 55.6, 107.7, 111.2, 124.3, 124.7, 129.0, 129.8, 148.4, 152.8, 159.8, 173.7. $C_{19}H_{22}N_2O_2$ (310.4): Calcd: C, 73.52; H, 7.14; N, 9.03. Found: C, 73.45; H, 7.08; N: 8.90.

2.16. *8,9-Dihydro-4-methoxy-7H-benzo[de]quinoline-2-carboxaldehyde (**19**)*

DIBAL-H (0.6 mL, 3.7 mmol) was added dropwise to a stirred solution of the methyl ester **16** (0.49 g, 1.91 mmol) in dry toluene (15 mL) at −78 °C. The mixture was stirred at this temperature for 25 min and then allowed to reach 0 °C. 1 N HCl (4.5 mL) was added and the resulting suspension was filtered through celite. The filtrate was diluted with water (10 mL) and extracted with AcOEt (3 × 50 mL). The combined organic phases are washed with saturated aqueous NaCl, dried over Na_2SO_4 and the solvents were evaporated *in vacuo* to give a brownish oil, which was purified by flash chromatography (cyclohexane/EtOAc 85:15) to afford 0.30 g of aldehyde **19** (yield 70%) as a clear viscous oil. ^1H NMR ($CDCl_3$): $\delta = 2.11$–2.19 (quintet, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2$), 3.02–3.08 (t, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2$), 3.27–3.33 (t, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2$), 3.98 (s, 3H, OCH_3), 6.95 (d, $J = 8.1$ Hz, 1H, H_{arom}), 7.25 (d, $J = 8.1$ Hz, 1H, H_{arom}), 8.77 (s, 1H, H_{arom}), 9.81 (s, 1H, C*H*O). ^{13}C NMR ($CDCl_3$): $\delta = 23.3$, 29.7, 34.4, 55.8, 108.6, 116.8, 116.9, 127.7, 128.2, 128.3, 130.1, 145.3, 154.5, 193.4. $C_{14}H_{13}NO_2$ (227.3): Calcd: C, 73.99; H, 5.76; N, 6.16. Found: C, 74.02; H, 5.74; N, 6.10.

*2.17. 8,9-Dihydro-4-methoxy-7H-benzo[de]quinoline-2-acetonitrile (**20**)*

A solution of *p*-tolouenesulfonylmethyl isocyanide (TosMIC) (92.5 mg, 0.47 mmol) in dimethoxyethane (DME) (0.5 mL) was added dropwise to a suspension of potassium *tert*-butoxide (107 mg, 0.96 mmol) in DME (0.5 mL) at −30 °C. After the addition, the mixture was cooled to −60 °C and a solution of the aldehyde **19** (100 mg, 0.44 mmol) in DME (1 mL) was cautiously added. The reaction was stirred at this temperature for 1 h and methanol (1.5 mL) was added. The mixture was allowed to thaw and then refluxed for 15 min. Upon completion of the reaction, the solvent was removed *in vacuo* and H_2O (1.5 mL) followed by acetic acid (0.1 mL) was added. The resulting suspension was extracted with dichloromethane (3 × 50 mL), the combined organics washed with saturated aqueous $NaHCO_3$ and NaCl and dried (Na_2SO_4). The solvent was evaporated under reduced pressure and the residue was purified by flash column chromatography (cyclohexane/AcOEt 85:15) to give 70 mg of the desired compound (67%) as a colorless viscous oil. ^1H NMR ($CDCl_3$): $\delta = 2.11$–2.17 (quintet, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2$), 3.01–3.04 (t, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2$), 3.16–3.19 (t, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2$), 3.94 (s, 3H, OCH_3), 3.99 (s, 2H, CH_2CN), 6.91 (d, $J = 8.1$ Hz, 1H, H_{arom}), 7.21 (d, $J = 8.1$ Hz, 1H, H_{arom}), 7.95 (s, 1H, H_{arom}). ^{13}C NMR ($CDCl_3$): $\delta = 23.4$, 26.6, 29.7, 34.2, 55.6, 108.2, 111.7, 125.0, 128.8, 129.8, 129.9, 149.1, 152.7, 160.7, 179.4.

