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Synthesis, Enantiomeric Resolution, and Structure–Activity Relationship Study of a Series of 10,11-Dihydro-5*H*-Dibenzo[a,d]cycloheptene MT_2 Receptor Antagonists

Gilberto Spadoni,[b] Annalida Bedini,[b] Giuseppe Diamantini,[b] Giorgio Tarzia,[b] Silvia Rivara,*[a] Simone Lorenzi,[a] Alessio Lodola,[a] Marco Mor,[a] Valeria Lucini,[c] Marilou Pannacci,[c] Alessia Caronno,[c] and Franco Fraschini[c]

Racemic N-*(8-methoxy-10,11-dihydro-5*H*-dibenzo[a,d]cyclohepten-10-ylmethyl)acetamide (compound **5**) was previously identified as a novel selective MT_2 antagonist fulfilling the requirements of pharmacophore and 3D QSAR models. In this study the enantiomers of **5** were separated by medium-pressure liquid chromatography and behaved as the racemate. Compound **5** was modified at the acylaminomethyl side chain and at position C8. The resulting analogues generally behaved as melatonin receptor antagonists (GTPγS test) with a modest degree of selectivity (up to 10-fold) for the MT_2 receptor. Changes at the amide side chain led to a decrease in binding affinity, whereas 8-acetyl and 8-methyl derivatives **12** and **11**, respectively, were as potent as the 8-methoxy parent compound **5**. Docking experiments with an MT_2 receptor model suggested binding modes consistent with the observed SARs and with the lack of selectivity of the enantiomers of **5**.

Introduction

After its discovery and initial characterization as an amphibian skin-lightening molecule,[1] melatonin (*N*-acetyl-5-methoxytryptamine, MLT) was soon recognized as a hormone of fundamental importance in the regulation of circadian and seasonal rhythms in a variety of animal species, including humans. MLT secretion by the pineal gland is controlled by the dark/light cycle through the hypothalamic suprachiasmatic nucleus (SCN), conveying information about the photoperiod to MLT binding sites in tissues and organs.[2] Besides the control of circadian rhythms, many other physiological and behavioral effects have been ascribed to MLT, such as the modulation of the cardiovascular and immune systems, and the influence over metabolism and hormone secretion.[3] Moreover, pharmacological administration of MLT has been related to a variety of effects, from the re-entrainment of disrupted circadian rhythms and sleep onset, to the treatment of headache disorders, depression, and cancer.[4] These effects are likely to be produced by some of the multiple mechanisms identified for MLT, such as the activation of membrane receptors, scavenging of free radicals and reactive oxygen species, and interaction with cellular components, like calmodulin.[5] In mammalian tissues MLT interacts with two high-affinity G-protein-coupled receptor subtypes, MT_1 and MT_2,[6] and it displays much lower affinity for the MT_3 binding site, characterized as the enzyme NRH–quinone reductase 2.[7] MT_1 and MT_2 receptors are widely distributed in the central and peripheral nervous system, as well as in peripheral organs.[8] The physiological role of these receptors has been partially elucidated, giving evidence that MT_1 is mainly involved in neuronal firing of the SCN, hormone secretion, and vasoconstriction, whereas MT_2 is responsible for phase shifts of circadian rhythms and vasodilation.[9]

Parallel to the investigation of the pathophysiological role of MT_1 and MT_2, various MLT receptor ligands have been developed.[10] The nonselective agonist ramelteon has been recently marketed as a new hypnotic drug, while other ligands are in clinical trials for the treatment of sleep disturbances or depression.[11] In the field of MLT receptor antagonists, different series of MT_2-selective compounds have been described,[12] whereas examples of MT_1-selective agents are still limited.[13] Starting from the analysis of molecular models of representative MT_2 antagonists (Figure 1), we advanced a hypothesis on the struc-

[a] *Prof. S. Rivara, Dr. S. Lorenzi, Dr. A. Lodola, Prof. M. Mor*
Dipartimento Farmaceutico, Università degli Studi di Parma
V.le G. P. Usberti 27A, Campus Universitario, 43100 Parma (Italy)
Fax: (+39) 0521-905006
E-mail: silvia.rivara@unipr.it

[b] *Prof. G. Spadoni, Dr. A. Bedini, Prof. G. Diamantini, Prof. G. Tarzia*
Istituto di Chimica Farmaceutica e Tossicologica
Università degli Studi di Urbino "Carlo Bo"
Piazza Rinascimento 6, 61029 Urbino (Italy)

[c] *Dr. V. Lucini, Dr. M. Pannacci, Dr. A. Caronno, Prof. F. Fraschini*
Dipartimento di Farmacologia, Chemioterapia e Tossicologia Medica
Università degli Studi di Milano, Via Vanvitelli 32, 20129 Milano (Italy)

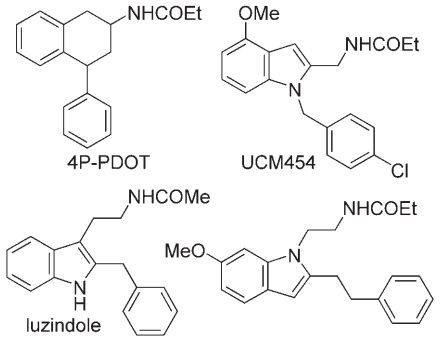
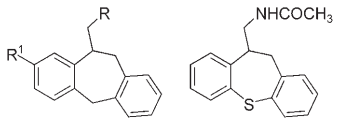


Figure 1. Representative MT_2-selective antagonists.

tural determinants for MT_2-selective antagonism.[14] In particular, the presence of a lipophilic substituent in a position corresponding to positions 1–2 of the indole nucleus of MLT and non-coplanar with the indole ring was identified as a key element for MT_2 selectivity and decreased intrinsic activity.[14] The hypothesis was tested by statistical analysis, by means of a 3D QSAR investigation on structure-selectivity and structure-intrinsic activity with an extended set of MLT receptor ligands.[15] The information obtained from these analyses was applied to the design of a new series of MT_2-selective ligands endowed with a novel tricyclic scaffold. The most interesting compounds thus identified were dihydrodibenzocycloheptene derivatives carrying an acylaminomethyl side chain and a methoxy substituent in suitable positions (for example, compound **5** in Table 1). According to our hypothesis, the skewed conformation of the tricyclic scaffold allows a proper arrangement of the pharmacophore elements, composed by the methoxyphenyl ring, which mimics the indole portion of MLT, the amide fragment, and the additional out-of-plane aromatic ring.[16] Docking studies with a homology model of the MT_2 receptor led us to the identification of a hydrophobic pocket where the non-coplanar lipophilic substituent typical of many MT_2 antagonists (such as the benzyl group of luzindole, the *p*-chlorobenzyl group of UCM454, or the phenyl ring of 4P-PDOT; Figure 1) could be accommodated. This pocket could also be occupied by one of the benzene rings of the dihydrodibenzocycloheptene nucleus.[17] The structural exploration of the tricyclic alkylamides was rather limited, and the compounds were tested as racemates, thus providing no information on their enantiomeric selectivity at the MT_1 and MT_2 receptors.[16] Herein we further explore the structure–activity relationships for this class of MLT receptor ligands, having synthesized a series of 10,11-dihydro-5*H*-dibenzo[a,d]cycloheptene analogues of **5** modified either at the amide side chain or at position 8 (Table 1). According to our modeling studies, position 8 should correspond to position 5 of the MLT indole ring, and we had previously found that an 8-methoxy group leads to an increase in binding affinity at both receptor subtypes, without any effect on selectivity or intrinsic activity.[16] To understand the role of the 8-position substituent, we explored the effects of a number of substituents differing in their electronic and lipophilic properties on the binding affinity and intrinsic activity toward MT_1 and MT_2. The amide function on the side chain was replaced by other groups to evaluate the importance of the NH fragment and the possibility of additional interactions. Moreover, separation of the enantiomers of **5** by medium-pressure liquid chromatography (MPLC) was performed, allowing an assessment of their enantiomeric selectivity.

