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Short Communication

Synthesis of new tricyclic melatoninergic ligands

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Abstract

We report the synthesis and biological evaluation of a series of new tricyclic analogs of the hormone melatonin, which act as probes of the constraints at the hormone's receptor site with regard to the lower *N*1-C2 region of the indole moiety of melatonin. Three of the new compounds, *N*-[2-(2-methoxy-6,7,8,9-tetrahydropyrido[1,2-*a*]indol-10-yl)ethyl]acetamide (**9**), and the respective propionamide **10** and butyramide **11**, are as potent as melatonin in the *Xenopus laevis* melanophore model.

Keywords: Tricyclic fused [1,2-*a*]indoles; Synthesis; *Xenopus laevis* melanophores; Melatonin agonists and antagonists

1. Introduction

The pineal hormone, melatonin, has been shown to have a physiological role in regulating seasonal breeding in photoperiodic species [1] and can entrain circadian rhythms in mammals [2]. In humans it has been suggested that melatonin might prove a valuable therapy for rhythm disorders, including seasonal affective disorders (SAD) [3], jet lag, and shift work problems [4]. Melatonin also inhibits dopamine release from amacrine cells within the retina [5], and can enhance vasoconstriction in the rat tail artery [6]. Many studies have also indicated an influence on immune function [7] and antioxidant actions [8].

A number of these effects are thought to be mediated through interaction with specific receptors and three distinct receptor subtype cDNAs have been isolated [9]. These encode high affinity receptors, which belong to the seven-transmembrane domain receptor family and which inhibit adenylyl cyclase *via* a pertussis toxin sensitive GTP-binding protein.

The understanding of the physiological and pathophysiological role(s) of melatonin in animals and man is hampered by the relatively small number of melatonin receptor agonists and antagonists available. In a series of studies during the last decade we have sought to understand how melatonin binds to and activates its receptor and to use the knowledge gained to design potent receptor agonists and antagonists, which will be useful tools for defining the full physiological and pathophysiological role of this hormone. Thus, several key interactions between ligand and receptor have been identified. The 5-methoxyl group and amide moiety, and their relative position, are critical to high affinity [10–17]. Positions 2 and 6 are also important for activity, and both are involved in the metabolism of melatonin [18,19].

In order to probe the constraints at the receptor site with regard to the lower *N*1-C2 region of the indole moiety of melatonin, we have recently reported the synthesis and biological activity of a number of 2-phenyltryptamines annulated on the [*a*] face of the pyrrole moiety by the introduction of one, two or three methylene groups (**1–3**) [20]. In the *Xenopus* melanophore pigment aggregation model compounds **1a–j** were found to exhibit agonistic activity while their

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congeners **3a–j** were antagonists. Molecules **2a–d** were antagonists and **2e–h** had agonist acivity at low concentrations but antagonized responses at high concentrations (Fig. 1). These findings suggest that the *Xenopus* melatonin receptor cannot accommodate an *N*-*n*-alkyl chain attached to a phenyl ring substituent with $n > 2$ (Fig. 1, compounds **3a–j**) in the required orientation to induce or stabilize the active receptor conformation.