*2.18. 8,9-Dihydro-4-methoxy-7H-benzo-2-[de]quinolinethanamine (**21**)*

A solution of the nitrile **20** (70 mg, 0.30 mmol) in dry benzene (1 mL) was added dropwise to a suspension of lithium aluminum hydride (0.05 g, 1.32 mmol) in dry ether (2 mL) at 0 °C. The mixture was then refluxed for 1.5 h, cooled to 0 °C and treated carefully with H_2O (2 mL). The resulting suspension was filtered through celite and the filtrate was extracted with AcOEt (3 × 50 mL). The combined organics were washed with a saturated aqueous NaCl solution and dried (Na_2SO_4). The solvent was removed *in vacuo* to give 50 mg of the desired amine as a yellowish oil, pure enough to be used as such in the following reactions.

*2.19. N-Acetyl 8,9-dihydro-4-methoxy-7H-benzo-2-[de]quinolinethanamine (**8a**)*

This compound was obtained as a colorless oil following general method 2.10. Yield 25%. Flash column chromatography eluent system: AcOEt/MeOH 95:5. ^1H NMR

($CDCl_3$): δ = 1.95 (s, 3H, CH_3CO), 2.11–2.17 (m, 2H, $CH_2CH_2CH_2$), 3.00–3.06 (m, 4H, $CH_2CH_2CH_2$, $ArCH_2CH_2NH$), 3.16–3.20 (t, *J* = 6.6 Hz, 2H, $CH_2CH_2CH_2$), 3.64–3.68 (q, *J* = 6.2 Hz, 2H, CH_2CH_2NH), 3.95 (s, 3H, OCH_3), 6.64 (bs, 1H, N*H*CO), 6.86–6.88 (d, *J* = 7.3 Hz, 1H, H_{arom}), 7.16–7.18 (d, *J* = 7.3 Hz, 1H, H_{arom}), 7.68 (s, 1H, H_{arom}). ^{13}C NMR ($CDCl_3$): δ = 21.0, 23.4, 29.3, 30.5, 31.9, 39.7, 55.6, 107.7, 112.0, 124.0, 129.0, 129.8, 129.9, 151.0, 152.5, 159.5, 170.1. $C_{17}H_{20}N_2O_2$ (284.4): Calcd: C, 71.81; H, 7.09; N, 9.85. Found: C, 71.75; H, 7.15; N, 9.80.

*2.20. N-Propanoyl 8,9-dihydro-4-methoxy-7H-benzo-2-[de]quinolinethanamine (**8b**)*

The title compound was obtained as a colorless viscous oil following general method 2.10. Yield 20%. Flash column chromatography eluent system: AcOEt/MeOH 97:3. ^1H NMR ($CDCl_3$): δ = 0.83–0.87 (t, *J* = 7.3 Hz, 3H, CH_3CH_2CO), 2.13–2.27 (m, 4H, $CH_2CH_2CH_2$, CH_2CONH), 3.01–3.12 (m, 4H, $CH_2CH_2CH_2$, $ArCH_2CH_2NH$), 3.17–3.20 (t, *J* = 6.1 Hz, 2H, $CH_2CH_2CH_2$), 3.63–3.68 (q, *J* = 6.1 Hz, 2H, CH_2CH_2NH), 3.95 (s, 3H, OCH_3), 6.77 (bs, 1H, N*H*CO), 6.87–6.89 (d, *J* = 7.9 Hz, 1H, H_{arom}), 7.16–7.18 (d, *J* = 7.9 Hz, 1H, H_{arom}), 7.69 (s, 1H, H_{arom}). ^{13}C NMR ($CDCl_3$): δ = 14.1, 22.7, 23.7, 29.3, 30.1, 31.9, 39.6, 55.8, 107.7, 111.9, 124.1, 128.2, 129.0, 129.8, 130.8, 152.6, 159.6, 172.0. $C_{18}H_{22}N_2O_2$ (298.4): Calcd: C, 72.46; H, 7.43; N, 9.39. Found: C, 72.36; H, 7.40; N, 9.30.

*2.21. N-Butanoyl 8,9-dihydro-4-methoxy-7H-benzo-2-[de]quinolinethanamine (**8c**)*