Table 1. Binding affinity (pK_i) and intrinsic activity (IA_r) of tricyclic compounds at MT_1 and MT_2 melatonin receptors stably expressed in NIH3T3 cells.



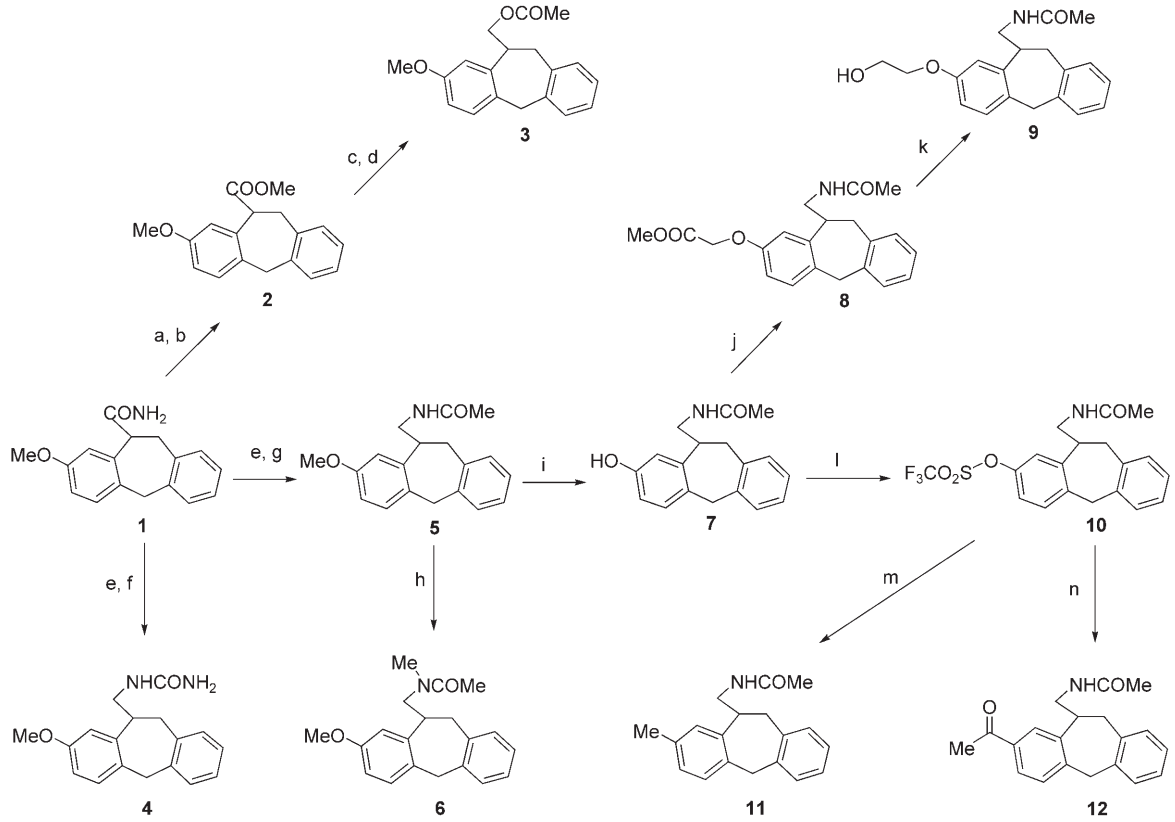
3–7, 9–13 **14**

Compd	R	R^1	Human MT_1 pK_i[a]	Human MT_1 IA_r[b]	Human MT_2 pK_i[a]	Human MT_2 IA_r[b]
MLT			9.50 ± 0.02	1.00 ± 0.01	9.69 ± 0.07	1.00 ± 0.03
3	$OCOCH_3$	OCH_3	6.85 ± 0.12	0.08 ± 0.03	7.95 ± 0.15	−0.10 ± 0.01
4	$NHCONH_2$	OCH_3	6.92 ± 0.09	−0.40 ± 0.04	8.01 ± 0.05	−0.19 ± 0.02
5	$NHCOCH_3$	OCH_3	7.57 ± 0.12	−0.21 ± 0.03	8.88 ± 0.04	−0.09 ± 0.02
(+)-**5**	$NHCOCH_3$	OCH_3	7.84 ± 0.06	−0.14 ± 0.01	8.93 ± 0.05	−0.07 ± 0.09
(−)-**5**	$NHCOCH_3$	OCH_3	7.74 ± 0.03	−0.37 ± 0.02	8.90 ± 0.20	−0.12 ± 0.01
6	$N(CH_3)COCH_3$	OCH_3	6.04 ± 0.04	0.02 ± 0.01	7.02 ± 0.02	−0.05 ± 0.02
7	$NHCOCH_3$	OH	6.38 ± 0.03	−0.18 ± 0.04	7.52 ± 0.02	−0.03 ± 0.03
9	$NHCOCH_3$	$O(CH_2)_2OH$	6.68 ± 0.18	−0.09 ± 0.03	7.59 ± 0.09	−0.02 ± 0.02
10	$NHCOCH_3$	OSO_2CF_3	6.36 ± 0.06	0.13 ± 0.01	6.60 ± 0.18	0.11 ± 0.01
11	$NHCOCH_3$	CH_3	7.70 ± 0.01	−0.05 ± 0.02	8.65 ± 0.04	−0.06 ± 0.01
12	$NHCOCH_3$	$COCH_3$	7.79 ± 0.01	−0.06 ± 0.02	8.87 ± 0.14	−0.03 ± 0.02
13[16]	$NHCOCH_3$	H	6.62 ± 0.06	0.03 ± 0.02	7.52 ± 0.04	−0.32 ± 0.07
14			7.25 ± 0.11	−0.02 ± 0.02	7.69 ± 0.34	0.32 ± 0.09

[a] pK_i values (± SEM) were calculated from IC_{50} values, obtained from competition curves by the method of Cheng and Prusoff,[36] and are the mean of at least three determinations performed in duplicate. [b] Relative intrinsic activity values (± SEM) were obtained by dividing the maximum analogue-induced G-protein activation by that of MLT.