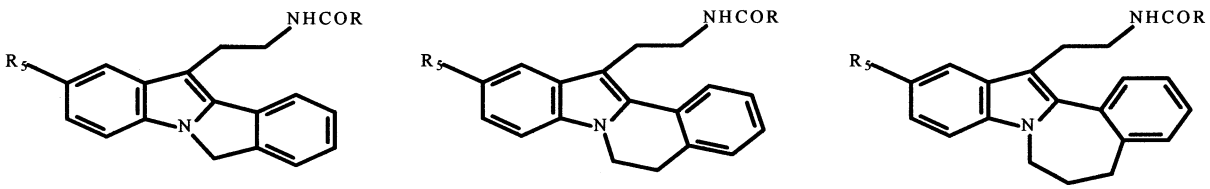
In the present study, we have extended this work to synthesize and evaluate a number of novel *N*-[2-(6,7,8,9-tetrahydropyrido[1,2-*a*]indol-10-yl)ethyl]alkanamides (**4-8**) and *N*-[2-(2-methoxy-6,7,8,9-tetrahydropyrido[1,2-*a*]indol-10-yl)ethyl]alkanamides (**9–13**) (Scheme 1). Similarly to compounds **1–3**, these analogs probe the constraints at the receptor site with regard to the lower *N*1-C2 region of the indole moiety in the absence though of the annulated 2-phenyl ring. The results indicate that the non-methoxy substituted compounds, **4–8**, are antagonists in the *Xenopus* melanophore pigment aggregation assay. In contrast, the new 2-methoxy analogs, **9–13**, are full agonists, unlike their analogous congeners, **2e–h**, which act as partial agonists (Fig. 1) [20].

2. Chemistry

The synthesis of **4–13** is shown in Scheme 1. The sulfone moiety at C-2 of indole (**14**) and 5-methoxyindole (**15**) was introduced by the method of Katritzky and Akutagava [21]. The derived compounds **16** and **17** were then *N*-alkylated to **18** and **19**, respectively, using 1,4-dibromobutane in the presence of potassium hydroxide in DMF as first described by Dehaen [22] and later modified by Caddick et al. [23]. The tricyclic molecules **20** and **21** were formed upon treatment of **18** and **19** with tributyltin hydride under radical conditions [23]. Aldehydes **22** and **23** were obtained by applying the Vilsmeier–Haack reaction [20] on compounds **18** and **19** and the sequence of the Henry reaction [20] followed by reduction [20] gave the amines **26** and **27**, which were not purified but were immediately acylated with the appropriate reagent [20] to give the desired *N*-[2-(6,7,8,9-tetrahydropyrido[1,2-*a*]indol-10-yl)ethyl]-alkanamides (**4–8**) [24] and *N*-[2-(2-methoxy-6,7,8,9-tetrahydropyrido[1,2-*a*]indol-10-yl)ethyl]alkanamides (**9–13**) [24].

3. Results and discussion

The agonist and antagonist potency of the new analogs was assessed in a well-established, specific model of melatonin action, the pigment aggregation response of *Xenopus laevis* melanophores [19,20,25]. All five non-methoxy substituted fused [1,2-*a*]indoles **4–8** had no agonist activity, but displayed weak antagonistic activity like luzindole, the most-commonly used commercially available melatonin receptor antagonist (Table 1). The lower antagonistic potency of the new analogs **4–8** compared to that of **2a–d** (cf. pIC_{50} values of **2a–d** in Fig. 1) may be due to the presence of the phenyl ring of the tetralin moiety. The phenyl ring in this position might enhance hydrophobic attachments. The 5-methoxy substituted (numbering with respect to the indole ring) molecules **9–13** were all full agonists and three of them, compounds **9–11**, were almost equipotent to melatonin (Table 1). Furthermore, the new molecules **9–13**, are much more potent agonists than **2e–h** (cf. pEC_{50} values of **2a–d** in Fig. 1) presumably because the extra aromatic ring in the latter reduces the ability of the receptor binding pocket to