Butyramide **8c** was obtained as a colorless viscous oil following general method 2.10. Yield 22%. Flash column chromatography eluent system: AcOEt/MeOH 97:3. ^1H NMR ($CDCl_3$): δ = 0.89–0.93 (t, *J* = 7.3 Hz, 3H, $CH_3CH_2CH_2CO$) 1.59–1.68 (sextet, *J* = 7.3 Hz, 2H, $CH_3CH_2CH_2CO$), 2.08–2.19 (m, 4H, $CH_2CH_2CH_2$, $CH_3CH_2CH_2CO$), 3.00–3.10 (m, 4H, $CH_2CH_2CH_2$, $ArCH_2CH_2CH_2$,NH), 3.16–3.19 (t, *J* = 6.1 Hz, 2H, $CH_2CH_2CH_2$), 3.64–3.69 (q, *J* = 6.1 Hz, 2H, CH_2CH_2NH), 3.94 (s, 3H, OCH_3), 6.66 (bs, 1H, N*H*CO), 6.86–6.88 (d, *J* = 7.3 Hz, 1H, H_{arom}), 7.16–7.18 (d, *J* = 7.3 Hz, 1H, H_{arom}), 7.67 (s, 1H, H_{arom}). ^{13}C NMR ($CDCl_3$): δ = 13.7, 19.2, 22.7, 23.5, 29.3, 30.5, 32.0, 39.6, 55.6, 107.8, 112.1, 124.0, 129.1, 129.8, 129.9, 151.0, 152.5, 159.4, 172.9. $C_{19}H_{24}N_2O_2$ (312.4): Calcd: C, 73.05; H, 7.74; N, 8.97. Found: C, 72.29; H, 7.70; N, 8.85.

*2.22. β-[8,9-Dihydro-4-methoxy-7H-benzo[de]quinolin-2-yl]propenonitrile (**22**)*

To a stirred solution of diethyl cyanomethylphosphonate (0.11 mL, 0.71 mmol) in THF (6.5 mL) was added potassium *tert*-butoxide (0.08 g, 0.71 mmol) and the resulting suspension was stirred at ambient temperature for 1 h. A solution of aldehyde **19** (0.09 g, 0.40 mmol) in THF (2 mL) was then added dropwise and the mixture stirred for 1 h. Upon completion of the reaction (TLC control, AcOEt), the solvent was removed *in vacuo* and the residue extracted with AcOEt (3 × 15 mL). The organic phase was washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure to give a brownish oil, which was purified by flash column chromatography (cyclohexane/AcOEt 80:20). The desired acrylonitrile **22** (72 mg, 73%) was obtained as a colorless viscous oil. ^1H NMR ($CDCl_3$): δ = 2.08–2.21 (quintet, *J* = 6.1 Hz, 2H, $CH_2CH_2CH_2$), 3.00–3.06 (t, *J* = 6.1 Hz, 2H, $CH_2CH_2CH_2$), 3.15–3.22 (t, *J* = 6.1 Hz, 2H, $CH_2CH_2CH_2$), 3.97 (s, 3H, OCH_3), 6.62–6.70 (d, *J* = 15.9 Hz, 1H, =C*H*CN), 6.92–6.96 (d, *J* = 8.0 Hz, 1H, H_{arom}), 7.27–7.31

(d, $J = 8.0$ Hz, 1H, H_{arom}), 7.44–7.52 (d, $J = 15.9$ Hz, 1H, Ar*CH*=), 7.82 (s, 1H, H_{arom}). ^{13}C NMR ($CDCl_3$): $\delta = 23.3$, 29.6, 34.4, 55.7, 98.4, 108.6, 115.9, 118.9, 126.2, 126.6, 128.0, 130.4, 143.3, 149.6, 153.5, 160.8. $C_{16}H_{14}N_2O$ (250.3): Calcd: C, 76.78; H, 5.64; N, 11.19. Found: C, 76.71; H: 5.60; N, 11.10.

2.23. 8,9-Dihydro-4-methoxy-7H-benzo-2-[de]quinolinopropanamine (**23**)

A solution of the nitrile **22** (40 mg, 0.16 mmol) in dry benzene (1 mL) was added dropwise to a suspension of lithium aluminum hydride (0.025 g, 0.66 mmol) in dry ether (1 mL) at 0 °C. The mixture was then refluxed for 2 h, cooled to 0 °C and treated carefully with H_2O (2 mL). The resulting suspension was filtered through celite and the filtrate was extracted with AcOEt (3 × 50 mL). The combined organics were washed with a saturated aqueous NaCl solution and dried (Na_2SO_4). The solvent was removed *in vacuo* to give the desired amine as a yellowish oil, pure enough to be used as such in the following reactions.

