MT_2 Selective melatonin receptor antagonists: design and structure-activity relationships

Silvia Rivara,[a,*] Marco Mor,[a] Simone Lorenzi,[a] Alessio Lodola,[a] Pier Vincenzo Plazzi,[a] Gilberto Spadoni,[b] Annalida Bedini,[b] and Giorgio Tarzia[b]

[a]Dipartimento Farmaceutico, Università degli Studi di Parma, Parco Area delle Scienze 27/A, I-43100 Parma, Italy

[b]Istituto di Chimica Farmaceutica e Tossicologica, Università degli Studi di Urbino "Carlo Bo", piazza Rinascimento 6, I-61029 Urbino, Italy

E-mail: silvia.rivara@unipr.it

Abstract

The neurohormone melatonin is involved in the regulation of many physiological functions, in particular those related to circadian and seasonal rhythms. In mammals, melatonin activates two GPCRs, named MT_1 and MT_2, and ligands of these receptors have been proposed for the treatment of different pathologies. In this article we describe the results of our researches in the field of melatonin receptor ligands, pointing the attention to the investigation of structure-activity relationships and to the development of novel MT_2 selective antagonists. Molecular modeling studies led us to formulate a hypothesis about the structural requirements for MT_2 selective antagonism. This hypothesis was supported by 3D-QSAR analysis, that allowed the definition of the molecular determinants correlated with binding affinity, receptor subtype selectivity and intrinsic activity. Three-dimensional models of the MT_1 and MT_2 receptors were built by homology modeling and they provided an explanation, at the receptor level, for the MT_2 receptor selectivity evidenced by the antagonists. The information obtained from our ligand-based and structure-based studies was exploited for the design of different series of potent and selective melatonin receptor antagonists with novel structures.

Keywords: melatonin, MT_2 receptor, selective antagonists, 3D-QSAR, drug design, homology modeling

Introduction

Melatonin (*N*-acetyl-5-methoxytryptamine) is a serotonin-derived neurohormone primarily secreted by the pineal gland with a circadian rhythm and reaching its highest plasma concentrations during the dark phase. The changing melatonin profile conveys information about

daylenght to the body physiology, and melatonin is known to modulate different aspects of cardiovascular, reproductive, retinal and immune system processes.[1]

In mammals, at least three different binding sites have been recognized for melatonin; the MT_1 and MT_2 membrane receptors belong to the GPCR family and are mainly coupled to inhibition of adenylyl cyclase; the lower affinity MT_3 binding site has been recently identified as the enzyme NRH:quinone oxydoreductase 2.[2,3]

In the last decade, the physiological role of the two receptor subtypes has been partially clarified,[4] thanks to the availability of MT_1 and MT_2 receptor ligands.[5] Indeed, there is still a need for new potent and subtype selective agonists and antagonists, not only to further characterize MT_1 and MT_2 receptors, but also as novel drugs. Ramelteon, a non-selective melatonin receptor agonist, has been recently approved for the treatment of insomnia,[6] and other potential therapeutical applications for melatonin and other MT_1-MT_2 ligands are currently under investigation. These compounds are mainly proposed for the treatment of alterations in sleep or in the phase of the circadian clock, migraine, depression, and Parkinson's disease.[7]

In this article we report and discuss our researches in the field of melatonin receptor ligands, which led us to the development of novel classes of MT_2 selective antagonists. This result was accomplished by the application of ligand-based and structure-based analyses, that allowed the rationalization of the structure-activity relationships (SARs). SAR results were successfully applied to the design of new compounds.