Chemistry

10,11-Dihydro-5*H*-dibenzo[a,d]-cycloheptene derivatives **3**, **4**, **6**, **7**, and **9**–**12** were prepared starting from the previously described compounds **1** and **5**.[16] As shown in Scheme 1, acidic hydrolysis of amide **1** and esterification of the resulting carboxylic acid gave the ester **2**, which, by reduction with $LiAlH_4$ and acylation of the intermediate alcohol with acetyl chloride, gave compound **3**. Urea **4** was prepared by a two-step procedure involving reduction of amide **1** ($AlCl_3$/$LiAlH_4$) and treatment of the intermediate amine with KCNO in the presence of acetic acid. Secondary amide **6** was synthesized via alkylation of **5** with methyl



Scheme 1. Reagents and conditions: a) 37% HCl, CH_3COOH, reflux, 20 h; b) MeOH, H_2SO_4, reflux, 18 h; c) $LiAlH_4$, THF, RT, 30 min; d) CH_3COCl, TEA, THF, RT, 18 h; e) $AlCl_3$, $LiAlH_4$, THF, reflux, 4 h; f) KCNO, CH_3COOH, H_2O/THF, RT, 4 h; g) Ac_2O, TEA, THF, RT; h) CH_3I, NaH, DMF, RT, 18 h; i) BBr_3, CH_2Cl_2, RT, 6 h; j) $ClCH_2COOCH_3$, NaH, DMF, −10 °C, 4 h; k) $LiAlH_4$, THF, 0 °C, 1 h; l) trifluoromethanesulfonic anhydride, Py, CH_2Cl_2, RT, 2 h; m) $[(CH_3)_4Sn]$, $[PdCl_2(Ph_3P)_2]$, LiCl, dioxane, DMF, 110 °C, 24 h; n) $[(CH_3)_4Sn]$, $[PdCl_2(dppf)]$, LiCl, DMF, molecular sieves, 90 °C, 20 h, CO atmosphere.

iodide and NaH. Demethylation of **5** with BBr_3 gave the hydroxy derivative **7** that was a test ligand as well as a key intermediate for the synthesis of derivatives **9**–**12**. O-Alkylation of **7** with methyl chloroacetate in the presence of NaH afforded the intermediate **8**, which was converted into the corresponding 2-hydroxyethoxy derivative **9** by reduction with $LiAlH_4$. The trifluoromethanesulfonate **10** was synthesized from **7** by treatment with trifluoromethanesulfonic anhydride in the presence of pyridine; the conversion of **10** into the 8-methyl and 8-acetyl derivatives **11** and **12** was achieved by palladium-catalyzed coupling reactions with tetramethyltin and tetramethyltin–CO, respectively, according to a procedure used for analogous transformations.[18]

Enantiomeric resolution

Milligram-scale enantiomeric separation of racemic **5** was obtained by MPLC, using triacetylcellulose (TAC) as the chiral stationary phase. This chiral phase was previously employed for the resolution of various racemates, especially aromatic pharmaceuticals,[19] and it was successfully used for the separation of enantiomeric mixture of MLT analogues.[20,21] Enantiomeric purity was verified and quantified using a prepacked Chiralcel OD-H column (cellulose tris-3,5-dimethylphenylcarbamate; 250×4.6 mm i.d.; 10 μm; Daicel Chemical Industries, France) and was further confirmed by recording the ^{1}H NMR spectra of the two collected fractions in the presence of europium tris-[3-(heptafluoropropylhydroxymethylene)-(+)-camphorate] [Eu-(hfc)$_3$]. The integration of the signal relative to the methoxy substituent showed that both fractions, (−)-**5** and (+)-**5**, are a mixture of enantiomers in a ratio of about 85:15.

Binding studies

The new derivatives **3**, **4**, **6**, **7**, **9**–**12**, **14** and the two enriched enantiomers (+)-**5** and (−)-**5** were evaluated as potential melatonin receptor ligands by binding experiments; the previously described racemate **5** was re-evaluated. Binding affinity was assessed in competition experiments with cloned human MT_1 and MT_2 receptors expressed in NIH3T3 rat fibroblast cells using 2-[^{125}I]iodomelatonin as the labeled ligand. The relative intrinsic activity (IA_r) was determined with the GTPγS test by measuring the direct activation of the G protein after binding of the tested compound to the cloned human MT_1 or MT_2 receptor. By convention, the natural ligand MLT has an efficacy (E_{max}) of 100%. Full agonists stimulate [^{35}S]GTPγS binding with a maximum efficacy close to that of MLT itself. If E_{max} is between 30 and 70% that of MLT ($0.3 < IA_r < 0.7$) the compound is considered a partial agonist, whereas if E_{max} is lower than 30% ($IA_r < 0.3$) the compound is considered an antagonist.[22]

Results and Discussion

Binding data for the newly synthesized tricyclic derivatives (Table 1) show moderate selectivity (~10-fold) towards the MT_2 receptor for the whole class. This trend does not apply to the least potent compound **10** and to the dihydrodibenzothiepine derivative **14**, which have a lower degree of selectivity. MT_1 and MT_2 binding data are quite correlated ($r = 0.91$ for compounds **3**–**7**, **9**–**12**, and **14**), suggesting a similar interaction mode of the compounds at the two receptors. Moreover, the GTPγS test reveals a common behavior, with intrinsic activity close to 0 for most derivatives and some with negative IA_r values. Compound **14** is the only weak partial agonist of the MT_2 receptor.

Modification of the amide function causes a general decrease in binding affinity at both receptor subtypes. Introduction of urea (as in **4**) or an ester group (as in **3**) decreases the binding affinity by about 10-fold. The effect of the ureido group is consistent with data from other series, in which ureido agonists and antagonists are usually 5–10-fold less potent than the corresponding amide analogues.[22–24] Compound **3** retains discrete binding affinity, even if published data for other ester derivatives would lead one to expect a remarkable loss of binding affinity, relative to their amide analogues. In fact, ethyl 2-(5-methoxyindol-3-yl)acetate[25] and ethyl 2-(7-methoxynaphthalen-1-yl)acetate[26] showed about 1000-fold lower affinity than MLT or agomelatine (*N*-[2-(7-methoxynaphthalen-1-yl)ethyl]acetamide), respectively, toward receptors expressed on native tissues. This discrepancy may be related to the different intrinsic activities of the parent amides, as compound **3** is an antagonist whereas MLT and agomelatine are agonists, and stereoelectronic requirements for ligand binding are likely to be different for antagonists and agonists. On the other hand, amide N-methylation, in compound **6**, causes a significant decrease in binding affinity. While receptor binding data for *N*-methyl-MLT are not available, this behavior cannot be generalized to all the classes of MLT receptor ligands, particularly at the MT_2 receptor; in fact, indanylpiperazine agonists,[27] tethahydroisoquinoline antagonists,[24] and GR128107,[28] in which the amide nitrogen atom is part of a ring structure (Figure 2), retain high MT_2 binding affinities.

The isostere dihydrodibenzothiepine derivative **14**, in which the bulky sulfur atom affects the geometry of the tricyclic structure, has an MT_2 pK_i value quite similar to **13**, but has lower MT_2 selectivity; for this reason, the dihydrodibenzothiepine scaffold was abandoned, and no derivatives of **14** were synthesized. Investigation into the role of position 8 was performed by the introduction of substituents with various electronic and lipophilic properties. The methoxy group of **5** was already observed to have a favorable effect on binding affinity at both receptor subtypes, whereas it does not affect intrinsic activity.[16] The electronic effect of substituents at position 8 was evaluated by introducing an electron-withdrawing acetyl group and a methyl group. Surprisingly, compounds **11** and **12** show MT_1 and MT_2 binding affinities similar to those of **5**, carrying the methoxy substituent (see below). The effect of hydrophilic substituents was also investigated, introducing hydroxy (as in **7**) and hydroxyethyloxy groups (as in **9**), having electronic properties similar to those of the methoxy group. On indole derivatives, the 5-hydroxyethyloxy group modulates intrinsic activity at the MT_1 and MT_2 subtypes differently, with 5-hydroxyethyloxy-*N*-acetyltryptamine (5-HEAT) being an MT_1 agonist and an MT_2 antagonist.[29] Hydrophilic substituents lead to a significant decrease in binding affinity, which is likely due to the preference for lipophilic groups. Compound **9** behaves as an antagonist, with no difference in intrinsic activity between receptor subtypes. Compound **10**, with the lipophilic trifluoromethanesulfonyloxy group, shows the lowest pK_i value at the MT_2 receptor.