2.24. N-Acetyl 8,9-dihydro-4-methoxy-7H-benzo-2-[de]quinolinopropanamine (**9a**)

This compound was obtained as a colorless oil following general method 2.10. Yield 20%. Flash column chromatography eluent system: AcOEt/MeOH 95:5. ^{1}H NMR ($CDCl_3$): $\delta = 1.95$ (s, 3H, CH_3CO), 2.10–2.18 (m, 2H, $CH_2CH_2CH_2$), 2.34–2.56 (bs, 2H, $CH_2CH_2CH_2NH$), 2.93–2.97 (t, $J = 7.3$ Hz, 2H, $ArCH_2CH_2CH_2$,NH), 2.99–3.02 (t, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2$), 3.19–3.22 (t, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2$), 3.28–3.33 (q, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2NH$), 3.95 (s, 3H, OCH_3), 6.58 (bs, 1H, N*H*CO), 6.87–6.88 (d, $J = 8.1$ Hz, 1H, H_{arom},), 7.15–7.17 (d, $J = 8.1$ Hz, 1H, H_{arom}), 7.68 (s, 1H, H_{arom}). ^{13}C NMR ($CDCl_3$): $\delta = 14.1$, 22.6, 23.3, 29.5, 33.3, 34.7, 39.2, 55.6, 108.7, 112.3, 124.5, 125.9, 129.5, 130.1, 151.4, 152.5, 159.2, 170.4. $C_{18}H_{22}N_2O_2$ (298.4): Calcd: C, 72.46; H, 7.43; N, 9.39. Found: C, 72.35; H, 7.40; N, 9.23.

2.25. N-Propanoyl 8,9-dihydro-4-methoxy-7H-benzo-2-[de]quinolinopropanamine (**9b**)

The title compound was obtained as a colorless viscous oil following general method 2.10. Yield 25%. Flash column chromatography eluent system: AcOEt/MeOH 95:5. ^{1}H NMR ($CDCl_3$): $\delta = 1.07$–1.15 (t, $J = 7.3$ Hz, 3H, CH_3CH_2CO), 1.90–2.40 (m, 6H, $CH_2CH_2CH_2$, $CH_2CH_2CH_2NHCO$, $CH_3C\mathit{H}_2CO$), 2.90–3.03 (m, 4H, $CH_2CH_2CH_2$, $ArC\mathit{H}_2CH_2CH_2$), 3.15–3.22 (t, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2$), 3.26–3.35 (quartet, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2NHCO$), 3.94 (s, 3H, OCH_3), 6.34 (bs, 1H, N*H*CO), 6.83–6.87 (d, $J = 8.1$ Hz, 1H, H_{arom}), 7.13–7.16 (d, $J = 8.1$ Hz, 1H, H_{arom}), 7.66 (s, 1H, H_{arom}). ^{13}C NMR ($CDCl_3$): $\delta = 10.0$, 23.5, 29.5, 29.7, 29.8, 34.0, 35.3, 39.1, 55.6, 107.8, 111.5, 123.8, 129.2, 129.7, 129.9, 152.5, 152.6, 159.3, 173.9. $C_{19}H_{24}N_2O_2$ (312.4): Calcd: C, 73.05; H, 7.74; N, 8.97. Found: C, 72.90; H, 7.65; N, 8.90.

2.26. N-Butanoyl 8,9-dihydro-4-methoxy-7H-benzo-2-[de]quinolinopropanamine (**9c**)

Butyramide **9c** was obtained as a colorless viscous oil following general method 2.10. Yield 20%. Flash column chromatography eluent system: AcOEt/MeOH 95:5. ^{1}H NMR ($CDCl_3$): $\delta = 0.86$–0.93 (t, $J = 7.3$ Hz, 3H, $C\mathit{H}_3CH_2CH_2CO$), 1.52–1.71 (sextet, $J = 7.3$ Hz, 3H, $CH_3C\mathit{H}_2CH_2CO$), 1.90–2.33 (m, 6H, $CH_2CH_2CH_2$, $CH_2CH_2CH_2NHCO$,

$CH_3CH_2CH_2CO$), 2.90–3.03 (m, 4H, $CH_2CH_2CH_2$, $ArCH_2CH_2CH_2$), 3.16–3.22 (t, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2$), 3.26–3.35 (quartet, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2NHCO$,), 3.94 (s, 3H, OCH_3), 6.36 (bs, 1H, N*H*CO), 6.84–6.87 (d, $J = 8.1$ Hz, 1H, H_{arom}), 7.13–7.17 (d, $J = 8.1$ Hz, 1H, H_{arom}), 7.66 (s, 1H, H_{arom}). ^{13}C NMR ($CDCl_3$): $\delta = 13.8$, 19.2, 23.4, 29.5, 34.0, 35.3, 38.8, 39.1, 55.5, 107.7, 111.5, 123.8, 124.0, 129.2, 129.7, 129.9, 152.4, 152.6, 159.3, 173.1. $C_{20}H_{26}N_2O_2$ (326.4): Calcd: C, 73.59; H, 8.03; N, 8.58. Found: C, 73.43; H, 7.98; N, 8.50.