Results and Discussion

Our interest in the field of melatonin receptor ligands was initially focused on the development of novel agonists and on the definition of the structural requirements for binding to and activating the melatonin receptors. We synthesized different series of melatonin analogues, obtaining very potent derivatives, such as 2-bromomelatonin.[8,9,10,11] Starting from a series of conformationally constrained compounds, we proposed a pharmacophore model for agonists and a bioactive conformation for the natural ligand.[12] A 3D-QSAR CoMFA analysis allowed the rationalization of the structure-activity relationships for more than one hundred agonists.[13] To investigate the effect of different mutual arrangements of the pharmacophoric elements on binding affinity and intrinsic activity, the structure of melatonin was modified by shifting the acylaminoalkyl side chain at position 2 of the indole ring and by inserting different substituents on the indole nitrogen. The contribution of the methoxy group in different positions of the indole nucleus was also evaluated (Figure 1). 2-*N*-Acylaminoalkylindole derivatives were characterized by reduced intrinsic activity, with compounds behaving as partial agonists or antagonists.[14,15] A quantitative structure-activity relationship study was performed by means of a Free-Wilson analysis, assessing group contributions to MT_1 and MT_2 receptor affinity and intrinsic activity. The most notable results were obtained for the alkyl chain length and the substituent at the indole nitrogen. Indeed, the presence of a monomethylene alkyl chain had little effect on binding

affinity, but a consistent reduction in intrinsic activity was observed at both receptors, with respect to the two methylene chain; a *N*-benzyl group lowered MT_1 and MT_2 intrinsic activity, but while it decreased the binding affinity at the MT_1 receptor, it increased the affinity at the MT_2 receptor. As a result, exploiting the findings of the Free-Wilson analysis, the potent and MT_2 selective antagonist UCM 454 was identified.

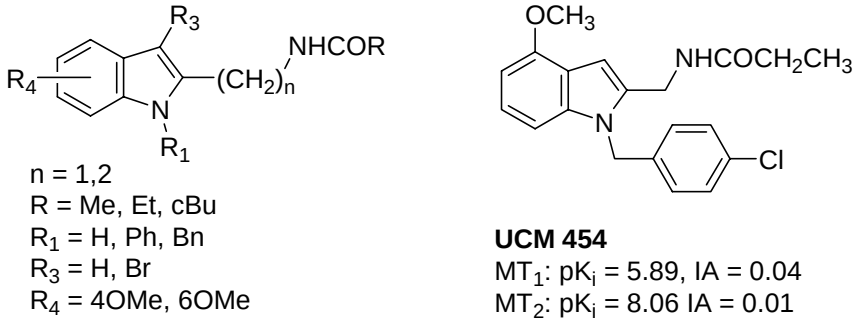


Figure 1. 2-*N*-Acylaminoalkylindole derivatives.

The comparison of the structural features of UCM 454 with those of other MT_2 selective antagonists, such as the well known luzindole and 4P-PDOT (Figure 2, left),[16] led us to formulate a hypothesis about the structural requirements for MT_2 subtype selectivity of antagonist compounds. These compounds are characterized by a substituent, in a position corresponding to positions 1 or 2 of melatonin, that can be located out-of-the-plane of the aromatic planar indole ring. At the receptor level, we can hypothesize the presence of a lipophilic cavity in the MT_2 subtype able to accommodate the out-of-plane group. The occupation of this additional pocket would lead to an antagonist behavior of the compounds and to selectivity for the MT_2 receptor, being not present in the MT_1 subtype (Figure 2, right). According to this hypothesis, compounds with a substituent out-of-the-plane of the molecule are characterized by a very low affinity for the MT_1 receptor.

