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Design and synthesis of potent *N*1-substituted indole melatonin receptor agonists†

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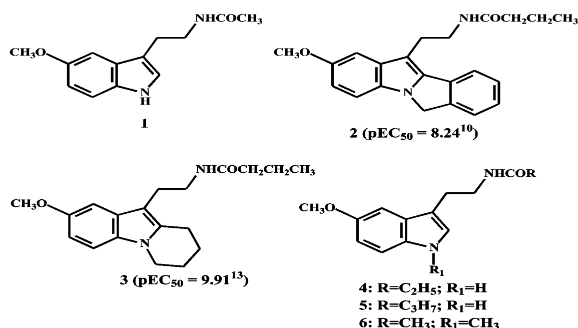
The design and expeditious synthesis of two new indole analogs with up to 5-fold potency of that of melatonin is described.

The pineal gland neurohormone melatonin (*N*-acetyl 5-methoxytryptamine, **1**) is synthesised and secreted mainly at night. It regulates seasonal changes in various aspects of physiology in photoperiodic species such as sheep, and, acting on specific receptors in the suprachiasmatic nucleus (SCN) of the hypothalamus, can entrain the circadian clock. Considerable interest has focused on its potential in treating the disordered circadian rhythms which occur in shift-work, jet-lag, and sleep disorders in the elderly.[1] In addition, melatonin may play a protective role in cancer by lengthening cell cycle times or decreasing the transcription of the estrogen receptor gene.[2,3]

Melatonin exerts these effects by activating specific, high affinity, G-protein-coupled membrane receptors.[4] Three distinct melatonin receptor subtypes have been cloned—MT_1, MT_2, and Mel_{1c}.[5] MT_1 and MT_2 receptor mRNA has been identified in several mammalian tissues, but the Mel_{1c} mRNA has not been found in mammals. Melatonin receptors have been subjected to a number of modelling studies based on both the amino acid sequence[6] and pharmacophore models[7,8] and a number of active conformations have been proposed. These models have been compared and assessed in a recent review.[9]

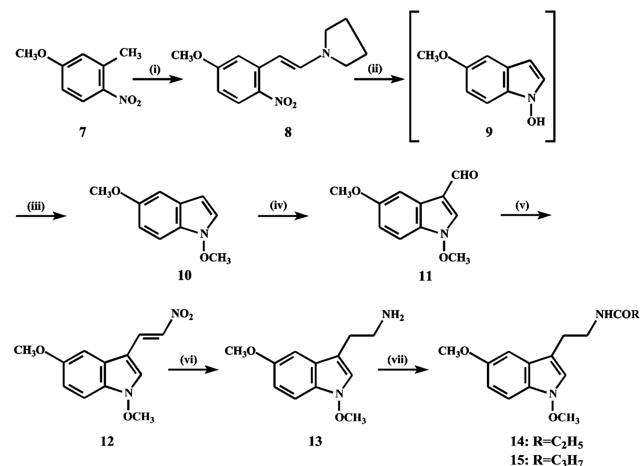
During the last decade we have sought to understand how melatonin interacts with its receptors. A number of structure–affinity relationships have been identified[10] and recently we and other researchers have proposed molecular models of the melatonin binding site: the key elements for high binding affinity are the presence and the relative spatial position of the methoxy group and the *N*-alkanamido chain linked to an appropriate spacer.[11,12] The proton donor NH indole is not essential whereas the presence of *N*1-alkyl substituents leads to an almost 45-fold decrease in agonist potency and in some cases even to antagonism. This has been attributed to unfavourable steric interactions at the lower part of the indole nucleus and specifically in the area between *N*1 and C-2.[12]



However, as we have recently shown by the synthesis of various potent agonists, *e.g.* the MT_2 selective agonist **2**[10] and its congener **3**,[13] the role of the lower part of the indole moiety in binding merits further research.

In our ongoing effort to probe the stereoelectronic requirements for optimal melatoninergic activity we report here the synthesis and biological activity of two *N*1-substituted indoles, compounds **14** and **15** Scheme 1. The rationale behind the design of the two new molecules was primarily based on molecular modelling studies, which have been recently conducted by our research group[10] and by Mor *et al.*[12] Thus, the presence of the 5-methoxy moiety in the parent structure has been retained in **14** and **15** as the oxygen of the 5-methoxy group serves as an electron donor for the formation of a hydrogen bond between melatonin and its receptor.[14] The change of the acetamido moiety to propanamido and butyramido in the C-3 side chain of **14** and **15** was performed as the latter two have been found to increase the agonist potency of melatonin by a factor of about 1.5 in the *Xenopus laevis* assay.[15] The second methoxy group was introduced at *N*1 in order to explore a possible synergism in potency with the 5-methoxyl and more importantly to probe its influence in binding *via* the non classical −I, +R effect it exerts to the aromatic indole nucleus *via* the *N*1 heteroatom.