2.27. N-Cyclopropanecarbonyl 8,9-dihydro-4-methoxy-7H-benzo-2-[de]quinolinopropanamine (**9d**)

The title compound was obtained as a colorless viscous oil following general method 2.10. Yield 25%. Flash column chromatography eluent system: AcOEt/MeOH 97:3. ^1H NMR ($CDCl_3$): $\delta = 0.64$–0.69 (m, 2H, CH_2 cycloprop.), 0.86–0.94 (m, 2H, CH_2 cycloprop.), 1.30–1.36 (m, 1H, C*H*CO), 1.96–2.03 (m, 2H, $CH_2CH_2CH_2$), 2.11–2.17 (m, 2H, $ArCH_2CH_2CH_2$) 2.95–2.99 (t, $J = 7.3$ Hz, 2H, $ArCH_2$) 3.00–3.03 (t, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2$), 3.20–3.23 (t, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2$), 3.31–3.36 (quartet, $J = 6.2$ Hz, 2H, CH_2NHCO), 3.95 (s, 3H, OCH_3), 6.46 (bs, 1H, N*H*CO), 6.86–6.88 (d, $J = 7.7$ Hz, 1H, H_{arom}), 7.15–7.17 (d, $J = 7.7$ Hz, 1H, H_{arom}), 7.68 (s, 1H, H_{arom}). ^{13}C NMR ($CDCl_3$): $\delta = 6.9$, 8.6, 12.9, 14.7, 23.4, 29.6, 33.7, 35.1, 39.3, 55.6, 107.9, 111.7, 124.0, 129.2, 129.7, 129.9, 152.5, 152.9, 159.3, 173.6. $C_{20}H_{24}N_2O_2$ (324.4): Calcd: C, 74.05; H, 7.46; N, 8.63. Found: C, 73.95; H, 7.40; N, 8.58.

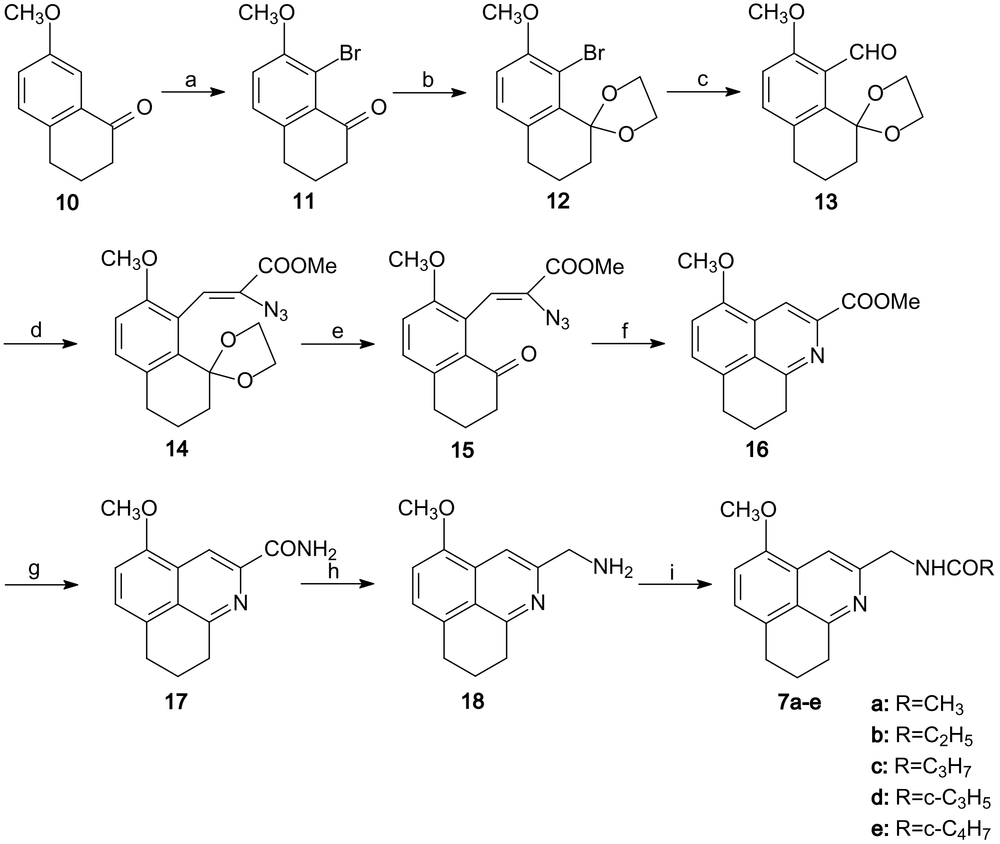
2.28. N-Cyclobutanecarbonyl 8,9-dihydro-4-methoxy-7H-benzo-2-[de]quinolinopropanamine (**9e**)

