ELSEVIER

www.elsevier.nl/locate/farmac

Il Farmaco 55 (2000) 184–187

IL FARMACO

Design and synthesis of melatonin receptors agonists and antagonists

Giorgio Tarzia [a,*], Giuseppe Diamantini [a], Marco Mor [b], Gilberto Spadoni [a]

[a] *Istituto di Chimica Farmaceutica e Tossicologica, Università degli Studi di Urbino, Piazza Rinascimento 6, I-61029 Urbino, Italy*
[b] *Dipartimento Farmaceutico, Università degli Studi di Parma, Viale delle Scienze, I-43100 Parma, Italy*

Abstract

We review our work towards the design and synthesis of high-affinity melatonin (*N*-acetyl-5-methoxytryptamine) agonist and antagonist compounds. High affinity melatonergic agonists were obtained by shifting the melatonin side chain from C_3 to N_1 of the indole ring system. Conversely, by moving the side chain from C_3 to C_2 it was possible to obtain melatonin antagonist compounds, albeit of moderate affinity.

Keywords: Design and synthesis; Melatonin receptors; Agonists and antagonists

1. Introduction

Melatonin (*N*-acetyl-5-methoxytryptamine, MLT (**1**) Fig. 1) is the principal hormone of the vertebrate pineal gland by which it is secreted during darkness [1]. Much has been claimed for its physiological role and therapeutic effects [2–10], but the situation has yet to be clarified [10].

Two distinct types of MLT receptors have been identified: one of high affinity (ML_1) and one of low affinity (ML_2) [11]. ML_1 (or Mel_1) receptors have been identified in and cloned from several species including humans [12–17] and are further classified into three subtypes Mel_{1A}, Mel_{1B} and Mel_{1C}; of these three receptor subtypes (G-protein coupled receptors) only Mel_{1A} and Mel_{1B} are present in mammals. Mel_{1A} and Mel_{1B} are now designated as mt_1 and MT_2 [18].

2. Discussion of the project and results

Our research focused initially on the design of several indole melatonin agonists to contribute to the knowledge of the mode of interaction of MLT with its receptors [19–23]. A number of structure–affinity relationships were identified, and recently we and other authors proposed molecular models of the putative melatonin binding site [21,22,24–28]. These groups produced several high-affinity non-selective melatonin agonists and it is now possible to reliably design this type of compounds. The situation is quite different in the case of the antagonists, as very few high-affinity competitive antagonists have been reported to date [12,29–36] and the lack of selective agonists and antagonists endowed with high affinity slows progress in the field. The structure of MLT is reported in Fig. 1.

In agreement with and in addition to previous findings the following results were obtained:

- Insertion of a suitable substituent (Br, Ph, COOR) at C_2 enhances affinity via interaction with complementary sites of the receptor [19,20,37].
- The substitution at C_4 with Br or at C_7 with OMe decreased the affinity.
- The size of the acyl group is important for the binding of the side chain to the receptor and in some cases (cycloalkyl groups) it decreases the intrinsic activity of the corresponding compounds [19–21,23,37,38].
- A 3D-QSAR model describing quantitative–affinity relationships within different classes of melatonergic ligands was derived by applying the CoMFA methodology; this model offers the possibility of

* Corresponding author. Tel.: +39-0722-2545; fax: +39-0722-2737.
E-mail address: gat@chim.uniurb.it (G. Tarzia)

0014-827X/00/$ - see front matter
PII: S0014-827X(00)00017-3

predicting affinities of designed compounds for the target MLT receptors [21].

- By using conformationally restricted MLT congeners (Fig. 1) it is seen that one of the bioactive conformations of the 3-ethylamido side chain of MLT is in a folded *gauche*/*anti* oriented form and is almost orthogonal to the plane of the indole [22].
- The 5-OMe group can be replaced by suitable substituents (i.e. 5-Br or 5-Cl) [23].
- The translocation of the ethylamido side chain from C_3 to N_1 resulted in high affinity MLT agonists that obey the same SARs ascertained for the natural ligand [37].
- The translocation of the melatonin side chain from C_3 to C_2 yields melatonin antagonists; the length of the amidoethyl side chain can be shortened or lengthened by one C unit [38].
- Relative to the 2-alkylamido compounds, suitable combinations of the OMe group (C_4- or C_6-) of the acyl residue and of the N-1 indole substituent enhance the binding affinity of the ligands for the melatonin receptors and, based on preliminary data, the MT_2 selectivity [37].
- The effects on binding affinity and efficacy deriving from variation of the acyl group are similar to those observed in the natural series of melatonergic agonists [37,38].
- In the case of the 2-alkylamido compounds, substitution with 3-Br does not lead to an increase of affinity [38].