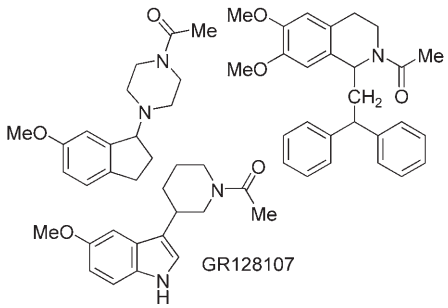


Figure 2. Melatonin receptor ligands lacking the amide H atom.

To investigate the putative binding modes of this class of compounds at the MT_2 receptor, an assessment of the enantiomeric selectivity for the lead compound was performed. The two enantiomers of compound **5** ((−)-**5** and (+)-**5**) were therefore separated by MPLC and individually tested. The two enantiomers have similar binding affinities for both receptors. The pK_i values reported in Table 1 for (−)-**5** and (+)-**5** are lower than those previously obtained for the racemate.[16] Considering inter-batch variability of binding data, all the new data reported in Table 1 were obtained in parallel experiments to allow proper structure–activity comparisons.

The similar affinities of (−)-**5** and (+)-**5** are consistent with the previously proposed pharmacophore and 3D QSAR models for MT_2 antagonists. In fact, poor selectivity had been suggested by the similar fitting (RMSD of the pharmacophore elements) obtained for the two enantiomers superposed with the reference antagonist 4P-PDOT.[16] The two enantiomers can be docked into the putative binding site of our previously developed 3D model of the MT_2 receptor,[17] both fulfilling the putative requirements for receptor binding and antagonist behavior. These requirements call for the formation of a hydrogen bond between the amide oxygen atom of the antagonist and the hydroxy group of Tyr183 from the second extracellular loop, resulting in a T-shaped interaction between the aromatic portion of the ligand and the NH group of His208 in transmembrane helix 5, and for a lipophilic group fitting into a hydrophobic pocket, the occupation of which is related to MT_2 selectivity and decreased intrinsic activity.[17] The skewed arrangement of the tricyclic structure of **5** allows the accommodation of the benzene ring within the hydrophobic cavity for both enantiomers (pose A, Figure 3 A). With respect to this hypothetical binding mode, the similar MT_2 affinities obtained for

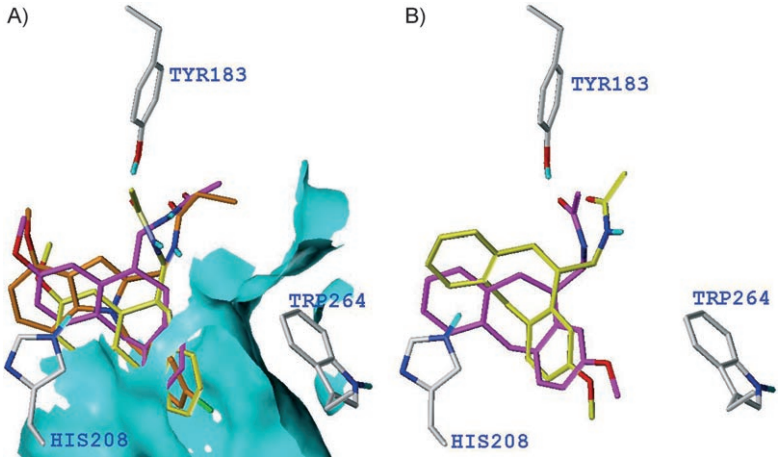


Figure 3. Energy-minimized conformations of the last step of a 1-ns MD simulation for the complexes between MT_2 receptor model and **5**. Pose A) (*R*)-**5** (purple C atoms) and (*S*)-**5** (yellow C atoms) docked in the MT_2 receptor with the methoxy substituent near His 208. UCM454 (orange C atoms) is shown for comparison. A section of the binding site surface colored in cyan is also represented to locate the lipophilic cavity. Pose B) (*R*)-**5** and (*S*)-**5** docked accommodating the methoxy substituent within the hydrophobic pocket.

5, **11**, and **12** seriously challenge the previous hypothesis on the importance of the T-shaped interaction between the aromatic portion of the antagonist, mimicking the indole nucleus of MLT, and His 208, as the electron-donor methoxy substituent should lead to a more stabilized interaction and to a higher binding affinity than the electron-withdrawing acetyl group. Automated docking into the putative MT_2 binding site, performed by the program Glide[30] starting from both enantiomers of each compound, showed that different poses are possible. Several docking solutions with the highest score (Emodel) did not fulfill one or more features of our previously proposed binding mode. This may be due to the wideness of the receptor cavity relative to the ligand structures, and it provided an initial indication that the homology model of the MT_2 receptor could not afford a quantitative prediction of ligand affinity. Focusing on the docking solutions in which a hydrogen bond is undertaken by the amide chain and the two benzene rings are close to His 208 and Trp 264, an alternative binding solution was found for all compounds with the exception of **10**. This solution (pose B) is characterized by the accommodation of the substituent at position 8 within the hydrophobic cavity near Trp 264, whereas the other benzene ring makes contact with His 208. In Figure 3 B the enantiomers of **5** are represented in the MT_2 receptor according to pose B, which is still consistent with the poor enantiomeric selectivity observed for **5**. The calculated Emodel values for compounds **3**–**7** and **9**–**14** do not correlate with their affinity values, independently from the pose or the enantiomer selected. This lack of correlation may be attributed to the inaccuracy of the modeled binding site or to the scoring function of the docking procedure. However, the two cited solutions are qualitatively consistent with our previous knowledge and can help the interpretation of some observed SARs. Pose B is consistent with the antagonist behavior of this series and with the lack of influence of the substituent at position 8 on intrinsic activity; in fact, for many series of MLT receptor ligands,[14,31,32] intrinsic activity is strongly affected by the presence of substituents in a position corresponding to position 5 of the indole ring of MLT, and in particular it is enhanced by a methoxy group.

Compound **9** behaves as an antagonist, with no differences in intrinsic activity between receptor subtypes due to the hydroxyethyloxy substituent, in contrast to what had been observed for MLT derivatives.[29] This is consistent with pose B, accommodating position 8 within the small lipophilic pocked lined by Trp 264, and providing circumstantial evidence of a SAR different from that known for 5-indole substituents. The poor MT_2 receptor affinity of compound **10** probably reflects the steric limitation of the lipophilic pocket. Docking experiments provided evidence that it could not be docked in the MT_2 receptor in pose B, suggesting an excessive volume of the trifluoromethanesulfonyloxy substituent. Although pose B showed a qualitatively better agreement with the SARs for this series of compounds, a better definition of their binding mode was not possible employing the homology model of MT_2 receptor. In fact, molecular dynamics (MD) simulations on the complexes built from both poses of (*R*)-**5** and (*S*)-**5** showed similar oscillations around the starting positions, with no significant change in ligand–receptor interactions and no evident differences among the four complexes.