This compound was obtained as a colorless viscous oil following general method 2.10. Yield 15%. Flash column chromatography eluent: AcOEt. ^1H NMR ($CDCl_3$): $\delta = 1.74$–2.34 (m, 11H, C*H*CO, 6H–CH_2 cyclobut., $CH_2CH_2CH_2$, $ArCH_2CH_2CH_2$), 2.90–3.04 (m, 4H, $CH_2CH_2CH_2$, $ArCH_2$), 3.19–3.26 (t, $J = 6.2$ Hz, 2H, $CH_2CH_2CH_2$), 3.26–3.35 (quartet, $J = 6.2$ Hz, 2H, CH_2NHCO), 3.94 (s, 3H, OCH_3), 6.10 (bs, 1H, N*H*CO), 6.80–6.88 (d, $J = 8.1$ Hz, 1H, H_{arom}), 7.13–7.17 (d, 1H, $J = 8.1$ Hz, H_{arom}), 7.67 (s, 1H, H_{arom}). ^{13}C NMR ($CDCl_3$): $\delta = 18.1$, 18.3, 23.4, 25.2, 29.6, 33.8, 35.2, 38.0, 39.0, 55.5, 107.8, 111.6, 123.8, 129.2, 129.7, 129.9, 152.4, 152.6, 159.3, 175.0. $C_{21}H_{26}N_2O_2$ (338.4): Calcd: C, 74.53; H, 7.74; N, 8.28. Found: C, 74.45; H, 7.70; N, 8.20.

3. Results

3.1. Chemistry

The strategy for the synthesis of the target molecules **7a–e** is shown in Scheme 1. 7-Methoxy-1-tetralone (**10**) was converted to aldehyde **13** following the route reported by Hickey et al. [32] and the aldehyde was then transformed to the *a*-azido ester **14** by the method of Allen and coworkers [33]. Treatment of **14** with dil. HCl in THF/H_2O gave the 1-oxo analog **15**, which was subsequently cyclized to the ester **16** upon treatment with triethyl phosphate (TEP) in benzene [32]. Reaction of **16** with ammonia gas in methanol at 0 °C gave the primary amide **17**, which was reduced with lithium

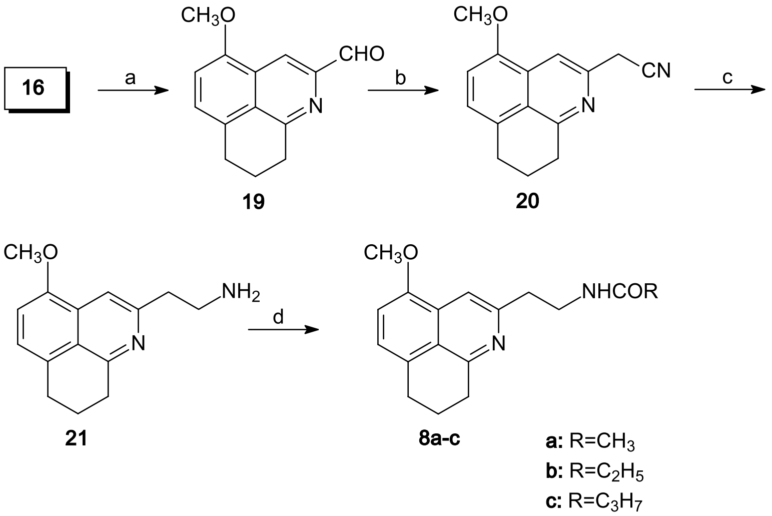


Scheme 1. Reagents and conditions: (a) NBS, CH_3CN; (b) $HOCH_2CH_2OH$, TsOH, $CH(OCH_3)_3$; (c) *n*-BuLi, DMF, THF, −78 °C; (d) N_3CH_2COOMe, MeONa, 5 °C; (e) dil. HCl, THF/H_2O; (f) TEP, benzene; (g) NH_3 (gas), MeOH; (h) $LiAlH_4$, THF, reflux; (i) $(RCO)_2O$ or RCOCl, Et_3N, CH_2Cl_2.