2.1. Notes on the in vitro testing methodologies

The evaluation of our compounds was performed according to the following models used in the following sequences.

2.1.1. Affinity

The affinity of several compounds prepared in this study was determined using 2-[^{125}I]iodomelatonin (100 pM) as a radioligand in competition binding analyses on cloned human mt_1 receptor subtype stably expressed in NIH3T3 mouse fibroblast cells [39]. The affinity of some of these compounds was evaluated prior to the cloning of human melatonin receptors, and it was done on quail optic tecta membranes [40].

2.1.2. Intrinsic activity

In vitro functional data were obtained by using the following assays: (a) effects on forskolin-stimulated cAMP accumulation; (b) effects of co-incubation with GTPγS on the IC_{50} values (GTPγS index) [37]; and (c) [^{35}S]GTPγS radioligand binding assay in N11-13M mouse fibroblast cells expressing human mt_1 melatonin receptor [38,39].

The relative intrinsic activity values were obtained by dividing the maximal G-protein activation of a test compound by that of MLT. Thus, the intrinsic activity of MLT or of full agonists is always equal or very close to 1, between 0 and 1 for partial agonists, and below 0 for inverse agonists.

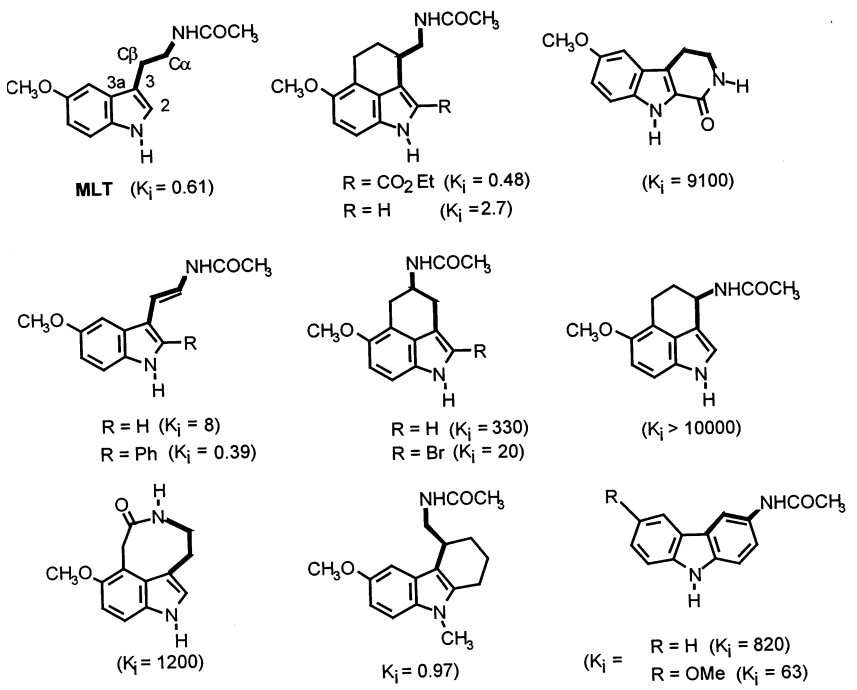

Fig. 1. Melatonin and conformationally restricted melatonin analogs (K_i = nM).

References

[1] R.J. Reiter, Pineal melatonin: cell biology of its synthesis and of its physiological interactions, Endocr. Rev. 12 (1991) 151–180.

[2] J. Arendt, S. Deacon, Treatment of circadian rhythm disorders, Melatonin Chronobiol. Int. 14 (1997) 185–204.

[3] I.V. Zhdanova, R.J. Wurtman, H.J. Lynch, J.R. Ives, A.B. Dollins, C. Morabito, J.K. Matheson, D.L. Schomer, Sleep-inducing effects of low doses of melatonin ingested in the evening, Clin. Pharmacol. Ther. (St. Louis) 57 (1995) 552–558.

[4] J. Arendt, D.J. Skene, B. Middleton, S.M. Lockley, S. Deacon, Efficacy of melatonin treatment in jet lag, shift work and blindness, J. Biol. Rhythms 12 (1997) 604–617.

[5] A. Oldani, L. Ferini-Strambi, M. Zucconi, B. Stankov, F. Fraschini, S. Smirne, Melatonin and delayed sleep phase syndrome: ambulatory polygraphic evaluation, NeuroReport 6 (1994) 132–134.

[6] M.A. Pappola, M. Sos, R.A. Omar, R.J. Bick, D.L.M. Hickson-Bick, R.J. Reiter, S. Efthimiopoulos, N.K. Robakis, Melatonin prevents death of neuroblastoma cells exposed to the Alzheimer peptide, J. Neurosci. 17 (1997) 1683–1690.