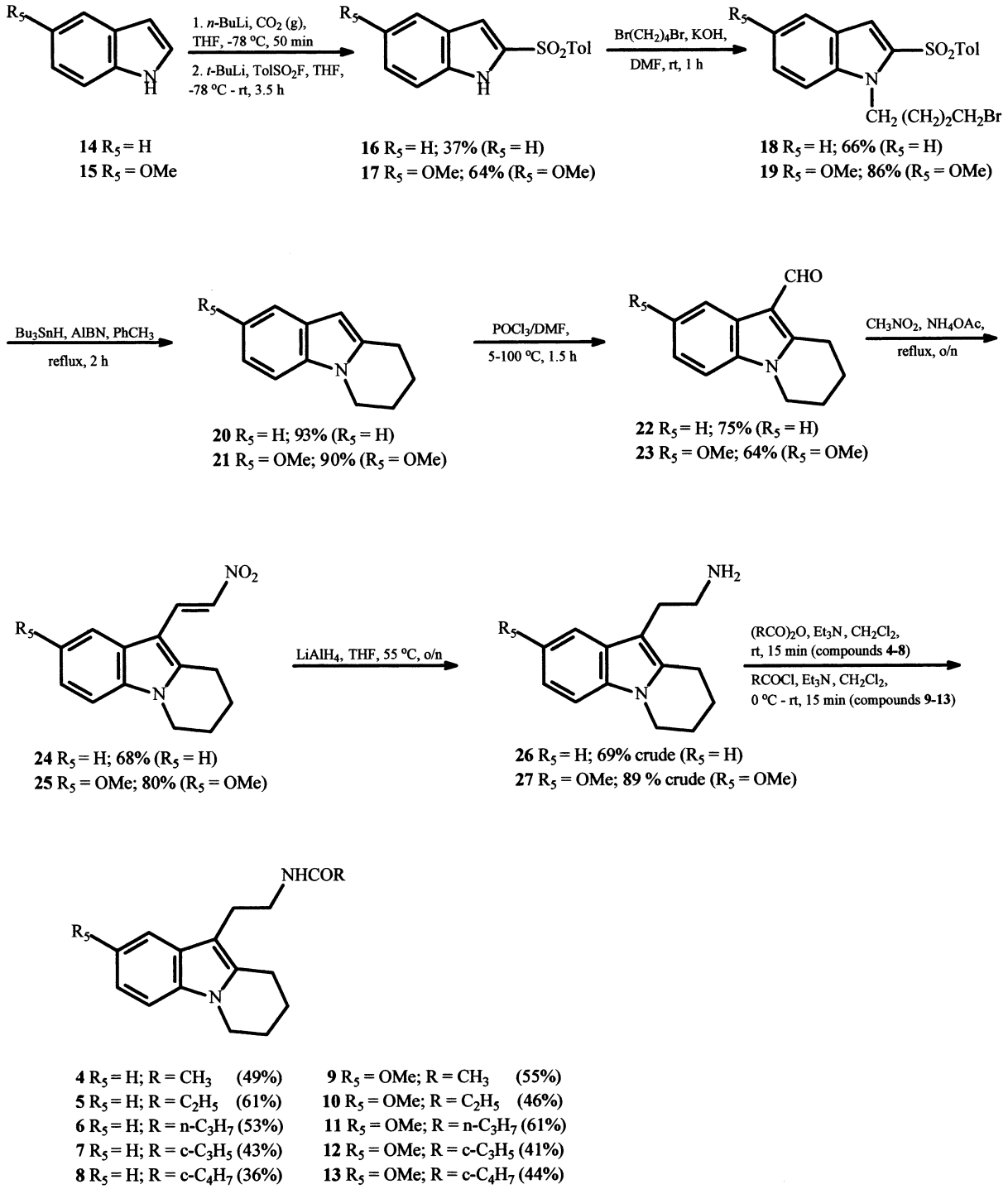


1a R_5 = H; R = CH_3
1b R_5 = H; R = C_2H_5
1c R_5 = H; R = n-C_3H_7
1d R_5 = H; R = c-C_3H_5
1e R_5 = OMe; R = c-C_4H_7
1f R_5 = OMe; R = CH_3
1g R_5 = OMe; R = C_2H_5
1h R_5 = OMe; R = n-C_3H_7
1i R_5 = OMe; R = c-C_3H_5
1j R_5 = OMe; R = c-C_4H_7