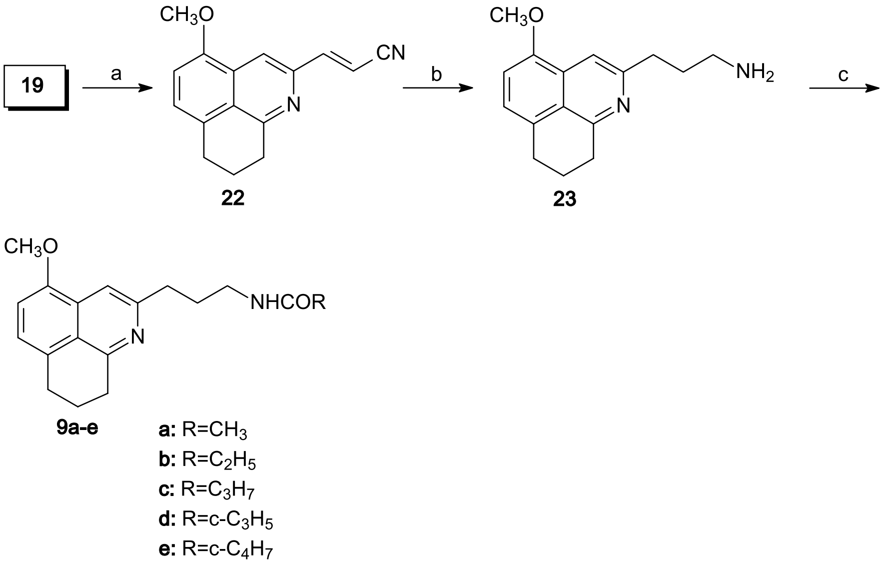
aluminum hydride in THF to amine **18**. Acylation of the latter with the required acid anhydride or acid chloride in dichloromethane in the presence of triethylamine, yielded the desired amides **7a**–**e**.

The synthesis of their congeners, **8a**–**c** (Scheme 2), involved the conversion of ester **16** to the aldehyde **19**, effected on treating the former with DIBAL-H at −78 °C in the presence of toluene, followed by the reaction of **19** with tosylmethyl isocyanide (TosMIC) in the presence of potassium *tert*-butoxide and 1,2-dimethoxyethane, reduction of the nitrile **20** with lithium aluminum hydride in the presence of benzene and diethyl ether (1:2) to the amine **21**, and immediate acylation with the appropriate reagent.

The synthesis of the new analogs **9a**–**e** (Scheme 3) involved a modified Wittig reaction on aldehyde **19** using diethyl cyanomethylphosphonate and potassium *tert*-butoxide in THF to give the *a*,β-unsaturated nitrile **22**, reduction with lithium aluminum hydride in the presence of benzene and diethyl ether (1:2) to the amine **23** and acylation of the latter with the required acid anhydride or acid chloride in dichloromethane in the presence of triethylamine.



Scheme 2. Reagents and conditions: (a) DIBAL-H, toluene, −78 °C; (b) TosMIC, *t*-BuOK, DME, −30 °C, −60 °C; (c) $LiAlH_4$, Et_2O/benzene, reflux; (d) $(RCO)_2O$ or RCOCl, Et_3N, CH_2Cl_2.



Scheme 3. Reagents and conditions: (a) $(EtO)_2P(O)CH_2CN$, *t*-BuOK, THF; (b) $LiAlH_4$, Et_2O/benzene, reflux; (c) $(RCO)_2O$ or RCOCl, Et_3N, CH_2Cl_2.

3.2. Pharmacological protocols

3.2.1. 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 [21,27–30]. 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. 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 to 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.

3.2.2. Binding affinity assay

The binding affinity of the analogs was determined in competition radioligand binding assays using 2-[^{125}I]-iodomelatonin (specific activity 2200 Ci/mol, Perkin Elmer, UK), as described previously [29] on the recombinant human MT_1 and MT_2 subtypes expressed in NIH 3T3 cells.

4. Discussion

It is apparent from the data presented in Table 1 that irrespectively of the size and shape of the R group, all of the new compounds with a single methylene unit in their side chains (**7a**–**e**) are all antagonists, although potency is low. Moreover, within this series, potency increases on changing the amine acylating group from methyl through cyclobutanecarbonyl, with **7e** the most potent. The cyclopropanecarbonyl congener **7d** exhibits a small improvement in binding affinity at MT_2, but not MT_1, having a 2.8-fold selectivity.