[7] D.E. Blask, S.T. Wilson, F. Zalatan, Physiological melatonin inhibition of human breast cancer cell growth in vitro: evidence for a glutathione-mediated pathway, Cancer Res. 57 (1997) 1909–1914.

[8] M. Lipartiti, D. Franceschini, R. Zanoni, M. Gusella, P. Giusti, C.M. Cagnoli, A. Kharlamov, H. Manev, Neuroprotective effects of melatonin, Adv. Exp. Med. Biol. 398 (1996) 315–321.

[9] K.S. Takaki, C.D. Mahle, A.J. Watson, Melatonergic ligands: pharmaceutical development and clinical applications, Curr. Pharm. Des. 3 (1997) 429–438.

[10] R.M. Hagan, N.R. Oakley, Melatonin comes of age?, Trends Pharmacol. Sci. 16 (1995) 81–83.

[11] M.L. Dubocovich, Melatonin receptors: are there multiple subtypes?, Trends Pharmacol. Sci. 16 (1995) 50–56.

[12] S.M. Reppert, D.R. Weaver, C. Godson, Melatonin receptors step into light: cloning and classification subtypes, Trends Pharmacol. Sci. 17 (1996) 100–102.

[13] P.J. Morgan, P. Barrett, H.E. Howell, R. Helliwell, Melatonin receptors: localization, molecular pharmacology and physiological significance, Neurochem. Int. 24 (1994) 101–146.

[14] T. Ebisawa, S. Karne, M.R. Lerner, S.M. Reppert, Expression cloning of a high-affinity melatonin receptor from *Xenopus* dermal melanophores, Proc. Natl. Acad. Sci. USA 91 (1994) 6133–6137.

[15] S.M. Reppert, D.R. Weaver, T. Ebisawa, Cloning and characterization of a mammalian melatonin receptor that mediates reproductive and circadian responses, Neuron 13 (1994) 1177–1185.

[16] S.M. Reppert, C. Godson, C.D. Mahle, D.R. Weaver, S.A. Slaugenhaupt, J.F. Gusella, Molecular characterization of a second melatonin receptor expressed in human retina and brain: the Mel_{1b} melatonin receptor, Proc. Nad. Acad. Sci. USA 92 (1995) 8734–8738.

[17] C. Mazzucchelli, M. Pannacci, R. Nonno, V. Lucini, F. Fraschini, B.M. Stankov, The melatonin receptor in the human brain: cloning experiments and distribution studies, Mol. Brain Res. 39 (1996) 117–126.

[18] M.L. Dubocovich, D.P. Cardinali, B. Guardiola-Le Maitre, R.M. Hagan, D.N. Krause, D. Sugden, P.M. Vanhoutte, F.D. Yocca, The IUPHAR Compendium of Receptor Characterization and Classification, IUPHAR Media, London, UK, 1998, pp. 187–193.

[19] E. Duranti, B. Stankov, G. Spadoni, A. Duranti, V. Lucini, S. Capsoni, G. Biella, F. Fraschini, 2-Bromomelatonin: synthesis and characterization of a potent melatonin agonist, Life Sci. 51 (1992) 479–485.

[20] G. Spadoni, B. Stankov, A. Duranti, G. Biella, V. Lucini, A. Salvatori, F. Fraschini, 2-Substituted 5-methoxy-*N*-acyltryptamines: synthesis, binding affinity for the melatonin receptor, and evaluation of the biological activity, J. Med. Chem. 36 (1993) 4069–4074.

[21] 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 CoMFA study, J. Med. Chem. 41 (1998) 3831–3844.

[22] G. Spadoni, C. Balsamini, G. Diamantini, B. Di Giacomo, G. Tarzia, M. Mor, P.V. Plazzi, S. Rivara, V. Lucini, R. Nonno, M. Pannacci, F. Fraschini, B.M. Stankov, Conformationally restrained melatonin analogues: synthesis, binding affinity for the melatonin receptor, evaluation of the biological activity and molecular modeling study, J. Med. Chem. 40 (1997) 1990–2002.

[23] G. Spadoni, C. Balsamini, A. Bedini, A. Carey, G. Diamantini, B. Di Giacomo, A. Tontini, G. Tarzia, R. Nonno, V. Lucini, M. Pannacci, B.M. Stankov, F. Fraschini, *N*-Acyl-5- and 2,5-substituted tryptamines: synthesis, activity and affinity for human mT_1 and MT_2 melatonin receptors, Med. Chem. Res. 8 (1998) 487–498.

[24] D. Sugden, N.W.S. Chong, D.F.V. Lewis, Structural requirements at the melatonin receptor, Br. J. Pharmacol. 114 (1995) 618–623.