2a R_5 = H; R = CH_3 (pIC_{50} = 5.05)
2b R_5 = H; R = C_2H_5 (pIC_{50} = 5.64)
2c R_5 = H; R = n-C_3H_7 (pIC_{50} = 6.35)
2d R_5 = H; R = c-C_4H_7 (pIC_{50} = 5.43)
2e R_5 = OMe; R = CH_3 (pEC_{50} = 7.90; pIC_{50} < 4)
2f R_5 = OMe; R = C_2H_5 (pEC_{50} = 8.22; pIC_{50} = 4.10)
2g R_5 = OMe; R = n-C_3H_7 (pEC_{50} = 8.03; pIC_{50} = 4.37)
2h R_5 = OMe; R = c-C_4H_7 (NA[a])

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 = OMe; R = c-C_4H_7
3f R_5 = OMe; R = CH_3
3g R_5 = OMe; R = C_2H_5
3h R_5 = OMe; R = n-C_3H_7
3i R_5 = OMe; R = c-C_3H_5
3j R_5 = OMe; R = c-C_4H_7

Fig. 1. Tetracyclic analogs **1–3** [20]: compounds **1a–j** are agonists in the *X. laevis* melanophore model; compounds **2a–d** are antagonists; compounds **2e–h** are partial agonists; compounds **3a–j** are antagonists. [a] See footnotes, Table 1, for explanation.



Scheme 1. Synthetic methodology followed for the preparation of the new analogs **9–13**.

recognize and bind these molecules. The relative decrease in agonist potency observed for compounds **12** and **13** compared to that of **9–11**, seems reasonable as the former bear the *N*-cyclopropanoyl and *N*-cyclobutanoyl groups, respectively, in their side-chain. These moieties are known to exert a detrimental effect on agonistic activity [18,26]. In the case of **12** and **13**, however, a shift to antagonism at the expense of their agonistic activity was not observed.

The findings reported herein for analogs **4–13** suggest that the absence or presence of the methoxy substituent in their skeleton is the critical factor in determining agonist or antagonist action. The results are in agreement with earlier data, showing that the

absence of the 5-methoxyl group in tryptamine derivatives, as in luzindole [27], or in 4-phenyl substituted tetralines, as in 4-phenyl-2-acetylaminotetralin [28], leads to antagonism. We attribute the difference in agonist/antagonist behavior between analogs **4–8** and **9–13** to a combination of stereoelectronic effects exerted by both the methoxyl group, where present, and the cyclohexane ring, annulated between *N*1 and C2 of the indole nucleus. This argument is reinforced by our earlier observations on compounds **2**, which, as mentioned previously, instead of a cyclohexane ring bear a tetralin moiety annulated between *N*1 and C2 [20].

In conclusion, the compounds reported herein are the first examples that utilize a fused cyclohexane at the lower *N*1-C2 region of the melatonin skeleton in order to probe the constraints at the receptor site. As these preliminary results merit further investigation we are currently engaged with the synthesis and biological evaluation of a new series of tricyclic compounds.

4. Materials and methods

Melanophore cells were grown in 96-well tissue culture plates, and growth medium [29,30] was replaced with 0.7 × L-15 culture medium 18 h before analogs were tested. Initial absorbance of cells (A_i, 630 nm) was measured in each well using a Bio-Tek microtiter plate reader (model EL3115, Anachem, UK), then cells were treated with the concentrations of the analogs indicated. 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} to 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.

Table 1
Agonistic and antagonistic activity of compounds **4–13** in the *X. laevis* melanophore assay

Comp.	R_5	R	Agonist (pEC_{50})	Antagonist (pIC_{50})
Melatonin			10.07	
Luzindole			NA [a]	5.61
4	H	CH_3	NA	3.75
5	H	C_2H_5	NA	4.24
6	H	C_3H_7	NA	3.43
7	H	*c*-C_3H_5	NA	3.71
8	H	*c*-C_4H_7	NA	3.22
9	OCH_3	CH_3	9.29	
10	OCH_3	C_2H_5	9.78	
11	OCH_3	C_3H_7	9.91	
12	OCH_3	*c*-C_3H_5	7.73	
13	OCH_3	*c*-C_4H_7	5.43	

[a] NA, no agonist or antagonist effect detected at 100 μM. Agonist and antagonist data on melanophores are the mean of triplicate experiments.