In contrast to the single methylene spacer series **7a**–**e**, as well as their analogous *N*1–C7 previously reported annulated counterparts **5a**–**c** ([22b], Fig. 1) which both had antagonist activity, lengthening the acyl group of the two methylene units analogs in the series **8a**–**c**, had a varied effect on binding and agonism/antagonism equilibrium. In this series, the acetamido congener **8a** acted as a partial agonist ($pEC_{50} = 5.84$% and 73% of maximal aggregation), but also antagonized (77%) melatonin induced aggregation at 100 μM. The propionamido analog **8b** also acted as a partial agonist (pEC_{50}=4.21, 66% of maximal), but its butyramido counterpart **8c** had no agonist action but was a full antagonist at 100 μM. Again, these analogs show a preference for the MT_2 receptor, with **8c** the most selective (13.2-fold).

The **9a**–**e** analogs also exhibited varying agonist/antagonist behaviour. Thus, the acetamido compound **9a** with a three methylene units spacer in its side chain, has no agonist activity, but is an antagonist (73% inhibition of the binding of melatonin), whereas the analogous **8a** molecule, with a shorter spacer, is a partial agonist. Comparison of MT_2 binding data for **8a** and **9a** shows that the increase in the length of the spacer of the side chain by a methylene group is unfavorable for interaction with the MT_2 receptor, so that **9a**, despite its low affinity has 10-fold selectivity for the MT_1 site. In the *Xenopus* model, the propionamido analog **9b**, is, like its congener **8b**, a partial agonist, but much more potent (21-fold). Conversely, in the same model, the butyramido compound **9c**, like the analogous ligand **8c**, has no agonist activity but is an antagonist. The introduction of a cyclopropanecarbonyl group, **9d**, gives a partial agonist, as does, but to a lesser extent,

Table 1
Melatoninergic activity of compounds **7a–e**, **8a–c** and **9a–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)	Human MT_2 (pK_i)	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
7a	CH_3	NA	24% inhibition[c]	5.07 ± 0.07	5.16 ± 0.07	1.2
7b	C_2H_5	NA	78% inhibition[c]	NT[d]	NT	—
7c	C_3H_7	NA	62% inhibition[c]	NT	NT	—
7d	c-C_3H_5	NA	86% inhibition[c]	4.66 ± 0.30	5.11 ± 0.05	2.8
7e	c-C_4H_7	NA	88% inhibition[c]	NT	NT	—
8a	CH_3	5.84 ± 0.01 (73%)[b]	77% inhibition[c]	5.13 ± 0.02	5.49 ± 0.10	2.3
8b	C_2H_5	4.21 ± 0.05 (66%)[b]	NA	4.46 ± 0.13	5.00 ± 0.06	3.5
8c	C_3H_7	NA	87% inhibition[c]	>4.00	5.12 ± 0.04	13.2
9a	CH_3	NA	73% inhibition[c]	5.02 ± 0.04	>4.00	0.10
9b	C_2H_5	5.54 ± 0.15 (52%)[b]	NA	NT	NT	—
9c	C_3H_7	NA	4.61 ± 0.11	NT	NT	—
9d	c-C_3H_5	5.85 ± 0.09 (92%)[b]	NA	4.94 ± 0.06	5.13 ± 0.04	1.6
9e	c-C_4H_7	$\geqslant 10^{-4}$ M (46%)[b]	NA	NT	NT	—

Agonist and antagonist data are the mean of triplicate experiments ± SEM.

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

[b] partial agonist; percentage full agonist action is shown in brackets.

[c] percentage inhibition of melatonin-induced (1 nM) aggregation at 100 μM.

[d] NT, not tested.

the incorporation of a cyclobutanecarbonyl group, **9e**. This relatively unusual finding (normally these two acylating groups favor antagonism, *cf*. compounds **6d** and **6e** (Fig. 1) [22b]) parallels our earlier results [23] on probes **4d** and **4e** (Fig. 1) and may be attributed to the unique stereoelectronic effects imposed on the side chains of **9d** and **9e** by the condensation of the cyclohexane nucleus between C1 and C8 of the isoquinoline ring; it seems that due to these factors the side chains of **9d** and **9e** more readily induce the receptor conformation needed for partial agonist action [22a].

5. Concluding remarks

In conclusion, the new compounds presented herein and in particular analogs **8a–c** and **9a–e**, nicely illustrate how small incremental changes in the size and shape of their pharmacophoric side chains can influence the functional response observed, presumably by affecting the dynamic equilibrium between agonist and antagonist conformations of the melatonin receptor.

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