[25] J.M. Jansen, S. Copinga, G. Gruppen, E.J. Molinari, M.L. Dubocovich, C.J. Grol, The high affinity melatonin binding site probed with conformationally restricted ligands — Part I. Pharmacophore and minireceptor models, Bioorg. Med. Chem. 4 (1996) 1321–1332.

[26] C.J. Grol, J.M. Jansen, The high affinity melatonin binding site probed with conformationally restricted ligands — Part II. Homology modeling of the receptor, Bioorg. Med. Chem. 4 (1996) 1333–1339.

[27] S. Sicsic, I. Serraz, J. Andrieux, B. Brémont, M. Mathé-Allainmat, A. Poncet, S. Shen, M. Langlois, Three-dimensional quantitative structure–activity relationship of melatonin receptor ligands: a comparative molecular field analysis study, J. Med. Chem. 40 (1997) 739–748.

[28] 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.

[29] M.L. Dubocovich, *N*-Acetyltryptamine antagonizes the melatonin induced inhibition of [^{3}H]dopamine release from retina, Eur. J. Pharmacol. 105 (1984) 193–194.

[30] M.L. Dubocovich, Luzindole (N-0774): a novel melatonin receptor antagonist, J. Pharmacol. Exp. Ther. 246 (1988) 902–910.

[31] P.J. Garratt, R. Jones, D.A. Tocher, D. Sugden, Mapping the melatonin receptor. Part 3. Design and synthesis of melatonin agonists and antagonists derived from 2-phenyltryptamines, J. Med. Chem. 38 (1995) 1132–1139.

[32] S. Copinga, G. Van Brummelen, W.J. Drijfhout, P.G. Tepper, C.J. Grol, A.S. Horn, M.L. Dubocovich, Synthesis and pharmacological evaluation of 4-aryl-2-amidotetralins: nonindolic melatonin-receptor agents, in: Proc. XIIIth Int. Symp. Med. Chem. (Paris, September 19–23, 1994).

[33] B. Guardiola-Le Maitre, P. Delagrange, Melatonin analogues: from pharmacology to clinical application, Eur. J. Med. Chem. 30 (1995) 643s–651s.

[34] M.L. Dubocovich, M.I. Masana, S. Iacob, D.M. Sauri, Melatonin receptor antagonists that differentiate between the human Mel_{1a} and Mel_{1b} recombinant subtypes are used to assess the pharmacological profile of the rabbit retina Ml_1 presynaptic heteroreceptor, Naunyn–Schmiedeberg's Arch. Pharmacol. 355 (1997) 365–375.

[35] D.J. Davies, P.J. Garratt, D.A. Tocher, S. Vonhoff, J. Davies, M.C. Teh, D. Sugden, Mapping the melatonin receptor. Part 5. Melatonin agonists and antagonists derived from tetrahydrocyclopent[*b*]indoles, tetrahydrocarbazoles and hexahydrocyclohept[*b*]indoles, J. Med. Chem. 41 (1998) 451–467.

[36] V. Leclerc, E. Fourmaintraux, P. Depreux, D. Lesieur, P. Morgan, H.E. Howell, P. Renard, D.H. Caignard, D. Pfeiffer, P. Delagrange, B. Guardiola-Le Maitre, 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.

[37] G. Tarzia, G. Diamantini, B. Di Giacomo, G. Spadoni, D. Esposti, R. Nonno, V. Lucini, M. Pannacci, F. Fraschini, B.M. Stankov, 1-(2-Alkanamidoethyl)-6-methoxyindole derivatives: a new class of potent indole melatonin analogues, J. Med. Chem. 40 (1997) 2003–2010.

[38] G. Spadoni, C. Balsamini, A. Bedini, G. Diamantini, B. Di Giacomo, A. Tontini, G. Tarzia, M. Mor, P.V. Plazzi, S. Rivara, R. Nonno, M. Pannacci, V. Lucini, F. Fraschini, B.M. Stankov, 2-[*N*-Acylamino(C_1–C_3)alkyl]indoles as MT_1 melatonin receptor partial agonists, antagonists, and putative inverse agonists, J. Med. Chem. 41 (1998) 3624–3634.

[39] R. Nonno, V. Lucini, M. Pannacci, C. Mazzucchelli, D. Angeloni, F. Fraschini, B.M. Stankov, Pharmacological characterization of the human melatonin Mel_{1a} receptor following stable transfection into NIH3T3 cells, Br. J. Pharmacol. 124 (1998) 485–492.

[40] B. Cozzi, B. Stankov, C. Viglietti-Panzica, S. Capsoni, N. Aste, V. Lucini, F. Fraschini, G.C. Panzica, Distribution and characterization of melatonin receptor in the brain of the Japanese quail, *Coturnix japonica*, Neurosci. Lett. 150 (1993) 149–152.