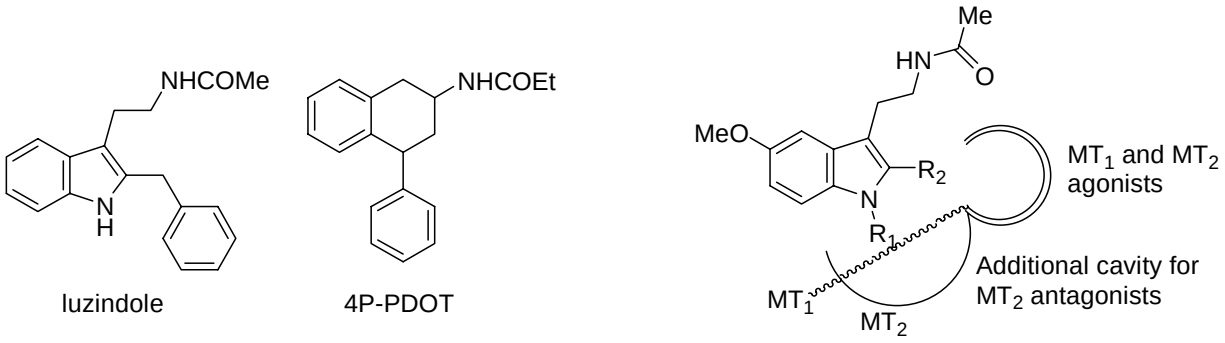


Figure 2. Left. MT_2 selective antagonists. Right. MT_1/MT_2 selectivity: the binding site hypothesis.

This hypothesis, initially based only on molecular superpositions, was supported by statistical analysis. A series of MT_1 and MT_2 antagonists, with known affinity at both receptor subtypes, was submitted to a 3D-QSAR CoMFA study to investigate their structure-activity relationships.[17] We found that MT_2 binding affinity could be enhanced by occupying the out-of-plane region surrounding positions 1 and 2 of melatonin and the region corresponding to the methoxy substituent of melatonin (Figure 3, left); these regions proved also to be highly correlated with the selectivity for the MT_2 receptor (Figure 3, center). Another CoMFA analysis was performed on a series of melatonin receptor ligands characterized by a gradual change in their intrinsic activities, shifting from agonist to partial agonist to antagonist behavior depending on the presence or the absence of certain structural features. The 3D-QSAR study highlighted an opposite effect on intrinsic activity of the methoxy group and the out-of-plane substituent. In fact, while the occupation of the region corresponding to the methoxy substituent led to an increase of intrinsic activity, the out-of-plane region was correlated to a decrease of intrinsic activity, thus leading to an antagonist behavior of the compounds (Figure 3, right). Therefore, these analyses provided a statistical validation of our previous hypothesis of an additional cavity at the MT_2 receptor, where the out-of-plane substituent of MT_2 selective antagonists could be accommodated.

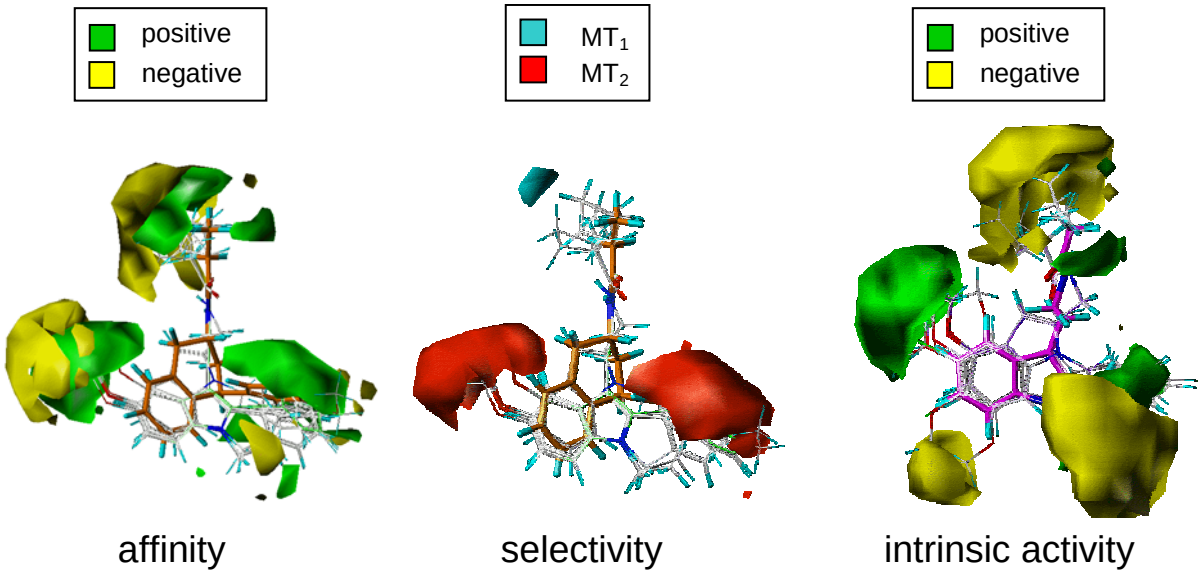
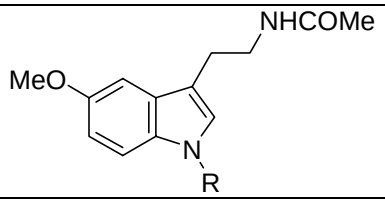