In conclusion, modulation of the dihydrodibenzocycloheptene structure by the introduction of different substituents at position 8 and at the side chain results in a series of MLT receptor antagonists with moderate selectivity for the MT_2 receptor. Substitution of the amide function with other functional groups leads to a decrease in binding affinity at both receptor subtypes. Introduction of lipophilic substituents at position 8 provides compounds with binding affinities similar to those of the previously described 8-methoxy derivative **5**. The enantiomers of **5** were separated and they do not show evidence for selectivity toward either of the receptor subtypes. Docking experiments into the MT_2 receptor model are consistent with this result, as both enantiomers could be docked into the MT_2 receptor with similar arrangements. Moreover, an alternate docking solution was proposed, suggesting the accommodation of the substituent at position 8 within a hydrophobic pocket. This solution provides a plausible explanation for the lack of effect of this substituent on the intrinsic activity of the compounds.

Experimental Section

Chemistry

Melting points were determined on a Büchi SMP-510 capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AC 200 spectrometer; chemical shifts (δ) are reported in ppm relative to the central peak of the solvent. Coupling constants (*J*) are given in Hz. EIMS spectra (70 eV) were taken on a Fisons Trio 1000 instrument. Only molecular ions (M^+)

and base peaks are given. IR spectra were obtained on a Nicolet Avatar 360 FTIR spectrometer; absorbances are reported in $\tilde{\nu}$ (cm^{-1}). Elemental analyses for C, H, and N are within 0.4% of the calculated values. Column chromatography purifications were performed under "flash" conditions using Merck 230–400 mesh silica gel. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} plates. *N*-(10,11-dihydro-dibenzo[b,f]thiepin-10-ylmethyl) acetamide (**14**) was purchased from Specs (The Netherlands). Triacetylcellulose (TAC), with a particle size of 15–25 μm, was purchased from Merck (Darmstadt, Germany). The two radioligands 2-[^{125}I]iodomelatonin (specific activity: 2000 Ci mmol^{-1}) and [^{35}S]GTPγS ([^{35}S]guanosine-5′-*O*-(3-thiotriphosphate); specific activity: 1000 Ci mmol^{-1}) were purchased from Amersham Pharmacia Biotech (Italy).

8-Methoxy-10,11-dihydro-5*H*-dibenzo[a,d]cycloheptene-10-carboxylic acid amide (1) was prepared as previously described.[16]

8-Methoxy-10,11-dihydro-5*H*-dibenzo[a,d]cycloheptene-10-carboxylic acid methyl ester (2). HCl (aq, 37%, 3 mL) was added to a suspension of amide **1** (0.40 g, 1.5 mmol) in glacial acetic acid (4.5 mL). The reaction mixture was held at reflux for 20 h, poured into H_2O and extracted 3× with EtOAc. The combined organic phases were washed with H_2O, dried (Na_2SO_4), and evaporated to yield the intermediate acid that was purified by flash chromatography (EtOAc as eluent); MS (EI) *m/z*: 268 ([*M*]$^+$), 222 (100); ^{1}H NMR ($CDCl_3$): δ = 3.32 (dd, 1H, *J* = 14.7, 5.0), 3.62 (dd, 1H, *J* = 14.5, 10.3), 3.74 (s, 3H), 3.89 (d, 1H, *J* = 15.0), 4.20 (dd, 1H, *J* = 5.3, 10.2), 4.26 (d, 1H, *J* = 15.3), 6.72 (dd, 1H, *J* = 2.8, 9.3), 6.73 (d, 1H, *J* = 2.8), 7.11–7.22 ppm (m, 5H).

H_2SO_4 (aq, 96%, 0.2 mL, 3.6 mmol) was added to a solution of the above acid (0.30 g, 1.12 mmol) in dry MeOH (15 mL). After being held at reflux while stirring overnight, the reaction mixture was evaporated under reduced pressure and the residue partitioned between H_2O and EtOAc. The combined organic phases were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. The crude residue was purified by flash chromatography (cyclohexane/EtOAc 8:2 as eluent); oil, 51% overall yield. MS (EI) *m/z*: 282 ([*M*]$^+$), 222 (100); ^{1}H NMR ($CDCl_3$): δ = 3.27 (dd, 1H, *J* = 4.9, 14.8), 3.61 (dd, 1H, *J* = 10.9, 14.6), 3.72 (s, 3H), 3.73 (s, 3H), 3.91 (d, 1H, *J* = 15.0), 4.20 (dd, 1H, *J* = 5.0, 10.6), 4.23 (d, 1H, *J* = 15.0), 6.65 (d, 1H, *J* = 2.8), 6.69 (dd, 1H, *J* = 2.8, 10.8), 7.09–7.24 ppm (m, 5H); IR (neat): $\tilde{\nu}$ = 1734, 1609 cm^{-1}.

(8-Methoxy-10,11-dihydro-5*H*-dibenzo[a,d]cyclohepten-10-ylmethyl)acetate (3). A solution of **2** (0.19 g, 0.7 mmol) in dry THF (2 mL) was added dropwise to a stirred ice-cooled suspension of $LiAlH_4$ (0.04 g, 1 mmol) in dry THF (2 mL) under N_2. Upon completion of the addition, the mixture was stirred at room temperature for 30 min. The unreacted $LiAlH_4$ was destroyed by careful addition of H_2O at 0 °C, and the resulting mixture was filtered through a celite pad. The filtrate was concentrated in vacuo, and the residue partitioned between H_2O and EtOAc. The combined organic phases were washed with brine, dried (Na_2SO_4), and evaporated to yield the crude desired product that was purified by flash chromatography (cyclohexane/EtOAc 1:1 as eluent); oil, 95% yield. MS (EI) *m/z*: 254 ([*M*]$^+$), 236 (100); ^{1}H NMR ($CDCl_3$): δ = 3.20 (dd, 1H, *J* = 9.5, 14.5), 3.31 (dd, 1H, *J* = 4.1, 14.4), 3.37 (m, 1H), 3.67 (dd, 1H, *J* = 7.0, 10.8), 3.76 (s, 3H), 3.82 (dd, 1H, *J* = 4.8, 10.8), 3.99 (d, 1H, *J* = 14.8), 4.09 (d, 1H, *J* = 14.8), 6.68 (dd, 1H, *J* = 2.7, 8.3), 6.71 (d, 1H, *J* = 2.7), 7.08–7.24 ppm (m, 5H); IR (neat): $\tilde{\nu}$ = 3396, 1608 cm^{-1}.