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References

[1] T.J. Bartness, J.B. Powers, M.H. Hastings, E.L. Bittman, B.D. Goldman, The timed infusion paradigm for melatonin delivery: what has it taught us about the melatonin signal, its reception, and the photoperiodic control of seasonal responses?, J. Pineal Res. 15 (1993) 161–190.
[2] R.J. Reiter, The melatonin rhythm: both a clock and a calendar, Experientia 49 (1993) 654–664.
[3] M.C. Blehar, A. Lewy, Seasonal mood disorders: consensus and controversy, J. Psychopharmacol. Bull. 4 (1990) 465–494.
[4] J. Arendt, D.J. Skene, B. Middleton, S.W. Lockley, S. Deacon, Efficacy of melatonin treatment in jet lag, shift work, and blindness, J. Biol. Rhythms 12 (1997) 604–617.
[5] M.L. Dubocovich, Characterization of a retinal melatonin receptor, J. Pharmacol. Exp. Ther. 234 (1985) 395–401.
[6] D.N. Krause, V.E. Barrios, S.P. Duckles, Melatonin receptors mediate potentiation of contractile responses to adrenergic nerve stimulation in rat caudal artery, Eur. J. Pharmacol. 276 (1995) 207–213.
[7] P.M. Liebmann, A. Wolfler, P. Felsner, D. Hofer, K. Schauenstein, Melatonin and the immune system, Int. Arch. Allergy Immunol. 112 (1997) 203–211.
[8] R.J. Reiter, D.X. Tan, J. Cabrera, D. D'Arpa, Melatonin and tryptophan derivatives as free radical scavengers and antioxidants, Adv. Exp. Med. Biol. 467 (1999) 379–387.
[9] S.M. Reppert, D.R. Weaver, C. Godson, Melatonin receptors step into the light: cloning and classification of subtypes, Trends Pharmacol. Sci. 17 (1996) 100–102.
[10] S. Kloubert, M. Mathé-Alainmat, J. Andrieux, S. Sicsic, M. Langlois, Synthesis of benzocycloalkane derivatives as new conformationally restricted ligands for melatonin receptors, Bioorg. Med. Chem. Lett. 8 (1998) 3325–3330.
[11] C.B. Heward, M.E. Hadley, Structure–activity relationships of melatonin and related indoleamines, Life Sci. 17 (1975) 1167–1177.
[12] D. Sugden, N.W.S. Chong, Pharmacological identity of 2-[^{125}I]iodomelatonin binding sites in chicken brain and sheep pars tuberalis, Brain Res. 539 (1991) 151–154.
[13] P. Depreux, D. Lesieur, H.A. Mansour, P. Morgan, H.E. Howell, P. Renard, D.H. Caignard, B. Pfeiffer, P. Delagrange, B. Guardiola, S. Yous, A. Demarque, G. Adam, J. Andrieux, Synthesis and structure–activity relationships of novel naphthalenic and bioisosteric related amidic derivatives as melatonin receptor ligands, J. Med. Chem. 37 (1994) 3231–3239.