Figure 3. Left: CoMFA steric contour plots for structure-affinity analysis performed on antagonist compounds at MT_2 receptor. 4P-PDOT is depicted in capped sticks with orange carbons. Center: CoMFA steric contour plots for MT_1/MT_2 selectivity analysis. Right: CoMFA steric contour plots for structure-intrinsic activity analysis at MT_2 receptor. Luzindole is shown in capped sticks with magenta carbons.

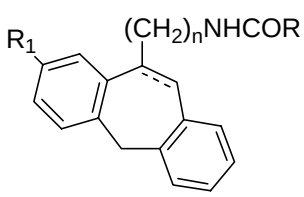
Keeping in account the 3D-QSAR results about the role of the out-of-plane substituent on intrinsic activity and receptor subtype selectivity, we applied our hypothesis to melatonin derivatives, testing, on cloned MT_1 and MT_2 receptors, some *N1*-indole-substituted melatonin derivatives which had been previously tested only on tissues expressing a mixture of receptor subtypes. As can be seen in Table 1, a methyl group was roughly tolerated, a planar phenyl ring lowered the binding affinity at both receptors, while the benzyl group led to a reduction of intrinsic activity, but it was well tolerated at the MT_2 receptor, providing some subtype selectivity. This can be explained by our findings, supposing that the benzyl group can be accommodated in the MT_2 receptor additional pocket, out-of-the-plane of the indole ring.

Table 1. MT_1 and MT_2 binding affinity and intrinsic activity of *N*-substituted melatonin derivatives.

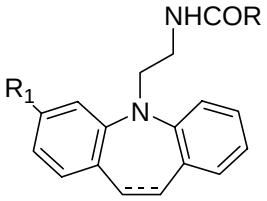


	MT_1		MT_2	
R	pK_i	IA	pK_i	IA
H	9.78	1.00	9.53	1.00
Me	8.65	1.06	8.76	0.98
Ph	6.74	0.85	6.87	0.53
Bn	6.85	0.07	8.19	-0.09

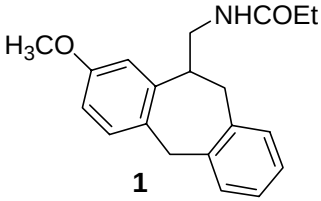
The information obtained from QSAR analyses was also exploited for the design of novel MT_2 selective antagonists, characterized by structures unrelated to those of known compounds. To this end, we looked for scaffolds able to fulfill the requirements for selective antagonism, and we selected some tricyclic dibenzo seven-membered structures which are characterized by a skewed, not coplanar, arrangement of the two benzene rings, and are therefore able to occupy the out-of-plane region. We thus prepared a series of dibenzocycloheptene and dibenzoazepine derivatives, bearing an acylaminoalkyl side chain, and a methoxy substituent in a position topographically correspondent to that of melatonin (Figure 4).[18]



n=1, 2
R= Me, Et, Pr
R_1= H, OMe



R= Me, Et
R_1= H, OMe



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MT_1: pK_i = 8.87, IA = -0.10
MT_2: pK_i = 9.79, IA = -0.15

Figure 4. Tricyclic antagonists.