The strategy for the synthesis of the new melatoninergic ligands **14** and **15** is shown in Scheme 1. As the known methods for the preparation of 1-methoxyindoles are laborious and multi step[16–18] we developed a new, efficient two step route to 1,5-dimethoxy-1*H*-indole (**10**), which is also an important precursor of the natural product 1,5-dimethoxygramine present in *Gymnocrantheria paniculata*.[19] Thus, after considerable experimentation with Leimgruber-Batcho's indole synthesis[20] we found that condensation of 3-methyl-4-nitroanisole (**7**) with



Scheme 1 *Reagents and conditions*: i, DMFDMA, pyrrolidine, reflux, 8 h; ii, Zn (1.5 equiv.) NH_4Cl, Et_2O, 7 h; iii, CH_3I, NaOH (10%), Aliquat® 336; iv, $POCl_3$, DMF, 45 °C; v, CH_3NO_2, $AcONH_4$, reflux, 3 h; vi, $LiAlH_4$, Et_2O, reflux; vii, $(RCO)_2O$, Et_3N, CH_2Cl_2.

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hot dimethylformamide dimethyl acetal (DMFDMA) in pyrrolidine, the presence of the latter being absolutely necessary, leads to the enamine **8**; subsequent reduction of the nitro group with zinc powder (1.5 equiv.) in the presence of ammonium chloride[18] gives the desired compound **10** after methylation of the *in situ* formed 1-hydroxyindole (**9**). Formylation of **10** under Vilsmeier–Haack conditions gives aldehyde **11**, which by the sequence of the Henry reaction, reduction with lithium aluminium hydride and subsequent acylation with propionic and butyric anhydride[10] leads to the target molecules **14** and **15** respectively in approximately 25% overall yield from 3-methyl-4-nitroanisole (**7**).

The biological activity of **14** and **15** was assessed in a well-established, specific model of melatonin action, the pigment aggregation response of *Xenopus laevis* melanophores.[21] In these cells many thousands of black pigment granules are distributed evenly throughout the cell, and addition of melatonin induces their rapid movement towards the centre of the cell. This response can be quantified by measuring the changes in light absorbance of the cells as the pigment concentrates near the cell centre. Table 1 shows the data obtained for the new ligands *N*-[2-(1,5-dimethoxy-1*H*-indol-3-yl)ethyl]propionamide (**14**) and *N*-[2-(1,5-dimethoxy-1*H*-indol-3-yl)ethyl]butyramide (**15**) and those reported for *N*-[2-(5-methoxy-1*H*-indol-3-yl)ethyl]propionamide[15] (**4**), *N*-[2-(5-methoxy-1*H*-indol-3-yl)ethyl]butyramide[15] (**5**) and *N*-[2-(5-methoxy-1-methyl-1*H*-indol-3-yl)ethyl]acetamide (**6**)[22] on *Xenopus laevis* melanophores. These results demonstrate that although the structural changes made in **14** and **15** constitute relatively minor interpositions onto the basic nucleus, the consequences are quite significant. Thus, compound **15** is almost 5-times more potent than melatonin, whereas the replacement of the methyl group at *N*-1, compound **6**, by the methoxy moiety, compounds **14** and **15**, enhances the agonist potency 1.3 times and 575 times, respectively. This trend, albeit marginal, is also observed in the case of compound **15** which is 3-times more potent than its non-*N*-OMe counterpart **5**. These findings are clearly consistent with the previously reported hypothesis which suggests that the lower part of the indole nucleus is an electrostatically favourable region for efficient binding to the receptor site.[12] However, the 129-fold decrease in the agonist potency of **14** compared to that of its congener **4** might suggest that the positive influence on the agonist potency exerted by the presence of the *N*-OMe group in the indole nucleus can be attributed to a synergistic effect caused by the presence of the *N*-OMe group and the size of the acyl group, R = C_3H_7 being optimal.

In summary, *en route* to the target molecules **14** and **15** we have developed a versatile method towards the synthesis of the biologically important 1,5-dimethoxy-1*H*-indole (**10**). Compounds **14** and **15** constitute important examples of potent *N*1-substituted indole melatonin receptor agonists, the activity of which seems to be drastically related to the size of the C-3 alkanamido chain. It will be interesting to compare the *in vivo* activity of analogs **14** and **15** and their selectivity at MT1 and MT2 receptor subtypes. Currently, efforts are underway to prepare various congeners of **14** and **15** with conformationally constrained side chains aiming at achieving an optimum fit at the melatonin receptor binding site.

Table 1 Agonist activity of the melatoninergic ligands **4**–**6** and the new compounds **14** and **15** in the *Xenopus laevis* melanophore assay

Compound	R	R_1	Agonist (pEC_{50})
Melatonin			10.7
14			8.10
15			10.75
4	C_2H_5	H	10.21
5	C_3H_7	H	10.24
6	CH_3	CH_3	7.99

Notes and references

† *Selected data* for **15**: ^1H NMR (200 MHz, $CDCl_3$) δ 0.87–0.92 (t, 3H, CH_3CH_2, J = 7.3) 1.58–1.63 (quintet, 2H, CH_2CH_2CO, J = 7.3), 2.06–2.10 (t, 2H, CH_2CO, J = 7.3) 2.86–2.89 (t, 2H, ArCH_2, J = 6.8), 3.53–3.58 (q, 2H, CH_2NH, J = 6.5), 3.83 (s, 3H, CH_3O) 4.01 (s, 3H, CH_3ON), 5.48 (br s, 1H, NHCO), 6.88–7.30 (m, 4H, H_{arom}); ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.7, 19.1, 25.3, 38.8, 39.4, 55.9, 65.7, 100.8, 108.6, 109.4, 112.9, 121.8, 122.7, 124.3, 154.5, 172.9. Anal. ($C_{16}H_{22}N_2O_3$) C, H, N.

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