[14] P.J. Garratt, S. Travard, S. Vonhoff, A. Tsotinis, D. Sugden, Mapping the melatonin receptor. 4. Comparison of the binding affinities of a series of substituted phenylalkylamides, J. Med. Chem. 39 (1996) 1797–1805.
[15] C. Navajas, T. Kokkola, A. Poso, N. Honka, J. Gynther, J.T. Laitinen, A rhodopsin-based model for melatonin recognition at its G protein-coupled receptor, Eur. J. Pharmacol. 304 (1996) 173–183.
[16] P.J. Garratt, R. Jones, S.J. Rowe, D. Sugden, Mapping the melatonin receptor. 1. The 5-methoxy group of melatonin is not an essential requirement for biological activity, Bioorg. Med. Chem. Lett. 4 (1994) 1555–1558.
[17] M. Mor, S. Rivara, C. Silva, F. Bordi, P.V. Plazzi, G. Spadoni, G. Diamantini, C. Balsamini, G. Tarzia, F. Fraschini, V. Lucini, R. Nonno, B.M. Stankov, Melatonin receptor ligands: synthesis of new melatonin derivatives and comprehensive comparative molecular field analysis (CoMFA) study, J. Med. Chem. 41 (1998) 3831–3844.
[18] P.J. Garratt, R. Jones, D.A. Tocher, D. Sugden, Mapping the melatonin receptor. 3. Design and synthesis of melatonin agonists and antagonists derived from 2-phenyltryptamines, J. Med. Chem. 38 (1995) 1132–1139.
[19] D. Sugden, Aggregation of pigment granules in single cultured *Xenopus laevis* melanophores by melatonin analogues, Br. J. Pharmacol. 104 (1991) 922–927.
[20] R. Faust, P.J. Garratt, R. Jones, L.-K. Yeh, A. Tsotinis, M. Panoussopoulou, T. Calogeropoulou, M.-T. Teh, D. Sugden, Mapping the melatonin receptor. 6. Melatonin agonists and antagonists derived from 6*H*-isoindolo[2,1-*a*]indoles, 5,6-dihydroindolo[2,1-*a*]isoquinolines, and 6,7-dihydro-5*H*-benzo[*c*]azepino [2,1-*a*]indoles, J. Med. Chem. 43 (2000) 1050–1061.
[21] A.R. Katrizky, K. Akutagava, Carbon dioxide: a reagent for the protection of nucleophilic centers and the simultaneous activation of alternative locations to electrophilic attack. Part I. A new synthetic method for the 2-substitution of 1-unsubstituted indoles, Tetrahedron Lett. 26 (1985) 5935–5938.
[22] W. Dehaen, A. Hassner, Annulation of heterocycles via intramolecular nitrile oxide-heterocycle cycloaddition reaction, J. Org. Chem. 56 (1991) 896–900.
[23] S. Caddick, K. Aboutayab, K. Jenkins, R.I. West, Intramolecular radical substitution reactions: a novel approach to fused [1,2-*a*]indoles, J. Chem. Soc., Perkin Trans. 1 (1996) 675–682.
[24] Satisfactory analytical data/mass spectra were obtained for all new compounds. The spectral properties were in accord with the assigned structures.
[25] D. Sugden, Effect of putative melatonin receptor antagonists on melatonin-induced pigment aggregation in isolated *Xenopus laevis* melanophores, Eur. J. Pharmacol. 213 (1992) 405–408.
[26] V. Leclerc, E. Fourmaintraux, P. Depreux, D. Lesieur, P. Morgan, H.E. Howell, P. Renard, D.-H. Caignard, B. Pfeiffer, P. Delagrange, B. Guardiola-Lemaitre, J. Andrieux, Synthesis and structure–activity relationships of novel naphthalenic and bioisosteric related amidic derivatives as melatonin receptor ligands, Bioorg. Med. Chem. 6 (1998) 1875–1887.
[27] M.L. Dubocovich, Luzindole (N-0774): a novel melatonin receptor antagonist, J. Pharmacol. Exp. Ther. 246 (1988) 902–910.
[28] M.L. Dubocovich, Melatonin receptors: are there multiple subtypes?, Trends Pharmacol. Sci. 16 (1995) 50–56.
[29] A. Daniolos, A.B. Lerner, M.R. Lerner, Action of light on frog pigment cells in culture, Pigment Cell Res. 3 (1990) 38–43.
[30] M.N. Potenza, M.R. Lerner, A rapid quantitative bioassay for evaluating the effect of ligands upon receptors that modulate camp levels in melanophore cell line, Pigment Cell Res. 5 (1992) 372–378.