These compounds were characterized by an antagonist behavior and by different potencies at the melatonin receptors. Dihydrodibenzocycloheptene derivatives with an acylaminomethyl side chain (n=1 in Figure 4) were the most potent ones. The methoxy substituent improved the potency of the compounds, although not MT_2 selectivity, nor intrinsic activity, thus evidencing a behavior peculiar to the series. One of the most interesting compounds was **1** in Figure 4, characterized by a high potency at MT_2 receptor, comparable to that of melatonin and among the highest reported for antagonists, with a moderate receptor selectivity. This compound can be easily superposed to other potent and MT_2 selective antagonists (i. e., luzindole, 4P-PDOT, UCM 454), thus being able to reproduce the spatial disposition of the pharmacophore elements defined for receptor antagonism. Moreover, due to the bent arrangement of the tricyclic scaffold, it is able to occupy both the regions of space correlated with MT_2 selectivity, according to our 3D-QSAR analysis, those of the methoxy substituent and of the out-of-plane group (Figure 3, center).

To further rationalize the structure-activity relationships previously obtained for melatonin receptor antagonists, we built by homology modeling three-dimensional models of the MT_1 and MT_2 receptors, starting from the crystallographic coordinates of bovine rhodopsin.[19] In particular, we were looking for an explanation at the receptor level of the role of the out-of-plane substituent in receptor selectivity and intrinsic activity. During the building process relevant modifications were applied to the rhodopsin template, in particular to the region of transmembrane (TM) 5, to allow the accommodation of the bulky MT_2 antagonists in the too narrow space available within the TM helices. Antagonists were thus docked within the putative binding site, and the complexes were submitted to a simulated annealing protocol to get mutual adjustments in the receptor and ligand structures, and then to molecular dynamics simulations to evaluate their stability. The putative interaction pattern was characterized by a T-shaped interaction between the electron-rich, aromatic nucleus of the antagonist and the NH group of His208 in TM5, by a hydrogen bond between the amide oxygen of the antagonist and the hydroxy group of Tyr183, belonging to the extracellular loop 2, and by the accommodation of the out-of-plane group within a hydrophobic pocket (Figure 5).

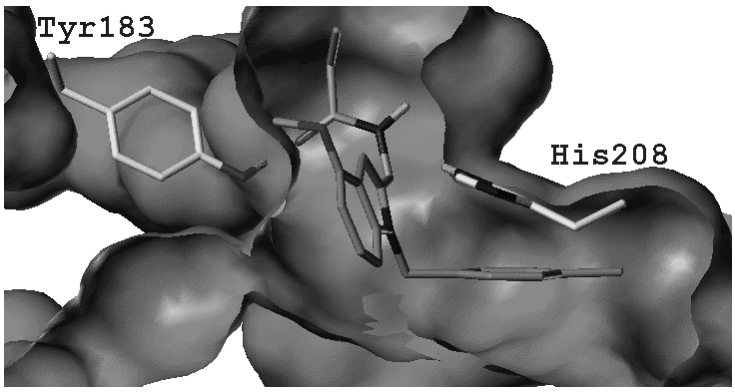

Figure 5. UCM 454 within the binding site of the MT_2 receptor model.

It is interesting to observe that some amino acids defining this hydrophobic pocket are different in MT_1 and MT_2 receptors and, in particular, the two bulkier MT_1 Ile115 and Met200 generated to a smaller cavity compared to the corresponding MT_2 residues Val128 and Ile213. This resulted in a worse accommodation of the out-of-plane group in the MT_1 receptor and in a reduced stability of the antagonist-receptor complexes, thus providing a possible explanation for the MT_2 selectivity observed for these antagonists. Moreover, the aromatic out-of-plane substituent was engaged in a π-π interaction with the indole ring of Trp264, belonging to the CWXP motif in TM6, that is known to be involved in the process of receptor activation. This finding may therefore be consistent with the antagonist behavior of the compounds. Different MT_2 selective antagonists, representative of the different classes of compounds reported in the literature, were docked within the receptor model. They comprised indole derivatives (i.e., *N*-benzylmelatonin), the tricyclic antagonist **1** and tetralin derivatives (i. e., 4P-PDOT), all of them sharing the same interaction pattern. The MT_2 receptor model was also tested for its ability to explain the structure-activity relationships found for a small series of UCM 454 derivatives, carrying different substituents at the indole nitrogen. The results obtained from molecular dynamics simulations performed on the receptor-ligand complexes were in qualitative agreement with the binding affinities experimentally measured. Therefore the MT_2 receptor model was also able to explain the SARs found for a series of structurally related derivatives.