Acetyl chloride (0.06 mL, 0.84 mmol) was added to a solution of the intermediate alcohol (0.18 g, 0.71 mmol) and TEA (0.11 mL, 0.8 mmol) in dry THF (5 mL), and the mixture was stirred at room temperature for 18 h. The reaction mixture was poured into H_2O and extracted 3× with EtOAc. The combined organic phases were washed with brine, dried (Na_2SO_4), and evaporated to give the crude desired product that was purified by flash chromatography (cyclohexane/EtOAc 8:2 as eluent); oil, 68% yield. MS (EI) *m/z*: 296 ([*M*]$^+$), 236 (100); ^{1}H NMR ($CDCl_3$): δ = 2.13 (s, 3H), 3.06 (dd, 1H, *J* = 14.6, 8.8), 3.38 (dd, 1H, *J* = 14.5, 4.2), 3.55 (m, 1H), 3.76 (s, 3H), 3.89 (d, 1H, *J* = 15.1), 3.93 (dd, 1H, *J* = 9.1, 10.8), 4.20 (d, 1H, *J* = 15.1), 4.36 (dd, 1H, *J* = 10.9, 5.0), 6.68 (dd, 1H, *J* = 2.8, 8.2), 6.73 (d, 1H, *J* = 2.7), 7.10–7.22 ppm (m, 5H); IR (neat): $\tilde{\nu}$ = 1738, 1608 cm^{-1}; anal. ($C_{19}H_{20}O_3$) C, H.

(8-Methoxy-10,11-dihydro-5*H*-dibenzo[a,d]cyclohepten-10-ylmethyl) urea (4). A solution of aluminum trichloride (0.20 g, 1.5 mmol) in dry THF (6 mL) was added dropwise to an ice-cooled stirred suspension of $LiAlH_4$ (0.16 g, 4.3 mmol) in dry THF (6 mL). After 10 min, a solution of the amide **1** (0.20 g, 0.75 mmol) in dry THF (6 mL) was added dropwise, and the resulting mixture was held at reflux for 4 h. After cooling to 0 °C, the excess hydride was cautiously destroyed with H_2O, the resulting mixture was filtered on celite, and the filtrate was concentrated in vacuo and partitioned between EtOAc and 2 N NaOH. The combined organic phases were washed with brine, dried (Na_2SO_4), and evaporated to afford the crude amine that was used without any further purification; oil. MS (EI) *m/z*: 253 ([*M*]$^+$), 178 (100); ^{1}H NMR ($CDCl_3$): δ = 2.06 (br s, 2H), 2.85–3.36 (m, 5H), 3.76 (s, 3H), 3.97 (d, 1H, *J* = 14.8), 4.11 (d, 1H, *J* = 14.8), 6.66 (dd, 1H, *J* = 2.7, 8.1), 6.71 (d, 1H, *J* = 2.7), 7.10–7.21 ppm (m, 5H).

A solution of KCNO (0.13 g, 1.55 mmol) in H_2O (0.6 mL) was added dropwise to a solution of the above crude amine (0.16 g, 0.63 mmol) in THF (3 mL), H_2O (0.8 mL), and glacial acetic acid (0.4 mL). The reaction mixture was stirred at room temperature for 4 h, then poured into H_2O and extracted with EtOAc. The combined organic phases were washed with brine, dried (Na_2SO_4), and evaporated to afford the crude urea that was purified by flash chromatography (EtOAc as eluent) and crystallization (EtOAc/MeOH/cyclohexane). White solid, mp: 198–199 °C; 48% overall yield. MS (EI) *m/z*: 296 ([*M*]$^+$), 236 (100); ^{1}H NMR ([D$_6$]acetone): δ = 3.02 (dd, 1H, *J* = 9.4, 14.9), 3.12–3.60 (m, 4H), 3.72 (s, 3H), 3.97 (d, 1H, *J* = 14.9), 4.07 (d, 1H, *J* = 14.6), 5.15 (br s, 1H), 5.84 (br s, 2H), 6.64 (dd, 1H, *J* = 2.8, 8.4), 6.83 (d, 1H, *J* = 2.8), 7.06–7.23 ppm (m, 5H); IR (Nujol): $\tilde{\nu}$ = 3454, 3338, 3272, 1647, 1578 cm^{-1}; anal. ($C_{18}H_{20}N_2O_2$) C, H, N.

***N*-(8-Methoxy-10,11-dihydro-5*H*-dibenzo[a,d]cyclohepten-10-ylmethyl)acetamide (5)** was prepared as previously reported.[16]

***N*-(8-Methoxy-10,11-dihydro-5*H*-dibenzo[a,d]cyclohepten-10-ylmethyl)-*N*-methylacetamide (6)**. Sodium hydride (60% in mineral oil, 0.03 g, 0.65 mmol) and methyl iodide (0.04 mL, 0.67 mmol) were added to a solution of **5** (0.15 g, 0.52 mmol) in dry DMF (5.6 mL). The reaction mixture was stirred at room temperature for 18 h, then poured into ice-cooled H_2O and extracted with EtOAc. The combined organic phases were washed with brine, dried (Na_2SO_4), and evaporated to give the crude desired product that was purified by flash chromatography (CH_2Cl_2/EtOAc 9:1 as eluent) and crystallization (Et_2O/petroleum ether). White solid, mp: 104 °C; 37% yield. MS (EI) *m/z*: 309 ([*M*]$^+$), 236 (100); ^{1}H NMR ([D$_6$]DMSO; registered at 120 °C): δ = 1.86 (s, 3H), 2.92 (s, 3H), 2.62–3.87 (m, 5H), 3.70 (s, 3H), 3.92 (d, 1H *J* = 15.0), 4.10 (d, 1H, *J* = 15.0), 6.66 (m, 2H), 7.05–7.18 ppm (m, 5H); anal. ($C_{20}H_{23}NO_2$) C, H, N.

***N*-(8-Hydroxy-10,11-dihydro-5*H*-dibenzo[a,d]cyclohepten-10-ylmethyl)acetamide (7)**. A solution of BBr_3 (1 M in CH_2Cl_2, 1.1 mL) diluted with dry CH_2Cl_2 (5 mL) was added dropwise to a solution of **5**

(0.32 g, 1.08 mmol) in dry CH_2Cl_2 (8 mL) at 0 °C, and the resulting mixture was stirred at room temperature for 6 h. After distillation of the solvent, the residue was partitioned between H_2O and EtOAc. The combined organic phases were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure to yield a crude residue that was purified by crystallization (Et_2O). White solid, mp: 218–220 °C; 76% yield. MS (EI) *m/z*: 281 ($[M]^+$), 222 (100); ^{1}H NMR ([D$_6$]acetone): $\delta = 1.93$ (s, 3H), 2.98 (dd, 1H, $J = 14.1$, 8.9), 3.06–3.42 (m, 3H), 3.57 (m, 1H), 3.92 (d, 1H, $J = 14.5$), 4.05 (d, 1H, $J = 14.9$), 6.55 (dd, 1H, $J = 2.5$, 8.1), 6.74 (d, 1H, $J = 2.5$), 7.00–7.19 (m, 5H), 7.30 ppm (br s, 1H); IR (Nujol): $\tilde{\nu} = 3388$, 3141, 1640, 1535 cm^{-1}; anal. ($C_{18}H_{19}NO_2$) C, H, N.