On the basis of the accurate definition of the spatial requirements for MT_2 binding, we designed new compounds, trying to arrange the pharmacophore elements in a different and original way. In particular, we connected two aromatic rings and the amide function through an acyclic scaffold, selecting a series of compounds able to fulfill the pharmacophore model developed for antagonists and to be successfully docked into the MT_2 receptor model, in at least one accessible conformation. As a way to generate novel structures, we applied an "open-chain analog" approach,[20] starting from our tricyclic antagonist **1** or from the tetralin 4P-PDOT, and selecting the compounds which fulfilled the previously cited requirements. We therefore decided to synthesize 2,3-diphenyl-propylamide derivatives, formally deriving from ring opening of the tricyclic antagonist **1**, and 3,3-diphenyl-propylamide derivatives, that can be considered acyclic

analogs of 4P-PDOT (Figure 6). Structure rigidification was obtained by the introduction of a double bond in the propyl chain, and the role of the methoxy group was also investigated. Moreover, we decided to evaluate some phenyl-ethylamide derivatives carrying a benzyl substituent in different positions, able to occupy the out-of-plane region. The pharmacological properties of these compounds are currently under investigation; if our working hypothesis is confirmed, these compounds will represent not only novel melatonin receptor ligands with innovative structures, but they will also provide a further characterization of structure-activity relationships, that proved to be of great utility in the process of drug design.

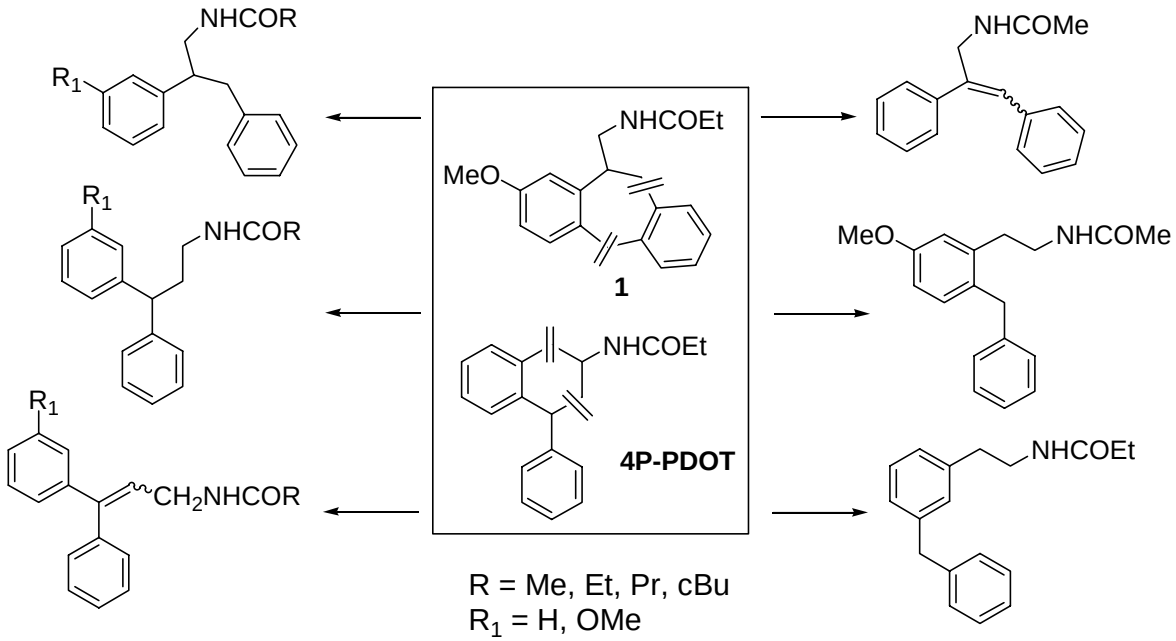

Figure 6. Non-cyclic melatonin receptor ligands.

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