(11-Acetylaminomethyl-10,11-dihydro-5*H*-dibenzo-[a,d]cyclohepten-2-yloxy)acetic acid methyl ester (8). Sodium hydride (80% in mineral oil, 0.03 g, 1.16 mmol) and methyl chloroacetate (0.08 mL, 0.91 mmol) were added to a stirred solution of **7** (0.12 g, 0.42 mmol) in dry DMF (1.6 mL) at −10 °C under N_2 atmosphere, and stirring was continued for a further 4 h. The mixture was poured into ice-cooled H_2O and extracted with EtOAc. The combined organic phases were washed with H_2O, dried (Na_2SO_4), and evaporated to give a crude residue that was purified by flash chromatography (EtOAc as eluent) and crystallization (Et_2O). White solid, mp: 146–9 °C; 81% yield. MS (EI) *m/z*: 353 ($[M]^+$), 221 (100); ^{1}H NMR ($CDCl_3$): $\delta = 1.95$ (s, 3H), 2.98 (m, 1H), 3.27–3.60 (m, 4H), 3.80 (s, 3H), 3.88 (d, 1H, $J = 15.0$), 4.17 (d, 1H, $J = 15.0$), 4.60 (s, 2H), 5.47 (br s, 1H), 6.66 (dd, 1H, $J = 2.7$, 8.2), 6.78 (d, 1H, $J = 2.7$), 7.11–7.22 ppm (m, 5H); IR (Nujol): $\tilde{\nu} = 3280$, 1736, 1643 cm^{-1}.

***N*-[8-(2-Hydroxyethoxy)-10,11-dihydro-5*H*-dibenzo-[a,d]cyclohepten-10-ylmethyl)acetamide (9)**. A suspension of **8** (0.10 g, 0.28 mmol) in dry THF (7 mL) was added dropwise to an ice-cooled stirred suspension of $LiAlH_4$ (0.02 g, 0.52 mmol) in dry THF (7 mL) under N_2. Upon completion of the addition, the mixture was stirred at 0 °C for 1 h, then H_2O was carefully added to destroy the unreacted $LiAlH_4$, and the resulting mixture was filtered through a celite pad. The filtrate was concentrated in vacuo, and the residue was partitioned between H_2O and EtOAc. The combined organic phases were washed with brine, dried (Na_2SO_4), and evaporated to yield the crude desired product that was purified by flash chromatography (EtOAc/MeOH 95:5 as eluent) and crystallization (CH_2Cl_2/Et_2O). White solid, mp: 135 °C; 80% yield. MS (EI) *m/z*: 325 ($[M]^+$), 266 (100); ^{1}H NMR ($CDCl_3$): $\delta = 1.94$ (s, 3H), 2.97 (dd, 1H, $J = 14.9$, 8.6), 3.29–3.66 (m, 4H), 3.87 (d, 1H, $J = 14.9$), 3.94 (m, 2H), 4.05 (m, 2H), 4.20 (d, 1H, $J = 15.2$), 5.55 (br s, 1H), 6.69 (dd, 1H, $J = 2.6$, 8.3), 6.81 (d, 1H, $J = 2.6$), 7.10–7.22 ppm (m, 5H); IR (Nujol): $\tilde{\nu} = 3276$, 3074, 1638, 1561 cm^{-1}; anal. ($C_{20}H_{23}NO_3$) C, H, N.

(11-Acetylaminomethyl-10,11-dihydro-5*H*-dibenzo-[a,d]cyclohepten-2-yl)trifluoromethansulfonate (10). Trifluoromethanesulfonic anhydride (0.21 mL, 1.25 mmol) was added dropwise to an ice-cooled suspension of **7** (0.20 g, 0.71 mmol) in dry CH_2Cl_2 (20 mL) and pyridine (0.25 mL, 3.1 mmol), and the resulting mixture was stirred at room temperature for 2 h. The reaction mixture was washed twice with 2 N HCl, once with a saturated solution of $NaHCO_3$, and once with brine. The organic phase was dried (Na_2SO_4) and evaporated to yield a crude product that was purified by flash chromatography (cyclohexane/EtOAc 3:7 as eluent), oil; 78% yield. MS (EI) *m/z*: 413 ($[M]^+$), 72 (100); ^{1}H NMR ($CDCl_3$): $\delta =$ 2.00 (s, 3H), 2.99 (dd, 1H, $J = 14.9$, 8.7), 3.28–3.58 (m, 3H), 3.39 (dd, 1H, $J = 3.9$, 14.9), 3.96 (d, 1H, $J = 15.1$), 4.25 (d, 1H, $J = 15.1$), 5.67 (br s, 1H), 7.03 (dd, 1H, $J = 2.6$, 8.2), 7.08 (d, 1H, $J = 2.5$), 7.12–7.31 ppm (m, 5H); anal. ($C_{19}H_{18}F_3NO_4S$) C, H, N.

***N*-(8-Methyl-10,11-dihydro-5*H*-dibenzo[a,d]cyclohepten-10-ylmethyl)acetamide (11)**. A stirred mixture of **10** (0.23 g, 0.56 mmol), tetramethyltin (0.16 mL, 1.11 mmol), bis(triphenylphosphine)palladium chloride(II) (0.02 g, 0.03 mmol), LiCl (0.07 g, 1.72 mmol), and 2,6-di-*tert*-butyl-4-methylphenol (a few mg) in dry dioxane (5 mL) and dry DMF (0.5 mL) was heated at 110 °C in a sealed flask for 24 h. The reaction mixture was filtered through a celite pad and the filtrate concentrated in vacuo. The residue was dissolved in EtOAc, washed with brine, dried (Na_2SO_4), and evaporated to give a crude product that was purified by flash chromatography (cyclohexane/EtOAc 3:7 as eluent) and crystallization (Et_2O/petroleum ether). White solid, mp: 127–8 °C; 67% yield. MS (EI) *m/z*: 279 ($[M]^+$), 220 (100); ^{1}H NMR ($CDCl_3$): $\delta = 1.95$ (s, 3H), 2.29 (s, 3H), 3.00 (dd, 1H, $J = 14.6$, 8.8), 3.30–3.66 (m, 3H), 3.35 (dd, 1H, $J = 3.9$, 14.7), 3.92 (d, 1H, $J = 15.1$), 4.19 (d, 1H, $J = 14.8$), 5.42 (br s, 1H), 6.95 (dd, 1H, $J = 2.5$, 8.1), 7.01 (d, 1H, $J = 2.5$), 7.08–7.22 pppm (m, 5H); IR (Nujol): $\tilde{\nu} = 3276$, 1638, 1561 cm^{-1}; anal. ($C_{19}H_{21}NO$) C, H, N.

***N*-(8-Acetyl-10,11-dihydro-5*H*-dibenzo[a,d]cyclohepten-10-ylmethyl)acetamide (12)**. A stirred mixture of **10** (0.20 g, 0.48 mmol), tetramethyltin (0.08 mL, 0.57 mmol), $PdCl_2$(dppf) (0.02 g, 0.03 mmol), LiCl (0.06 g, 1.47 mmol), molecular sieves (4 Å, 50 mg) and 2,6-di-*tert*-butyl-4-methylphenol (a few mg) in dry DMF (4 mL) was heated at 90 °C for 20 h under a CO atmosphere. The reaction mixture was filtered (celite), the filtrate concentrated in vacuo and partitioned between H_2O and EtOAc. The organic phase was washed with brine, dried (Na_2SO_4), and evaporated to give a crude product that was purified by flash chromatography (EtOAc as eluent). Beige solid, 10% yield. MS (EI): *m/z*: 307 ($[M]^+$), 69 (100); ^{1}H NMR ($CDCl_3$): $\delta = 1.98$ (s, 3H), 2.57 (s, 3H), 3.02 (dd, 1H, $J = 14.3$, 8.5), 3.38 (dd, 1H, $J = 3.8$, 14.6), 3.47–3.60 (m, 3H), 4.01 (d, 1H, $J =$ 14.9), 4.27 (d, 1H, $J = 14.9$), 5.48 (br s, 1H), 7.10–7.33 (m, 5H), 7.72 (dd, 1H, $J = 2.5$, 7.9), 7.80 ppm (dd, 1H, $J = 2.5$); anal. ($C_{20}H_{21}NO_2$) C, H, N.

Preparative enantiomeric separation and determination of enantiomeric excess

Semipreparative separation of the racemate **5** was carried out in a Büchi borosilicate column, (40 × 3.0 cm i.d.), filled with a slurry of TAC (100 g) in EtOH/H_2O 6:4 and packed following a previously described procedure.[20] Solvent delivery system: Gilson 307 pump; mobile phase: EtOH/H_2O 6:4; flow rate: 1.0 mL min^{-1}; column temperature: 25 °C; sample concentration: 4% (*w/v* in EtOH); amount injected: 30 mg. Detection at 280 nm was carried out using a Waters Lamba-Max Model 480 tunable absorbance detector. $[\alpha]_D^{25}$ values were determined on a PerkinElmer 241 at 25 °C.

As the chromatogram did not show a clear separation of the two enantiomers, each fraction was examined by analytical chiral HPLC [Chiralcel OD-H column (cellulose tris-3,5-dimethylphenylcarbamate; 250 × 4.6 mm i.d.; 10 μm; Daicel Chemical Industries, France; mobile phase: *n*-hexane/*i*PrOH, 4:1; flow rate: 1 mL min^{-1}] to decide which fractions should be collected or recycled onto the semipreparative column. Collected fractions were evaporated under reduced pressure, and the residue was purified by filtration on silica gel (cyclohexane/EtOAc 7:3 as eluent) and crystallization from Et_2O/petroleum ether. The enantiomeric purity obtained for the two collected fractions [(−)-**5**, e.r. 87:13; $[\alpha]_D^{20} = -25°$ ($c = 0.142$, $CHCl_3$); (+)-**5**, e.r. 85:15; $[\alpha]_D^{20} = +24°$ ($c = 0.078$, $CHCl_3$)] were sufficient for a first evaluation of their MLT receptor affinity.

Receptor binding experiments

Binding affinities of compounds were determined using 2-[^{125}I]iodomelatonin as the labeled ligand in competition experiments on cloned human MT_1 and MT_2 receptors expressed in NIH3T3 rat fibroblast cells. The characterization of NIH3T3-MT_1 and -MT_2 cells has already been described in detail.[33,34] With the exception of (+)-**5** and (−)-**5**, all the compounds were tested as racemic mixtures. Membranes were incubated for 90 min at 37 °C in binding buffer (Tris-HCl 50 mM, pH 7.4). The final membrane concentration was 5–10 μg of protein per tube. The membrane protein level was determined in accordance with a previously reported method.[35] 2-[^{125}I]Iodomelatonin (100 pM) and various concentrations of the new compounds were incubated with the receptor preparation for 90 min at 37 °C. Nonspecific binding was assessed with 10 μM MLT; IC_{50} values were determined by nonlinear fitting strategies with the program PRISM (GraphPad Software Inc., San Diego, CA, USA). The pK_i values were calculated from the IC_{50} values in accordance with the Cheng–Prusoff equation.[36] The pK_i values are the mean of at least three independent determinations performed in duplicate.

The intrinsic activity of the compounds was evaluated through [^{35}S]guanosine-5′-*O*-(γ-thiotriphosphate) ([^{35}S]GTPγS) binding in NIH3T3 cells stably transfected with human MT_1 or MT_2 receptors, as previously described.[33,34,37] Nonspecific binding was defined using GTPγS (10 μM). The data of [^{35}S]GTPγS binding experiments are given as the percentage of basal binding (basal stimulation is the amount of [^{35}S]GTPγS specifically bound in the absence of the compounds); basal binding was fixed at 100%. Under optimized conditions,[33,34] in cell lines expressing human MT_1 or MT_2 receptors, MLT (100 nM) produced a concentration-dependent stimulation of basal [^{35}S]GTPγS binding, with a maximal stimulation above basal levels of 370 and 250% in MT_1 and MT_2, respectively. Full agonists increased the basal [^{35}S]GTPγS binding in a concentration-dependent manner, like the natural ligand MLT, whereas partial agonists increased it to a much lesser extent; antagonists are without effect. To optimize [^{35}S]GTPγS binding and measure without uncertainty the percent variation in [^{35}S]GTPγS binding evoked by the test compounds relative to MLT, compounds were added at three different concentrations (one concentration was equivalent to 100 nM MLT, another was 10-fold less, and a third, 10-fold greater). The equivalent concentration was estimated on the basis of the ratio of the affinity of the test compound over that of MLT. It was assumed that at the equivalent concentration the test compound occupies the same number of receptors as MLT at 100 nM. All measurements were performed in triplicate. The relative intrinsic activity values (IA_r) were determined by dividing the maximum net stimulation of [^{35}S]GTPγS binding of a test compound by that of MLT, as measured in the same experiment.

Docking and MD simulations

Docking studies were performed by Glide 3.5,[30] employing a previously developed MT_2 receptor model.[17] Ligand geometry was optimized using the MMFF94s force field,[38] implemented in Macromodel 8.6,[39] to a gradient of 0.01 kJ(mol Å)$^{-1}$. As Glide does not perform conformational analysis on 7-membered rings, the various conformations of the 10,11-dihydro-5*H*-dibenzo[a,d]cycloheptene scaffold were built after a simulated annealing performed by Sybyl 7.2[40] (force field MMFF94s, 100 cycles from 700 to 0 K); four conformations of the tricyclic nucleus were generated for each enantiomer, having the torsion angles ϕ_1 (C5a–C9a–C10–C11) = 13°, −13°, 69° or −69° and ϕ_2 (C4a–C11a–C11–C10) = 69°, −69°, 13° or −13°, respectively. Protein structure was subjected to the Protein Preparation procedure implemented in Glide, selecting a neutralization zone of 10–20 Å around the ligand, docked within the putative binding site. Docking experiments were performed starting from minimum-energy conformations of the ligands placed in arbitrary positions, within a region centered on amino acids His 208, Trp 264, and Tyr 183, using enclosing and bounding boxes of 16 and 14 Å on each side, respectively. van der Waals radii of the protein atoms were not scaled, whereas van der Waals radii of the ligand atoms with partial atomic charge lower than |0.15| were scaled by 0.8. Standard precision mode was applied, with the amide bond of the ligand maintained fixed in *anti* disposition. Poses with a Coulomb–van der Waals score greater than 10 kcal mol^{-1} were rejected, while the other docking solutions were ranked according to their Emodel value. The Emodel is a ligand–receptor complex energy score that combines different contributions, the value of which is related to the likelihood of the ligand conformation and orientation within the binding site.

The best scoring solutions for poses A and B of (*R*)-**5** and (*S*)-**5** were merged into the MT_2 receptor model and the complexes were submitted to geometry optimization, with MMFF94s force field to an energy gradient of 0.01 kJ(mol Å)$^{-1}$, with fixed protein backbone. MD simulations were performed on the complexes to evaluate their stability, with MMFF94 force field,[41] a time step of 1 fs for 1 ns after equilibration for 100 ps at 310 K, with fixed protein backbone.

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